FOOD CHEMICAL CONTAMINANTS

Aflatoxin B_1 in Common Egyptian Foods

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Samples of common Egyptian foods (17 nuts and seeds, 10 spices, 31 herbs and medicinal plants, 12 dried vegetables, and 28 cereal grains) were collected from markets in Cairo and Giza. A portion of each sample was extracted with chloroform, and the concentrated extract was cleaned by passing through a silica gel column. Aflatoxin B_1 was determined by reversed-phase liquid chromatography with UV detection. The highest prevalence of aflatoxin B_1 was in nuts and seeds (82%), followed by spices (40%), herbs and medicinal plants (29%), dried vegetables (25%), and cereal grains (21%). The highest mean concentration of aflatoxin B_1 was in herb and medicinal plants (49 ppb), followed by cereals (36 ppb), spices (25 ppb), nuts and seeds (24 ppb), and dried vegetables (20 ppb). Among nuts and seeds, the prevalence of aflatoxin B_1 was highest (100%) in watermelon seeds, in-shell peanuts, and unshelled peanuts. The lowest prevalence and concentrations were in hommos (garbanzo beans). The highest concentrations of aflatoxin B_1 were detected in foods that had no potential for field contamination but required drying during processing and storage, such as pomegranate peel, watermelon seeds, and molokhia.

Aflatoxin B_1 is one of the most potent chemical carcinogens known (1). The acute toxic effects of aflatoxins on humans and various animal species have been fully documented (2). Aflatoxin B_1 produces cancer in the liver and other organs in many animal species (3). Epidemiological studies in Africa (4, 5) and Southeast Asia (6) have shown strong correlations between the rate of human liver cancer and the level of aflatoxin contamination in the daily diet. However, efforts to monitor aflatoxin B_1 in food and to increase public awareness of proper storage and processing practices have been limited.

A study in India on mycotoxins in dried fruits and nuts (such as coconuts, raisins, almonds, walnuts, date palms, cashews, groundnuts, and pistachios) showed that the highest percentage of fungi occurs during the rainy season (7). Another Indian study found that common spices such as coriander, cardamon, cumin, pippali, and emblic are contaminated with aflatoxin B_1 at levels above the tolerance level set by the World Health Organization (8).

A study in Sudan showed that aflatoxin production was highest in groundnuts stored at 30°C and 15% moisture (9). Another study found an association between high initial contamination of grain sorghum and increased aflatoxin B_1, B_2, G_1, and G_2 production during storage (10). A study in Tanzania showed that aflatoxin B_1 is present in >50% of milk samples tested (11). A study in Algeria indicated that peanuts produced there are highly contaminated with aflatoxins (12).

Few studies are available on the stability and level of mycotoxins in processed food, dried fruits, dried vegetables, poultry liver, poultry feed, and animal tissue. One study in India of stored and cooked pearl millet (*Pennesetum typhoides*) showed a high incidence and alarming levels of aflatoxin B_1 in this food regularly consumed in tribal communities. Approximately 65% of stored and 42% of cooked millet samples contained various combinations of aflatoxins (13).

In Egypt, 4 of 4 locally produced hard cheeses and 2 of 5 locally made milk powders were contaminated with aflatoxin M_1 at levels considered harmful by international safety standards (14). In controlled studies, aflatoxins B_1, B_2, G_1, and G_2 were found in plasma, milk, and feces of lactating Egyptian goats after 24 h of aflatoxin feeding. Body weight and milk yield decreased significantly during the feeding period (15, 16). The aflatoxin contamination decreased the nutritive value of the goats' milk and affected its suitability for cheese processing and for manufacture of condensed and dried milk (17, 18). Aflatoxin contamination also leads to quantitative and qualitative changes in protein synthesis, resulting in decreased protein content of meat and milk from farm animals and decreased pro-

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tein synthesis in liver and muscle tissues of broiler chicken (19, 20).

The objective of this study was to collect information on the levels and distribution of aflatoxin B_1 in common Egyptian foods. The data will provide an estimate of the potential risk from consuming aflatoxin B_1 daily. Cairo was selected as the site for this investigation to reflect the pattern and levels of contamination in an active consumer market of about 13 million people.

Experimental

Sampling

Samples were randomly selected from those available to the public in 3 districts in Cairo, Egypt: El Atarin, Dokki Square, and Giza Market. No particular preference was used in selecting samples or locations. About 500–1000 g samples were obtained, transferred into zip-lock bags, and kept refrigerated until analysis, except for a 20 h period during travel from Egypt to the United States, which took place during the winter. Samples were refrigerated upon arrival in the United States until analysis.

Reagents and Materials

(**a**) *Organic solvents.*—Chloroform, methanol, water, and acetonitrile were all liquid chromatographic (LC) grade (Fisher Scientific, Fair Lawn, NJ).

(**b**) *Acid-washed Celite.*—A filtering aid (Sigma Chemical Co., St. Louis, MO).

(**c**) *Column chromatography materials.*—Silica gel, 100–200 mesh (Fisher Scientific). Anhydrous granular sodium sulfate (Matheson, Coleman, and Bell, Raleigh, NC).

(**d**) *Aflatoxin standards.*—Aflatoxin B_1 solid (Sigma), 99% pure, was used to prepare stock solutions in methanol without further analysis or purification. A standard solution (Supelco, Inc., Bellfonte, PA) contained aflatoxins B_1, G_1, B_2, and G_2 at 1, 0.3, 1, and 0.3 μg/mL, respectively.

Apparatus

(**a**) *Extraction and concentration apparatus.*—A Waring blender was used to reduce bulk samples before extraction. A Burrell wrist-action shaker (Burrell Corp., Pittsburgh, PA) was used to shake samples during extraction. The extract was evaporated on a Buchler Instrument rotoevaporator (Fisher Scientific). A Meyer N-Evap analytical evaporator (Organomation Associates, Shewsbury, MA) was used for final extract concentration.

(**b**) *Column chromatography.*—22 × 300 mm glass tubes with fritted glass disks and Teflon stopcocks were used for column chromatography cleanup.

(**c**) *LC.*—An ISCO LC unit consisting of a Model 2350 dual-pump gradient system and a Model V^4 variable wavelength UV detector (ISCO, Inc., Lincoln, NE). ISCO ChemResearch software loaded on a Model Vectra ES/12 Hewlett-Packard computer (Hewlett-Packard, Palo Alto, CA) was used for system control, data collection, and analysis. A Model 470 Waters scanning fluorescence detector (Waters, Millipore, Milford, MA) was also used on-line with the UV detector.

Liquid/Liquid Extraction

Liquid extraction and cleanup procedures were similar to those published by the International Union of Pure and Applied Chemistry (IUPAC) and AOAC INTERNATIONAL for analysis of aflatoxins in peanuts and peanut products (21). About 100–200 g sample was blended in a Waring blender and sieved through a 1 mm sieve. Larger particles were blended again to a mesh size of 1 mm. From the blended sample, 50 g was transferred to a 500 mL conical flask along with 25 g Celite, 10 mL water, and 250 mL chloroform. The flask was stoppered with a glass-joint stopper, loaded in the Burrell shaker, and shaken at medium speed for 30 min. The contents were then filtered through Whatman No. 1 filter paper. The extraction flask was washed with three 3 mL portions of chloroform, and the washings were filtered and combined with the initial extract. The filter residue was discarded in a biohazard waste bag, and the filtrate was reserved for column chromatography.

Column Chromatography Cleanup

Chromatographic columns were prepared by initially packing 10 g anhydrous sodium sulfate into a 22 × 300 mm glass tube with a fritted glass disk. Chloroform was added to 10 g silica gel (100–200 mesh) to create a slurry, which was added to the chromatographic column. The stopcock was opened to allow the silica gel packing to settle while the excess chloroform drained. During draining, another 10 g anhydrous sodium

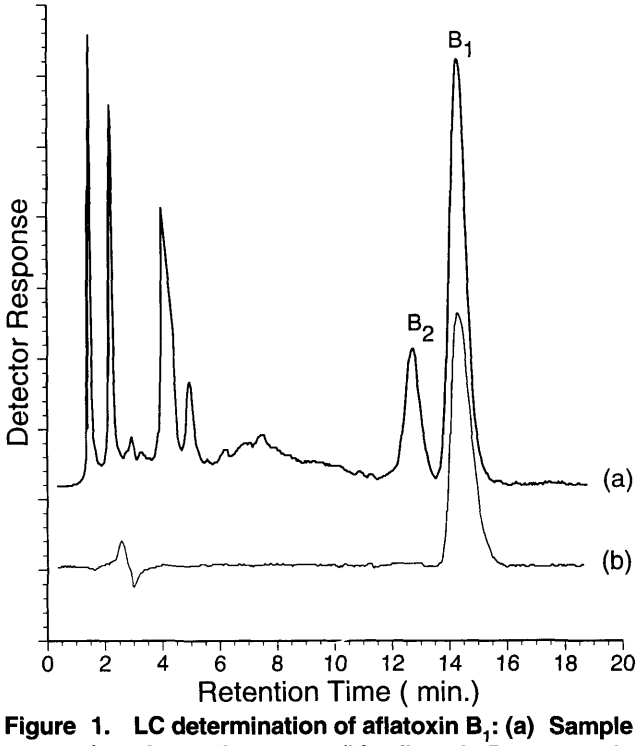

Figure 1. LC determination of aflatoxin B_1: (a) Sample extract from in-shell peanuts; (b) aflatoxin B_1 standard, 1.5 μg/mL.

Table 1. Levels and frequency of detection of aflatoxin B_1 in common Egyptian foods

Food group	No. of samples	Frequency of detection, %	Measured range, ppb	Mean concentration, ppb
Nuts and seeds	17	82.4	4–74	24
Spices	10	40.0	10–46	25
Herbs and medicinal plants	31	29.0	24–105	49
Dried vegetables	12	25.0	7–28	20
Cereal grains	28	21.4	6–92	36

sulfate was added to the top of the silica gel, to prevent column from drying. The total volume of the extract was recorded, and 50 mL portions were loaded onto the column and allowed to filter through at a rate of ca 1 drop/s. The extract on the column was rinsed with 150 mL hexane, followed by 150 mL ether to remove fats and oils. Both effluents were discarded. Aflatoxin was eluted with 150 mL 3% methanol in chloroform. The eluate was transferred to a 500 mL round-bottom flask and evaporated to dryness on the rotoevaporator. The residue was dissolved in methanol and transferred to a 15 mL graduated conical tube in five 1 mL methanol rinses. The methanol solution was evaporated to near dryness and redissolved in 1 mL LC mobile phase.

Laboratory spikes were used to verify aflatoxin recovery and effectiveness of the cleanup procedure. Samples with no detectable levels of aflatoxin B_1 were used as blanks and as matrix spikes.

LC Analysis

The analytical column was Supelcosil LC-18, 25 cm × 4.6 mm, 5 µm packing (Supelco, Inc.). The guard column was Supelcoguard LC-18, 25 cm × 4.6 mm, 5 µm packing. The analytical conditions were as follows: column temperature, 25°C; mobile phase, acetonitrile–methanol–water (20 + 20 + 60); flow rate, 1.0 mL/min; pressure, 2700 psi; UV detector wavelength, 365 nm; 0.2 AUFS. Retention time was ca 14.5 min for aflatoxin for B_1. A Model 470 Waters scanning fluorescence detector set at an excitation wavelength of 365 nm and an emission wavelength of 455 nm was used occasionally to verify the identity of the aflatoxin B_1 peak.

Results and Discussion

A chromatogram for LC determination of aflatoxin B_1 in a contaminated sample of in-shell peanuts is shown in Figure 1. An overlay of an LC chromatogram for an aflatoxin B_1 standard is shown for comparison. The extraction method, column chromatography cleanup, and LC parameters were adjusted to allow separation and identification of aflatoxins (G_1, G_2, B_2, and B_1) without interference from sample matrix. A known standard of aflatoxin B_1 and a standard mixture of aflatoxins (B_1, B_2, G_1, and G_2) were used routinely to verify complete separation and identification of the aflatoxin components.

The lower limit of detection for 30 µL injections in the LC–UV system was about 2 ng. For quantitation of aflatoxin B_1, a 4-level (5–50 ng) calibration curve was constructed and used with each set of ≤10 samples. A 1-level (25 ng) calibration

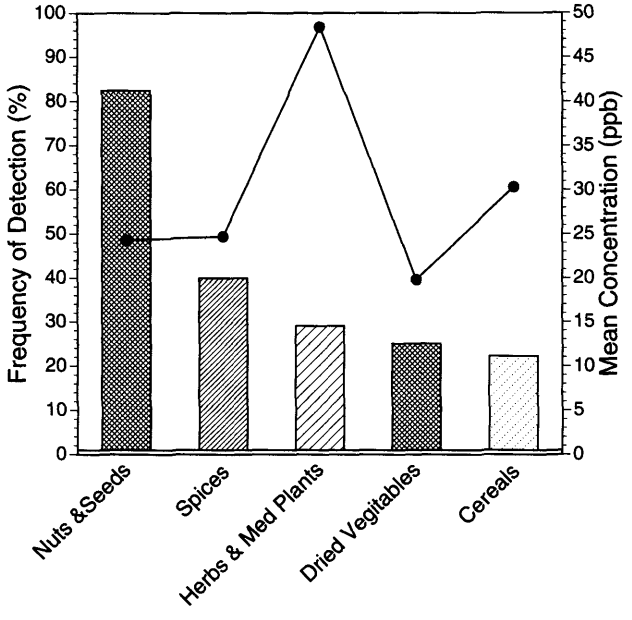

Figure 2. Level (line) and frequency (bars) of detection of aflatoxin B_1 in common Egyptian foods.

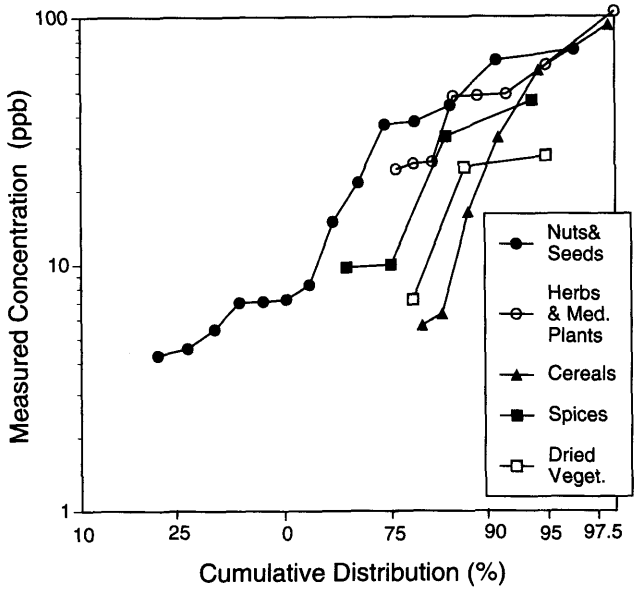

Figure 3. Cumulative distribution of aflatoxin B_1 concentrations in each food group.

Table 2. Projected geometric mean (GM) values of food groups based on log normal distribution of the measurable samples

Food group	Log normal goodness of fit, R^2	Projected GM, ppb	Standard error of GM, %	Group GSD[a]
Nuts and seeds	0.939 ($p < 0.001$)	10.0	8	3.7
Spices	0.923 ($p = 0.001$)	12.0	18	2.9
Herbs and medicinal plants	0.916 ($p < 0.01$)	0.7	71	13.0
Dried vegetables	0.873 ($p < 0.1$)	5.1	50	4.5
Cereal grains	0.700 ($p < 0.4$)	2.9	230	4.4

[a] GSD, geometric standard deviation.

check was conducted on every 5 samples. Recovery of laboratory spikes was about 100%. Average recovery for spiked corn matrixes was 95% with a standard deviation of 6.35 ($n = 4$). These recoveries are consistent with those of previous studies using similar extraction and cleanup procedures (22).

Results are summarized in Table 1 and Figure 2. Aflatoxin B_1 was detected most frequently in nuts and seeds, followed by spices, herbs and medicinal plants, dried vegetables, and cereal grains. Highest concentrations were found in herbs and medicinal plants, followed by cereal grains, nuts and seeds, spices, and dried vegetables.

Figure 3 shows the cumulative distribution of measurable results versus the logarithm of the concentration of aflatoxin B_1. Most samples within each group, except nuts and seeds, had aflatoxin B_1 levels below the detection limit. To estimate the mean of all samples within the group, log normal distribution parameters were determined from the best-fit line for each set of measured data (Figure 3). The goodness of fit, R^2 statistics, and p values <0.01 shown in Table 2 indicate that the validity of this assumed log normal distribution is good when aflatoxin B_1 is detectable in at least 6 samples. The higher p values (lower significance) of R^2 for dried vegetables and cereal grains are due to the small number of samples and do not indicate a statistically poor fit. The steeper slope of the data for herbs and medicinal plants (Figure 3) is reflected in the high geometric standard deviation (GSD) for this group and the consequently low projected geometric mean (GM) when extrapolated from the 29% of measurable data. GM values in Table 2 are all lower than their corresponding arithmetic means in Table 1 because of extrapolation beyond measurable limits of the analytical method and differences in the assumed distribution between the 2 tables. Despite the larger standard error of the projected GMs for the last 2 groups, the projected GMs provide a good working estimate of central concentration values and could be used for future monitoring or risk assessment studies.

The 17 samples of nuts and seeds consisted of watermelon seeds, in-shell peanuts, peanuts off-shell, pumpkin seeds, and hommos (garbanzo beans), which are consumed in large quantities as snacks. All products in this group usually are roasted and lightly salted before being sold to consumers. The levels and frequency of detection of aflatoxin B_1 in this group are compared in Figure 4. Aflatoxin B_1 was detected in all samples of watermelon seeds (5/5), pumpkin seeds (3/3), in-shell peanuts (3/3) peanut off-shell (2/3) and hommos (1/3). The highest mean concentration (36 ppb) was found in in-shell peanuts, followed by watermelon seeds (35 ppb).

Spices commonly used in Egyptian foods were analyzed: coriander (4 samples), red pepper (2 samples), black pepper (2 samples), and cinnamon (2 samples). The 2 cinnamon samples gave the highest prevalence and concentration (10 and 46 ppb) of aflatoxin B_1. Aflatoxin B_1 was found in 1 red pepper sample (10 ppb) and in 1 black pepper sample (33 ppb). No aflatoxin was found in the coriander samples.

The herbs and medicinal plants listed in Table 3 consist of 31 types of dried parts, stems, roots, flowers, seeds, or fruits widely used in hot drinks (e.g., karkadie, helba, and kerfa) or in folk medicinal preparations. Aflatoxin B_1 was detected in karkadie (24 ppb), halfabar (64 ppb), rawind (48 ppb), khashab keena (49 ppb), misht ballot (26 ppb), pomegranate peel (105 ppb), somowa (26 ppb), and salamakka (48 ppb). Dried pomegranate peel is widely used as an anthelmintic.

The 12 samples of dried vegetables consisted of beans (6 samples), freek (3 samples), and molokhia (3 samples). The

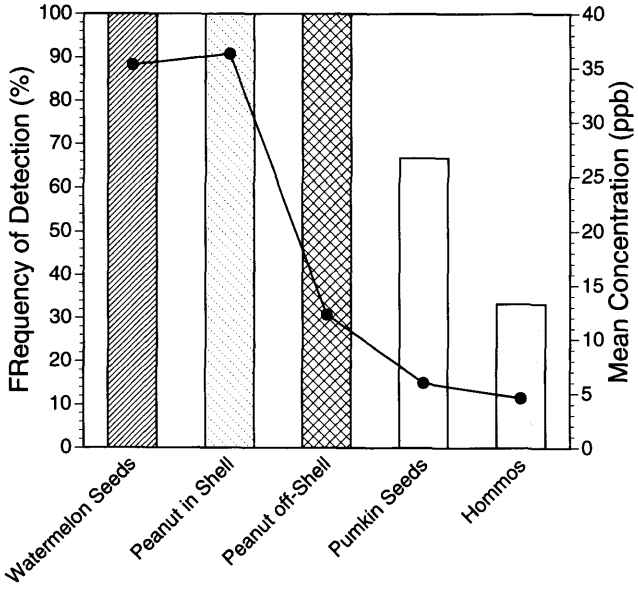

Figure 4. Levels (line) and frequency (bars) of detection of aflatoxin B_1 in seeds and nuts.

Table 3. Aflatoxin B_1 in herbs and medicinal plants examined in this study

Common Egyptian name (English name)	Scientific name	Aflatoxin B1 concn, ppb
Karkadie (roselle)	*Hibiscus sabdarifa* L.	24
Halfa bar (camel's hay)	*Cymbopogon proximus*	64
Kaff Mriam (St. Mary's flower)	*Anstatica hierochuntica* L.	ND[a]
Rawind	*Rheum officinalis*	48
Kababa seini (cubeba fruits)	*Piper cubeba* L.	ND
Khashab keena (cinchona bark)	*Cinchona ledgeriana*	49
Misht ballot	*Quercus infectoria*	26
Ganzabeel (ginger)	*Zingiber officinale*	ND
Khashab morr	*Picrasma excelse*	ND
Kesher romman (pomegranate peel)	*Puncia granatum*	105
Khlingan	*Alpinia galnga*	ND
Morr (myrrh)	*Comiphora molmol*	ND
Somowa (cleme)	*Cleome droserifolia*	26
Mermaria	*Salvia* sp.	ND
Kafora	*Eucalyptus* sp.	ND
Helba (fenugreek)	*Trigonella foenum groecum* L.	ND
Moghat (glossostemon root)	*Glossostemon bruguieri* D.C.	ND
Salamakka (senna pods)	*Cassia obocolladon*	48

[a] ND, none detected.

highest prevalence (2/3) and concentration (26 ppb) of aflatoxin B_1 were found in dried molokhia (dried leaves used for preparing a green soup). Aflatoxin B_1 was also detected in 1 freek sample. No aflatoxin was detected in any of the dried lentil and fava bean samples.

The 28 samples of cereal grains consisted of white maize (19 samples) and wheat (9 samples), which are used in different proportions (up to 50:50) for preparing the most popular Egyptian bread. The prevalence of aflatoxin B_1 in maize was 6/19, at concentrations of 6–92 ppb. The prevalence in wheat was 2/9, at concentrations of 6–22 ppb.

The study indicates that a significant portion of aflatoxin B_1 in the Egyptian diet could come from nonessential foods (e.g., seeds and peanuts), food additives (spices), and medicinal plants. Most of these nonessential dietary components, as well as dried vegetables, are probably infested with *Aspergillus flavus* during drying and storage.

For example, contamination of watermelon seeds is expected to occur during drying, storage, and processing (salting and roasting for consumption), because *A. flavus* is not known to contaminate watermelon fruits. The prevalence of aflatoxin B_1 in pumpkin seeds is lower (2/3) and the concentration is much lower (6–7 ppb) than that found in watermelon seeds, probably because of the limited need for drying and processing pumpkin seeds compared to watermelon seeds.

Cinnamon bark (*Cinnamonum zeylanicum*) and pomegranate peel demonstrate the effect of drying on aflatoxin B_1 levels. The thick bark and peels may be contaminated during drying. This type of contamination can be prevented with proper drying, packaging, and storage. However, proper procedures are difficult to practice in a developing country, where small agricultural producers may not be aware of the aflatoxin problem nor do they have the means or training to implement proper drying and storage. Therefore, it is important to establish a monitoring program to create public awareness of the potential hazard by training public health professionals and food inspectors.

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