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

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Abstract

BACKGROUND: Aflatoxins (AFs) are fungal secondary metabolites produced by Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius, which are toxic to humans and animals. AFs are produced in many types of foods and feeds, and are commonly found in dry commodities. The International Agency for Research on Cancer has classified AFs as a Group I carcinogen. A. flavus and A. parasiticus produce aflatoxins, which are mutagenic and carcinogenic. AFs are produced in various countries and are a worldwide problem that affects both food safety and agricultural economies. 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 AFB1, 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 AFB1 ranged 1–3.78 ng/g and the maximum level (ML) detected was 113 ng/g.

AFs are primarily produced by strains of Aspergillus parasiticus, A. parasiticus, A. nomius, Aspergillus pseudotamarii, and Aspergillus bombycis. All of these species are found in the soil [1]. The four major AFs commonly isolated from foods and feeds are AFs B1, B2, G1, and G2. A. parasiticus 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 niger, A. bombycis, and A. parasiticus produce all four major AFs. AFs M1 and M2 are hydroxylated metabolites of AFB1, and B1, respectively, and are produced in milk-producing animals [12, 13]. AFM1 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 AFM1 in human breast milk from Victoria, Australia, and Thailand. AFM, 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 AFB1, 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 from 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

- Samples were 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
- Processing as they were already “ready to eat.”

| Food commodities categorized to following groups |
|-----------------------------------------------|
| Food category                                  | Included samples |
| Bakery                                        | Bread (white, brown, and bran) |
|                                               | Toast white, Tamis (white and brown), rusk (white and brown), cornflakes |
| Cereal                                         | Rice (white and brown), and spaghetti (white and brown), oats |
| Legumes                                       | Fava bean, lentil, and chickpea |
| 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) |

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].

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

Standards of AFM1 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, (AfM1) 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 2000x 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 AFM\(_1\) by HPLC.

**Determination of AFM\(_1\) 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.

**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\(_\alpha\) were converted into other derivatives, AFB\(_\alpha\) and AFG\(_\alpha\), respectively, (AFG\(_\gamma\) and AFB\(_\gamma\)) had low fluorescence properties, therefore, and they were converted to G\(_\alpha\) and B\(_\alpha\), 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\(_\alpha\) 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], for AFs G\(_\alpha\), B\(_1\), G\(_\beta\), and B\(_\beta\) 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, in chloroform L (9.93 mg/ml). An AFM, standard as injected every ten injections as a quality control. AFM, was stored at −20°C in a sylillated vial wrapped in aluminum foil. Since AFs B, B, G, G, and M 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:

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

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.

\[\text{LOQ} = 10s+X\]

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, B, G, and G) and M 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 (AFB, AFB, AFG, and AFG) 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, AFB, AFG, and AFG) 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, AFB, AFG, and AFG) in samples collected from Jeddah region, respectively.

The obtained results of Table 1 indicated that the incidence rate % of bakery 0% and legumes but in coffee 62%, cereals 7%, nuts 27.3, and dairy products 13.3% for AFs (AFB, AFB, AFG, and AFG) 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 | 0/51 (0%)          | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | AFB< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | AFG< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | TAF< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
| Cereals rice (white and brown), spaghetti (white and brown), oats | AFB< | 5/72 (7%)          | 0.46 | 3.58 | 0.160 | 0.79 | 0.000 | 0.00 | 0.00 | 1.03 |
|                                          | AFB| -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | AFG| -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | TAF | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
| Legumes bread (white, brown, and bran)   | AFB| 0/28 (0%)          | 0.46 | 5.38 | 0.186 | 0.89 | 0.000 | 0.00 | 0.00 | 1.26 |
|                                          | AFB< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | AFG< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | TAF | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
| Coffee Arabic Turkish                    | AFB< | 5/8 (62.5%)        | 0.29 | 0.93 | 0.37 | 0.36 | 0.372 | 0.71 | 0.00 | -  | -  |
|                                          | AFB| -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | AFG| -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | TAF | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
| (Nuts) walnut cashew peanut pistachio almond | AFB | 27/99 (27.3%)    | 0.19 | 482.4 | 11.14 | 60.5 | 0.000 | 0.000 | 0.000 | 0.66 | 12.3 |
|                                          | AFB< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | AFG< | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
|                                          | TAF | -                  | -    | -    | -    | -   | -      | -   | -  | -  | -  |
| Daily products milk, liquid yogourt, yogourt, fresh milk | AFM< | 4/50 (13.3%)  | 0.06 | 0.93 | 0.061 | 0.01 | 0.06 | 0.00 | -  | -  | -  |

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

Table 2: Aflatoxin detection range in dry food commodities

| Aflatoxins μg/kg | Incidence rate (%) | AFs | AF< | AF< | AF< | AF< | AF< | AF< | AF< |
|------------------|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|
|                  |                    |      | Min | Max | Mean | ±SD | Median | IQR | 10 | 90 | 95 |
| LOD<             | 222                | 86.0 | 242 | 93.8 | 93.0 | 246 | 93.0 | 93.0 | 221 | 85.7 |
| LOD<<2           | 21                 | 8.1  | 15  | 5.8  | 5.5  | 14  | 5.4  | 8    | 3.1 | 20 | 7.76 |
| <=4              | 7                  | 2.7  | 1   | 0.4  | 0.0  | 0   | 0.0  | 0    | 0   | 4  | 1.55 |
| >4<20            | 4                  | 1.6  | 0   | 0.0  | 0    | 0   | 0    | 0    | 4   | 9  | 3.5 |
| >20              | 4                  | 1.6  | 0   | 0.0  | 0    | 0   | 0    | 0    | 4   | 4  | 1.55 |
| Total            | 258                | 100.0 | 258 | 100.0 | 100.0 | 258 | 100.0 | 100.0 | 258 | 100.0 |

LOD: Limit of quantitation, LOD: Limit of detection, AFB: Aflatoxin B<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>, AFG<sub>3</sub>, AFG<sub>4</sub>, and TAF.<sub>1</sub> Total aflatoxins.

Discussion

Aflatoxins are hepatocarcinogens and have been classified as Class 1 human carcinogen [3]. The average daily intake of AFB<sub>1</sub> 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...
In our results found that in walnuts AFB1 exceed in the average daily intake of AFB, 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, and G. The detection range in dry food commodities sample was contaminated with AFs ranged from 0.036 to 482.4 for AFB, and AFG, 0.035–87.1 μg/kg when the samples (n = 258) were analyzed the positive samples 36 and 18 for AFs, B, and G contained the incidence rat% 14.34 and 7%, respectively.

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

| No. of replicated samples | AFG₁ | AFB₁ | AFG₂ | AFB₂ |
|--------------------------|------|------|------|------|
| 1                        | 79.0 | 95.22| 53.25| 63.1 |
| 2                        | 78.4 | 87.28| 53.25| 72.6 |
| 3                        | 78.4 | 89.66| 60.0 | 65.13|
| Mean                     | 78.46| 90.72| 55.5 | 66.49|

AFG₁: Aflatoxin G₁, AFB₁: Aflatoxin B₁, AFG₂: Aflatoxin G₂, AFB₂: Aflatoxin B₂, AFG₁: Aflatoxin M₁.

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 M₁ in fluid milk

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

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
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 AFB₁ 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, AFB₁, 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 μg/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 AFB₁, and 5–40 ng/mL for (AFB₁, AFB₂, AFG₁, and AFG₂), 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 AFB₁, AFB₂, AFG₁, and AFG₂ were below 0.1 μg/kg (AG₁ and AG₂ 0.04 μg/kg; and AB₁ and AB₂ 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 is 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.

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