Aflatoxin contamination in peanuts commercially available in Sri Lanka

D.M.M.P. Dissanayake and Pathmalal M. Manage

Department of Zoology,
University of Sri Jayewardenepura,
Nugegoda, 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 Aspergillus 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 vendors 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 AFB₁ and AFB₂ whereas, *A. parasiticus* produces two additional toxins AFG₁ and AFG₂ (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 aflatoxin accumulation is shown in peanuts (ground nuts) and is used in commercial preparation of aflatoxin (Dutta and Das, 2000; Coden et al., 1963). Aflatoxin B₁ 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 μg kg⁻¹ (15 ppb) 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 outbreak 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, cholestasis, hepatoma, acute hepatitis, hemorrhage, jaundice, cirrhosis in malnourished children and Kwashiorkor victims (Groupman et al., 1988). In 1993, the International Agency for Research on Cancer (IARC) classified AFB₁ 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.
Aflatoxin contamination in peanuts (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 aflatoxin 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 AFB1, AFB2, AFG1 and AFG2 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 \( \text{AFB}_1, \text{AFB}_2, \text{AFG}_1, \text{and AFG}_2 \)

\[
\mu g/\text{kg} = B \times Y \times S \times V / Z \times X \times W
\]

where,

- \( B \) = average area of \( \text{AFB}_1 \) peaks in test solution,
- \( Y \) = Concentration of \( \text{AFB}_1 \) standard (\( \mu g/\text{kg} \)),
- \( S \) = Volume of \( \text{AFB}_1 \) standard (\( \mu l \)),
- \( V \) = Final volume of test solution (\( \mu l \)),
- \( Z \) = Average area of \( \text{AFB}_1 \) peak in standard solution,
- \( X \) = Spotted volume of test solution (\( \mu l \)),
- \( W \) = 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 below.

\[
C = A \times Mw \times 1000 / E
\]

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, in spot a, b, and c (Plate 1). Densitometric analysis of AFB, 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, at high percentage during the extraction and cleanup procedures (Table 1). In contrast, analysis of standard AFB, (200 ppb) by chloroform water extraction and sep pack cartridge clean up procedure gave a higher level than 100 ppb (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, ) 3. Chloroform water extraction, silica gel mini column/sep pac cartridge clean up.

Table 1: Method validation for densitometric analysis of AFB,  

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

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 AFB₁ 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 AFB₁ in peanuts in Colombo area was $889 \pm 1154 \, \mu g \, kg^{-1}$ (ppb). The mean concentration of AFB₁ in the presence study was $1.98 \pm 4.28 \, \text{ppb}$.

Table 2: Mean concentrations of AFB₁ from Acetone/Water and Chloroform/Water extraction for method validation

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

In this study, two samples out of eight were contaminated with AFB₁ greater than 1 ppb and only one sample has 12 ppb (Table 4). The level of AFB₁ 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 (15 ppb) levels of aflatoxin B₁ 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 AFB₁ in commercially available peanuts in Colombo city.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of samples tested</th>
<th>No. of samples within the tolerance levels</th>
<th>Mean (ppb)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombo⁴ (1984)</td>
<td>28</td>
<td>6</td>
<td>$889 \pm 1154$</td>
<td>ND - 4500</td>
</tr>
<tr>
<td>Colombo⁵ (2004)</td>
<td>8</td>
<td>7</td>
<td>$1.98 \pm 4.27$</td>
<td>$0.002 - 12.5$</td>
</tr>
</tbody>
</table>
Aflatoxin contamination in peanuts


Measurement of aflatoxins contamination in the Present study. ND – Not detected (minimum detectable level is 5μg/kg)

Table 4: Mean concentrations of AFB\textsubscript{1} from densitometric and equation analysis.

<table>
<thead>
<tr>
<th>TLC No.</th>
<th>Total concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
</tr>
<tr>
<td>a</td>
<td>0.04</td>
</tr>
<tr>
<td>b</td>
<td>0.20</td>
</tr>
<tr>
<td>c</td>
<td>0.02</td>
</tr>
<tr>
<td>d</td>
<td>0.49</td>
</tr>
<tr>
<td>e</td>
<td>12.52</td>
</tr>
<tr>
<td>h</td>
<td>0.32</td>
</tr>
<tr>
<td>J</td>
<td>1.41</td>
</tr>
</tbody>
</table>

In 1984, Samarajeewa documented that the percentages of samples contaminated with aflatoxin AFB\textsubscript{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 manufacturers of processed peanuts to prevent or reduce such contamination. In the present study all the samples were with very low contamination of AFB\textsubscript{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\textsubscript{1} contamination was very low or insignificant. Thus, unnecessary doubt is not needed regarding AFB\textsubscript{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.
Acknowledgments

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References

Adibian, R.E. (1992) Microbial food poisoning, Department of Clinical Microbiology, University of Sheffield Medical School, UK, 2-6,85.


