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Original article

Seasonal variation and health implications due to aflatoxins in nuts sold in Riyadh region

Variations saisonnières et implications cliniques des aflatoxines contenues dans les graines de fruits à coque de la région de Riyadh

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Abstract

This study aimed to assess the health risk from aflatoxins (AF) in nuts commercialized in Riyadh, Kingdom of Saudi Arabia (KSA), across two seasons (summer 2016 to winter 2017). Nut samples including pistachios, walnuts, cashews, and almonds, were analyzed using high-performance liquid chromatography (HPLC) with fluorescence detection. For samples collected in summer, the highest ranges were found in pistachios (0.35–7.13 $\mu\text{g/kg}$), walnuts (0.50–4.39 $\mu\text{g/kg}$), cashews (0.21–1.40 $\mu\text{g/kg}$) and almonds (0.05–1.04 $\mu\text{g/kg}$). AFs were detected in 30 (67%) of the summer samples, with a mean concentration of $1.00 \pm 1.25 \mu\text{g/kg}$. For samples collected in winter, the highest ranges were found in pistachios (0.26–10.76 $\mu\text{g/kg}$), cashews (0.16–2.08 $\mu\text{g/kg}$), almonds (0.25–1.56 $\mu\text{g/kg}$), and walnuts (0.27–1.14 $\mu\text{g/kg}$). AFs were detected in 39 (100%) of the winter samples, with a mean concentration of $1.05 \pm 1.66 \mu\text{g/kg}$. All the samples analyzed had AF levels below the Saudi regulatory limit of 20 $\mu\text{g/kg}$. However, estimation of the margin of exposure and risk of hepatocellular carcinoma (HCC) revealed that pistachio and walnut, particularly in summer, may pose health risks to consumers. Further, the total AF content of three samples exceeded the limit stated in European regulations.

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Keywords: Seasonal investigation; Nut products; Aflatoxin; Dietary exposure; Margin of exposure; Hepatocellular carcinoma

Résumé

Ce travail a pour but d'évaluer les risques sanitaires liés aux aflatoxines (AFs) contenues dans les fruits à coque commercialisés dans la région de Riyadh, Royaume d'Arabie Saoudite, au cours de deux saisons (été 2016 et hiver 2017). Les échantillons de fruits à coque comprenant des pistaches, des noix de Grenoble, des noix de cajou et des amandes, ont été analysés par technique de chromatographie liquide à haute performance (HPLC) avec détection par fluorescence. Les échantillons récoltés en été donnaient les taux les plus élevés d'aflatoxines dans les pistaches (0,35–7,13 $\mu\text{g/kg}$), les noix de Grenoble (0,50–4,39 $\mu\text{g/kg}$), les noix de cajou (0,21–1,40 $\mu\text{g/kg}$) et les amandes (0,05–1,04 $\mu\text{g/kg}$). Les AFs ont pu être détectées dans 30 échantillons d'été (67 %) avec une concentration moyenne de $1,00 \pm 1,25 \mu\text{g/kg}$. Parmi les échantillons d'hiver les concentrations les plus élevées se rencontraient dans les pistaches (0,26–10,76 $\mu\text{g/kg}$), les noix de cajou (0,16–2,08 $\mu\text{g/kg}$), les amandes (0,25–1,56 $\mu\text{g/kg}$) et les noix de Grenoble (0,27–1,14 $\mu\text{g/kg}$). Les AFs furent détectées dans 39 (100 %) échantillons d'hiver à une concentration moyenne de $1,05 \pm 1,66 \mu\text{g/kg}$. Tous les échantillons analysés avaient des taux d'AFs inférieurs aux taux de 20 $\mu\text{g/kg}$ légalement reconnu en Arabie Saoudite. Cependant la prise

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en compte des marges d'exposition et du risque de carcinome hépatocellulaire (HCC) révèlent que la consommation de pistaches et de noix de Grenoble, plus particulièrement en été, peut exposer à des problèmes sanitaires. Par ailleurs le contenu total en AFs de 3 échantillons dépasse les limites de la législation européenne.

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Mots clés : Exploration saisonnière ; Fruits à coque ; Aflatoxine ; Exposition nutritionnelle ; Limite d'exposition ; Carcinome hépatocellulaire

1. Introduction

Nuts have a high nutritional value, with 60–70% oil and 17% protein, and they contain substantial amounts of phenolic compounds, fatty acids, amino acids and antioxidants. Nut consumption reduces the risk of cardiovascular disease, gallstones, obesity and weight gain, and possibly reduces the risk of diabetes mellitus among women [1,2]. Nevertheless, nuts are cultivated in hot and humid regions and are therefore exposed to toxigenic fungi and aflatoxin (AF) production [3,4]. In KSA, nuts are used in many foods and drinks, such as morning tea and fruit salad. The most commonly consumed nuts are almonds, pistachios, cashews and walnuts. In KSA, 16 M kg of nuts are consumed each year (Customs Department of the Ministry of Finance). These nuts are imported from multiple countries, including Iran, China, Syria, Turkey, USA, India, Sudan and Egypt [5].

AFs (B1, B2, G1, and G1) are metabolites of *Aspergillus flavus* and *A. parasiticus* secreted in food cereals and feedstuff. The presence of AFs in food can lead to serious health problems, including food poisoning and liver cancer [6]. Moreover, acute toxicity from aflatoxicosis can result in death. In 2004, in eastern Kenya, there was an outbreak of aflatoxicosis (314 cases) caused by the consumption of contaminated food; 68% of these patients died as a result [39].

A literature survey identified only a limited number of reports on the occurrence of AFs in dried food and nuts in the KSA. The reported incidence of AF contamination ranges from below the limit of detection to highly contaminated. These studies were conducted in multiple cities, including the holy city of Makkah and Al-Dawadmi. AFs were found in chestnut (20–60 µg/kg) and peanut and sunflower seeds (1.7–8.5 µg/kg). Of 264 samples analyzed, AFs were detected in 70 (at 1.0–110 µg/kg) [5,7–9], whereas Alhussaini [10] reported 20 samples free of mycotoxin. These studies suggest the importance of continuous monitoring of AFs in nuts in the KSA. The Joint FAO/WHO Expert Committee on Food Additives and the European Food Safety Authority recommended use of the margin of exposure (MOE) approach to determine the risk of genotoxic and carcinogenic substances such as AF. In our study we aimed to assess the occurrence of AF in cashews, pistachios, almonds and walnuts, which are widely sold in Riyadh, and accordingly to determine dietary exposure and risk of hepatocellular carcinoma (HCC).

2. Materials and methods

2.1. Sampling

A total of 84 nut samples were randomly collected over two years from different supermarkets and grocery shops in Riyadh. These samples were taken to the laboratory and stored at –20 °C before analysis. Of the samples, 45 were cashew nuts ($n = 11$), pistachio nuts ($n = 11$), almond nuts ($n = 11$) and walnuts ($n = 12$) collected in the summer season (May 2016). The remaining 39 samples (cashew nuts, $n = 10$; pistachio nuts, $n = 9$; almond nuts, $n = 11$; and walnuts, $n = 9$) were collected in the winter season (January 2017).

2.2. Extraction

AFs were extracted using AOAC [11] official method 970.45 (BF method) as described previously [12]. Briefly, 5 g of sample and 2 g sodium sulphate were placed in a blender jar. 100 mL of extraction solvent (methanol:water [55:45, v/v]) was then added to the jar, which was securely stoppered and blended at medium speed for 3 min. The extract was filtered through filter paper and 25 mL of the filtrate was transferred to a separation funnel, to which 20 mL of hexane was added. The contents were gently shaken and the upper hexane layer discarded. 25 mL of chloroform was added to the separation funnel and the contents were gently shaken and left to stand. The entire lower layer was transferred into a conical flask. Extraction with chloroform was repeated. The combined extract was then concentrated to approximately 2 mL using a vacuum rotary evaporator at 60 °C and then transferred into a screw-capped borosilicate vial and evaporated to dryness on a hotplate at 80 °C.

2.3. Derivatization

For derivatization, 150 µL of trifluoroacetic acid was added to the dry film which was left for 30 min in a dark environment. For HPLC analysis, 850 µL of acetonitrile:water (1:9 v/v) was added to the film.

2.4. Separation and quantification

Separation was performed using an HPLC apparatus (Perkin Elmer, USA) consisting of a fluorescence detector, binary

analytical pump, vacuum degasser and auto-sampler equipped with a 100- μ L loop. A reverse-phase C18 (150 mm $\times$ 4.6 mm i.d and 5 μ m particle size) chromatographic column was used. Excitation and emission wavelengths were set at 360 and 440 nm, respectively, using the super high sensitivity mode in the fluorescence detector. The isocratic mobile phase was water:methanol:acetonitrile (60:20:20, v/v/v), set to run at a flow rate of 1 mL/min.

For quantification, we used standard concentrations of AFB1, AFG1, AFB2 and AFG2 at 0.98, 0.88, 0.31, and 0.32 μ g mL⁻¹, respectively, obtained from Supelco. After derivatization, the AF standard concentrations used for calibration were adjusted to: 0.0198, 0.0594, 0.099 and 0.198 ng 20 μ L⁻¹ for AFG1; 0.0176, 0.0528, 0.088 and 0.176 ng 20 μ L⁻¹ for AFB1; 0.0064, 0.0192, 0.032 and 0.064 ng 20 μ L⁻¹ for AFG2; and 0.0062, 0.0186, 0.031 and 0.062 ng 20 μ L⁻¹ for AFB2.

The limit of detection (LOD) was calculated in Microsoft Excel according to the International Council for Harmonisation (ICH) Guidelines using the following equation:

$$\text{LOD} = 3.3 * \text{Standard deviation of response} / \text{slope of regression line}$$

2.5. Assessment of chronic exposure and cancer risk

The daily intake of AF in nuts and the associated HCC risk were calculated as previously described [6]. The average nut consumption was reported by the Arab Center for Nutrition as 20 g per person per day with the average body weight per person being taken as 60 kg. The percentage prevalence of hepatitis B virus (HBV) was found to be 8.3% [13]. This was used to assess the cancer risk for the total population in the Riyadh region of 5,839,672 in 2015, as documented by the General Authority for Statistics, KSA. The HCC risk was estimated to be 0.01/10⁵/year/ng AFB1/kg body weight for HBV-negative subjects and 0.3/10⁵/year/ng AFB1/kg body weight for HBV-positive subjects [14].

$$\text{Dietary exposure} = [\text{mean consumption (g)} \\ \times \text{concentration } (\mu\text{g/kg})] / \text{body weight (kg)}.$$

The MOE is the ratio between the benchmark dose lower confidence limit causing a 10% proliferation in tumor formation in the Fisher rat (BMDL₁₀), and the average exposure dose [15]. Benford et al. estimated the BMDL₁₀ of developing HCC as 250 ng/kg bw/day for AFB1 [16].

$$(\text{MOE} = \text{BMDL}_{10} / \text{total intake})$$

3. Statistical analysis

Statistical analysis was performed using Microsoft Excel version 2013. Average concentrations and standard deviations were calculated for all parameters. Significance differences were calculated using Student's *t*-test. Differences between seasons and parameters were declared if the *P*-value was <0.05.

4. Results

4.1. Aflatoxin analysis

The AFs were extracted using an approved protocol ([11] AOAC official method 970.45, BF method) for AFs in peanuts and peanut products and were then separated and quantified by HPLC with fluorescence detection. The correlation coefficient (linear regression) was equal to 1 for each AF standard. The LOD of each AF was equal to 0.008, 0.005, 0.002 and 0.002 ng 20 μ L⁻¹ for AFG1, AFB1, AFG2 and AFB2, respectively.

4.2. Incidence and levels of aflatoxin in nut samples in summer

Of the 45 nut samples analyzed, AF was detected in 30 (67%). The mean concentration was 1.00 ± 1.25 μ g/kg with a range of 0.05–7.13 μ g/kg and median of 4.33 μ g/kg. Thus, no samples (0%) had total AF levels above the Saudi limit (20 μ g/kg) but 2 samples (4.4%) had levels higher than the European Commission limit (4 μ g/kg). The results showed that AFs are prevalent in nuts sold in Riyadh. Table 1 gives the minimum, maximum, average, median and standard deviation values for AFB1, AFG1, AFB2, AFG2 and total AF.

4.3. Incidence and levels of aflatoxin in nut samples in winter

AF was detected in the range of 0.16–10.76 μ g/kg in all samples analyzed. The mean concentration was 1.05 ± 1.66 μ g/kg with a median of 0.69 μ g/kg. Thus, only one sample (10.76 μ g/kg – 2.6%) had total AF level higher than the European Commission limit. Table 2 gives the full results for AFB1, AFG1, AFB2, AFG2 and total AF.

4.4. Aflatoxin in nuts across the two seasons

Of the 84 nut samples analyzed, AF was detected in 69 (82%). The mean concentration was 1.03 ± 1.49 μ g/kg with a range of 0.05–10.76 μ g/kg and a median of 0.69 μ g/kg. Thus, no sample containing AF was above the Saudi limit, but three samples (3.6%) had levels higher than the European Commission limit. AFB1 was the most abundant toxin, with an average concentration of 0.64 ± 20.68 μ g/kg. Of 49 samples containing AFB1, 3 samples had levels higher than 2 μ g/kg [adopted by the European Union [17] as the permitted level for AFB1 in 2010], followed by AFB2 with a mean concentration of 0.55 ± 1.25 μ g/kg, AFG2 (mean: 0.51 μ g/kg), and AFG1 (mean: 0.29 μ g/kg).

4.5. Incidence and levels of aflatoxin in different nut types in summer

Table 2 shows the distribution of AFs in the different nut types. The order of overall mean contamination is pistachio nuts (1.77 ± 2.39 μ g/kg) > walnuts (1.35 ± 1.13 μ g/kg) > cashews (0.63 ± 0.41 μ g/kg) > almonds (0.55 ± 0.34 μ g/kg). It is noteworthy that the AF concentration in pistachio and walnuts was

Table 1

Distribution and levels of AFs ($\mu\text{g/kg}$) in nut samples ($n = 45$) from Riyadh, Saudi Arabia (summer, 2016).

Statistic	AFG1	AFB1	AFG2	AFB2	Total aflatoxin
Number of samples with AFs detected	14	23	23	8	30
% contamination	31	51	51	18	67
Minimum value	0.05	0.14	0.23	0.07	0.05
Maximum value	0.61	4.28	1.11	2.29	7.13
Average value	0.26	0.73	0.56	0.43	1.00
Median value	0.21	0.33	0.56	0.18	0.73
SD	0.17	1.11	0.22	0.75	1.25

AF: aflatoxins.

Table 2

Distribution and levels of AFs ($\mu\text{g/kg}$) in nut samples ($n = 39$) from Riyadh, Saudi Arabia (winter, 2017).

Statistic	AFG1	AFB1	AFG2	AFB2	Total aflatoxin
Number of samples with AFs detected	13	26	36	8	39
% contamination	33	67	92	21	100
Minimum value	0.09	0.16	0.13	0.07	0.16
Maximum value	0.70	6.01	1.76	4.76	10.76
Average value	0.32	0.55	0.47	0.68	1.05
Median value	0.27	0.25	0.39	0.10	0.69
SD	0.19	1.13	0.35	1.65	1.66

AF: aflatoxins.

Table 3

Aflatoxin levels ($\mu\text{g/kg}$) in samples of nuts imported into Riyadh, KSA, with individual focus on type (summer, 2016).

Nut type	<i>n</i>	AFB1		AFG1		AFB2		AFG2		Total AF	
		nc	Range	nc	Range	nc	Range	nc	Range	nc	Range
Cashews	11	8	0.14–0.49	7	0.10–0.30	2	0.07–0.11	7	0.23–0.79	11	0.21–1.40
Pistachios	11	5	0.33–4.28	2	0.26–0.61	1	2.29	5	0.35–1.11	7	0.35–7.13
Almonds	11	2	0.21–0.46	2	0.05–0.11	–	–	4	0.52–0.76	6	0.05–1.04
Walnuts	12	8	0.27–3.85	3	0.27–0.54	5	0.17–0.21	7	0.50–0.83	9	0.50–4.39

KSA: Kingdom of Saudi Arabia; AF: aflatoxins; *n*: total number of analyzed samples; nc: number of contaminated samples.

slightly higher than in cashew nuts and almonds. Thus, a sample of walnuts and other pistachio nuts had an AF content higher than the EU permissible limit while the remainder of samples were below the Saudi and EU regulatory limits (Table 3).

4.6. Incidence and levels of aflatoxin in different nut types in winter

The distribution of AFs in the different nut types is shown in Table 4. The order of mean contamination is as follows: pistachio nuts ($1.85 \pm 3.36 \mu\text{g/kg}$) > cashews ($1.02 \pm 0.74 \mu\text{g/kg}$) almonds > ($0.75 \pm 0.35 \mu\text{g/kg}$) > walnuts ($0.65 \pm 0.27 \mu\text{g/kg}$). For the pistachio nuts, one sample had an AF level exceeding the EU limit. AFs detected in other nut samples were found to be below the Saudi and EU regulatory limits (Table 4).

4.7. Incidence and levels of aflatoxin in different nut types across the two seasons

For the distribution of AFs in the different nut types the order of mean contamination across the two periods of analysis was: pistachio nuts ($1.86 \pm 2.97 \mu\text{g/kg}$) > walnuts

($1.16 \pm 0.95 \mu\text{g/kg}$) > cashews ($0.83 \pm 0.62 \mu\text{g/kg}$) > almonds ($0.67 \pm 0.36 \mu\text{g/kg}$).

4.8. Chronic exposure and cancer risk

HCC risk and chronic exposure to AFs in different types of nuts for the Riyadh population across two seasons are presented in Table 5. Clearly, pistachio nuts and walnuts may pose some health hazards to the average consumer, particularly HBV carriers. Conversely, cashews and almonds do not pose health problems to the average consumer.

Fig. 1 shows the MOE. The closer the value was to 600 or over, the safer the exposure level, and vice versa [16,18]. Although the MOE is not an absolute quantity it gives an adequate indication of the level of concern. It is known that larger MOEs mean safer chronic exposure and vice versa. In 2005, the EFSA suggested that an MOE of 10,000 or more was representative of the limit of safe exposure; however, along with the International Life Sciences Institute, European Branch and WHO, the EFSA recommended setting special MOE levels [18]. Accordingly, the MOE of AFB₁ was taken as ranging from 100 to 600, based on the study by Wogan et al. (1974) [16,40].

Table 4

Aflatoxin levels ($\mu\text{g/kg}$) in samples of nuts imported into Riyadh, KSA, with focus on individual type (winter, 2017).

Nut type	<i>n</i>	AFB1		AFG1		AFB2		AFG2		Total AF	
		nc	Range	nc	Range	nc	Range	nc	Range	nc	Range
Cashews	10	7	0.16–0.89	2	0.40–0.55	–	–	9	0.17–1.76	10	0.16–2.08
Pistachios	9	8	0.18–6.01	4	0.09–0.70	1	4.76	7	0.13–0.57	9	0.26–10.76
Almonds	11	5	0.19–0.69	3	0.18–0.43	4	0.09–0.12	11	0.25–0.87	11	0.25–1.56
Walnuts	9	6	0.18–0.24	4	0.11–0.27	3	0.07–0.09	9	0.2–0.79	9	0.27–1.14

KSA: Kingdom of Saudi Arabia; AF: aflatoxins; *n*: total number of analyzed samples; nc: number of contaminated samples.

Table 5

Estimated daily intake (EDI) (ng/kg bw/day) and risk in terms of HCC cases attributed to aflatoxin in different types of nuts for the total population of Riyadh, KSA, over two seasons (summer 2016 to winter 2017).

	Cashews	Pistachios	Almonds	Walnuts
Summer (2016)				
EDI	0.21	0.59	0.18	0.45
HCC cases in HBsAg+/10 ⁵ individuals	0.063	0.177	0.054	0.135
HCC cases in HBsAg–/10 ⁵ individuals	0.002	0.006	0.002	0.005
HCC cases in total HBsAg+ population	0.305	0.858	0.262	0.654
HCC cases in total HBsAg– population	0.112	0.316	0.096	0.241
HCC cases in total population	0.418	1.174	0.358	0.895
Winter (2017)				
EDI	0.34	0.62	0.25	0.22
HCC cases in HBsAg+/10 ⁵ individuals	0.102	0.186	0.075	0.066
HCC cases in HBsAg–/10 ⁵ individuals	0.003	0.006	0.003	0.002
HCC cases in total HBsAg+ population	0.494	0.902	0.364	0.320
HCC cases in total HBsAg– population	0.182	0.332	0.134	0.118
HCC cases in total population	0.676	1.234	0.497	0.438

KSA: Kingdom of Saudi Arabia

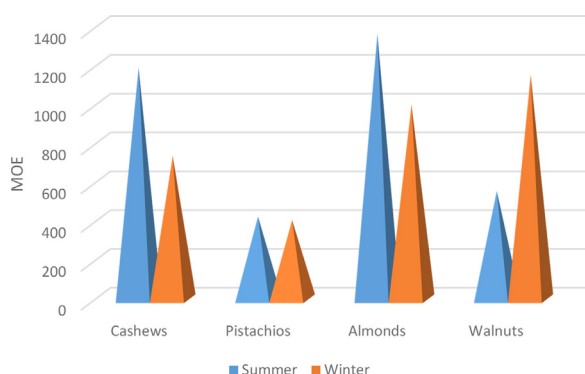


Fig. 1. Margin of exposure (MOE) for developing hepatocellular carcinoma, based on a BMDL₁₀ level of 250 ng/kg bw/day for total aflatoxin in different types of nuts commercialized in Riyadh, Kingdom of Saudi Arabia, across two seasons (summer 2016 to winter 2017). The closer the value to 600 or above, the safer the exposure level, and vice versa.

5. Discussion

The present investigation demonstrated that the level of AFB1 in the samples for the two seasons was higher than other AFs, without significant correlation ($P > 0.05$). This is not unexpected, since it is well known that it is the most commonly found in nature. Its toxicity is irrefutable as it can be metabolized by the cytochrome P450 enzymes to form 8,9 epoxide. This species causes DNA damage and hence liver cancer [19–21].

The incidence of AF and seasonal effects in general showed a weak correlation ($P > 0.05$). This might be because the summer temperature in Riyadh is high, while the relative humidity of the atmosphere is low. In winter, the opposite is true. The most suitable environment for the growth of *Aspergillus* species and the secretion of AF is relatively high moisture and temperature. Seasonal effects might be more evident in a more longitudinal survey study. However, it is noteworthy that the AF concentration in walnuts in summer was significantly higher than in winter ($P < 0.05$). These levels found in walnuts are slightly lower than those reported previously in the holy city of Makkah by Al Tawila et al. (2013). These differences might be due to the local environment or source of the samples. It is also lower than concentrations reported in China, Malaysia and Morocco [22,23,41], but comparable to those reported in Turkey, Pakistan and Qatar [24–27].

Pistachio has been shown to have a higher mean concentration of AF than other nuts, although no significant correlation has been reported ($P > 0.05$). AF incidence in pistachio is consistent with previous reports for AFs in commercially sold pistachio nuts in the holy city of Makkah, Pakistan, Spain, Qatar, Iran and Turkey [5,25,27–31,42], but lower than the level reported in Morocco and Pakistan [22,32]. The AF levels detected in cashew and almond nuts were found to be consistent with earlier reports of AFs in cashew and almond nuts in Saudi Arabia, Algeria, Turkey, Pakistan, Qatar, Benin and Brazil [5,25,27,33–37],

but lower than the levels reported in Pakistan and Sierra Leone [32,38].

Nuts are stored in distribution centres in Riyadh at room temperature while walnuts are kept refrigerated. These conditions could affect toxin levels in imported nuts. However, the present study suggests that *Aspergillus* in walnut and pistachio is able to grow during transportation and storage, as well as in the climatic conditions prevailing in the Riyadh region.

6. Conclusion

This study examined the dietary exposure and cancer risk attributed to AFs for four different types of nuts (walnuts, almonds, cashews and pistachios) in the Riyadh region, KSA. The samples were collected across two seasons (summer, 2016 and winter, 2017). Statistical analysis showed that the evidence for a seasonal effect is very limited. Percentage contamination was up to 82% for the samples analyzed. The AF content of pistachio nuts was slightly higher than that of the other nut samples. Although no samples contained AF at concentrations higher than the acceptable limit stipulated in the Saudi regulations, the consumption of pistachio nuts and walnuts contaminated with AF may represent health risks for the average overall consumer. Nonetheless, the MOE was found to be higher than 600 for almonds, cashews nuts and walnuts collected in winter, indicating safe exposure. We recommend further studies addressing mycotoxin exposure in the KSA.

Disclosure of interest

The author declares that he has no competing interest.

References

- [1] Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. *Br J Nutr* 2006;96(S2):S52–60. <http://dx.doi.org/10.1017/BJN20061864>.
- [2] Yang J. Brazil nuts and associated health benefits: a review. *LWT-Food Sci Technol* 2009;42:1573–80.
- [3] Adetunji MC, Aliko OP, Awa NP, Atanda OO, Mwanza M. Microbiological quality and risk assessment for aflatoxins in groundnuts and roasted cashew nuts meant for human consumption. *J Toxicol* 2018. <http://dx.doi.org/10.1155/2018/1308748>.
- [4] Elzupir AO, Alamer AS. Quantitative cancer risk of aflatoxin in peanut butter and vegetable oils: Sudan case study. *Toxin Rev* 2015;33:202–5.
- [5] El tawila MM, Neamatallah A, Serdar SA. Incidence of aflatoxins in commercial nuts in the holy city of Makkah. *Food Control* 2013;29:121–4.
- [6] Elzupir AO, Alamer AS, Dutton MF. The occurrence of aflatoxin in rice worldwide: a review. *Toxin Rev* 2014;34:37–42.
- [7] Abdel-Gawad KM, Zohri AA. Fungal flora and mycotoxins of six kinds of nut seeds for human consumption in Saudi Arabia. *Mycopathologia* 1993;124:55–64.
- [8] Alwakeel SS, Nasser LA. Microbial contamination and mycotoxins from Nut in Riyadh, Saudi Arabia. *Am J Food Technol* 2011;6:613–30.
- [9] Elzupir AO, Alamer AS, AlRajhi M, Idriss H. Assessment of health risks from aflatoxins in rice commercialized in Riyadh, Kingdom of Saudi Arabia. *Qual Assur Safety Crops Foods* 2018;10:255–60.
- [10] Alhussaini MS. Mycobiota and Mycotoxins of Nuts and Some Dried Fruits from Saudi Arabia. *J Am Sci* 2012;8:525–34.
- [11] AOAC. Official method 970. 45 aflatoxins in peanuts and peanut products BF method. 17th ed. USA: AOCS–AOAC method; 2000.
- [12] Elzupir AO, Younis YMH, Fadul MH, Elhussein AM. Determination of aflatoxins in animal feed in Khartoum State, Sudan. *J Anim Vet Adv* 2009;8:1000–3.
- [13] Al-Wayli HM. Prevalence of hepatitis B surface antigen in a Saudi hospital population. *Saudi Med J* 2009;30:448–9.
- [14] WHO. Evaluation of certain food additives and contaminants. Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 884. Geneva: WHO; 1999.
- [15] WHO/IPCS. Environmental health criteria 239: principles for modelling dose–response for the risk assessment of chemicals. Geneva: World Health Organization; 2009. http://apps.who.int/iris/bitstream/10665/43940/1/9789241572392_eng.pdf [Accessed on 22/12/2016].
- [16] Benford D, Leblanc JCR, Setzer W. Application of the margin of exposure (MoE) approach to substances in food that are genotoxic and carcinogenic. Example: Aflatoxin B1 (AFB1). *Food Chem Toxicol* 2010;48:S34–41.
- [17] European Union. Commission regulation No. 165/2010 of 26 February 2010 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Off J Eur Comm* 2010;L50:8–12.
- [18] Benford D, Bolger PM, Carthew P, Coulet M, DiNovi M, Leblanc JC, et al. Application of the margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food Chem Toxicol* 2010;48:S2–24.
- [19] Ostry V, Malir F, Toman J, Grosse Y. Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Res* 2017;33(1):65–73.
- [20] Oyedele OA, Ezekiel CN, Sulyok M, Adetunji MC, Warth B, Atanda OO, et al. Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. *Int J Food Microbiol* 2017;251:24–32.
- [21] Peromingo B, Rodríguez M, Delgado J, Andrade MJ, Rodríguez A. Gene expression as a good indicator of aflatoxin contamination in dry-cured ham. *Food Microbiol* 2017;67:31–40.
- [22] Juan C, Zinedine A, Molto JC, Idrissi L, Manes J. Aflatoxins levels in dried fruits and nuts from Rabat-Sale area, Morocco. *Food Control* 2008;19:849–53.
- [23] Wang Y, Nie J, Yan Z, Li Z, Cheng Y, Chang W. Occurrence and co-occurrence of mycotoxins in nuts and dried fruits from China. *Food Control* 2018;88:181–9.
- [24] Golge O, Hepsag F, Kabak B. Determination of aflatoxins in walnut sujuk and Turkish delight by HPLC–FLD method. *Food Control* 2016;59:731–6.
- [25] Abdulkadar AHW, Al-Ali A, Al-Jedah J. Aflatoxin contamination in edible nuts imported in Qatar. *Food Control* 2000;11:157–60.
- [26] Luttfallah G, Hussain A. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. *Food Control* 2011;22:426–9.
- [27] Masood M, Iqbal SZ, Asi MR, Malik N. Natural occurrence of aflatoxins in dry fruits and edible nuts. *Food Control* 2015;55:62–5.
- [28] Cano-Sancho G, Sanchis V, Marín S, Ramos AJ. Occurrence and exposure assessment of aflatoxins in Catalonia (Spain). *Food Chem Toxicol* 2013;51:188–93.
- [29] Cheraghali AM, Yazdanpanah H, Doraki N, Abouhossain G, Hassibi M, Ali-abadi S, et al. Incidence of aflatoxins in Iran pistachio nuts. *Food Chem Toxicol* 2007;45:812–6.
- [30] Dini A, Khazaeli P, Roohbakhsh A, Madadlou A, Poureanmdari M, Setoodeh L, et al. Aflatoxin contamination level in Iran's pistachio nut during years 2009–2011. *Food Control* 2013;30:540–4.
- [31] Hepsag F, Golge O, Kabak B. Quantitation of aflatoxins in pistachios and groundnuts using HPLC–FLD method. *Food Control* 2014;38:75–81.
- [32] Iqbal SZ, Mehmood Z, Asi MR, Shahid M, Sehar M, Malik N. Co-occurrence of aflatoxins and ochratoxin A in nuts, dry fruits, and nut products. *J Food Safety* 2018;38:e12462.
- [33] Ait Mimoun N, Arroyo-Manzanares N, Gámiz-Gracia L, García-Campaña AM, Bouti K, Sabaou N, et al. *Aspergillus* section Flavi and aflatoxins in dried figs and nuts in Algeria. *Food Addit Contam: Part B* 2018;11(2):119–25.
- [34] Lamboni Y, Frisvad JC, Hell K, Linnemann AR, Nout RM, Tamo M, et al. Occurrence of *Aspergillus* section Flavi and section Nigri and aflatoxins in raw cashew kernels (*Anacardium occidentale* L.) from Benin. *LWT-Food Sci Technol* 2016;70:71–7.

- [35] Milhome MAL, Lima CG, Lima LK, Lima FAF, Sousa DOB, Nascimento RF. Occurrence of aflatoxins in cashew nuts produced in northeastern Brazil. *Food Control* 2014;42:34–7.
- [36] Nile SH, Park SW, Khobragade CN. Occurrence and analysis of aflatoxin M1 in milk produced by Indian dairy species. *Food Agric Immunol* 2016;27(3):358–66.
- [37] Yilmaz I, Aluc M. Determination of aflatoxin levels in cashews on Turkish markets. food. In: 9th Baltic Conference on Food Science and Technology “Food for Consumer Well-Being”. 2014. p. 321–3.
- [38] Sombie JI, Ezekiel CN, Sulyok M, Ayeni KI, Jonsyn-Ellis F, Krska R. Survey of roasted street-vended nuts in Sierra Leone for toxic metabolites of fungal origin. *Food Addit Contam: Part A* 2018 [just-accepted].
- [39] Azziz-Baumgartner E, Lindblade K, Gieseke K, Schurz-Rogers H, Kieszak S, Njapau H, et al. Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. *Environ Health Perspect* 2005;113:1779–83.
- [40] Wogan GN, Paglialunga S, Newberne PM. Carcinogenic effects of low dietary levels of aflatoxin B1 in rats. *Food Chem Toxicol* 1974;12:681–5.
- [41] Leong YH, Ismail N, Latif AA, Ahmad R. Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. *Food Control* 2010;21:334–8.
- [42] Set E, Erkmen O. The aflatoxin contamination of ground red pepper and pistachio nuts sold in Turkey. *Food Chem Toxicol* 2010;48:2532–7.