

The Occurrence of Aflatoxins in Peanuts in Supermarkets in Ahvaz, Iran

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Abstract

A human's exposure to aflatoxins occurs quite easily due to the fact that all one has to do is consume food containing this chemical. Due to the adverse effects of aflatoxins such as immune deficiency and carcinogenicity on humans, the analyzing of them in food items is necessary for assuring the health of consumers. This research was necessary because there is a lack of supply of data for aflatoxins in Ahvaz, Iran. For this purpose the peanut samples were purchased from some of existing supermarkets in Ahvaz and analyzed for the determination of aflatoxins concentration in peanuts by a TLC scanner. In total, 59.26% of samples were contaminated with aflatoxins, 14.8% of samples were found to contain above of 20 ppb, above the maximum level of total aflatoxins permitted in Iran.

Keywords: aflatoxin, TLC scanner, peanut

1. Introduction

Mycotoxins are a closely related group of secondary fungal metabolites. Aflatoxins are one of the most studied mycotoxins that are produced by *aspergillus flavus* and *aspergillus parasiticus* (Koehler et al., 1975; Wood, 1992; Wilson & King, 1995; Efuntoye, 1999; Yong & Cousin, 2001; Ezekiel et al., 2012). Among the various mycotoxins, aflatoxins have been the subject of intensive research because they are potent carcinogens and have many adverse effects on their consumers (Pohland, 1993; Boutrif, 1998; Hoogenboom et al., 2001; Wild & Gong, 2010). The human liver is the main target organ for aflatoxins. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and this leads to necrosis or liver cell death (Hussein & Brasel, 2001). The mechanism contributing to cell necrosis is the reaction of aflatoxin metabolites with different cell proteins, which leads to inhibition of carbohydrate and lipid metabolism and protein synthesis (Fink-Grenmels, 1999; Wild & Turner, 2002). The major members of aflatoxins are designated as B₁, B₂, G₁ and G₂ (Clifford et al., 1967). Among them B₁ is one of the most potent environmental mutagens and carcinogens known (Wogan & Newberne, 1967; Butler et al., 1969; Wong & Hsieh, 1976; Groopman et al., 1988; Eaton & Gallagher, 1994). B₂ and G₂ are less toxic dihydro derives of B₁ and G₂ (Clifford et al., 1967). Human exposure to aflatoxins may occur by consumption of foods that have been contaminated by certain strains of *A. flavus* or *A. parasiticus* during growth, harvest, or storage (Alpert et al., 1971; Vasanthi & Bhat, 1998; Lewis et al., 2005). According to The Food and Agricultural Organization (FAO), 25% of the world's food products are affected by mycotoxins, most of them are aflatoxins (Leslie et al., 2008). Nearly all agricultural products are potentially subjects to the contamination of aflatoxins. No one in the scientific society can easily forget the tragedy of acute aflatoxicosis in Kenya. This was one of the most severe cases of human aflatoxin poisoning in history, with a death total of 125, with a total of 317 cases due to ingestion of contaminated maize (Lewis et al., 2005). Ahvaz is a city in the south of Iran that has a climate suitable for growing fungi in agriculture products and therefore a mycotoxins challenge should always be considered. Peanut and peanut products which are widely utilized for food or food products would seem to constitute the highest risk of aflatoxin contamination (Sorenson et al., 1984). Many methods for measuring the levels of aflatoxins in commodities have been established such as gas chromatography, High Performance Liquid Chromatography (HPLC), Enzyme-Linked Immunosorbent assay (ELISA), Enzyme Multiplied Immunoassay Technique (EMIT), High Performance Thin Layer chromatography (HPTLC) and TLC

(Thin Layer Chromatography) scanner. Among these methods the TLC scanner method was found to be simple, robust, economic, and can be considered as an alternative to modern methods (Stroka et al., 2000). Due to the above factors, in this study for measuring levels of aflatoxins, TLC scanner method was used.

Objectives: This study was carried out to evaluate aflatoxins (B_1 , B_2 , G_1 and G_2) levels in raw and roasted peanuts of some existing supermarkets in Ahvaz.

2. Materials

2.1 Chemicals, Reagents, and Materials

All glassware in this study, before use, were soaked in dilute sulfuric acid (2M) for several hours, then rinsed extensively with distilled water to remove all traces of acid (checked by using pH paper), because acid may cause loss of aflatoxins. Fluted filter paper (24 cm diameter) was from Vicam Company, USA. Hexane, CH_3OH , benzene, $CHCl_3$, acetone and acetonitrile and toluene HPLC grade, Silica gel 60G and sodium chloride was purchased from Merck Co. Standard Aflatoxin B_1 , B_2 , G_1 and G_2 purchased from Biochemical Co. in Australia (1 mg of each) (Cunniff, 1995).

2.1.1 Working Standard Solution

The working standard mixture was made of aflatoxins B_1 , B_2 , G_1 , and G_2 in toluene - acetonitrile 98:2 containing 2 ppb of aflatoxins B_1 and G_1 and 1ppb G_2 and B_2 respectively.

2.1.2 Instruments

Camag TLC Scanner Switzerland 3, high speed Blender jar, USA, YO-2410000 Thermo Scientific Precision Eight-hole Steaming Bath,

3. Method of Extraction

In order to determine the aflatoxin's concentration in peanut, sample sizes were determined with G*Power software according to Large effect Size, then 27 samples were purchased from different supermarkets in different areas in Ahvaz. Each sample was ground into powder, stored in a glass bottle and kept in a freezer prior to analysis. Two 100 g of each of milled sample in Erlenmeyer, 500 ml hexane, 250 ml methanol 55% and 4 g NaCl were added and blended 1 min at high speed and then stood 30 min undisturbed in the jar. Aliquot 25 ml aqueous methanol phase was transferred into a 250 ml separator, and then 25 ml $CHCl_3$ was added to that and shaken 30-60 seconds. $CHCl_3$ layer was transferred to Erlenmeyer and evaporated to dryness on steam bath. Residue was quantitatively transferred to a vial with $CHCl_3$ and evaporated to dryness. Then residue was dissolved in 200 μ l benzene- acetonitrile (98:2). This mixture and the solutions of aflatoxin standards were spotted on the plate coated silica gel and developed with $CHCl_3$ -acetone (90:10) as mobile phase. T.L.C plate was removed, dried at room temperature, and scanned with T.L.C scanner equipped by densitometer. Aflatoxins concentration was determined from fluorescent intensity on thin layer chromatograms (Cunniff, 1995). The TLC scanner equipped with a mercury lamp was used to scan the developed plate at the wavelength of 366 nm (Stroka et al., 1999). The identification and quantification of fully separated aflatoxins was performed by comparison with aflatoxin standards using densitometry (Figure 1).

4. Results

The results are shown in Tables (1), (2), (3) and some of them in Figures (1). The results of this study show that in total, 59.26% of samples were contaminated with aflatoxins, 45.12% of samples were contaminated with B_1 at levels ranging from 0.37 to 3.7 μ g/kg, 14 / 32% of samples were contaminated with B_2 ranging from 0.2 to 42.05 μ g/kg, 18.25% of samples were contaminated with G_1 ranging from 0.11 to 225.8 μ g/kg and 22.31% samples were contaminated with G_2 ranging from 0.04 to 15.2 μ g/kg. Table 1 shows the mean and range of the investigated aflatoxins concentration in the peanut samples.

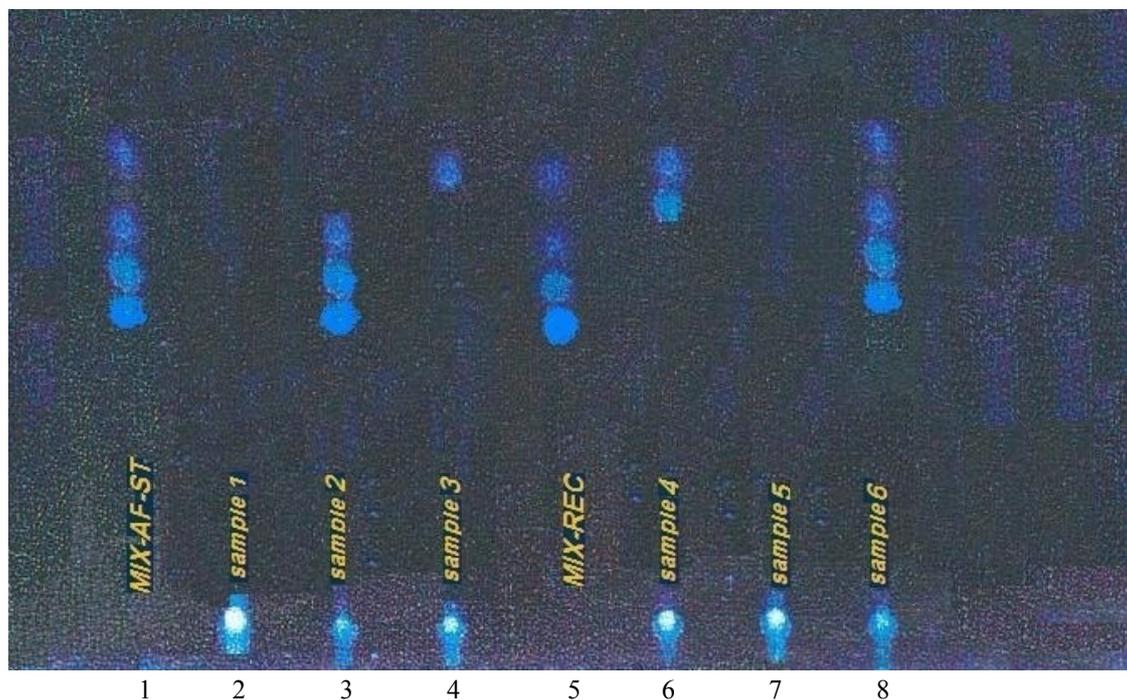


Figure 1. Florescent image of spotted samples on TLC

1: Mixture std B₁, B₂ G₁ and G₂, 5: mixture standard for recovery, 2, 3, 4, 6, 7, 8: spots of different peanut samples.

Table 1. Aflatoxins concentration in peanut in some of supermarkets Ahvaz – Iran ($\mu\text{g}/\text{kg}$)

Aflatoxin	Min	Max	Mean	Precision (RSD* %)
B ₁	0.37	3.7	0.305	6
B ₂	0.2	42.05	3.238	2.6
G ₁	0.11	225.8	12.074	6.7
G ₂	.04	15.2	0.665	5.4

*Relative Standard deviation.

LOD (limit of detection) and LOQ (limit of quantification) of aflatoxin contamination levels in peanut were determined (Table 2). LODs ranged from 0.1 to 0.2 ng/g for aflatoxins while the LOQs were found to be from 0.2 to 0.3 ng/g.

Table 2. The values of LOD and LOQ of aflatoxins by TLC scanner (ng/g)

Aflatoxins	LOD	LOQ
B ₁	0.6	1.5
B ₂	0.2	0.6
G ₁	0.6	1.5
G ₂	0.2	0.7

Also recovery was done and the results of recovery showed in Table 3.

Table 3. The values of recovery of aflatoxins

Aflatoxins	Recovery
B ₁	90.3
B ₂	91
G ₁	92
G ₂	90.7

5. Discussion

In developing countries, many individuals are chronically exposed to low levels of aflatoxins in their diet (Wild & Gong, 2010). At the same time, developed countries have satisfied-developed condition for controlling internal food quality standards; people in many of developing countries have no managed food systems and diet quality in their country. Unfortunately, very little information is available regarding the contamination of peanuts and peanut products in Ahvaz, so in this research we measured the aflatoxin B₁, B₂, G₁, G₂ in peanuts collected from existing supermarkets in Ahvaz and then analyzed them with the TLC scanner.

The results show that 14.8% of samples were found to contain above maximum levels of total aflatoxins residues to be permitted. In Iran, concentrations of up to 20 ppb are permitted in human foods (Lusas, 1979). The percent of concentration of aflatoxin B₁ is more than all of them and was 45.12%. Extraction was done well, aflatoxins were identified and determined and results of Recovery were in acceptable range, 90.3-92%.

In 2012 Siah Shadbad et al. determined aflatoxins B₁, B₂, G₁, G₂, in nuts (almond, walnut, seeds of apricot, sunflower seeds kernel, sesame seed, peanuts, pistachio, hazelnuts and cashews samples) of some of confectionaries in Tabriz by ELISA and HPLC methods. The results of their study showed that 33% of investigated peanuts were contaminated by aflatoxins at concentration less than 5 ppb (total aflatoxins) which was less than the maximum permitted level for total aflatoxins (20 ppb) in Iran. Comparison of data in this study with our research shows that the percent of contaminated peanuts with aflatoxins were less than our results in Ahvaz.

In 2010 Bakhiet et al. measured aflatoxin levels in 60 stored peanut in Sudan by TLC technique. Thirty five samples of the 60 stored (58.33%) gave positive readings with TLC technique, and in culture, *Aspergillus flavus* was isolated from twenty six samples (43.33%). The concentration of aflatoxin B₁ in these samples was ranged from low to very high, in range of (17.57-404.00 ppb kernel) and average concentration of aflatoxin B₁ was 23.7 ppb (Bakhiet and Musa). In our research, the percent of contaminated samples with aflatoxin B₁ (59.26%) are similar to investigated peanuts in this study (58.33%) and the average concentration of aflatoxin B₁ in our research (0.305 ppb) is lower than the investigated peanuts in this study (23.7 ppb).

In 2003 Younis et al. determined aflatoxin contamination in 400 samples of peanut products. The percentage of aflatoxin contamination was 2%, 64%, 14% and 11% for peanut kernels, peanut butter, peanut cake, and roasted peanuts respectively. The highest aflatoxin levels were detected in peanut kernels (3-8 ppb), while peanut cakes and roasted peanuts showed a moderate level of contamination (7-10 ppb), and (4-12 ppb) respectively. Within all contaminated samples and irrespective of the peanut type, the presence of aflatoxin B₁ was always predominant, followed by G₁, B₁ and G₂ (Younis & Malik, 2003). According to these results, in our research the incident of aflatoxins contamination in samples (58.33%) is more than this study and the concentration of aflatoxin B₁ in both studies are more than other aflatoxins.

In 2009 Carlos A. F. et al determined aflatoxin levels in peanut products traded in the Northeast region of São Paulo, Brazil by HPLC. For this purpose they collected 240 samples of peanut products traded in the cities of Araras, Leme, Pirassununga and Porto Ferreira from June 2006 to May 2007. The samples were analyzed for aflatoxins B₁ and G₁ by high performance liquid chromatography. Results showed 44.2% of the samples tested positive for aflatoxins at levels of 0.5 to 103.8 µg·kg⁻¹. Average concentrations of aflatoxins B₁ and G₁ in investigated peanut samples were 0.21 and 23.6 ppb respectively. Nine of the positive samples (3.7% of the analyzed samples) had total aflatoxin concentrations (B₁+B₂+G₁+G₂) higher than the limit established by Brazilian regulations (20 ppb) (Oliveira et al., 2009). In our research, the percent of contaminated samples with aflatoxins (58.33%) is more than investigated peanuts this study (44.2%) and the average concentration of aflatoxin B₁ in this study (0.21 ppb) is lower than the mean concentration of aflatoxin B₁ in our study (0.305 ppb) but the average concentration of aflatoxin G₁ in this study (23.6 ppb) is more than average concentration of aflatoxin G₁ (112.95 ppb) in our study.

In 2010, Hong et al. (2010) determined aflatoxins B₁ and B₂ in peanuts and corn based products by HPLC and fluorescence detector. Aflatoxins B₁ and B₂ were detected in 5 out of the 9 peanut samples and 5 out of the 11 corn based products that it means 59.87% of samples. and at levels ranging from 0.2 to 101.8 ppb (Hong G and Yusof 2010). In our research the incident of aflatoxin contamination in samples (59.26%) are similar to this study (59.87%). but the mean concentration of aflatoxin B₁ in peanut in our research (0.305 ppb) is lower than ones in this study.

Ali et al did a survey study in the Philippines in 1999 on peanut-based products. The study revealed that 60% of the samples were positive for aflatoxin B₁ in range of 1.00-244ppb (Ali et al., 2005). The average concentration of aflatoxin B₁ was 21.3 ppb. In our research the percent of contaminated samples with aflatoxin B₁ (59.26%) are similar to that in this study (60%). but the average concentration of aflatoxin B₁ in our research (0.305 ppb) is lower than the average concentration of aflatoxin B₁ in this study.

Siame et al. (1998) did a study in Botswana, Africa reported that the levels of aflatoxin B₁ in a range of (0.8-16.00 µg/Kg) for the raw shelled peanut samples and average of it was 0.8 ppb. and for peanut butter were (3.2-16.00 µg/Kg) and average was 9.6 ppb (Siame et al., 1998). In our research the average concentration of aflatoxin B₁ (0.305 ppb) is more than the average concentration of aflatoxin B₁ in raw shelled peanut samples in this study (0.8 ppb).

In Tokyo, Japan, Tabata et al. (1993) found that several peanut products were contaminated by aflatoxin B₁ in a range of (0.4-21.7 µg/Kg) and the average of this contamination was 11.5 ppb (Tabata et al., 1993). The mean concentration of aflatoxin B₁ in peanut from Ahvaz (0.305 ppb) was lower than investigated peanuts in Tabata's study (11.5 ppb).

6. Conclusion

In recent decades with the growing concept of human development worldwide, the issue of security and safety of food products have been discussed and generated special attention from the United Nations (Carvalho, 2006). With the development of communication and increased globalization, the need of cooperation is clear and ignoring the problems is not possible. The World Trade Organization (WTO) is the only global international organization dealing with the rules of trade between nations and next trades in future will be regulated by this organization (Aruoma, 2006). Many regulations have been established in countries to protect the consumer from the harmful effects of pollutants. Aflatoxin is one of the most important pollutants (Heathcote & Hibbert, 1978). Today aflatoxins have been one of the most important global concerns regarding contamination of food products (Selim, 2010). There is need for continuous monitoring of residues of aflatoxins or their metabolites in our food, and the environment to ensure that consumers are not being exposed to unacceptable levels of these natural toxins. This also would enable us to be aware of the trend in the levels of the environment and build up a database upon which future regulatory legislations could be decided.

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