

Technical Note

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Comparison of Aflatoxins contamination in oil seeds, peanut, nuts and their products

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Abstract

This study aimed to evaluate the aflatoxins occurrence in 478 samples of peanut, nuts, oil seed and their products in Iran market over three years. Based on the results, the mean concentrations of aflatoxin B1 and aflatoxin total ($\mu\text{g}/\text{kg}$) in these products were as follows respectively: peanut (9.1, 13.8), almond slices (7.4, 8.2), walnut (0.372, 0.372), hazelnut (1.2, 1.2), almond (13.35, 15.2), cashew nut ($<\text{LOQ}$, $<\text{LOQ}$), apricot kernel nut (31.4, 31.4), mixed nut (2.41, 2.6), sesame seed (1.88, 1.96), watermelon seed (50.25, 55.4), sunflower seed (2.92, 3.11), pumpkin seed ($<\text{LOQ}$, $<\text{LOQ}$), soybean ($<\text{LOQ}$, $<\text{LOQ}$), peanut butter (13.3, 17.54), pistachio butter (10.18, 11.65), sesame butter (0.6, 0.9), hazelnut butter ($<\text{LOQ}$, $<\text{LOQ}$), and almond butter (1.01, 1.72). The maximum aflatoxin B1 and aflatoxin total contamination levels ($\mu\text{g}/\text{kg}$) detected in these samples were: watermelon seeds (275, 296.72), peanut (171.95, 259.95), almond slices (250.3, 266.55), almond (156, 184.4), apricot kernel nut (243.87, 243.87), peanut butter (80.7, 101) sunflower seed, (60, 69.37), sesame seed (42.12, 44.45), respectively. The results of this investigation have shown considerably high levels of aflatoxins in peanut, some nuts, oil seed and their products' samples, and emphasize the need to monitor the levels of these contaminants in food on a regular basis. Contamination in other oilseed samples was not considerable according to maximum limits of aflatoxins in the Iranian national standards and European regulation.

Keywords mycotoxins, aflatoxins, peanut, nut, oil seeds

1. Introduction

Tree nuts, peanut, and their products are popular snacks and food ingredients in many parts of the world. Preventing chronic diseases and protecting against oxidative stress can be achieved through their consumption as significant sources of nutrients and phytochemicals [1]. Also, they are a source of intake of fiber that helps to reduce the occurrence of obesity and cardiovascular diseases [2]. The world is using more peanuts and tree nuts than ever before because of the health advantages related to these nuts. Peanuts are the most consumed groundnut while almond is the most consumed tree nut [3]. Peanut

butter and other nut butter, a food paste made primarily from dry roasted peanuts or nuts, are popular food products worldwide too. Oil seeds and oil seed meals are good sources of proteins [4]. They are consumed as nutritional products, natural foods, and premium snack foods worldwide.

Aflatoxins are a group of toxins that are produced by certain fungi. Four kinds of these toxins include aflatoxin B1, B2, G1, and G2 are most commonly found on crops such as cereals, peanuts, oil seed, and tree nuts [5].

Individuals can be exposed to aflatoxins by eating contaminated plant products or by consuming meat or dairy



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products from animals that eat contaminated feed.

Aflatoxin contamination is a concern in some parts of the world. For example, a study conducted in Zimbabwe found that 80% of raw peanut samples were contaminated with aflatoxins [6].

Exposure to aflatoxins because of consuming contaminated food is considered the second largest environmental risk factor for liver cancer development [7-9]. It is estimated that approximately 5–28% of HCC occurrences in the world are attributed to aflatoxin exposure [10].

Also, long-term exposure to aflatoxins in food can have several health effects, including liver damage, immune system suppression, malnutrition, rye syndrome, and kwashiorkor [11, 12]. So presence of aflatoxins in food are significant health hazard to consumers. Many factors including environmental conditions, fungal species, kind of products, and storage conditions are important for aflatoxin production in different countries [13]. Aflatoxin levels in food products should be limited to the lowest practical level due to their high level of toxicity in people and many animals.

Tree nuts, oil seeds, peanuts, peanut butter, and other nut butter are susceptible to aflatoxin contamination that inhibits the body's ability to fight diseases. It has been found as a factor in child stunting [14, 15] Peanut butter and other nut butter may be more contaminated than the nut grain because, unlike with the grain, visually inspecting peanut butter and other nut butter is nearly impossible, especially when it is broken, shriveled, and has insect damage. Also, moldy nuts or peanuts have a higher chance to contaminate with aflatoxins and cannot be separated visually by buyers or consumers [16]. The incidence of aflatoxins in peanuts, tree nuts, peanut butter, tree nut butter, and oil seeds has been studied in several research articles. Results of a study about peanut and peanut butter collected from an informal market in Zimbabwe revealed that 60% of the samples of raw peanuts were contaminated with total aflatoxins ranging from 0.75 to 426.4 µg/kg, while 100% of the samples of peanut butter contained total aflatoxins ranging from 4.7 to 435.0 µg/kg [6].

According to the findings of a different study, 35% of the peanut products purchased in Indonesian traditional markets and supermarkets between 2001 and 2002 were contaminated with aflatoxins at levels ranging from 5 µg/kg to 870 µg/kg [17]. Results of research in Iran in 2021 showed that contamination of aflatoxin B1 in peanuts was high in Mashhad and Tehran proveniences compared with the other cities [18].

A total of 1089 samples of peanuts and peanut products were collected between 2011 and 2017 and tested for aflatoxin to determine the exposure of Taiwan's population to aflatoxin from these sources. Aflatoxins were found in

25% of them [19].

59% of raw peanuts with shells, 55% of raw peanuts without shells, 61% of roasted peanuts with shells, 68% of roasted peanuts without shells, 50% of peanut butter, and 42% of peanut cookies were found to be contaminated with aflatoxins out of a total of 198 peanut and peanut products that were commercially available in major cities of Punjab, Pakistan [20]

A study in Turkey monitored aflatoxins levels in hazelnut samples obtained from exporter companies over 3 years. A total of 3188 samples of raw and processed hazelnuts were analyzed. The total aflatoxins level of the contaminated samples ranged from 0.07 to 43.59 µg/kg for roasted hazelnut kernels and between 0.02 to 78.98 µg/kg for hazelnut kernels [21].

Results of aflatoxins analysis of peanut butter in Zambia in the 3-year survey from 2012 to 2014 revealed that aflatoxin contamination is a common occurrence in peanut butter [22].

In a study in Pakistan retail markets, 744 samples of vegetable seeds and oil seeds (soybean, sunflower, canola, olive, corn, and mustard) were collected and tested for aflatoxin determination. In this investigation, 56.7% samples of imported and 57.0% samples of local edible seeds were observed to be contaminated with aflatoxin [23].

In the production of tree nut butter, the nuts are typically roasted before being ground into a paste. Roasting can help to reduce aflatoxin levels, but it is not always effective in eliminating them.

There are regulated aflatoxin levels in food products in many countries and international organizations. Over 100 nations have set the maximum limit of aflatoxins in human food or animal feed [24]. In the EU, maximum levels for aflatoxins and certain other contaminants in food are set in Regulation [25] and subsequent amendments.

In Iran, maximum limits for aflatoxin B1 and the sum of the aflatoxins B1, B2, G1, and G2 (total aflatoxins) in peanut, tree nuts, and their products are 8 µg/kg and 10 µg/kg respectively. In oil seeds, these maximum limits are 5 µg/kg and 15 µg/kg for aflatoxin B1 and total aflatoxins respectively [26]. Thus, the objective of this study was to investigate about results of the analysis of aflatoxin contamination in oil seed, peanut, tree nut, and their butter in Iran. The results of the study can influence policy direction on the management of such high-risk food products.

2. Methods

According to the national standard of Iran, for sample preparation, all of the aggregated samples collected should be milled and passed from a sieve with a one mm screen and collected in sealed bags until analysis. All

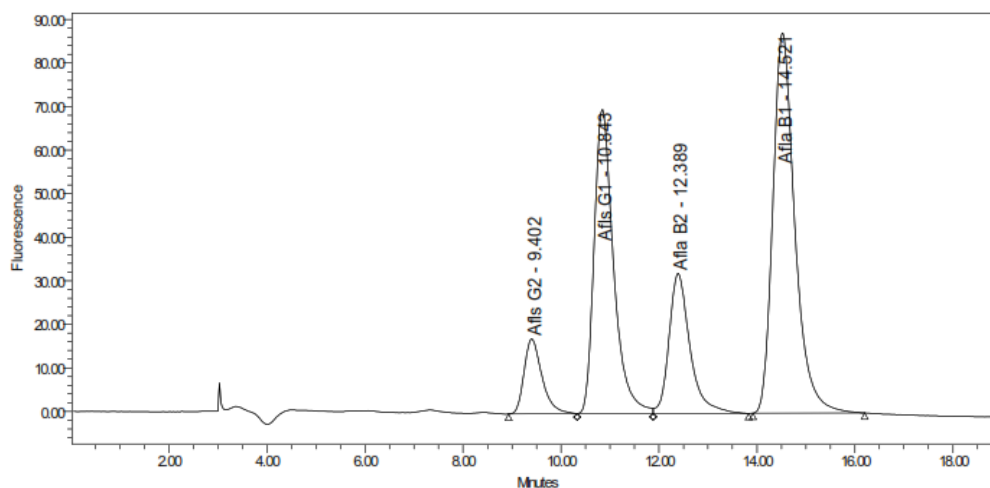


Fig 1. An example of the standard chromatogram of aflatoxins

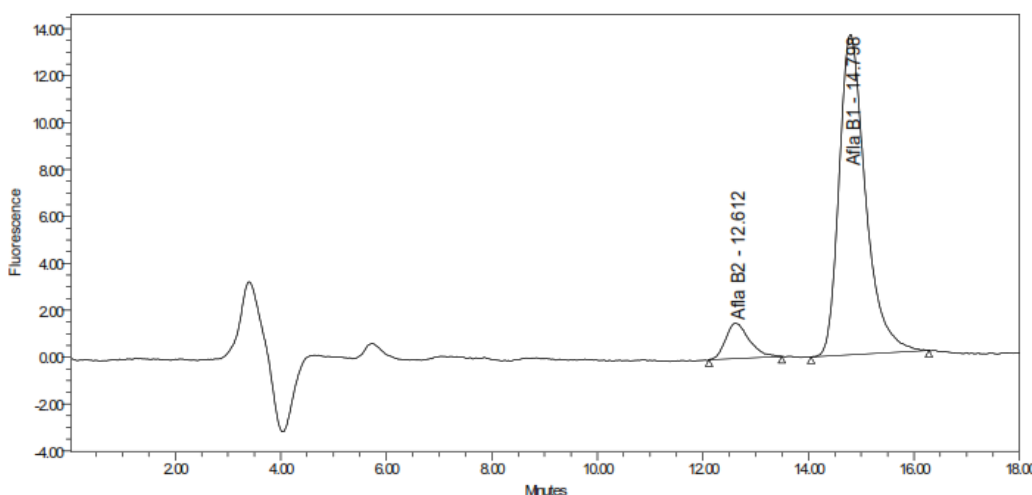


Fig 2. An example of the chromatogram of aflatoxins in a contaminated sample

samples were analyzed by food testing laboratories accredited according to ISO/ IEC 17025 defined by Iran's accreditation center in terms of maintaining and establishing the quality management system of ISO/ IEC 17025. Additionally, all laboratories have used the same method, which is based on Iran's national standard [27]. This standard has been validated by the European Union. According to ISO/IEC 17025 standards, selectivity, linear working range, matrix effects, recovery, precision, limit of detection (LOD), and limit of quantification (LOQ) were determined.

Daily recoveries were calculated by using certified reference materials or spiking blank samples. If the results of the recoveries were according to guidelines for establishing numeric values for the criteria of the fully validated method described in the codex manual [28], results were reported. Also, $R^2 \geq 0.99$ and LOQ not more than $0.3 \mu\text{g}/\text{kg}$ for each aflatoxin reported in all these laboratories.

2.1. Extraction method and aflatoxins determination

According to the national standard, for aflatoxins determination, after sample preparation and homogenization, all samples are defatted by N-Hexane. Then extraction is done with methanol: H₂O (80:20 v/v). After filtering, dilution with phosphate-buffered saline (PBS), clean up by passing the extracts through the immunoaffinity column (IAC), and drying with a gentle vacuum, methanol is passed from the column for elution. After dilution of the extract with deionized water, aflatoxins in extract were detected and determined by High performance liquid chromatography (HPLC). Aflatoxins are detected at the excitation wavelength of 395 nm and emission wavelength of 435 nm after derivatization with a Kobra Cell or Photochemical reactor of UV light. An example of the standard chromatogram of aflatoxins and a contaminated sample are shown in Figures 1 and 2, respectively.

Table 1. Occurrence of aflatoxin B1 in peanut and tree nut samples

sample	Total number of samples	% of Positive samples (more than LOQ)	Contamination (µg/kg) Mean Max		No. of samples with aflatoxin B ₁ more than the national maximum limit (8 µg/kg)
peanut	61	55 (33 samples)	9.1	171.95	9
almond slices	54	53.7 (29 samples)	7.4	250.3	4
almond	25	36 (9 samples)	13.35	156	3
cashew nut	5	0	<LOQ	<LOQ	0
walnut	48	4 (2 samples)	0.372	3.42	0
hazelnut	4	25 (one sample)	1.2	3.9	0
Mixed nut	134	6.7 (9 samples)	2.41	249.2	3
apricot kernel nut	8	50 (4 samples)	31.4	243.87	1

Table 2. Occurrence of total aflatoxins in peanut and tree nut samples

sample	Total number of samples	% of Positive samples (more than LOQ)	Contamination (µg/Kg) Mean Max		No. of samples with total aflatoxins more than the national maximum limit (10 µg/kg)
peanut	61	55 (33 samples)	13.8	259.95	9
almond slices	54	53.7 (29 samples)	8.2	266.55	4
almond	25	36 (9 samples)	15.2	184.4	3
cashew nut	5	0	<LOQ	<LOQ	0
walnut	48	4 (2 samples)	0.372	3.42	0
hazelnut	4	25 (one sample)	1.2	3.9	0
mixed nut	134	6.8 (9 samples)	2.6	271.1	2
apricot kernel nut	8	50 (4 samples)	31.4	243.87	1

3. Results and Discussion

Peanuts, nuts, oil seeds, peanut butter, and nut butter are popular worldwide because of their varied attributes such as desirable taste, high nutritional value as well as some health benefits.

These products are valued as a dessert and dry fruit and are used in ice-creams and confectionary products. Also,

aflatoxin contamination of peanut, tree nuts, oil seeds, and their products is a significant food safety-related problem worldwide. Aflatoxin infectivity in food poses a hazard to human health and has been recognized as a foremost limitation to trade [29]. One of the limitations to the role of these products in human nutrition and health is their susceptibility to aflatoxin contamination.

Table 3. Occurrence of aflatoxin B1 in oil seed samples

sample	Total number of samples	% of Positive samples (more than LOQ)	Contamination (µg/Kg)		No. of samples with aflatoxin B ₁ more than the national maximum limit (5 µg/kg)
			Mean	Max	
Sesame seed	50	16 (8 samples)	1.88	42.12	4
Watermelon seed	8	62.5 (5 samples)	50.25	275	3
sunflower seed	24	8.3 (2 samples)	2.92	6.11	1
pumpkin seed	6	0	< LOQ	< LOQ	0
soybeans	4	0	< LOQ	< LOQ	0

Table 4. Occurrence of total aflatoxins in oil seed samples

sample	Total number of samples	% of Positive samples (more than LOQ)	Contamination (µg/Kg)		No. of samples with total aflatoxins more than the national maximum limit (15 µg/kg)
			Mean	Max	
Sesame seed	50	16 (8 samples)	1.96	44.45	2
Watermelon seed	8	62.5 (5 samples)	55.4	296.72	3
sunflower seed	24	8.3 (2 samples)	3.11	69.37	1
pumpkin seed	6	0	< LOQ	< LOQ	0
soybeans	4	0	< LOQ	< LOQ	0

Since these products are used as food as well as food ingredients, regulatory limits have been established by INSO [26].

In this survey, results of aflatoxins analysis of 478 samples of peanut, tree nuts, oil seeds, and their products revealed that although the mean of aflatoxins in some samples was

below the maximum national or international limits, and aflatoxins were not detected in 73% (350 samples), there were limited samples with high amount aflatoxins.

Among the samples, the highest average levels of aflatoxins were determined in peanut, apricot kernel, peanut butter, and watermelon seed. The average and maximum amount of aflatoxin B1 and total aflatoxin contamination

Table 5. Occurrence of aflatoxin B1 in peanut butter and tree nut butter samples

sample	Total number of samples	% of Positive samples (more than LOQ)	Contamination (µg/Kg)		No. of samples with aflatoxin B ₁ more than the national maximum limit (8 µg/kg)
			Mean	Max	
Peanut butter	14	93 (13 samples)	13.3	80.07	5
Pistachio butter	13	77 (10 samples)	10.18	80	2
Sesame butter	15	13 (2 samples)	0.6	4	0
Hazelnut butter	3	0	< LOQ	< LOQ	0
Almond butter	2	50 (1 samples)	1.01	1.72	0

Table 6. Occurrence of total aflatoxins in peanut butter and tree nut butter samples

sample	Total number of samples	% of Positive samples (more than LOQ)	Contamination (µg/Kg)		No. of samples with total aflatoxins more than the national maximum limit (10 µg/kg)
			Mean	Max	
Peanut butter	14	93 (13 samples)	17.54	101	3
Pistachio butter	13	77 (10 samples)	11.65	90.74	2
Sesame butter	15	13 (2 samples)	0.9	9	0
Hazelnut butter	3	0	< LOQ	< LOQ	0
Almond butter	2	50 (1 samples)	1.72	2.11	0

of peanut and tree nuts samples have been shown in Table 1 and Table 2, respectively. Table 3 and Table 4 show the mean and maximum of aflatoxin B1, and total aflatoxin contamination in oil seeds samples and aflatoxins contamination in peanut butter and nut butter have been mentioned in Table 5 and Table 6.

Watermelon seed, almond, peanuts, and peanut butter had the highest average detected levels of aflatoxin B1 at 50.25, 13.35, 9.1, and 13.3 µg/Kg respectively. About the

average of total aflatoxins, it was detected at 55.4, 15.2, 13.8, and 17.54 in watermelon seed, almond, peanuts, and peanut butter respectively. In walnut, hazelnut, sesame butter, and almond butter, aflatoxin contamination was lower than the maximum national limit according to INSO 5925 and EU regulations. Pumpkin seed, soya bean, cashew nut, and hazelnut butter had no detected levels of aflatoxins. Aflatoxins were detected in 55.3% of peanut butter and nut butter samples.

Other studies about aflatoxin contamination are also in agreement with the findings of these results. For example, about 99% of notifications of US food products contaminated with aflatoxins that were exported to the EU market were related to aflatoxin contamination in almond, peanut, and pistachio nuts [30].

In a systematic search from 2000 to 2020, aflatoxin B1 had the most frequency in nut samples. The mean concentration of total aflatoxins and aflatoxin B1 was reported for peanuts at 37.85, 32.82 µg/kg, almonds at 3.54, 3.93 µg/kg, walnuts at 42.27, 22.23 µg/kg, and for hazelnut at 17.33, 10.54 µg/kg respectively [31]. Good Agricultural practices such as planting improved varieties of plants that are resistant to stress and drought, prevention of kernel damage during harvesting, time and way of drying of them before storage, good storage practices such as controls of storage temperature and humidity, and implementation of good manufacturing practices in the food production chain is very important to produce healthy products.

A three-year survey of aflatoxins in peanut, tree nuts, oil seeds, and their products from 2020 to 2022 indicated that the levels of aflatoxins especially aflatoxin B1 in these products can be hazardous. Although consuming some seeds such as watermelon seeds is not important in daily consumption basket, but even consuming a small amount of them can introduce a large amount of aflatoxin into the body. High contamination in nut butter and peanut butter can be the reason for using unhealthy primary products to prepare them. In addition to the fact that the quality of the kernels in butter and creams is not visible. These products can be used in confectionery products and consumed by children too much. So occurrence of aflatoxins in them can be a serious health concern for them. Therefore, it is necessary to control the quality and aflatoxins in these food products to protect the health of consumers.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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