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Research article

Occurrence of aflatoxins in nuts and peanut butter imported to UAE

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ABSTRACT

Nuts are an important food group that contributes to maintaining health; however, they can be a source of exposure to aflatoxins. This study was conducted from 2017 to 2021 to assess the incidence of aflatoxins in nuts and nut products imported to the UAE from 57 countries. Associations between container type and processing technique and aflatoxin levels were also analyzed. A total of 5401 samples of pistachios, peanuts, peanut butter, and mixed nuts were examined using HPLC-FLD analysis in conjunction with immunoaffinity cleanup. In nuts, non-conformity was detected in samples imported from 32 different countries. Mean aflatoxin values for the non-compliant samples ranged from 81.0 to 92.7 μg/kg in pistachios, peanuts, and mixed nuts. A significant difference ($p < 0.05$) was found between mean aflatoxin levels in samples of peanut butter (29.3 μg/kg) compared to the other types of nuts. Nuts packed in containers made of fabric material had the highest mean aflatoxin levels of 108.1 μg/kg, while 29.7 μg/kg was the lowest mean level and was detected in nuts packed in glass. Ground samples had the highest aflatoxin levels (158.9 μg/kg) among processed products. This report will be valuable as a reference document in developing approaches to control nut importation and for establishing procedures that prevent food safety risks due to aflatoxin exposure. A need was underlined for the regulating authority to audit companies importing nuts, ensure safe practices are in place, and establish standards to minimize contamination and prevent the need for product rejection at the border.

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1. Introduction

Nuts have multiple health benefits; they contribute to prevention of anemia, coronary disease, cholesterol regulation, and are involved in the proper functioning of the body organs due to their elevated content of various macronutrients, micronutrients and their dietary fiber content [1]. Nuts are known to be one of the most susceptible food groups to mycotoxin contamination [2]. Mycotoxins have been recognized as the greatest dietary risk factor for chronic illnesses and these exceed those caused by food additives, synthetic contaminants, pesticides residues, and plant toxins [3]. It has been proven that the secondary metabolites of fungal toxins can affect the immune and reproductive systems as well as have a direct effect on the liver by causing disease [4,5]. The International Agency for Research on Cancer (IARC) has classified aflatoxins as Group I carcinogens [6]. All countries that export food and feed products to Europe are subject to monitoring by the EU rapid alert system (RASFF), which is an online database of serious human health risks from imported food/feed. RASFF recently reported that aflatoxins were responsible for 89% of 5045 notifications for food and 439 notifications for feed [7]. The European Food Safety Authority (EFSA) categorized the contribution of major food groups to dietary exposure of humans to aflatoxins. The EFSA (2020) reported that of the major food groups contributing to aflatoxin B_1 exposure, the most frequently implicated food group for all ages was "grain and grain-based products". However, it is notable that nuts belong to another major food group "legumes, nuts, and oilseeds" where peanuts contributed 24% of the adult aflatoxin exposure in most surveys. High aflatoxin B_1 values were detected in other types of nuts including pistachios [2,8], as well as in the food group "herbs, spices, and condiments", but the contribution of the latter group to aflatoxin B_1 exposure was considered insignificant due to the minimal quantities that are consumed. Nuts may be present as an ingredient in the "sugar and confectionary" food group and can be an important contributor to aflatoxin B_1 exposure of children and adolescents. The "animal and vegetable fats and oils" food group caused considerable aflatoxin B_1 exposure to toddlers and other children due to consumption of peanut butter in European countries, and this contributed 39% of the exposure in toddlers [2]. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the contribution of peanuts to aflatoxin exposure worldwide has been estimated to be more than 10% of the total [9]. A global systematic review found that although there are disparities among countries, on average 8.9 g of nuts are consumed daily [10]. A daily quantity of 30 g nut consumption has been proposed in dietary guidelines in order to reduce the risk of cardiovascular disease [11]. Although it is advisable to increase nut consumption, a greater mycotoxin exposure could affect vulnerable groups [1]. A recent risk assessment study conducted in North-Central Nigeria found that chronic aflatoxin B_1 exposure from peanuts resulted in a risk of 247, 99, and 41 new liver cancer cases/year/100,000 children, adolescents, and adults, respectively [12].

The United Arab Emirates (UAE) imports more than 85 thousand metric tons of nuts yearly from 57 countries [13]. To control the exposure to aflatoxins, maximum limits have been set by Codex Alimentarius for various types of nuts: 15 μg/kg for peanuts, 10 μg/kg for ready-to-eat almonds, pistachios, brazil nuts, and hazelnuts, while 15 μg/kg is prescribed for the latter 4 nuts that are intended for further processing [14]. The reliance on Codex-established limits is uneven between countries. For example, some commodities are regulated by EU legislation, whereas stricter limits have been set for other products. Other countries have established their own maximum residue limits (MRLs) including Australia, Japan, China, Canada, and the USA [10]. As for the UAE, MRLs for aflatoxins established in the UAE'S GSO 193:2021 on contaminants and toxins in food and feed were based on Codex standards with some modification. A concentration of 10 μg/kg was established for both ready-to-eat pistachios and those intended for further processing, whereas 15 μg/kg was permitted for both ready-to-eat nuts and for peanuts, mixed nuts, and peanut butter intended for further processing.

The risk of contamination with mycotoxins can occur during cultivation, processing, transporting, or storage [15]. For instance, the occurrence of damage while harvesting or handling seeds or by birds and insects promotes the growth of aflatoxin-producing fungi [4]. The warmer the climate, the greater the chance of contamination by these secondary metabolites [16]. It is anticipated that an increase in pre-harvest aflatoxin contamination may result from global warming [7]. Specifically, an ambient temperature of 25–30 °C and a moisture content greater than 16% are favorable conditions for the production of mycotoxins [17]. Taking peanuts as an example, periods of rain that may occur after their uprooting, because of their need for an extended time to dry will promote aflatoxin contamination [18].

In addition to natural factors that contribute to contamination, processing steps including packaging, can influence the incidence of aflatoxins [15]. A number of studies have investigated the incidence of aflatoxin residues in nuts. In India, aflatoxins were found to be common in 154 samples of nuts and dried fruits including cashew nuts, pistachios, and almonds, and as much as 442.8 ng/g was detected in pistachios using an enzyme-linked immuno-sorbent assay (ELISA) method [1]. Relying on RIDASCREEN FAST immune-enzymatic competitive assays, Macri et al. (2021) found that aflatoxin levels exceeding the maximum limits were observed in 14.1% of 64 samples collected in Romania. Furthermore, polyethylene terephthalate (PET) was observed to be the most suitable plastic packaging material for maintaining the quality and the safety of nuts, where aflatoxin levels of most nuts packaged in PET contained about 1 μg/kg (mean = 1.64 ± 0.37 μg/kg). When other types of plastics (polyethylene, low-density polyethylene, and polypropylene) were considered it was found they showed higher mean concentrations of 3.16 ± 0.77, 8.63 ± 2.44, and 5.69 ± 1.62 μg/kg, respectively. Another study performed in Kenya involved the collection and ELISA analysis of peanuts from 1263 vendors. Results indicated that 37% of all samples exceeded the 10 μg/kg limit set by the Kenya Bureau of Standards (KEBS); however, this reached 69% for peanut butter packed in plastic jars and 68% for peanuts packaged in plastic jars [19].

Information on the prevalence and health risk of aflatoxins in the UAE and Gulf countries are still lacking. The present study investigated the incidence of aflatoxins in nuts imported to the UAE via its ports between 2017 and 2021. A secondary objective was to analyze the associations between container type and processing technique and aflatoxin levels.

2. Materials and methods

Trained analysts in the food chemistry laboratories accredited for the analyses using recognized international standards and validated methods, prepared samples and conducted the aflatoxin analysis according to ISO/IEC 17025:2017 (Food Chemistry Laboratories, Dubai Municipality, Dubai, UAE).

2.1. Sample collection

Sampling of nuts and nut products was conducted from 2017 to 2021 on a daily basis from 6 food warehouses located in the Dubai ports, Dubai Emirate. A total of 5401 samples of different types of nuts and nut products were collected. Authorized food inspectors from the Dubai municipality conducted sampling of nut products. Samples were analyzed within 24–48 h of their arrival. The present study focused on the types of nuts where aflatoxin analysis was part of their routine testing. Four major subcategories were defined: mixed nuts, peanuts, peanut butter, and pistachios. The methods of sampling for the official control of level of mycotoxins established by the EU Directive 2006/401/EC [20] were followed.

2.2. Chemicals and reagents

All chemicals, reagents and solvents were analytical grade including phosphate buffered saline (PBS) tablets and hexane, which were purchased from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, unless otherwise stated. Separate aflatoxin standards for B_1, B_2, G_1, and G_2 were procured from Romer Labs Division Holding GmbH, Getzersdorf, Austria at concentrations in acetonitrile of 2 μg/ml (B_1 and G_1) and 0.5 μg/ml (B_2 and G_2). A mix standard (Supelco Inc., Bellefonte, PA, USA), immune-affinity columns (R-Biopharm AG, Darmstadt, Germany), analytical grade acetonitrile and methanol (Panreac Applichem, Dubai, UAE) were used in this study. Milli-Q ultra-pure water was prepared by a water purification and dispensing unit (Merck, Milli-Q® IQ Element Water Purification, MA, USA).

2.3. Standards preparation

Individually purchased aflatoxins were used to prepare mixed standard solutions at 10 μg/kg for aflatoxins B_1 and G_1, and 3 μg/kg for aflatoxins B_2 and G_2 as high aflatoxin levels. These concentrations were further diluted 5 times to reach low concentrations. Alternately, the purchased mix standard was diluted accordingly with methanol.

2.4. Sample preparation

A total of 5 kg of each sample was ground into fine powder using a mill (Blixer-2, Robot Coup, France), then thoroughly mixed to establish homogeneity. The extraction started by blending 100 g sample with 250 ml of the extraction solvent acetonitrile: water (60:40 v/v) using an explosion-proof blender at 8000 rpm (Waring, USA) for 1–3 min, then filtered using Whatman #1 filter paper. Then 250 ml hexane was mixed with the filtrate as a defatting agent and extracted using a separatory funnel, discarding the upper layer. Ten ml of the sample was evaporated to dryness with a stream of N_2. Then 100 ml of PBS solution was added to the sample residues and mixed using an ultra-sonic water bath (Decom, F53000, UK). The clean-up was initiated by loading 20 ml of PBS to condition the immuno-affinity column, followed by the diluted sample and then eluted at a rate of 5 ml/min. The column was washed with distilled water after elution. Then 5 ml methanol was loaded on the column and allowed to pass through it by gravity to elute the toxin into a glass vial which was ready for injection.

2.5. HPLC conditions

To determine aflatoxins B_1, B_2, G_1 and G_2, a Waters Alliance separation module 2695 HPLC system (Waters, Milford, MA, USA) was used. This consisted of a fluorescence detector and quaternary pump capable of delivering 1 ml/min of mobile phase with a standard injection syringe delivering variable injection volumes. A 20 μl sample of supernatant was injected into a reverse phase LiChrosorb RP18 (4.6 × 250 mm, 5 μm) column attached to a Chromguard RP18 guard column (Waters, Milford, MA, USA) and a post column derivatization unit. The temperature was set at 40 °C using a column thermostat. A flow rate of 1 ml/min with a mobile phase containing acetonitrile/methanol/water in a ratio of 20:20:60 was applied. Detection was conducted at wavelengths of 360 nm excitation and 420 nm emission. The injection separation of all 4 peaks was achieved within 18 min of run time.

2.6. Quality control and quality assurance

All analytical laboratories in the Dubai Municipality conform to the general requirements of ISO/IEC 17025:2017 regarding the competence to carry out tests and/or calibrations, including sampling. Quality control procedures were documented. Validation methods and quality control procedures for aflatoxin analyses in food were followed by the procedures of the European Union [20]. The limit of quantification (LOQ) was considered to be the lowest concentration that could be quantified with acceptable routine recoveries of 60.0–140.0%. The acceptable limits and mean recovery precision were ±2 × RSD (%) and included the adjusted repeatability or intra-laboratory data [20]. When the concentration of aflatoxin residues in nuts and nut product samples exceeded the

MRLs, the sample was re-analyzed to confirm the results.

Recovery experiments were carried out with the CEN/AOAC method (2012) that uses a mixture of Acetonitrile/Water (60:40) as extraction solvent mixture, immuno-affinity column cleanup, and HPLC-FLD determination with post-column derivatization [21].

Immuno-affinity column recovery was checked by adding 1 ml of aflatoxin mix standard solution to phosphate buffered saline solution, the solution was passed then through the immuno-affinity column followed by a washing step with 20 ml of distilled water. Aflatoxins have been extracted by passing 5 ml of Methanol through the column and analyzed.

The recovery was calculated using the following formula:

(Measured aflatoxin concentrations/spiked standard concentration)*100 (1)

2.6.1. Acceptance criteria was 90–110% for aflatoxins

Sample recovery experiments were conducted by spiking a known amount of aflatoxins to the samples with known weight. Three spiking levels (1, 2.5 and 10 μg/kg for AFB1, & AFG1; 0.3, 0.75 and 3 μg/kg for AFB2 and AFG2) where used by adding an appropriate amount of aflatoxin standard solutions to the blank nut samples. The spiked samples were extracted, cleaned-up, derivatized, and analyzed.

Recovery percentage was calculated using the following formula:

%Recovery = (W*(Cs–Cu)*100)/(V*standard concentration) (2)

where:

W = weight of sample taken
Cs = aflatoxin concentration in sample
Cu = concentration of unspiked sample
V = volume of standard added

Twenty spiked samples at each level were injected to calculate the mean Recoveries.

Acceptance criteria for recoveries is 80–120% for Aflatoxin B1 and G1 and 70–130% for Aflatoxin B2 and G2.

Calibration curves were prepared between six points of 0.5–10 μg/l for Aflatoxin B_1, G_1 and 0.2–3.0 μg/l for Aflatoxin B_2, G_2 with linear response regression ($R^2 > 0.99$).

2.7. Statistical analysis

Aggregate functions were performed using Microsoft Excel 2013. Analysis of variance (ANOVA) was used to compare the type of nuts and type of packaging for the level of aflatoxins by using IBM SPSS Statistics (version 26) software. Statistical differences were tested at ($p < 0.05$). Aflatoxin values were measured, calculated and reported for the non-conforming samples only. The accredited laboratory and regulatory authority held results for compliant samples in confidence and data were not made public.

The various types of packaging materials observed in the present study were clustered into 5 major groups for effective comparison: metal, paper, fabric, plastic, and glass:

1. Metal: aluminum (foil tray, tin, aerosol, can, foil bag, foil packet, foil sachet, pouch), canister, doypack bags, metal (can, tin), and steel (bottle).
2. Paper: cardboard (bins, box, packet, bottle), carton, paper (bag, packet), and tetra pack
3. Fabric: coir netting, cotton bag, jute bag, and non-woven fabric bag.
4. Plastic: plastic (bottle, box, bucket, can, container, drum, jar, net, packet, pot, pouch, sachet, sack, tray), and foam box.
5. Glass.

Table 1
Results of in-house validation of the HPLC-FLD method for aflatoxins.

Analyte	Spike level	Mean Recovery (%) (n=20)	RSD (%)	LOD (μg/kg)	LOQ (μg/kg)
Aflatoxin B1	1,2.5 and 10 (μg/kg)	96.1	3.8–6.6	0.23	0.7
Aflatoxin B2	0.3,0.75, 3.0 (μg/kg)	94.4	2.6–4.3	0.1	0.3
Aflatoxin G1	1,2.5 and 10 (μg/kg)	94.3	2.5–5.6	0.23	0.7
Aflatoxin G2	0.3,0.75, 3.0 (μg/kg)	97.2	3.5–5.7	0.1	0.3

RSD: relative standard deviation of repeatability; LOD: limit of detection; LOQ: limit of quantification

3. Results and discussion

3.1. Analytical method performance

Triplicate analyses were performed using three different spike levels for each of aflatoxin B_1, B_2, G_1 and G_2 standards and the results are reported in Table 1. Mean aflatoxin recoveries were found to be 96.1, 94.4, 94.3 and 97.2%, respectively. The mean sample recoveries ranged between 80 and 120% for aflatoxin B_1 and G_1 and 70–130% for aflatoxin B_2 and G_2. These mean recoveries were considered acceptable as per the criteria established in the EU Regulation 519/2014 for aflatoxins [22]. Limits of detection (LODs) ranged from 0.1 (aflatoxin B_2 and G_2) to 0.23 μg/kg (aflatoxin B_1 and G_1), while limits of quantification ranged from 0.3 (aflatoxin B_2 and G_2) to 0.7 μg/kg (aflatoxin B_1 and G_1) (Table 1).

3.2. Incidence and concentrations of aflatoxins in different nuts

The incidence and concentrations of aflatoxins in the non-compliant samples studied are shown in Table 2. Of the 5401 samples examined, 4935 (91.4%) conformed to the UAE aflatoxin standards of <10 μg/kg for pistachios and <15 μg/kg for mixed nuts, peanuts, and peanut butter. Although mixed nut samples were in the lowest numbers (119) compared to the other types of nuts, they showed the highest degree of compliance (95.8%), and were followed by peanut butter (95.3%), pistachios (90.2%), and peanuts (88.2%) (Table 2). Among non-conforming samples, peanuts represented the greatest percentage (11.8%) and also had the highest mean aflatoxin concentrations (92.7 ± 82.3 μg/kg). These were followed by pistachios (82.4 ± 85.9 μg/kg) at 9.8% of non-conforming samples, mixed nuts (81.0 ± 50.6 μg/kg) at 4.2% non-conforming samples, and then peanut butter (29.3 ± 18.9 μg/kg) at 4.7% (Table 2). These means were well above the UAE standard, where the limit for the mean percent of aflatoxin for non-compliant peanut butter was 195%, while that for non-compliant pistachios, peanuts, and mixed nut samples ranged from 540 to 824%. The maximum aflatoxin levels for peanuts and peanut butter were 377 and 106.5 μg/kg, respectively. A previous study from Taiwan showed that 267 of 1089 peanut and peanut products (25% of total samples) imported from 2011 to 2017 were contaminated with aflatoxins and that 34 samples (3.1%) exceeded the limit, where 441 μg/kg was the maximum aflatoxin concentration [23]. In the current study, 8.2% (289 samples) of samples exceeded the aflatoxin concentration limit (15 μg/kg) which was higher than the previous study conducted in Taiwan. A global systematic review from 2000 to 2020 found that maximum aflatoxin levels were 530 μg/kg for peanuts and 245.6 μg/kg for pistachios [10]. In Qatar, 2.5% of nut samples exceeded the limits (total = 119) and 532.2 μg/kg was the maximum aflatoxin value detected [5], which was close to the maximum concentration found in the present study (551 μg/kg).

3.3. Incidence and concentrations of aflatoxins in respect to packaging materials

Table 3 shows the incidence and concentrations of aflatoxins in respect to the packaging materials. Fabric material had the highest number of non-compliant samples and highest aflatoxin concentrations (28.6% and 108.1 ± 88.5 μg/kg, respectively), followed by paper (11.4% and 81.7 ± 79.5 μg/kg), plastic (7.9% and 70.6 ± 78.2 μg/kg), metal (4.1% and 63.5 ± 57 μg/kg), and then glass (2.3% and 29.7 ± 17.4 μg/kg, respectively). Only the differences in aflatoxin content of samples packaged in either glass and fabric materials were significant ($p < 0.05$), although controversial results were found when the association between aflatoxins and types of packaging for peanuts was investigated in Kenya [19]. The latter authors found that plastic jars had the highest number of non-compliant samples (68.1%), followed by metal tins (44.4%), paper (41.5%), PVC bags (40.7%), propylene bags (36.2%), plastic basins (27.4%), and reed baskets (22.1%). When the two sets of results in plastic jars were compared, the frequency of non-conformity in Kenyan samples was much higher; where aflatoxin levels in 68/1264 samples (5.4%) exceeded the limits. The maximum aflatoxin concentrations in metal and paper packages (216.3 and 400 μg/kg, respectively) were higher than in the study conducted in Kenya (36 and 147 μg/kg, respectively), whilst the maximum aflatoxin levels for all types of plastics (22,064 μg/kg) was substantially higher than the result

Table 2
Incidence and concentrations of aflatoxins in non-compliant nut and nut products rejected at UAE borders

Type of sample	No. of compliant products (%)	No. of non-compliant products (%)	Mean (μg/kg)*	Range (μg/kg)*	Median (μg/kg)*	Difference from the limit (%)*
Mixed nuts (n = 119)	114 (95.8%)	5 (4.2%)	81 ± 50.6[d]	13.4–150.7	85.8	540[§]
Peanuts (n = 1726)	1522 (88.2%)	204 (11.8%)	92.7 ± 82.3[d]	11.6–377	59.3	618.3[§]
Peanut butter (n = 1795)	1710 (95.3%)	85 (4.7%)	29.3 ± 18.9[e]	10.7–106.5	22	195.2[§]
Pistachios (n = 1761)	1589 (90.2%)	172 (9.8%)	82.4 ± 85.9[d]	10.5–551	51.4	824.4[§§]
Total (n = 5401)	4935 (91.4%)	466 (8.6%)	77.1 ± 79.3	10.5–551	40.8	NA

Concentrations followed by the different letters are significantly different ($p < 0.05$).
NA: Not applicable.
* Aflatoxin concentrations were reported for only the non-compliant samples.
§ Difference was calculated based on the limit of 15 μg/kg
§§ Difference was calculated based on the limit of 10 μg/kg;

Table 3
Incidence and concentrations of aflatoxins in non-compliant products by product packaging

Type of packaging	No. of non-compliant products (%)	Mean (μg/kg)*	Range (μg/kg)*	Median (μg/kg)*
Metal (n = 617)	25 (4.1%)	63.5 ± 57^{bc}	11.7–216.3	35.6
Paper (n = 1175)	134 (11.4%)	81.7 ± 79.5^{bc}	10.5–400	50.8
Fabric (n = 175)	50 (28.6%)	108.1 ± 88.5^{b}	16.4–377	75.9
Plastic (n = 3175)	251 (7.9%)	70.6 ± 78.2^{bc}	10.7–551	36
Glass (n = 258)	6 (2.3%)	29.7 ± 17.4^{c}	12.9–57.8	22.1
Loose item (n = 1)	0	0	0	0

Concentrations followed by the different letters are significantly different ($p < 0.05$).
* Aflatoxin concentrations were reported for only the non-conformed samples.

found in current study (551 μg/kg).

The predominant use of fabric packaging was partly responsible for the increased fungal growth in peanuts observed during a study in India. This was observed by Darko et al. (2018) when peanuts were packed in woven polypropylene sacks as compared to hermetic packs, hermetic packs with oxygen absorbers, and evacuated hermetic packs (means of 8.01×10^4, 1.07×10^3, 14.55, and 57.82 CFU/g, respectively). Fabric materials are not airtight, exposing nuts or other types of food to moisture which can facilitate mycotoxin production by proliferating fungi. When results from vacuum-packed samples tested in the current study were examined, the aflatoxin concentrations of 14 vacuum-packed pistachio and peanut samples imported from China, Turkey, Iran, and the USA were found to be below the UAE standard (10 and 15 μg/kg for pistachios and peanuts, respectively) (data not shown). This was consistent with the Indian results, where the overall mean of vacuum-packed samples was 6.02 ± 1.04 μg/kg [24]. It is worth noting that more than half of the studied samples (58.8%) were packaged in the various types of plastic containers previously mentioned. Aflatoxins are UV light sensitive and UV can slightly reduce aflatoxin concentrations, which suggests that the light permeability of plastic packaging can contribute to maintaining nut quality and safety. However, the growth of aflatoxin-producing fungi like *Aspergillus flavus* is generally triggered by visible light (VIS), allowing both the inner and the outer nut layers to be infected [15]. Besides light, moisture and gas permeability through packaging film material depends on the type of plastic used which may influence aflatoxin contamination. A study conducted by John et al. (2019) found a difference in aflatoxin concentrations among different types of plastic packaging. They experimented with three types of plastic packages: low-density polyethylene (LDPE), polypropylene (PP), and polyethylene-laminated aluminium (PELA). According to their results, PELA, which is composed of a multi-layered structure, was the most suitable packaging material for protecting peanuts against aflatoxin contamination since it is a durable barrier to heat and oxygen [25]. The current study showed that the incidence and contamination of aflatoxins was lower in nuts packaged in metal than plastic materials. Similarly, a recent study found that aluminum foil packaging had a greater effect on slowing down aflatoxin formation in chili powder than polypropylene [26].

3.4. Incidence and concentrations of aflatoxins in respect to the processing methods

Fig. 1 shows the incidence and concentrations of aflatoxins as influenced by processing methods. Ground nut samples had the highest mean aflatoxin levels (158.9 μg/kg) which was approximately twice that of the sliced nut samples (81.62 μg/kg). These were

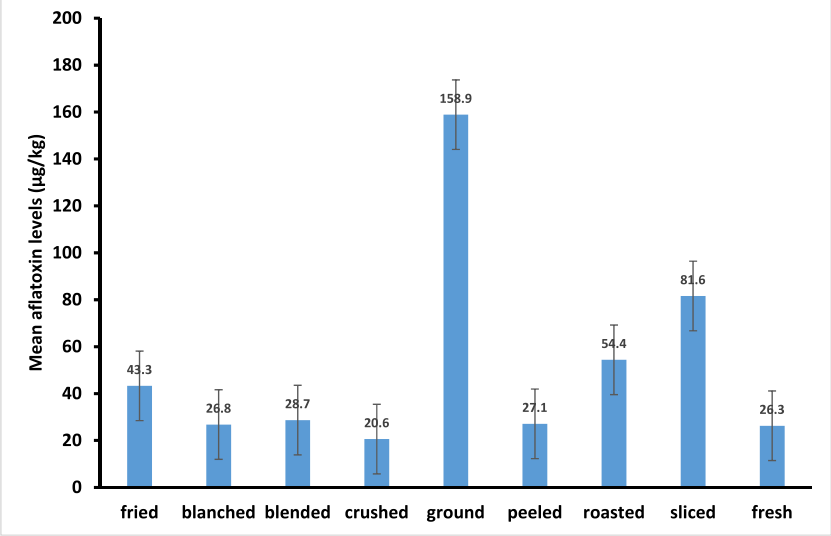


Fig. 1. Mean aflatoxin levels in the various studied types of fresh and processed non-compliant products.

ground peanuts where 12 of 21 samples had aflatoxin levels above 150 μg/kg (186–304 μg/kg). A study by Bisht & Menon (2017) on ginger found that whole roots had the least aflatoxin, but unlike the results of the present study, the sliced dried ginger had greater contamination than the ground dried ginger [27]. The effect of almond processing was studied by Zivoli et al. (2014) and it was found that grinding nuts distributed aflatoxin concentrations throughout the samples, supporting the results of the current work [28]. This seemed to be a logical explanation for observations following analysis of sliced nut samples that appeared to be affected by fungal growth or aflatoxin cross-contamination. Blanching was shown to slightly reduce the aflatoxin levels of almonds [28] and this was explained by the low water solubility of these toxins. Though the blanched samples of the current study had low aflatoxin levels (26.83 μg/kg), these levels cannot be attributed to the blanching step alone since there were a number of relevant variables, some unmonitored that could have influenced the outcome, including the country of origin, packaging, type of nuts, presence of other ingredients, storage time and conditions of storage.

Results of peanut butter analyses in the current study showed a very low mean concentration (mean = 29.3 ± 18.9 μg/kg; max = 106.5 μg/kg) compared to other nut products (Table 2). It was thought that peanut butter processing steps were responsible for the low concentrations. The difference between mean aflatoxins detected in peanut butter with those detected in each of peanut, pistachio, and mixed nuts was considered significant ($p < 0.05$), while no significant differences were found among the last three mentioned types. In previous work, Mupunga et al. (2014) found that 17% (3/18) of peanut samples imported by Zimbabwe were contaminated with aflatoxins at a mean level ranging between 6.6 and 622.1 μg/kg. Additionally, 10/11 peanut butter samples contained from 6.1 to 247 μg/kg with a mean level of 75.7 μg/kg aflatoxin [29]. Despite the small number of samples in that study, aflatoxin levels in peanut butter were much lower than that of peanuts in each of the studies, taking into consideration that the levels of the Zimbabwe study targeted samples above and below the limit. In the current study mean aflatoxin in non-compliant peanut butter was 70% lower than that of non-compliant peanuts. This could be explained by the lower peanut content of peanut butter as it contains other ingredients (e.g. salt, sugar, hydrogenated fat) or by the effect of processing [29,30]. It is known that mycotoxins are stable in food products [31], however, reaching the melting point of aflatoxins (237–289 °C) by cooking or dry roasting could partially destroy them [32]. The effect of processing has been investigated in previous studies. Siwela et al. (2011) studied the carryover of aflatoxins during peanut butter processing using HPLC analysis. Results showed that 89% of the initial aflatoxin concentration was reduced during production. In particular, half of the initial concentration was reduced after roasting at 160 °C, about the third was reduced after blanching and de-skinning steps, and an 11% reduction occurred after grinding [32]. It is to be noted that even after processing, the mean aflatoxin levels of the present work were above the limits for both peanuts and peanut butter, being at 618.3% and 195.2% of the limit, respectively, which indicates an alarming issue for the exporting countries. In contrast to the findings of Siwela et al. (2011), aflatoxin levels of fried and roasted samples were higher than that of non-heated, blended, crushed, peeled, or fresh samples. Since products that have been heat-treated should have reduced aflatoxin levels [33], it is probable that recontamination occurred during their subsequent movement through the food distribution chain and storage until they were analyzed.

3.5. Incidence and concentrations of aflatoxins in respect to exporting countries

The collected samples were imported from 57 countries. Table 4 shows that products imported from 25 of the 57 countries complied with UAE standards. The incidence of aflatoxins in the remaining 32 countries are shown in Fig. 2. The highest mean aflatoxin levels for non-conforming pistachios were those imported from Syria (200.2 μg/kg) and Afghanistan (152.2 μg/kg). The three highest mean aflatoxin levels above 200 μg/kg for peanuts were found in samples imported from Rwanda, Sri Lanka, and Chad (216, 205.9, 204.8 μg/kg, respectively). Products imported from Uganda, Thailand, and Brazil had mean aflatoxin levels between 150 and 200 μg/kg for peanuts and were 180.9, 179.1, and 150 μg/kg, respectively. The highest mean aflatoxin level in non-compliant mixed nuts was found in samples imported from Lebanon (111.3 μg/kg), while that for peanut butter was seen in samples imported from Uganda (48.9 μg/kg) (Fig. 2). Despite these extremely high aflatoxin levels in products imported from different countries, one must also consider the percent of non-compliant samples relative to the total number of imported products. For instance, only 4/64 (6.3%) of Syrian pistachio samples were non-compliant, 1/40 (2.5%) samples of Brazilian peanuts, 74/949 (7.8%) of Indian peanut butter, 2/93 (5.4%) of Italian pistachios, 3/50 (6%) and 4/83 (4.8%) of Lebanese mixed nuts and peanuts were non-conforming among the imported products during the 5 years of the study. Of course, this may negatively affect motivation to resolve potential chronic problems within these nut exporting countries. Nonetheless, the 100% of non-conformity found in peanut butter samples imported from Uganda and in peanut samples imported from Chad, Sri Lanka, and Rwanda for example, is unlikely an indicator of uncontrolled exportation

Table 4
Number of compliant products from countries that did not have any non-compliant products

Country	No. of samples	Country	No. of samples	Country	No. of samples
Algeria	1	Egypt	10	New Zealand	74
Australia	7	France	49	Oman	2
Bangladesh	3	Germany	80	Poland	3
Bosnia	2	Greece	13	Saudi Arabia	29
Bulgaria	3	Indonesia	3	Slovenia	3
Canada	78	South Korea	1	South Africa	70
Czech Republic	6	Mexico	5	Switzerland	5
Denmark	2	Netherlands	58	United Kingdom	179
Uzbekistan	3				

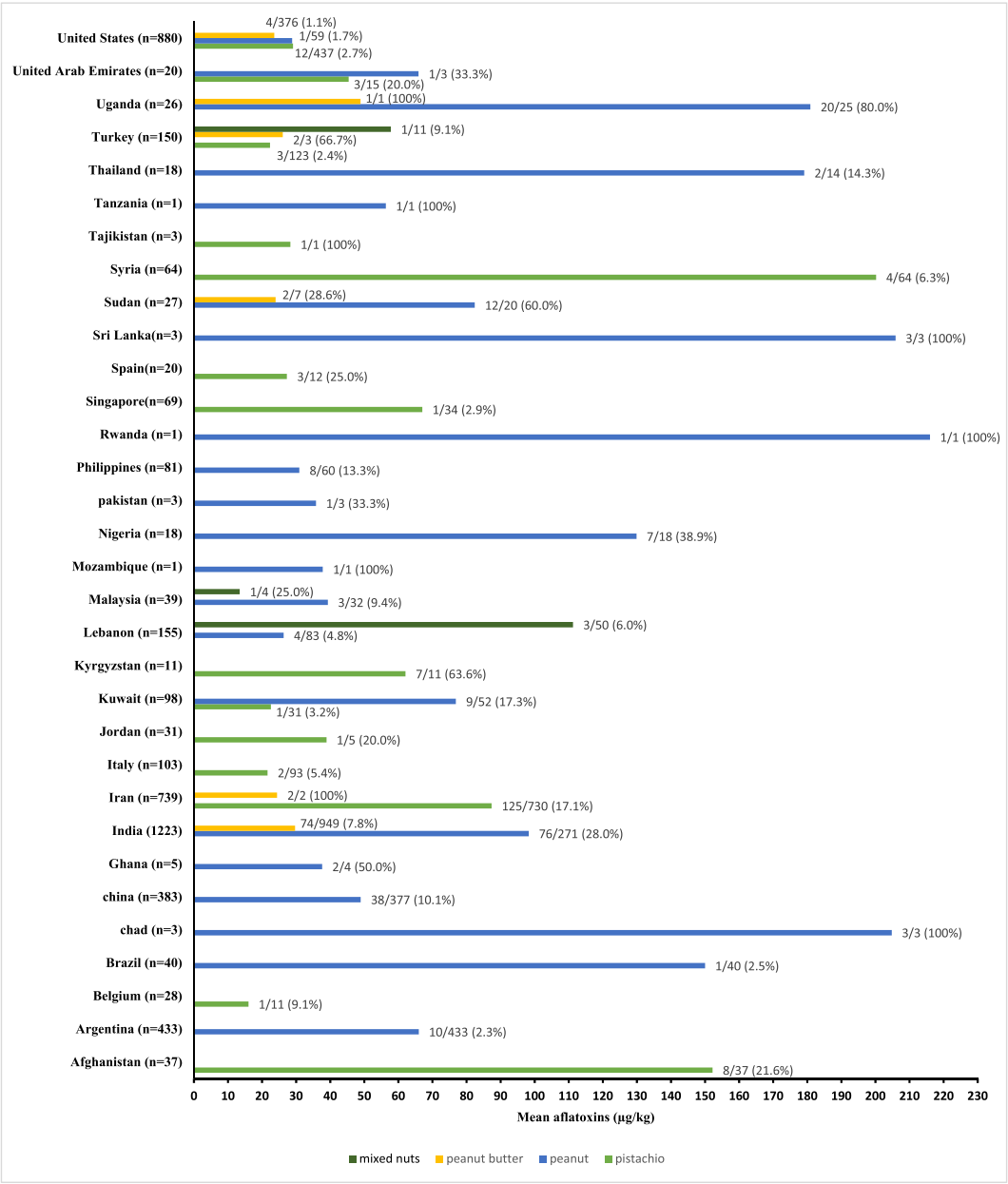


Fig. 2. Incidence and concentrations of aflatoxins in non-compliant nut samples among exporting countries. n: number of products.

due to the small number of samples (<5 samples in 5 years) (Fig. 2). A study from Taiwan found that in peanut and peanut products that were imported from the 6 top peanut exporting countries: China, Vietnam, Indonesia, Thailand, the USA, and the Philippines, 0 (0%), 13 (4%), 10 (13%), 2 (2%), 2 (2%), and 1 (1%) samples, respectively, exceeded aflatoxin limits [23]. In the present study, 38/377 of peanut samples (10%) imported from China, 2/14 (14.3%) from Thailand, 1/59 (1.7%) peanut and 4/376 (1.1%) samples of peanut butter from the USA, and 8/60 (13.3%) samples of peanuts imported from the Philippines exceeded the limits, while the three peanut samples from Indonesia were acceptable. Several factors play a role in aflatoxin contamination since it may vary annually and with respect to location and cultivation practices [23]. Regarding the USA specifically, a recent study analyzed the EU rapid alert system (RASFF) notifications for aflatoxins in food and feed exported from the USA between the years 2010–2019. The study revealed that of the USA mycotoxin notifications for foods, 98.9% were for aflatoxins in almonds, pistachios, and peanuts [7]. The UAE is the sixth largest tree nut export market of the USA, where the annual export in 2019 was estimated at US $439 million. In the current study, only 13 of 496 peanut and pistachio samples (2.6%) imported from the USA were non-compliant.

Besides packaging, processing, and climatic factors, additional variables can contribute to aflatoxin levels in nut products. Ingredients often used in processed samples such as spices, seasoning, cereals, beans, and dried fruits have been shown to be susceptible

to aflatoxin contamination and could serve as an aflatoxin source [2–5,17,34].

4. Conclusion

The current work investigated the incidence of aflatoxins in nuts imported to the UAE from 57 countries over 5 years (2017–2021). Aflatoxin levels exceeding MRLs were detected in products imported from both developing and developed countries, indicating a pervasive worldwide risk. About 8.6% of the total number of imported nut samples were rejected with an overall aflatoxin mean of 77.1 ± 79.3 μg/kg. Regarding packaging materials, products packed in glass had the lowest mean aflatoxin concentration (29.7 ± 17.4 μg/kg), while products packed in fabric materials had the highest mean aflatoxin level (108.1 ± 88.5 μg/kg). The type of processing had an effect on aflatoxin levels where ground samples had the highest mean level (158.9 μg/kg). Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and relying on modern technologies, in addition to monitoring storage conditions should be used by exporting countries to reduce losses from rejected shipments [35]. Additionally, it is apparent from the present findings that steps are required by regulatory agencies to establish minimum standards/specifications for packaging that is used for imported edible nuts and nut products to mitigate health risks from aflatoxins. Packaging of nuts to optimize safe shelf life should minimize oxygen permeability, prevent sunlight or moisture entry, and be uniformly adopted by countries expecting to ship products to the UAE. Since aflatoxin concentrations of the compliant samples in the present study were not available from authorized laboratory reports, comprehensive aflatoxin risk management advice cannot be provided at present. In the future, a focus on deoxynivalenol (DON) assessment should be developed, being the most widespread mycotoxin in food and feed specially in cereals and their products, and since humans are sensitive to its exposure according to a recent review [36]. DON is usually co-occurred with related molecules such as Culmorin which has been identified as emerging mycotoxin, or with its metabolites including its masked versions [37]. This fact highlights the need to consider DON detection crucial.

Author contribution statement

Tareq Osaili; Reyad Obaid: Conceived and designed the experiments; Wrote the paper.
Wael A. M. Bani Odeh, Ahmed A.S. A. Al Ali, Maryam S. Al Sallagi: Conceived and designed the experiments.
Manar Al Ayoubi, Nada El Darra: Analyzed and interpreted the data; Wrote the paper.
Vaidehi Garimella, Fatema Saeed Bin Bakhit: Performed the experiments; Contributed reagents, materials, analysis tools or data.
Richard Holley: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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