Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Apr 15; 8(E):117-126.
https://doi.org/10.3889/oamjms.2020.4643
eISSN: 1857-9655
Category: *E - Public Health*
Section: *Public Health Disease Control*

Aflatoxins Contamination of Human Food Commodities Collected from Jeddah Markets, Saudi Arabia

Mahmoud El Tawila[1], Serdar Sadeq[2], Alrasheedi Amani Awad[3], Jamil Serdar[4], Mohamed Hussein Fahmy Madkour[5], Mohamed M. Deabes[6]*

[1]Department of Environmental Science, Faculty of Meteorology, Environment and Arid land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia; [2]Ministry of Health Saudi Arabia, Public Health Ministry of Health, Health Programs and Chronic Disease, Saudi Arabia; [3]Department of Food Nutrition, Faculty of Home Economics, King Abdulaziz University, Jeddah, Saudi Arabia; [4]Ministry of Health Saudi Arabia, Alzahir Primary Health Care Centre;Saudi Arabia; [5]Faculty of Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia; [6]Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Giza, Egypt

Edited by: Sasho Stoleski
Citation: Mahmoud ET, Sadeq S, Awad AA, Madkour MHF, Deabes MM. Aflatoxins Contamination of Human Food Commodities Collected from Jeddah Markets, Saudi Arabia. Open Access Maced J Med Sci. 2020 Apr 15; 8(E):117-126. https://doi.org/10.3889/oamjms.2020.4643
Keywords: Recurrent pituitary adenoma; Giant pituitary adenoma; Transcranial; Endoscopic transsphenoidal
***Correspondence:** Mohamed M. Deabes, Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Giza, Egypt. E-mail: mydeabes@yahoo.com
Received: 19-Jan-2020
Revised: 05-Feb-2020
Accepted: 18-Mar-2020

Funding: This research did not receive any financial support
Competing Interests: The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflicts of interest.

Abstract

BACKGROUND: Aflatoxins (AFs) are fungal secondary metabolites produced by *Aspergillus flavus*. They contaminate of dietary food with AFs is a worldwide problem that affects both food safety and agricultural economies.

AIM: The aim of this study was designed to investigate the AFs contents of human food commodities mostly consumed in Jeddah, Saudi Arabia.

METHODS: The study was designed *in vitro*, contents in six food categories. A total of 288 samples were collected from 78 different markets in Jeddah. AFs were determined by high-performance liquid chromatography with fluorescence detector using immunoaffinity column clean-up.

RESULTS: The results indicated that the incidence rate 27.3% of nut samples collected from Jeddah, were contaminated with AFB_1, AFB_2. The concentrations of AFs (AFB_1, AFB_2, AFG_1, and AFG_2) were ranged from 0.19–482.4, 0.09–3.34, 0.19–87.1, to 0.09–579 µg/kg in the nut samples.

CONCLUSION: The results demonstrate the importance of routine monitoring of AFs contamination in various dry foods for human consumed should be performed regularly and the nuts contained high levels of AFs. The legal regulations must be unauthorized for human consumption to control the health risks associated with AFs.

Introduction

Aflatoxins (AFs) are secondary metabolites produced by fungi of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* that are toxic to humans and animals. AFs are the most important mycotoxins with regard to occurrence, toxicity, and impact on human health and trade in the world [1].

Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, and sometimes resulting in death [2]. The International Agency for Research on Cancer has classified AFB_1 as a Group I carcinogen, primarily affecting liver [3]. On the other side, the long-term exposure to AFB_1 caused genotoxicity and hepatocellular carcinoma [4], [5]. Natural occurrence of AF in nuts has been studied in various countries. According to a report from Mexico, 2.2% of pistachio nut samples analyzed contained AF higher than 20 ng/g [6]. In Sweden, 9.5% pistachio nut samples contained AFB_1 higher than 2 ng/g [7]. According to Ministry of Agriculture and Rural Affairs, Republic of Turkey [8], analysis of 523 pistachio nut samples in Turkey the mean of AFB_1 ranged 1–3.78 ng/g and the maximum level (ML) detected was 113 ng/g.

AFs are primarily produced by strains of *A. flavus*, *A. parasiticus, A. nomius* [1], *Aspergillus pseudotamarii* [9], and *Aspergillus bombycis* [10]. All of these species are found in the soil [1]. The four major AFs commonly isolated from foods and feeds are AFs B_1, B_2, G_1, and G_2. *A. flavus* and *A. pseudotamarii* produce only B AFs. They lack the ability to synthesize G AFs due to 0.8- to 1.5-kb deletion in the 28-gene AF biosynthesis cluster [11]. *Aspergillus nominus*, *A. bombycis*, and *A. parasiticus* produce all four major AFs. AFs M_1 and M_2 are hydroxylated metabolites of AFB_1 and B_2, respectively, and are produced in milk-producing animals [12], [13]. AFM_1 has been detected in raw milk from cows and water buffaloes in Iran at

high concentrations exceeding the maximum tolerance limit of the European Union/Codex Alimentarius Commission (50 ng/L) [13]. El-Nezami *et al*. [14] have reported on the presence of AFM_1 in human breast milk from Victoria, Australia, and Thailand. AFM_1 was detected at high concentration putting infants at risk of contamination.

The ingestion of AFs from contaminated food has led to serious health complications in humans [15], [16], [17]. Therefore, different countries have implemented strict regulations for AFs in food and feed to maintain the health of individuals [18]. The safe limit of AFs lies in the range of 4–30 µg/kg for human consumption. The European Union has the strictest standard level with AFB_1 and total AFs not beyond 2 µg/kg and 4 µg/kg, respectively, in any product meant for direct consumption [19], [20]. Similarly, the maximum acceptable limit set for AFs in the United States is 20 µg/kg [21]. In Saudi Arabia suggests a ML of total AF 10–15 µg/kg depends on type of food [22]. Besides this, various innovative technologies and control strategies are applied for pre- and post-harvest management of AFs to enhance sustainable agricultural productivity [21].

Therefore, dry food should be routinely tested for the presence of AFs before entering the market. To this end, our study was designed to investigate the AFs contents of food commodities mostly consumed in Jeddah, Saudi Arabia.

Material and Methods

Sample collection

During the period July to December 2018, 34 different kinds of food commodities samples were collected randomly form Jeddah city, from different municipalities including: Alaziziya, Al Sharafiya, New Jeddah, Almatar, Aljamia, Albalad, Historical Jeddah, Aljanoob, Obhor, and Buriman. The total number of collected samples is 288 (258 dry foods and 30 dairy products) samples. With taking into consideration that most food commodities included in this study did not require additional processing as they were already "ready to eat." However, for not ready to eat food such as rice and spaghetti, samples were further processed using established cooking techniques to represent "ready to eat" foods.

Samples collection technique

Dry food commodities

- To achieve fairness of the food sample collection, samples were collected for same food's group/category such as brand, country of origin, canned and filled, furthermore: Samples collected from different markets and mixed well together to get a homogeneous sample
- Samples were collected from 78 different markets and shops
- 200–300 g of each sample was collected
- Each sample was collected in sterile plastic bag for specimen collecting
- All dry food kept in a dry and cool area, temperature ranged 10–15°C, to prevent spoilage and swelling until analyzing time
- The most food commodities included in this study did not require additional
- Processing as they were already "ready to eat."

Dairy products

- Commercial pasteurized, ultra-high-temperature processing milk, fresh milk, and cheese samples were collected in this study
- Milk samples purchased from supermarkets from different municipalities in Jeddah city
- Different milk brands were collected including (Saudia, Alrabee, Almari, Alsafi, and Fresh milk)
- Dairy products simples collected 1 or 2 days prior the analysis day, and kept in the refrigerator temperature of 2–4°C
- Samples were prepared in accordance with the Association of Official Analytical Chemists (AOAC) Official Method 49.3.07 for milk and milk products by AOAC Official Method 991.31 [23].

Food commodities categorized to following groups	
Food category	Included samples
Bakery	Bread (white, brown, and bran)
	Toast white, *Tamis
	*Samoli (white and brown), rusk (white and brown), cornflakes
Cereal	Rice (white and brown), and spaghetti (white and brown), oats
Legumes	Fava bean, lentil, and chickpeas
Nuts	Walnut, cashew, peanut, pistachio, almond, hazelnut, and mix nuts
Coffee	Arabic, and Turkish
Dairy products	Packed (milk, liquid yogurt, and yogurt), fresh milk, cheese (white, and arish)

*Samoli Bread: is a long thin loaf of French beard, locally named Samoli, and made of flour, salt, yeast, and oil *Tamis bread: (Tamis) is an old Arabian famous bread which baked in a unique ways. Tamis is just basic bread with melted sugar and sesame seeds

Determination of AFM_1 in milk products by high-performance liquid chromatography (HPLC) with using immunoaffinity column (IAC) for cleanup

Standards of AFM_1 solution (0.5 µg/mL) were supplied by Sigma/Aldrich Chemicals Co (St. Louis, USA). Acetonitrile (ACN), n hexane and methanol, of HPLC-grade, were supplied by Sigma Chemical Company (St. Louis, MO, USA). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). IAC for AFM_1 (AflaM1) were purchased from VICAM (Milford, MA, USA). HPLC gradient grade methanol and

ACN, and sodium chloride were purchased from Merck (Darmstadt, Germany). AflaTest-P IAC were purchased from VICAM (Milford, MA 01757, USA) for cleanup and isolation of AFs extracted from samples.

AF standard

The preparation of AF standard was carried out according to the AOAC [20]. Crystals of AFs B_1, B_2, G_1, and G_2 were diluted using benzene-ACN (98:2 v/v) to obtain a concentration of 8–10 µg/ml (stock solution).

Extraction of AFM_1 by IAC

Milk samples were analyzed for the presence of AFM_1 using an IAC for cleanup and HPLC with fluorescence detection for determination based on the method of Dragacci *et al*. [24]. The milk samples (50 mL) were centrifuged at 2000× *g* for 15 min and the upper fat layer was discarded. The skimmed milk was passed through an IAC (AflaM1) (VICAM) at a rate of about 1–2 drops/second. The column was washed with purified water (10 mL) to remove extraneous non-specific material. Following, the AFM_1 eluted with 2.5 mL ACN-methanol (3:2; v/v). The eluate was evaporated to dryness using a stream of N_2 for the determination of AFM1 by HPLC.

Determination of AFM1 by HPLC

Derivatization

The derivative of residue from above by adding 200 µL hexane and 200 µL trifluoroacetic acid to dry residue in vial. Shake on vortex mixer ca 5–10 s. Let mixture sit for 10 min at 40°C, in heating block or bath; then evaporate to dryness under nitrogen on steam bath or heating block (<50°C). Add 2 mL water-ACN (75 + 25) to vial to dissolve residue and mix well using vortex mixer for LC analysis.

- Determination of AFs in dry food
- Sample extraction.

Twenty-five grams of finally ground sample were mixed with 5 g salt sodium chloride (NaCL) and place in blender jar. A 125 mL methanol:water (60:40) was added for extraction AFs from nuts, while in case of another samples were extracted using 200 ml methanol:water (80:20). After covering the jar, blending was carried out at high speed for 1 min. The extract was poured into fluted filter paper, and the filtrate was collected in a clean vessel.

Extract dilution

Pour 20 mL filtered extract into a clean vessel. Dilute extract with 20 mL of purified water and mix well. Filters dilute extract through glass microfiber filter into a clean vessel.

Immunoaffinity chromatography

Pass 10 mL filtered diluted extract (10 mL = 1 g sample equivalent) completely through AflaTest-P affinity column (Vicam) at a rate of about 1–2 drops/second until air comes through column. Pass 10 mL of purified water through the column at a rate of about 2 drops/second. Elute affinity column by passing 1.0 mL HPLC grade methanol through column at a rate of 1–2 drops/second and collecting all of the sample eluate (1 mL) in a glass vial. Evaporated to dryness using a stream of N_2 to be determined using HPLC as following.

Determination of AFs by HPLC

Derivatization

The derivatives of samples and standard were done as follow 100 µl of trifluoracetic acid (TFA) and were added to samples and mixed well for 30 s and the mixture stand for 15 min. 900 µl of water:ACN (9:1 v/v) were added and mixed well by vortex for 30 s and the mixture was used for HPLC analysis. In this step of reconstitution of the dry film, AFB_1 and AFG_1 were converted into other derivatives, AFB_2a and AFG_2a, respectively, (AFG_1 and AFB_1) had low fluorescence properties, therefore, and they were converted to G_2a and B_2a, which had high fluorescence properties, using (TFA).

HPLC conditions

HPLC (Agilent 1100 series) equipped with a fluorescence detector (G 1321A) analysis was carried out with a liquid chromatograph equipped with solvent delivery systems (Agilent Technologies, Inc. 200 Regency Forest Drive, Suite 330 Cary, NC 27511 USA) system containing a G1322A Vacuum Degasser, a G1312A binary pump and a reverse-phase analytical column packed with C_{18} material (Agilent ZORBAX, DB-5 µm, 150 mm × 4.6 mm). The mobile phase consisted of water:acetonitril:methanol (240:120:40), according by Deabes *et al*. [25], [26] Eshak *et al*. [27], El-Soud *et al*. [28], and Deabes *et al*. [$29_{a,b}$], for AFs G_1, B_1, G_2, and B_2, but for M1 the isocratic system with water:methanol:ACN 66:17:17. Separation was performed at 40°C temperature at a flow rate of 1.0 ml/min; the injection volume was 50 µl for both standard solutions and sample extracts by autosampler (G1329A). The detection was performed using fluorescence detector and was operated at an excitation wavelength of 360 nm and an emission wavelength of 440 nm. For AFM_1, the detection was performed using fluorescence detector and was operated at an wavelength of 365 nm for excision and 435 nm for emission. AFs concentration in samples was determined from the standard curve, using peak area for quantitation AOAC [23].

The concentrations of AFM_1 in milk were estimated from a standard curve 5.0–25 ng/ml methanol,

prepared from AFM_1 in chloroform L (9.93 mg/ml). An AFM_1 standard as injected every ten injections as a quality control. AFM_1 was stored at –20°C in a sylilated vial wrapped in aluminum foil. Since AFs B_1, B_2 G_1, G_2, and M_1 are a carcinogen, care was exercised to avoid personal exposure and proper decontamination procedures with 10% sodium hypochlorite were used.

Validation method

Accuracy

The accuracy of the method was studied by recovery studies. The accuracy of the method was determined by percentage recovery of AFs in the spiked sample at three concentration levels. The resultant samples were then analyzed (replicated 3 times) and the average percentage recoveries were calculated as:

$$\text{Recovery } (\%) = \frac{\text{Actual amount of AFs } (\text{ng/g}) \text{ quantity}}{\text{Initial amount of AFs } (\text{ng/g}) \text{ were added}} \times 100$$

The samples were examined by HPLC after extraction according to AOAC method 991.31 [23]. The averages of 20 analysis results and their standard deviations and limit of detection (LOD) and limits of quantitation (LOQ) values were obtained according to Eqs. (1) and (2) for each experiment by analyzing three samples with two injections at a time in the HPLC under the chromatographic conditions mentioned above by the ICH guidelines (ICH Q2(R1), [30] and NATA Technical Note 17 [31].

LOD

The lowest concentration of working solution of the analyst was further diluted with (ACN:water 1:9, v/v) to yield a series of appropriate concentrations. LOD of the method was determined by injecting progressively low concentrations of the standard solutions and S/N ratio for each concentration was observed. The concentration having signal-to-noise ratio nearly three has been found as LOD.

LOQ

The lowest concentration of working solution of the analyst was further diluted with ACN:water 1:9, v/v to yield a series of appropriate concentrations. LOQ of the developed method was determined by injecting progressively low concentrations of the standard solutions and observed S/N ratio (signal-to-noise ratio) of each concentration. The LOQ for investigated compound was established at signal-to-noise ratio approaching nearly to 10.

LOD is expressed as the analyst concentration corresponding to:

a. Mean value of the blank sample + 3 s
b. 0 + 3 s or the mean value of the blank sample + 4.65 s.

LOQ = 10s+X

where:
s = standard deviation for the blank or blank fortified with an analyst samples
X = measured value.

Statistical analysis

Descriptive statistics were calculated of the studied groups. Therefore, a Mann–Whitney U-test was used to determine the significance of the difference. A Kruskal–Wallis test was used to test the significance of the differences among the three samples levels, where a value of α = 0.05 was considered to indicate statistical significance.29 SPSS, version 22 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis according to IBM Corp. [32].

Results

In the present study, a total of 288 (258 dry foods and 30 dairy products) samples were detect AFs (B_1, B_2, G_1, and G_2) and M_1 in dairy products by HPLC.

A review of monitoring studies on the occurrence of AFs in food products has demonstrated that AFs are still being found frequently in food products at levels that are of significant concern for consumer protection [33], [34].

It is worthy to mention that the current investigation was carried out to determined the AFs (AFB1, AFB2, AFG1, AFG2) contamination levels in samples (Human Food Commodities) were obtained from Jeddah, markets. Then, the obtained data are recorded in Tables 1 and 2.

The obtained results of Tables 1 and 2 indicated that the % of incidence 7% in cereals samples. The concentrations of AFs (AFB_1, AFB_2, AFG_1, and AFG_2) ranged from 0.46–5.83, 0.0–0.0, 0.67–1016, to 0.0–0.0 µg/kg in the cereals samples collected from Jeddah, respectively.

The obtained results of Table 1 indicated that the incidence rat % of bakery 0% and legumes but in coffee 62%, cereals 7%, nuts 27.3, and dairy products 13.3% for AFs (AFB_1, AFB_2, AFG_1, and AFG_2) in samples collected from Jeddah region, respectively. The highly percentage of AFs was found in nuts. The results indicated that the incidence rate 27.3% of nut

Table 1: Distribution of food groups commodities obtained from Jeddah markets for aflatoxin contamination µg/kg

Food groups	AFs	Incidence rate (%)	AFs concentrations µg/kg						Percentiles		
			Min.	Max.	Mean	±SD	Median	IQR	10	90	95
Bakery bread (white, brown, and bran)	AFB_1	0/51 (0%)	-	-	-	-	-	-	-	-	-
	AFB_2		-	-	-	-	-	-	-	-	-
	AFG_1		-	-	-	-	-	-	-	-	-
	AFG_2		-	-	-	-	-	-	-	-	-
	TAF		-	-	-	-	-	-	-	-	-
Cereals rice (white and brown), spaghetti (white and brown), oats	AFB_1	5/72 (7%)	0.46	5.38	0.160	0.79	0.000	0.00	0.0	0.00	1.03
	AFB_2		-	-	-	-	-	-	-	-	-
	AFG_1		0.67	1.16	0.025	0.16	0.000	0.00	0.0	0.00	0.0
	AFG_2		-	-	-	-	-	-	-	-	-
	TAF		0.46	5.38	0.186	0.89	0.000	0.00	0.0	0.00	1.26
Legumes bread (white, brown, and bran)	AFB_1	0/28 (0%)	-	-	-	-	-	-	-	-	-
	AFB_2		-	-	-	-	-	-	-	-	-
	AFG_1		-	-	-	-	-	-	-	-	-
	AFG_2		-	-	-	-	-	-	-	-	-
	TAF		-	-	-	-	-	-	-	-	-
Coffee Arabic Turkish	AFB_1	5/8 (62.5%)	0.29	0.93	0.37	0.36	0.372	0.71	0.0	-	-
	AFB_2		-	-	-	-	-	-	-	-	-
	AFG_1		-	-	-	-	-	-	-	-	-
	AFG_2		-	-	-	-	-	-	-	-	-
	TAF		0.29	0.93	0.37	0.36	0.372	0.71	0.0	-	-
(Nuts) walnut cashew peanut pistachio almond hazelnut	AFB_1	27/99 (27.3%)	0.19	482.4	11.14	60.5	0.000	0.036	0.0	2.66	12.3
	AFB_2		0.09	3.34	0.11	0.41	0.000	0.00	0.0	0.29	0.78
	AFG_1		0.19	87.1	2.11	11.6	0.000	0.00	0.0	0.47	1.88
	AFG_2		0.09	45.4	1.24	6.45	0.000	0.00	0.0	0.14	0.87
	TAF		0.09	579.4	14.7	76.7	0.000	0.19	0.0	4.53	12.7
Dairy products milk, liquid yogurt, yogurt), fresh milk, cheese (white, arish)	AFM_1	4/30 (13.3)	µg/ml						0.1	0.07	0.1
			0.06	0.1	0.061	0.01	0.06	0.00			

Min: Minimum, Max: Maximum, SD: Standard division, IQR: Interquartile range.

Table 2: Aflatoxin detection range in dry food commodities

Aflatoxins µg/kg	Incidence rate (%)									
	AFB_1		AFB_2		AFG_1		AFG_2		TAFs	
	n	%	n	%	n	%	n	%	n	%
<LOD	222	86.0	242	93.8	240	93.0	246	95.3	221	85.7
LOD<-<2	21	8.1	15	5.8	14	5.4	8	3.1	20	7.75
2<-<4	7	2.7	1	0.4	0	0.0	0	0.0	4	1.55
4<-<20	4	1.6	0	0.0	0	0.0	1	0.4	9	3.5
>20	4	1.6	0	0.0	4	1.6	3	1.2	4	1.55
Total	258	100.0	258	100.0	258	100.0	258	100.0	258	100.0

LOQ: Limits of quantitation, LOD: Limit of detection, AFG_1: Aflatoxin G_1, AFB_1: Aflatoxin B_1, AFG_2: Aflatoxin G_2, AFB_2: Aflatoxin B_2. TAFs: Total aflatoxins.

samples collected from Jeddah, was contaminated with AFs B_1 and B_2, while 20% from were contaminated with AFs G_1 and G_2. The concentrations of AFs (AFB_1, AFB_2, AFG_1, and AFG_2) were ranged from 0.19–482.4, 0.09–3.34, 0.19–87.1, to 0.09–579 µg/kg in the nut samples collected from Jeddah markets.

Figure 1a and b shows the HPLC chromatogram of AFs (AFG_1, AFB_1, AFG_2, and AFB_2) separation of standards and high concentration of AFs in walnut. The highly percentage of AFs was found in walnuts, with risk levels 100% with a concentrations of AFs (AFB_1, AFB_2, AFG_1, and AFG_2) ranged from 84.447–482.380, 0.052–3.337, 20.008–87.142, to 12.732–45.40 "µg/kg" while in Pistachio (0.716–4.865, 0.01.744, 0.0–0.296, and 0.0–0.871 "µg/kg," respectively).

In a survey study of peanut products in North America, 19% of 1416 samples examined were contaminated with an average level of 1 µg/kg AFB_1 [35]. In Thailand, 49% of 216 samples contained AFB_1 at an average level of 424 µg/kg [36]. In parts of India, 100% of maize samples have been found contaminated with AF in the range of 6250–15600 µg/kg [37].

The results in Table 2 illustrated that the rat of incidence of contaminated samples 86.0%, 93.8, 93.0, and 95.3 in <LOD in the total of 222 samples for AFs B_1 and B_2, G_1 and G_2, respectively, while with LOD< - <2 in the total of 21 samples for AFs B_1 and B_2, G_1 and G_2 found that incidence % of contaminated samples 8.1, 5.8, 5.4, and 3.1, respectively. The total of AFs concentration µg/kg B_1, B_2 G_1, and G_2 was contaminated in dry food samples (n = 258) incidence rat of 100%.

The obtained data (Tables 3 and 4 and Figure 2a-e) illustrated that the mean recovery percentages of all tested AFs (AFB, AFB_2, AFG_1, and AFG_2) extracted by the first former method from spiked samples.

The average recoveries of AFs spiked on levels (5 µg/kg) in peanut were 79.0% for AFB_1; 76.5% for AFB_2; 83.7% for AFG_1, and 67.2% for AFG_2 and M_1 89.73.

Discussion

AFs are hepatocarcinogens and have been classified as Class 1 human carcinogen [3]. The average daily intake of AFB_1 in the high-risk area was 184.1 µg. hepatitis B can act synergistically with AFs to increase the risk of hepatocellular carcinoma [38]. According to the World Health Organization, chronic hepatitis B virus infection occurs more frequently (high infection >8%) in developing world including Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Sahara Africa, the Amazon Basin, parts of the Middle East, the Central Asian Republics, and some countries in Eastern Europe, while the rest of Europe infection rates are below 1% and less than

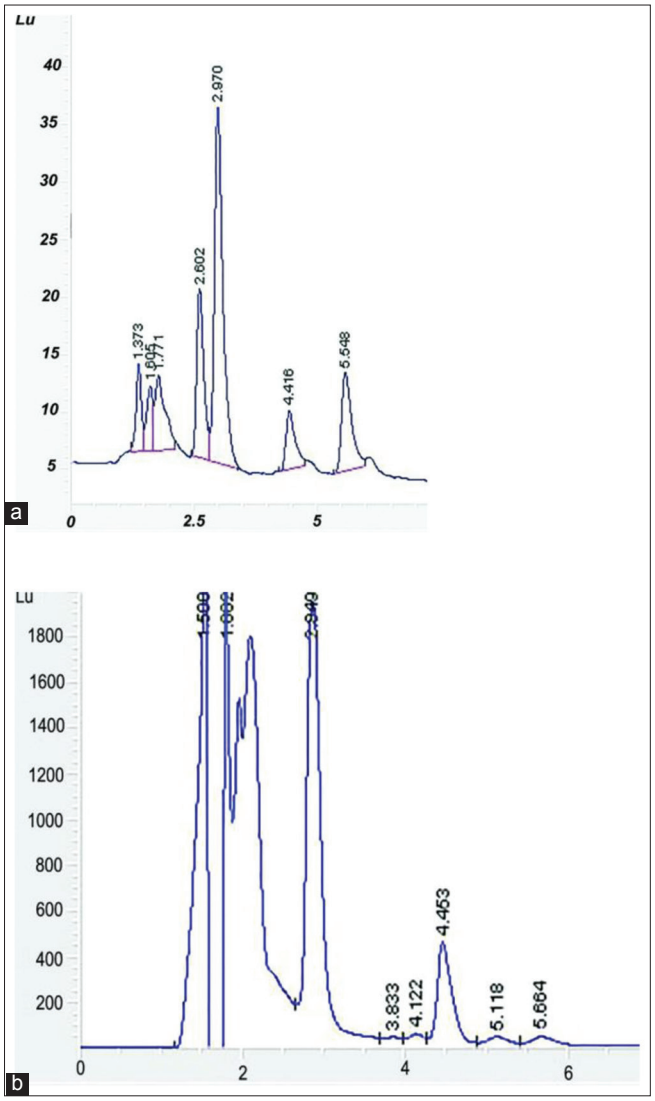

Figure 1: High-performance liquid chromatography chromatograms (a) standard of aflatoxins (AFs), (b) nut sample +AFs. R.T: Retention time: AFG_1 (2.4), AFB_1 (2.9), AFG_2 (4.4), AFB_2 (5.5)

20% of the population is ever exposed to hepatitis B virus infection [39]. Overall, epidemiological studies of human populations exposed to diets naturally contaminated with AFs revealed an association between the high incidence of liver cancer in Africa and elsewhere and dietary intake of AFs [40].

Table 3: Recovery rate of validated HPLC method for aflatoxins in food matrices

No. of replicated samples	AFG_1	AFB_1	AFG_2	AFB_2
1	79.0	95.22	53.25	63.1
2	78.4	87.28	53.25	72.6
3	78.0	89.66	60.0	65.13
Mean	78.46	90.72	55.5	66.49

AFG_1: Aflatoxin G_1, AFB_1: Aflatoxin B_1, AFG_2: Aflatoxin G_2, AFB_2: Aflatoxin B_2, AFM_1: Aflatoxin M_1.

Often up to 1 in 10 of the population in sub-Saharan Africa are infected with hepatitis B and C, AF intake raise the risk of liver cancer by more than ten-fold compared to the exposure of both hepatitis alone [41].

Table 4: Recovery rate of validated HPLC method for aflatoxins M_1 in fluid milk

Sample	Spike level (ng/mL)	Recovery (%)
Fluid milk	5	89.73

In our results found that in walnuts AFB1 exceed in the average daily intake of AFB_1 the high-risk more than 184.1 µg set by Turner *et al*. [38], it is average 271.70 µg. The obtained results of Tables 5 illustrated the incidence rat % were contaminated with AFs B_1 and G_1. The detection range in dry food commodities sample was contaminated with AFs ranged from 0.036 to 482.4 for AFB_1 and AFG_1 0.035–87.1 µg/kg when the samples (n = 258) were analyzed the positive samples 36 and 18 for AFs, B_1 and G_1 contained the incidence rat% 14.34 and 7%, respectively.

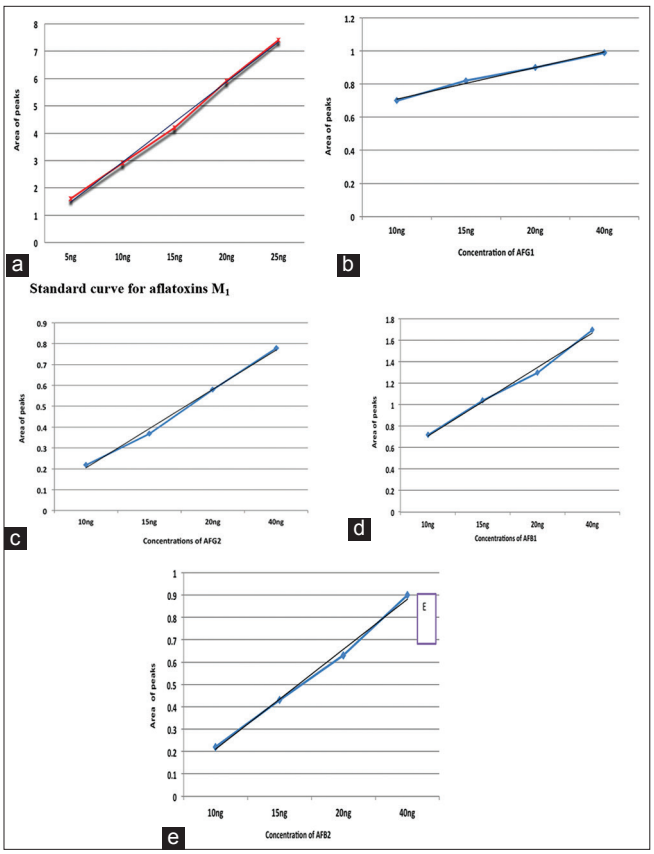

Figure 2: Calibration curves of FM_1 (a); AFG_1 (b); AFB_1 (c); AFG_1 (d); AFB_2 (e). AF: Aflatoxin

A review of monitoring studies on the occurrence of AF in food products has demonstrated that AFs are still being found frequently in food products at levels that are of significant concern for consumer protection [33], [34]. The occurrence of AFs in dried fruits and nuts was surveyed in the study by Luttfullah and Hussain, [42] in Pakistan. They found the percentage of contamination for total AFs in the samples such as in dried apricot (20%), dates (10%), dried figs (50%), dried mulberries (26%), and raisins (20%), while in apricot kernels (26%), almonds without shell (30%), walnuts with shell (40%), walnuts without shell (70%), peanut with shell (40%), peanuts without shell (50%), pistachios with shell (20%), pistachios without shell (50%), and pine nuts with shell (20%). The highest contamination levels of AFs were found in one peanut sample (14.5 mg/kg) and one pistachio sample (14 mg/kg). Molds of the genus *Aspergillus* frequently decay the kernel of pistachio nuts [43]. On the other

Table 5: Aflatoxins detection range in dry food commodities

AFs	Aflatoxin contamination (Ve+) µg/kg								
	Incidence rate (%)	Mean ± SD	Min.	Max.	Detection rang				
					AF<2 (%)	AF>2 (%)	AF>4 (%)	AF>10 (%)	AF> 20 (%)
AFB_1	36/258 (14.34)	4.3 ± 37.7	0.036	482.4	21 (58.3)	15 (41.6)	8 (22.2)	6 (16.6)	4 (11.1)
AFG_1	18/258 (7)	0.82 ± 7.2	0.035	87.1	14 (77.7)	4 (22.4)	4 (22.4)	4 (22.4)	4 (22.4)
TAF	37/258 (14.34)	5.8 ± 47.9	0.045	579.4	20 (54.1)	17 (45.9)	13 (35.1)	6 (16.2)	4 (10.8)

AFG_1: Aflatoxin G_1, AFB_1: Aflatoxin B_1, AF: Aflatoxin, TAF: Total aflatoxin, Min: Minimum, Max: Maximum, SD: Standard division.

Table 6: LOD and LOQ values of validated HPLC method for aflatoxins

Found concentrations	AFG_1 Initial concentration was added (5 ng/g)	AFB_1 Initial concentration was added (45 ng/g)	AFG_2 Initial concentration was added (5 ng/g)	AFB_2 Initial concentration was added (15 ng/g)
Actual amount (quantity)	3.95	42.85	2.13	9.47
Actual amount (quantity)	3.92	39.28	2.13	10.89
Actual amount (quantity)	3.9	40.35	2.4	9.77
Mean	3.9±0.02	40.83±1.8	2.22±0.15	10.04±07

Mean±SD (n=3), SD: Standard division.

hand, pistachio nuts are among the commodities with the highest risk of AF contamination [44].

Table 7: Limit of detection and limit of quantitation values of validated HPLC method for aflatoxins in peanut

Validation paramter	AFG_1	AFB_1	AFG_2	AFB_2	AFM_1
LOD	0.05	0.03	0.02	0.07	0.06
LOQ	0.4	0.3	0.2	0.2	0.42

LOQ: Limits of quantitation, LOD: Limit of detection, AFG_1: Aflatoxin G_1, AFB_1: Aflatoxin B_1, AFG_2: Aflatoxin G_2, AFB_2: Aflatoxin B_2, AFM_1: Aflatoxin M_1.

AF contamination in some edible dry fruits and nuts has been reported by Abdel-Hafez and Saber [45] and Singh *et al*. [46]. AFs were detected in 90% of hazelnut samples (25–175 mg/kg) and 75% of walnut samples (15–25 mg/kg). In a survey of peanut products in North America, 19% of 1416 samples examined were contaminated with an average level of 1 µg/kg [35] in Thailand, 49% of 216 samples contained AFB_1 at an average level of 424 µg/kg [36]. AFs are present in food chain consumption of AF in many parts of the world varies from 0 to 30,000 ng/kg/day [47]. Some factors such as high temperature and low moisture can result in cracks in the seed and subsequent invasion by the fungus. Temperature and moisture are the dominant factors that affect AF contamination of corn. Environmental conditions most favorable for maximum growth and AF production by *A. flavus* are temperatures >30°C, maximum relative humidity of >85%, and water activity of 0.98–0.99 [48]. Thus, *A. flavus* can infect with proper moisture/temperature conditions during storage almost any stored product [49]. AF formation in groundnut is favored by prolonged end of season drought and associated elevated temperature [50]. Cereals and cereal-based products are the major foods for human consumption worldwide [51]. Among cereals, rice and corn are mostly contaminated by AFs in natural conditions due to changes in agricultural practices. The occurrence of AFs is common in wide varieties of food include peanuts, nuts, figs, corn, rice, spices, and dried fruits [52]. It has been shown that among the tested cereals, 37.6% were at least contaminated by any of the AFs [53]. Although rice is not the high-risk commodity for AFs contamination, AFB_1 besides other mycotoxins have been found in rice from China, Egypt, India, Iran, Malaysia, Nepal, Pakistan, the Philippines, United Kingdom, and the United States [42], [54]. Palumbo *et al*. [55] found that AFs in rice of the highest mean concentrations of AFB1 n = 124; mean 3.1–3.3 µg/kg; max: 91.7 µg/kg. In our results, the total AFs were detected in some foods above the acceptable limits set by the European Union 4 ug/kg could be attributed to some suitable factors such as pH, nutrient composition, moisture content water activity, as well as external factors as temperature, relative humidity, soil properties, insects, and rodents attack [56], [57]. Atanda *et al*. [56] also suggested that these factors, however, do not work in solitude. Therefore, two or more factors may have to be met before fungal growth and corresponding toxin production can be effected.

Fungal infestation, growth, and AF development are linked principally to water activity (Aw). This observation is attributable to incorrect drying which display stored cereals and legumes to growth of mycotoxigenic fungi such as *Aspergillus* species which is conjectured to also increase with storage time [51], [58], [59].

Validation

The calibration curves, in the ranged of 5–25 ng/mL for AFM_1 and 5–40 ng/ml for (AFB_1, AFB_2, AFG_1, and AFG_2), were linear in the studied working.

The good accuracy and precision results are obtained in Tables 4, 6 and 7. The LOD was calculated by the ICH guidelines [30] for those concentrations that provide a signal-to-noise ratio of 3:1. The obtained LOD values for AFB_1, AFB_2, AFG_1, and AFG_2 were below 0.1 µg/kg (AG_2 and AG_1 0.04 µg/kg; and AB_1 and AB_2 0.02 µg/kg). The LOQs were set and experimentally confirmed at level of 1 µg/kg. These limits are well below established by the Codex MLs for food commodities.

Conclusion

This study shows that the incidence of AFs contamination in food commodities are consuming in Jeddah. The results demonstrate the importance of routine monitoring of AFs contamination in various food commodities should be performed regularly and the

nuts contained high levels of AFs. The legal regulations must be unauthorized for human consumption to control the health risks associated from AFs. Good processing, handling, transportation, storage system, and the source of production to imported for local market in Saudi Arabia can reduce the exposure to AFs.

Acknowledgments

All authors are highly grateful to the authority of the respective King Abdulaziz University, Jeddah, Saudi Arabia, for their support in doing this research would like to express Food Nutrition Department, Faculty of Home Economics, King Abdulaziz University, Jeddah, Saudi Arabia. A Project Funded 1-18-04-009-0007 we would like to thank for their financial support.

References

1. Wilson DM, Mubatanhema W, Jurjevic Z. Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. Adv Exp Med Biol. 2002;504:3-17. https://doi.org/10.1007/978-1-4615-0629-4_2
   PMid:11922097
2. Bathnagar D, Garcia S. *Aspergillus*. In: Labbe RG, Garcıa S, editors. Guide to Foodborne Pathogens. New York: John Wiley and Sons; 2001. p. 35-49.
3. International Agency for Research on Cancer. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Amines and Mycotoxins. IARC Monographs on Evaluation of Carcinogenic Risk to Humans. Lyon, France: International Agency for Research on Cancer; 1993. p. 56. https://doi.org/10.1016/0003-2670(94)80328-5
4. Theumer MG, Henneb Y, Khoury L, Snini SP, Tadrist S, Canlet C, *et al*. Genotoxicity of aflatoxins and their precursors in human cells. Toxicol Lett. 2018;287:100-7. https://doi.org/10.1016/j.toxlet.2018.02.007
   PMid:29421331
5. IARC. Aflatoxins, IARC Monographs on the Evaluvation of Carcinogenic Risks on Humans. Mono100-23. Lyon, France: IARC; 2012. Available from:https://monographs.iarc.fr/ENG/Monographs/%E2%80%A6/mono100F-23.pdf.
6. Joint FAO/WHO Expert Committee on Food Additives. 49th Meeting of the Joint FAO/WHO Expert Committee on Food Additives. In: Safety Evaluation of Certain Food Additives and Contaminants: Aflatoxins WHO Food Additives Series No. 40. Geneva: World Health Organization; 1998. p. 359-469. https://doi.org/10.1191/096032799678839888
7. Thuvander A, Moller T, Barbieri HE, Jansson A, Salomonsson AC, Olsen M. Dietary intake of some important mycotoxins by the Swedish population. Food Addit Contam. 2001;18(8):696-706. https://doi.org/10.1080/02652030121353
   PMid:11469326
8. Ministry of Agriculture and Rural Affairs, Republic of Turkey. Data on Aflatoxins in Hazelnuts, Pistachios, Walnuts and Almonds during 1998-2002. Beijing, China: Ministry of Agriculture and Rural Affairs; 2002.
9. Ito Y, Peterson SW, Wicklow DT, Goto T. *Aspergillus pseudotamarii*a new aflatoxin producing species in *Aspergillus* section Flavi. Mycol Res. 2001;105:233-9. https://doi.org/10.1017/s0953756200003385
10. Peterson SW, Ito Y, Horn BW, Goto T. *Aspergillus bombycis*, a new aflatoxigenic species, *A. nomius*. Mycologia. 2001;93:689-703. https://doi.org/10.1080/00275514.2001.12063200
11. Ehrlich KC, Chang PK, Yu J, Cotty PJ. Aflatoxins biosynthesis cluster gene cypA is required for G aflatoxin formation. Appl Environ Microbiol. 2004;70:6518-24. https://doi.org/10.1128/aem.70.11.6518-6524.2004
    PMid:15528514
12. Lanyasunya TP, Wamae LW, Musa HH, Olowofeso O, Lokwaleput IK. The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. Pak J Nutr. 2005;4(3):162-9.
13. Rahimi E, Bonyadian M, Rafei M, Kazemeini HR. Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. Food Chem Toxicol. 2010;48(1):129-31. https://doi.org/10.1016/j.fct.2009.09.028
    PMid:19786054
14. El-Nezami HS, Nicoletti G, Neal GE, Donohue DC, Ahokas JT. Aflatoxin M1 in human breast milk samples from Victoria, Australia and Thailand. Food Chem Toxicol. 1995;33(3):173-9. https://doi.org/10.1016/0278-6915(94)00130-g
    PMid:7896226
15. Fung F, Clark RF. Health effects of mycotoxins: A toxicological overview. J Toxicol Clin Toxicol. 2004;42(2):217-34.
    PMid:15214629
16. Binder EM, Tan LM, Chin LJ, Handl J, Richard J. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim Feed Sci Technol. 2007;137:265-82. https://doi.org/10.1016/j.anifeedsci.2007.06.005
17. Sherif SO, Salama EE, Abdel-Wahhab MA. Mycotoxins and child health: The need for health risk assessment. Int J Hyg Environ Health. 2009;212(4):347-68. https://doi.org/10.1016/j.ijheh.2008.08.002
    PMid:18805056
18. Juan C, Ritieni A, Mañes J. Determination of trichothecenes and zearalenones in grain cereal, flour and bread by liquid chromatography tandem mass spectrometry. Food Chem. 2012;134(4):2389-97. https://doi.org/10.1016/j.foodchem.2012.04.051
    PMid:23442700
19. European Commission. Commission Regulation (EC) No 1126/2007. of 28 September 2007 Amending Regulation (EC) no 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Fusarium toxins in maize and maize products. Off J Eur Union 2007;255:14-7. https://doi.org/10.5771/9783845266466-1032-1
20. Wu F. Mycotoxin reduction in Bt corn: Potential economic, health, and regulatory impacts. Transgenic Res. 2006;15:277-89. https://doi.org/10.1007/s11248-005-5237-1
    PMid:16779644
21. Prietto L, Moraes PS, Kraus RB, Meneghetti V, Fagundes CA, Furlong EB. Post-harvest operations and aflatoxin levels in rice (*Oryza sativa*). Crop Protect 2015;78:172-7. https://doi.org/10.1016/j.cropro.2015.09.011
22. Saudi Food and Drug Authority. Contaminants and Toxins in Food and Feed; 2019. Available from: https://www.resources.selerant.com/food-regulatory-news/saudi-government-publishes-a-new-draft-standard-on-contaminants-and-toxins.
23. AOAC Official Methods of analysis of AOAC International. Published 19thedby AOAC International Suite 500 481 North

Fredrick Avenue Gaithersburg, Maryland, USA. Ch. 49. Washington: Association of Official Analytical Chemists; 2012.

24. Dragacci S, Grosso F, Gilbert J. Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin M1 in liquid milk: Collaborative study. J AOAC Int. 2001;84(2):437-43. https://doi.org/10.1093/jaoac/84.2.437

    PMid:11324608

25. Deabes MM, Aboelsoud NH, Taha L. *In vitro* Inhibition of growth and aflatoxin B_1 production of *Aspergillus flavus* strain (ATCC 16872) by various medicinal plant essential oils. Maced J Med Sci. 2011;4(4):345-50. https://doi.org/10.3889/mjms.1857-5773.2011.0190

26. Deabes MM, Darwish HR, Abdel-Aziz KB, Farag IM, Nada SA, Tewfik NS. Protective effects of *Lactobacillus rhamnosus* GG on aflatoxins-induced toxicities in male albino mice. J Environ Anal Toxicol. 2012;2:2-9. https://doi.org/10.4172/2161-0525.1000132

27. Eshak MG, Deabes MM, Farrag AH, Farag IM, Stino FK. Effect of ozone-treated aflatoxin contaminated diets on DNA damage, expression of androgen and androgen receptor genes, and histopathological changes in Japanese quail. Glob Vet. 2013;11(1):1-13.

28. Abou El-Soud NH, Deabes MM, Abou El-Kassem L, Khalil M. Chemical composition and antifungal activity of *Ocimum basilicum* L. essential oil. Maced J Med Sci. 2015;3(3):374-9. https://doi.org/10.3889/oamjms.2015.082

    PMid:27275253

29. a. Deabes MM, Wagdy KB, Attallah AG, El-Desouky TA, Naguib K. Impact of silver nanoparticles on gene expression in *Aspergillus flavus* producer aflatoxin B1. Maced J Med Sci. 2018;6(4):600-5. https://doi.org/10.3889/oamjms.2018.117

    PMid:29731923. b. Deabes MM, Amra HA, El-Damaty EM, Rowayshed GH. Natural Co-occurrence of aflatoxins, cyclopiazonic acid, and their production by *Aspergillus flavu*s isolates from corn grown in Egypt. Adv Clin Toxicol. 2018;3(3):1-10. https://doi.org/10.23880/act-16000136

30. International Conference on Harmonization. Harmonized tripartite guideline, validation of analytical procedures, text and methodology. Geneva, Switzerland: International Conference on Harmonization; 2006. p. 12e4. https://doi.org/10.1007/springerreference_83218

31. NATA Technical Note 17. Guidelines for the Validation and Verification of Quantitative and Qualitative Test Methods. Australia: National Association of Testing Authorities. 2013. p. 17-8.

32. IBM Corp. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp; 2013.

33. Scott PM, Lawrence GA. Determination of aflatoxins in beer. J AOAC Int. 1997;80(6):1229-34.

    PMid:9419863

34. Stroka J, Anklam E. New strategies for the screening and determination of aflatoxins and the detection of aflatoxin-producing moulds in food and feed. Trends Anal Chem. 2002;21(2):90-5. https://doi.org/10.1016/s0165-9936(01)00133-9

35. Stoloff L. Aflatoxins an overview. In: Rodricks JV, Hesseltine CW, MehIman MA, editors. Mycotoxins in Human and Animal Health. Park Forest South, Illinois: Pathotos Publishers; 1977. p. 7-28.

36. Shank RC, Gordon JE, Wogan GN, Nondasuta A, Subhamani B. Dietary aflatoxins and human liver cancer. III. Field survey of rural Thai families for ingested aflatoxins. Food Cosmet Toxicol 1972;10:71-84. https://doi.org/10.1016/s0015-6264(72)80048-8

37. Krishnamachari KA, Bhat RV, Nagarajan V, Tilak TB. Investigations into an outbreak of hepatitis in parts of Western India. Indian J Med Res. 1975;63(7):1036-48.

    PMid:1213790

38. Turner PC, Mendy M, White H, Fortuin M, Hall AJ, Wild CP. Hepatitis B infection and aflatoxin biomarker levels in Gambian children. Trop Med Int Health. 2000;5(12):837-41. https://doi.org/10.1046/j.1365-3156.2000.00664.x

    PMid:11169271

39. European Food Safety Authority. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelsnuts and pistachios and derived products. EFSA J. 2007;446:1-127. https://doi.org/10.2903/j.efsa.2007.446

40. Turner PC, Sylla A, Diallo MS, Castegnaro JJ, Hall AJ, Wild CP. The role of aflatoxins and hepatitis viruses in the etiopathogenesis of hepatocellular carcinoma: A basis for primary prevention in Guinea Conakry, West Africa. J Gastroent Hep. 2002;17:441-8. https://doi.org/10.1046/j.1440-1746.17.s4.7.x

    PMid:12534775

41. Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ Health Pers. 2003;111:217-20. https://doi.org/10.1289/ehp.5753

42. Luttfullah G, Hussain A. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. Food Control. 2011;22:426-9. https://doi.org/10.1016/j.foodcont.2010.09.015

43. Mojtahedi H, Rabie CJ, Lubben A, Steyn M, Danesh D. Toxic aspergilli from pistachio nuts. Mycopathol. 1979;67(2):123-7.

    PMid:481560

44. Pittet A. Natural occurrence of mycotoxins in foods and feeds-an updated review. Rev Med Vet. 1998;149:479-92.

45. Abdel-Hafez, A 2nd, Saber SM. Mycoflora and mycotoxin of hazelnut (*Corylus avellana* L.) and walnut (*Juglans regia* L.) seeds in Egypt. Zentralblatt Mikrobiol. 1993;148:137-47. https://doi.org/10.1016/s0232-4393(11)80117-4

46. Singh PK, Shukla AN. Survey of mycoflora counts, aflatoxin production and induced biochemical changes in walnut kernels. J Stored Prod Res. 2008;44:169-72. https://doi.org/10.1016/j.jspr.2007.10.001

47. Denning DW. Aflatoxin and human diseases. In: Davies DM, deGlanville H, editors. Adverse Drug Reactions and Acute Poisoning Reviews. Vol. 4. Oxford, UK: Oxford University Press; 1987. p. 175-209.

48. Payne GA, Hagler WM, Adkins CR. Aflatoxin accumulation in inoculated ears of field-grown maize. Plant Dis. 1998;72:422-4. https://doi.org/10.1094/pd-72-0422

49. Payne GA. Aflatoxin in maize. Crit Rev Plant Sci. 1992;10(5):423-40.

50. Rachaputi NR, Wright GC, Kroschi S. Management practices to minimise pre-harvest aflatoxin contamination in Australian groundnuts. Austr J Exp Agric. 2002;42:595-605. https://doi.org/10.1071/ea01139

51. Temba MC, Njobeh PB, Kayitesi E. Storage stability of maize groundnut composite flours and an assessment of aflatoxin B1 and ochratoxin a contamination in flours and porridges. Food Control. 2017;71:178-86. https://doi.org/10.1016/j.foodcont.2016.06.033

52. Martinez-Miranda MM, Rosero-Moreano M, Taborda-Ocampo G. Occurrence, dietary exposure and risk assessment of aflatoxins in arepa, bread and rice. Food Control. 2019;98:359-66. https://doi.org/10.1016/j.foodcont.2018.11.046

53. Andrade PD, Caldas ED. Aflatoxins in cereals: Worldwide occurrence and dietary risk assessment. World Mycotoxin J. 2015;8:415-31. https://doi.org/10.3920/wmj2014.1847

54. Tanaka K, Sago Y, Zheng Y, Nakagawa H, Kushiro M. Mycotoxinsin rice. Int J Food Microbiol. 2007;119(1-2):59-66.

    PMid:17913273.

55. Palumbo R, Crisci A, Venâncio A, Abrahantes JC, Dorne JL, Battilani P, *et al*. Occurrence and co-occurrence of mycotoxins in cereal-based feed and food microorganisms. Microorganisms. 2020;8:741-17. https://doi.org/10.3390/microorganisms8010074

PMid:31947721

56. Atanda SA, Pessu PO, Agoda S, Isong IU, Adekalu OA, Echendu MA. Fungi and mycotoxins in stored foods. Afr J Microbiol Res. 2011;5(25):4373-82. https://doi.org/10.5897/ajmr11.487

57. Smith LE, Stasiewicz M, Hestrin R, Morales L, Mutiga S, Nelson RJ. Examining environmental drivers of spatial variability in aflatoxin accumulation in Kenyan maize: Potential utility in risk prediction models. Afr J Food Agric Nutr Dev. 2016;16(3):11086-5. https://doi.org/10.18697/ajfand.75.ilri09

58. Reiter E, Vouk F, Bohm J, Razzazi-Fazeli E. Aflatoxins in rice a limited survey of products marketed in Austria. Food Control. 2010;21:988-91. https://doi.org/10.1016/j.foodcont.2009.12.014

59. Sarker M, Ibrahim M, Aziz N, Punan M. Application of simulation in determining suitable operating parameters for industrial scale fluidized bed dryer during drying of high impurity moist paddy. J Stored Prod Res. 2015;61:76-84. https://doi.org/10.1016/j.jspr.2014.12.004