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Aflatoxin contamination in peanuts commercially available in Sri Lanka

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Abstract

Peanut is one of the oil seeds available in Sri Lanka. Peanuts and peanut products are considered as popular food items among all age groups of population, especially in urban areas. People prefer to buy peanut based food items as snacks. Peanut is shown to be a good substrate for the growth of *Aspergiillus* sp. and for the production of aflatoxins. Aflatoxins are potent teratogenic, mutagenic, and carcinogenic mycotoxin proven to be contained in food and feed. Present work was carried out as a preliminary study to determine the level of aflatoxin contamination in commercially available roasted and fried peanut samples collected from venders in Colombo city, Sri Lanka. Samples were tested for aflatoxins by the CB method of the Association of Official Analytical Chemists. Degree of aflatoxin contamination was determined by densitometric evaluation. For the method validation of aflatoxin analysis, two extraction and cleanup procedures were performed. Chloroform/water extraction and Sep Pac cartridge clean up procedure were selected as the most reliable methods. Results of the study showed that 6.6 % of the tested peanut samples were moderately contaminated (12.5 ppb) and the remaining was fallen into the category of uncontaminated according to the FAO and WHO standards. Overall result of the study showed that the samples were not significantly contaminated and unnecessary doubt on aflatoxin contamination is not needed. However, effective constant monitoring, surveillance and control programs on aflatoxins contamination are important to protect consumers from aflatoxin toxicities. Therefore, screening programs which prevents unsuitable products entering to the market are recommended to evaluate the contamination.

Key words: Peanut, aflatoxin, method validation, *Aspergillus flavus, A. parasiticus*

Introduction

Aflatoxins are one of the most potent toxic and carcinogenic metabolites produced by certain fungi; *Aspergillus flavus* and *Aspergillus parasiticus* (Davis & Diener 1983) in or on foods and feeds (Gopalan *et al.,* 1972). Major toxin produced by *A. flavus* are AFB_1 and AFB_2 whereas, *A. parasiticus* produces two additional toxins AFG_1 and AFG_2 (Davis & Diener, 1983) in addition to above. Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and human in the whole world. Recent information shows that some food items such as maize, peanut, copra and other oily seeds are more susceptible for aflatoxin contamination (Dutta and Das, 2000). Among them, very high level of afalatoxin accumulation is shown in peanuts (ground nuts) and is used in commercial preparation of aflatoxin (Dutta and Das, 2000; Coden *et.al.,* 1963). Aflatoxin B_1 is the most important aflatoxin as it grows on peanuts and grains before or after harvesting and also under poor storage conditions (Coden *et.al.,*1983). The occurrence of aflatoxins is also influenced by certain environmental factors. Hence, the extent of contamination will vary with geographic location, agricultural and agro economic practices and the susceptibility of commodities to fungal invasion during pre-harvest, storage and processing period (Dawson, 1991) often exceeds 15 $\mu g\ kg^{-1}$ (15ppb) which is the tolerable level of contamination established by for human consumption the upper limits initially established by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) (Adriabn, 1992).

The toxicity may cause acute or chronic diseases in human (Dawson, 1991) and out break of the aflatoxin hepatitis in human were reported in India in 1975 and Kenya in 1982 (Bhat, 1991). Aflatoxin is also associated with hepatocellular damage, necrosis, cholestosis, hepatoma, acute hepatitis, hemorrhage, jaundice, cirrhosisn in malnourished children and Kwashiorkor victims (Groupman *et. al.,* 1988). In 1993, the International Agency for Research on Cancer (IARC) classified AFB_1 and a mixture of aflatoxins as carcinogens that can cause cancer in human (Groupman *et. al.,* 1988). In tropical countries, it has been estimated that 25% or more of the food were contaminated with aflatoxin especially due to drought stress and insect damage (Codner *et.al.,* 1983). This necessitated a detailed study of the aflatoxin problem in relation to peanut products which have the highest risk of aflatoxin contamination.

In Sri Lanka cultivation and consumption of peanut are increased due to its high nutritional value. Peanut oil is a good substitute for coconut oil for cooking purposes and its pressed cake is used as a nutrient component in animal feeds

(Samarajeewa, 1984). In addition, peanut products such as fried peanuts and roasted peanuts are popular food items among people living in urban areas and peanut butter and other sweet preparations are important food items for human. Recently, preparation of peanut products has become popular home based self employment and there is a tendency for poor quality products to be introduced to the market. Thus, it is important to determine the level of aflatoxin contamination in peanuts and its products to implement suitable preventive measures against any toxic effects because, significant levels of contamination will create health problems. Therefore, the present preliminary survey was carried out to determine the most reliable method to analyze aflatoxins contamination levels of commercially available peanuts in Pettah region in the Colombo city, Sri Lanka.

Materials and Methods

Sample collection

Roasted and fried peanut samples (250g) were collected randomly from twenty peanut retailers in Pettah region, Colombo city, during July 2003. Samples were stored at 0 – 2 °C for pending assay. Corning and quartering method was employed to composite samples. Finally 50g was ground and from which 10g was taken for aflatoxin analysis (Whitarer *et.al.,* 1995).

Analysis

Two methods, namely Modified Association of Official Analytical Chemist, Contaminated Branch Method (AOAC, CB, 2000) and Aqueous Acetone Method (AOAC, 2000) were employed for the extraction of aflatoxins (Horwitz, 2000, Kauouri *et. al.,* 1999, Park *et. al.,* 1994). As aflatoxins are present in very low concentrations and due to the presence of lipids and other substances, a strong concentration and clean up procedures were necessary to make possible detection. A silica gel mini column cleanup (AOAC, 2000) and lead acetate cleanup (AOAC, 2000) procedures were employed separately for cleaning up (Horwitz, 2000). Extraction was done using chloroform. The extract was evaporated under the reduced pressure at <55 °C. The dried extract was then dissolved in 1 ml of chloroform, transferred to a 2 ml vial and it was kept well stopered to prevent evaporation. Estimation of aflatoxin AF B_1, AFB_2, AFG_1 and AFG_2 were done by Thin Layer Chromatographic (TLC) densitometric method (Park *et. al.,* 1994). 20µl aliquots of purified sample and a mixture of standard aflatoxins were spotted along side and Chloroform: Acetone: Water (88: 11.5: 05) solvent system was used. Dried TLC plates were examined under 365nm UV light. Visual estimation was difficult as fraction range was ±20% under ideal conditions. Therefore, developed plates were subjected to densitometric scanning

at 365nm using an auto-fluorescence type densitometer. Each spot was recorded as a peak and the concentration of each spot was calculated by the equation given on AOAC 49. 2. 19 (2000).

Concentrations of AFB_1, AFB_2, AFG_1 and AFG_2

$$\mu g/kg = B \times Y \times S \times V / Z \times X \times W$$ where,

B = average area of AFB_1 peaks in test solution, Y = Concentration of AFB_1 standard (µg/kg), S = Volume of AFB_1 standard (µl), V = Final volume of test solution (µl), Z = Average area of AFB_1 peak in standard solution, X = Spotted volume of test solution (µl), W = Sample represented by test solution (g)

Method validation for Aflatoxin analysis

Two extractions and clean up procedures were considered in order to determine the most suitable and reliable method for aflatoxin analysis. The concentration of the given individual aflatoxin solution was determined by the spectrometric method (AOAC, 49; 03/971, 22, 2000), using the equation given bellow.

$$C = A \times Mw \times 1000 / E$$

Where; C = Concentration, A = Absorption, Mw = Molecular weight, E = Molar absorption

The aflatoxin free sample was spiked with 200 ppb aflatoxin standard. The toxin was extracted by chloroform water extraction with Sep Pack Cartridge clean up procedure and acetone water extraction with lead acetate clean up procedures. The results of visual and densitometry evaluation were used to select the most reliable method.

Identification of aflatoxin

Four clearly identifiable spots were observed in reference standard. The patterns of the spots in test solution were compared with spots given by standard aflatoxin mixture.

Quantification

Quantification of aflatoxin was done by measuring intensity of fluorescence of the aflatoxin spots in the TLC plate by using a auto-fluorescence type densitometer, (Irradiation 356 nm, emission at 420- 460 nm), AOAC 49.2.19/980.20). Fifteen samples were analyzed by TLC visual estimation and eight of them were analyzed by the densitometric scanning.

Results and Discussion

Acetone water extraction and lead acetate clean up procedure were encountered to prevent problems due to the presences fatty materials, pigments and other impurities in the extracts. Additional centrifugation step was needed to remove unwanted particles as extra bands due to impurities were observed other than AFB_1 in spot a, b, and c (Plate 1). Densitometric analysis of AFB_1 by acetone water extraction standard showed that the concentration of aflatoxins were lower than 50 ppb in all spikes indicating the removal of standard AFB_1 at high percentage during the extraction and cleanup procedures (Table 1). In contrast, analysis of standard AFB_1 (200 ppb) by chloroform water extraction and sep pack cartridge clean up procedure gave a higher level than 100ppb (Table 1). The results indicated that the recovery of the latter method was greater than 50% (Table 1) and unwanted bands were not detected at **g, h** and **i** positions (Plate 1).

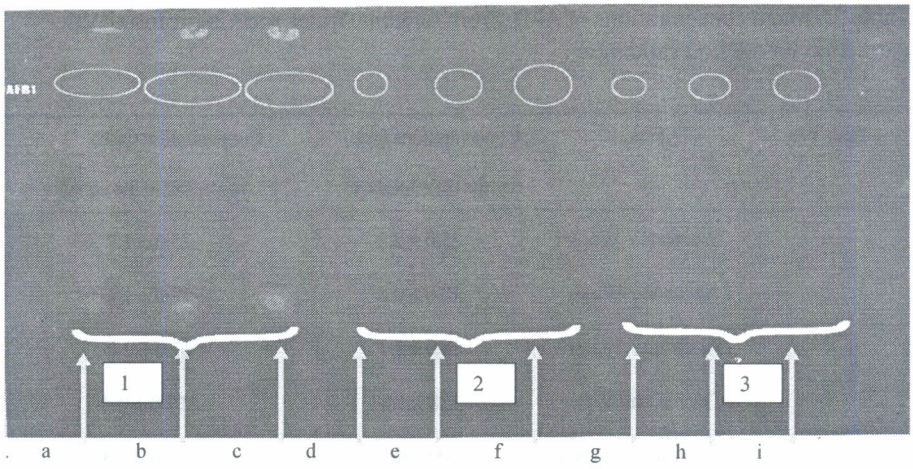


Plate 1. The separation of test and standard samples for method validation 1. Acetone water extraction, lead acetate cleanup, 2. Aflatoxin B1 standard (AFB_1) 3. Chloroform water extraction, silica gel mini column/sep pac cartridge clean up.

Table 1: Method validation for densitometric analysis of AFB_1

Spot No.	Method	Vol.(µl)	Dis. (mm)	Concentration (ppb) (by the densitometer)			Concentration (ppb) (According to the equation)		
				T1	T2	T3	T1	T2	T3
a	Acetone/water	5	15	15.5	28.3	31.1	15.5	28.3	31.1
b	Acetone/water	10	32	11.5	21.0	23.1	11.5	21.0	23.1
c	Acetone/water	15	49	11.2	20.4	22.4	11.2	20.2	22.4
h	Chloroform/water	10	134	86.1	156.8	172.0	86.1	156.8	172.0
i	Chloroform/water	15	151	65.4	119.0	117.1	98.0	178.5	195.7

T1, T2, T3 triplicate sample for each analysis

Thus, the overall results showed that the chloroform water extraction and Sep Pac Cartridge clean up procedure are more efficient than acetone water extraction and lead acetate clean up method (Table 1). Therefore, the chloroform water extraction and Sep Pack Cartridge clean up method was selected (AOAC CB) to analyze aflatoxin.

The levels of aflatoxin AFB_1 in peanuts from Colombo city detected by the densitometric concentration method and the calculation method lower than the (Table 2) previously reported data (Table 3). It has been documented by Samarageewa (1984) that the mean level of AFB_1 in peanuts in Colombo area was 889 ± 1154 $\mu g\ kg^{-1}$ (ppb). The mean concentration of AFB_1 in the presence study was 1.98 ± 4.28 ppb.

Table 2: Mean concentrations of AFB_1 from Acetone/Water and Chloroform/Water extraction for method validation

Spot No.	Method i	Concentrations (ppb) (by the densitometer)	Concentration (ppb) (According to the equation)
a	Acetone / Water	25.0 ± 2.2	25.0 ± 1.7
b	Acetone / Water	18.6 ± 0.8	18.6 ± 1.2
c	Acetone / Water	18.0 ± 1.3	17.0 ± 2.0
h	Chloroform/ Water	138 ± 1.6	157.0 ± 0.4
i	Chloroform/ Water	100 ± 0.7	157.0 ± 0.4

In this study, two samples out of eight were contaminated with AFB_1 greater than 1ppb and only one sample has 12 ppb (Table 4). The level of AFB_1 contamination in remaining sample was below 1 ppb. Therefore, in this study it was estimated that 6.6% of the tested samples were fallen into the category of moderately hazards (15ppb) levels of aflatoxin B_1 for human consumption according to the FAO and WHO standard (Adriabn, 1992). However, in the numbers of samples tested were low due to the limited facilities and chemicals available for this study.

Table 3: Mean concentrations of AFB_1 in commercially available peanuts in Colombo city.

Location	No. of samples tested	No. of samples within the tolerance levels	Mean (ppb)	Range
Colombo[a] (1984)	28	6	889 ± 1154	ND - 4500
Colombo[b] (2004)	8	7	1.98 ± 4.27	0.002 – 12.5

[a]*Measurement of aflatoxins contamination before Samarajeewa* (1984), [b] *Measurement of aflatoxins*

contamination after Samarajeewa (1984).

[b]*Measurement of aflatoxins contamination in the Present study, ND – Not detected (minimum detectable level is 5µg/kg)*

Table 4: Mean concentrations of AFB_1 from dencitometric and equation analysis.

TLC No.	Total concentration (ppb)	
	Concentration	**Equation**
a	0.04	0.04
b	0.20	0.20
c	0.02	0.02
d	0.49	0.61
e	**12.52**	**13.79**
h	0.32	0.27
J	1.41	1.37

In 1984, Samarajeewa documented that the percentages of samples contaminated with aflatoxin AFB_1 at hazards levels of human consumption (above 100 ppb) in Colombo city (Table 3). Thereafter, public awareness programs were made through talks and announcements, through the press, seminars and radio news to educate on aflatoxin contamination. Awareness programs were also organized for the manufactures of processed peanuts to prevent or reduce such contamination. In the present study all the samples were with very low contamination of AFB_1. This may be due to the improvement of storage facilities, manual removal of contaminated peanuts from the original lot and preventing moulding in the field. Educating warehouse owners on proper handling and storing of raw peanuts, better sealing procedures and other precautionary measures must have led to minimize moulding during storage. It was also found in the study that the decreasing tendency of entering highly contaminated peanuts into the local market is due to the fact that such peanut cannot be sold; as consumers and manufactures would not buy or process such peanuts. As a result, the AFB_1 contamination was very low or insignificant. Thus, unnecessary dought is not needed regarding AFB_1 contamination in roasted and fried peanuts in the market. However, continuous monitoring program is strongly recommended to evaluate the aflatoxin levels, of commercially available peanuts and its products in the future to be protected people from aflatoxin toxicity.

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