Journal of Human Environment and Health Promotion
Print ISSN: 2476-5481 Online ISSN: 2476-549X

Evaluation of Aflatoxins Levels in Nuts in Selected Military-owned Chain Stores in Tehran, Iran

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ARTICLE INFO

Article type:
Original article

Article history:
Received: 24 September 2022
Revised: 20 October 2022
Accepted: 10 November 2022

DOI: 10.52547/jhehp.8.4.181

Keywords:

Aflatoxin
Nut
Iran
HPLC
Chain store

ABSTRACT

Background: Aflatoxins are classified as definitive or possible human carcinogens. Nuts and nutty products are among the most frequent aflatoxin-contaminated foodstuffs. The aim of this study was to assess the levels of aflatoxins (B_1, B_2, G_1, G_2, total) in different nuts, including pistachio, almond, cashew, hazelnut obtained from selected supermarket chains in Tehran city.
Methods: A total of 20 nut samples were collected from eight randomly selected chain stores, and the levels of aflatoxins in the samples were evaluated using an immunoaffinity column and quantified by high-performance liquid chromatography technique (HPLC).
Results: Among 20 tested nuts, only one sample (roasted hazelnut) was contaminated with the aflatoxins (AG_1) at a level of 0.34 µg/kg, while the tested mycotoxins were not detected in the other samples.
Conclusion: Our findings indicated that different parameters such as climatic conditions, implementing strict regulations, improvement in harvest and post-harvest practices alongside rigorous surveillance by the Department of Public Health and Preventive Medicine (AJA University of Medical Sciences) on the quality of food materials may explain the low incidence of aflatoxins in this study.

1. Introduction

Aflatoxins are a group of toxic metabolites produced by some species of fungus Aspergillus (mainly *Aspergillus parasiticus* and *Aspergillus flavus*) [1]. Among the known 14 different types of aflatoxins, aflatoxin B_1 (AB_1), B_2 (AB_2), G_1 (AG_1), and G_2 (AG_2) are naturally occurring toxins in foods [2]. Acute and chronic aflatoxin poisonings occur via exposure to the toxins; however, chronic disease is more common [3]. Nausea, abdominal pain, diarrhea, fever, acute liver damage, coma, and death are the main characteristics of acute aflatoxicosis. This type of disease often occurs in Southeast Asia and Africa. Chronic aflatoxicosis results from exposure to low or moderate levels of aflatoxins. Chronic disease is mainly characterized by symptoms like chronic hepatitis, jaundice, cirrhosis, hepatomegaly, and liver cancer [4]. AB_1 is recognized as a human carcinogen and is the most common aflatoxin in contaminated food samples compared to the other three types. AB_2, AG_1, and AG_2 are classified as possible human carcinogens and are not generally reported

How to cite: Rezaeigolestani M, Dabbagh Moghaddam A, Misaghi A, Hashemi M. Evaluation of Aflatoxins Levels in Nuts in Selected Military-owned Chain Stores in Tehran, Iran. *J Hum Environ Health Promot.* 2022; 8(4): 181-5.

in the absence of AB_1 [5,6]. It has been shown that these mycotoxins can commonly contaminate a wide range of foodstuffs such as spices, nuts, rice, etc. [7]. Nuts and nutty products are among the most frequent aflatoxin-contaminated foods. Based on the national and EU standards, the maximum acceptable level of total aflatoxins in nuts is set at 15 µg/kg and 3 15 µg/kg, respectively [8,9]. Various factors related to nut products, including water activity, nutrients, pH of the product, manufacturing process, harvesting and post-harvesting conditions, etc., altogether provide convenient conditions for fungal proliferation. Therefore, as these food products are consumed daily as part of the ingredients of some foods and snacks, regular monitoring should be implemented to evaluate the level of aflatoxins contamination in nut products. Based on the official data, more than 800,000 million tons of different types of nuts are annually consumed in Iran, and per capita, nut consumption amounts to 10 kilograms, four times as much as the global average [10]. This highlights the importance of monitoring the nut contamination with aflatoxin-producing fungus in the country. Although several efforts have been made to investigate the degree of aflatoxins contamination in different foodstuffs in Iran, the importance of the issue makes it necessary to assess the occurrence of aflatoxins in food products consistently. In the present study, levels of aflatoxins in different types of nuts supplied in a selection of military-owned supermarket chains in Tehran city were assessed and quantified by HPLC analysis.

2. Materials and Methods

2.1 Study area

This study was conducted in eight randomly selected military supermarket chains in Tehran, Iran. Figure 1 depicts the location of the stores in Tehran city.

2.2 Sampling

Since nuts were unavailable in some of the studied stores for sale, a total of 20 nut samples were collected randomly, including pistachio (4), almond (5), cashew (3), hazelnut (3), and walnut (5). These nuts are commonly consumed snacks in Iran. The sampling plan was according to the Iranian national standard (No. 13534) [11] and Food Standards Agency, UK [12]. In brief, a random sample of 10 packages (100 g) of each nut was obtained and then thoroughly pulverized and mixed using a Waring blender. Before sample preparation, pistachios were dehulled. The prepared nut samples were stored at 4 °C until analysis.

2.3 Chemicals

Aflatoxin standards were purchased from Sigma (USA). Stock solutions of the aflatoxins with concentrations of 10,000 µg/ml were prepared in methanol and stored in the secured conditions at 20 °C in the dark. The toxins working solutions were prepared before use by diluting the stock solutions in the same solvent. All chemicals were analytical grade and the solvents used were of the highest purity.

2.4 HPLC determination of aflatoxin

The extraction and quantification of aflatoxins by HPLC analysis followed the method described by Mazaheri (2009) and ISIRI 6872 [13,14]. In order to extract mycotoxins, the nut samples were extracted with 200 ml of methanol/water (80 ml/20 ml). The extract was filtered with Whatman filter paper (No. 4) and diluted with deionized water. Then, after filtering with a glass microfiber filter, 75 mL of the filtrate was passed through the aflatest immunoaffinity columns (IAC) at a rate of about 1-2 drops/s. The immunoaffinity column was washed with water (15 mL) at the same flow rate. Final elution was completed by passing 0.5 ml of HPLC-grade methanol (at the same rate) through the column. The second portion of methanol (1 mL) was applied after 1min and collected. Finally, the eluate was diluted with deionized water and used for HPLC analysis. The levels of aflatoxins were determined by a reversed-phase HPLC system (Agilent 1100 chromatograph; Agilent Corporation, Santa Clara, CA, USA) equipped with a fluorescence detector with post-column derivatization (PCD) involving bromination. Mobile and stationary phases were water-methanol-acetonitrile (60:30:20, v/v/v) and 350 µl of nitric acid (4 mol/l), and 120 mg of KBr with a rate of 1 ml/min, and C-18 column (Nova-pack 2.1 × 100 mm: 3 µm) respectively. The fluorescence detector was operated at an emission wavelength of 435 nm and an excitation wavelength of 365 nm. One hundred µl of the prepared diluted samples were injected into HPLC in each round of quantification. To evaluate the reliability of the results obtained by HPLC analysis, linearity, the limit of detection (LOD), and the limit of qualification (LOQ) were determined. A five-point calibration curve was constructed for each aflatoxin using aflatoxin standards of 0.1-100 µg/kg. Accuracy was assessed by determining the aflatoxins recoveries. The recoveries of the studied mycotoxins were recorded by analyzing a blank pistachio sample (roasted) spiked at 2 µg/kg for each AB1 and AG1 and 1 µg/kg for each AB2 and AG2. After solvent evaporation (in a fume hood at 25 °C for ~ 1 h), the same extraction and analysis procedures described above were applied to the spiked samples. LOQ and LOD were calculated by spiked samples based on a signal-to-noise ratio of 3:1 for LOD and 5:1 for LOQ [8].

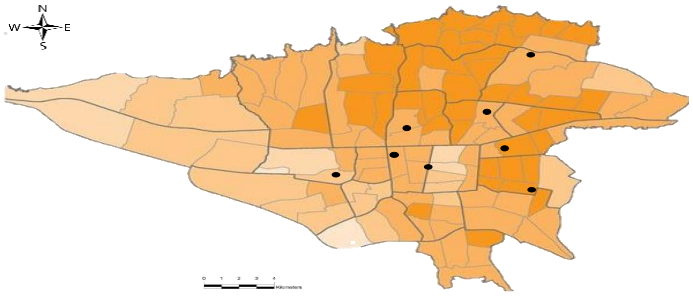

Figure 1: Sampling points (indicated with ●) in different districts of Tehran city, Iran. Source of the population density map: Atlas of Tehran metropolitan

3. Results and Discussion

The presence and levels of aflatoxins, including AB_1, AB_2, AG_1, AG_2, and total, were checked and quantified by HPLC analysis. Table 1 summarizes the validation results (LOD, LOQ, linearity, and recovery) on HPLC Method. The correlation coefficient, r^2 of the standard curves, ranged between 0.997-0.999, confirming the linearity of the graphs. When the AB_1 and AG_2 were spiked at 2 (µg/kg), and AB_2 and AG_2 1 (µg/kg), the recoveries obtained for the pistachio sample (roasted) ranged between 92.7, and 112.3%. The LOD of AB_1, AB_2, AG_1, and AG_2 were 0.12, 0.05, 0.14, and 1 (µg/kg), and LOQ were 0.3, 0.24, 0.3, and 0.21 (µg/kg), respectively. These results showed that the HPLC analysis adopted in the present study was reliable and acceptable. The presence of the toxins and the level of contaminations in the tested nut samples are shown in Table 2. As it can be seen, among the 20 tested nuts, only one sample (roasted hazelnut) was contaminated with the aflatoxins (AG_1) at a level of 0.34 µg/kg, while the tested mycotoxins were not detected in the other samples (by the HPLC analysis used in this study). A lot of research has investigated the occurrence of aflatoxins in different foodstuffs, including cereals, dairy products, nuts, and nutty products [7]. Nuts and nutty products are always recognized as possible dietary sources of aflatoxins. Among the studies conducted on determining aflatoxins in nuts, important investigations have been published from Iran, mainly on pistachios [15-17]. Unlike our findings, almost all of the works that existed in the literature, especially in Iran, reported a significant percentage of aflatoxin contaminations in nuts. In a study by Dini et al. (2013), 3181 commercial pistachios (raw) nut lots were tested for aflatoxins from 2009-2011 by the HPLC method [17]. Although the tested samples were intended for export and require a higher degree of quality, 1927 samples (23.5%) were contaminated with the mean value of 2.42-14.7 µg/kg and 428 (5.22%) sub-samples were above the European Union (EU) maximum tolerable level (8 µg/kg), while our samples were almost free of contamination. However, the authors indicated that in comparison with the data reported for the product in 2002-2003 (~50% contamination) [16], the level of pistachio contamination was decreased via improvement in hygienic conditions of harvesting and post-harvesting practices and cultivation of pistachios in Iran. In another study, ELISA and HPLC methods collected 142 nut samples, including walnut, almond, pistachio, hazelnut, etc., and tested them for aflatoxins [18]. They reported that 28.1% of pistachios, 7.1% of cashews, and 5.1% of walnuts (total of 13 cases) were contaminated (>15 ppb), and AB1 was the highest detected aflatoxin. Apart from these studies, a comprehensive review of aflatoxins in food products in Iran in 2016 showed that these toxins were found in many food products, including nuts [15]. All of the surveyed nut samples had some levels of aflatoxin contamination, which may indicate the great quality of the tested nuts in the present study. The incidence of aflatoxins in food products depends on many factors, such as season of the year, food type, geographical location, and post-harvest practices [19]. One of the main factors influencing the incidence of aflatoxins in foods is the geographical location and climatic condition, where the humid and warm weather provides an optimal situation for fungal proliferation and developing toxins [20]. In this regard, the research on aflatoxins in foodstuffs is mainly associated with the area with the aforementioned conditions, like Malaysia [21]. Iran has mostly a hot, dry climate, especially in the central parts, where the main nuts farmlands are (like pistachios farms in Kerman and Rafsanjan) [17,22]. Therefore, compared to tropical regions, the risk of aflatoxins production is much lower due to lower relative humidity. Higher levels of aflatoxins were frequently observed in different food products in African countries, most likely due to unacceptable storage conditions, consumer and farmer awareness, favorable climatic conditions for mold proliferation, technological hurdles, and poverty [7]. The lower levels of aflatoxins in the countries like Iran might also be linked with several strategies indicated in the literature [7,16,17]. These strategies, including implementation and adoption of strict regulations and standard limits versus aflatoxins, consumer and producer awareness, and improvement in post-harvest technologies, have been followed and emphasized by Iranian authorities for decades, and their positive impacts were reported by several authors [16,17]. In this concern, our finding revealed that regarding aflatoxin contamination, the quality of the supplied nuts in the surveyed stores was great and far lower than the national (15 µg/kg for total aflatoxins) and EU tolerable limits (2 µg/kg for total aflatoxins) set for nuts [8,9,17].

Table 1: Validation of aflatoxin determination by HPLC analysis

Toxin	LOD[a] (µg/kg)	LOQ[b] (µg/kg)	Calibration curve	r^2	Recovery (%)[c]
AB_1	0.12	0.3	y = 3.52x + 12.22	0.997	98.7
AB_2	0.05	0.24	y = 9.83x + 3.67	0.999	95.9
AG_1	0.14	0.3	y = 18.2x + 1.36	0.998	92.7
AG_2	0.1	0.21	y = 6.71x + 4.32	0.999	112.3

[a] Limit of detection
[b] Limit of quantification
[c] Aflatoxins recoveries were employed for determination of accuracy. Recoveries were determined by spiking 2 (µg/kg) AB_1 and AG_1, and 1 (µg/kg) AB_2 and AG_2 to the pistachio sample (roasted).

4. Conclusion

In conclusion, our findings indicated that several factors, such as climatic conditions, implementation of strict regulations, and improvement in post-harvest practices, may cause the low incidence of aflatoxins in nuts in Iran. Besides the factors above, rigorous surveillance by the Department of Public Health and Preventive Medicine (AJA University of Medical Sciences) on the function of related food suppliers and the quality of food materials in those supermarket chains decreases the occurrence of food-related hazards.

Table 2: Occurrence of aflatoxins in different types of nuts by HPLC analysis

Sample type	Analyzed sample	Number of positive	Aflatoxins[a] (µg/kg)				
			Total aflatoxin[b]	AB1	AB2	AG1	AG2
Pistachio (raw)	1	0	<LOD[c]	<LOD	<LOD	<LOD	<LOD
Pistachio (roasted)	3	0	<LOD	<LOD	<LOD	<LOD	<LOD
Almond (raw)	1	0	<LOD	<LOD	<LOD	<LOD	<LOD
Almond (roasted)	4	0	<LOD	<LOD	<LOD	<LOD	<LOD
Cashew (raw)	2	0	<LOD	<LOD	<LOD	<LOD	<LOD
Cashew (roasted)	1	0	<LOD	<LOD	<LOD	<LOD	<LOD
Hazelnut (raw)	1	0	<LOD	<LOD	<LOD	<LOD	<LOD
Hazelnut (roasted)	2	1	0.17 ± 0.24	<LOD	<LOD	0.17 ± 0.24	<LOD
Walnut	1	0	<LOD	<LOD	<LOD	<LOD	<LOD
Walnut (sliced)	4	0	<LOD	<LOD	<LOD	<LOD	<LOD

[a] Data are expressed as mean ± standard deviation.
[b] Total aflatoxin was represented by the summation of aflatoxin B_1, B_2, G_1 and G_2 levels
[c] Below the limit of detection

Authors' Contributions

Mohammadreza Rezaeigolestani, Arasb Dabbagh Moghaddam: Study design; Manuscript writing. Ali Misaghi, Mohammad Hashemi: collection and analysis of the data and the text revision.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The author gratefully acknowledges financial support from AJA University of Medical Sciences. (Project code: 91000449).

References

1. Farah Nadira A, Rosita J, Norhaizan ME, Mohd Redzwan S. Screening of Aflatoxin M1occurrence in Selected Milk and Dairy Products in Terengganu, Malaysia. *Food Control [Internet].* 2017; 73: 209-14. Available from: http://dx.doi.org/10.1016/j.foodcont.2016.08.004.

2. Cole RJ, Cox RH. 1-The Aflatoxins. *Handbook of Toxic Fungal Metabolites [Internet].* 1981: 1-66. Available from: http://www.sciencedirect.com/science/article/pii/B9780121797607500061.

3. Benkerroum N. Chronic and Acute Toxicities of Aflatoxins: Mechanisms of Action. *Int J Environ Res Public Health [Internet].* 2020 Jan 8; 17(2): 423. Available from: https://pubmed.ncbi.nlm.nih.gov/31936320.

4. Gholami-Shabani M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Aflatoxins and Aflatoxigenic Fungi in Iran: A Systematic Review of the Past, Present, and Future. *Mycol Iran [Internet].* 2017; 4(2): 65-84. Available from: https://mij.areeo.ac.ir/article-116760.html.

5. JECFA. The Safety Evaluation of Certain Food Additives and Contaminants: Aflatoxins. The Forty-Ninth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, Series No. 40. Geneva, Switzerland. 1998: 259-469.

6. IARC. International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans/World Health Organization. *International Agency for Research on Cancer.* 2012: 9-562.

7. Ismail A, Gonçalves BL, de Neeff DV, Ponzilacqua B, Coppa CFSC, Hintzsche H, et al. Aflatoxin in Foodstuffs: Occurrence and Recent Advances in Decontamination. *Food Res Int.* 2018; 113: 74-85.

8. Chun HS, Kim HJ, Ok HE, Hwang JB, Chung DH. Determination of Aflatoxin Levels in Nuts and their Products Consumed in South Korea. *Food Chem.* 2007; 102(1): 385-91.

9. ISIRI. Food & Feed-Mycotoxins-Maximum Tolerated Level. National Standard No. 5925. Institute of Standards and Industrial Research of Iran. Karaj, Iran. 2001.

10. Ministry of Agriculture. Iran's Annual Nut Production Tops 1m Tons [Internet]. Financial Tribune. 2023. Available from: https://financialtribune.com/articles/economy-domestic-economy/60857/iran-s-annual-nut-production-tops-1m-tons#:~:text=‟Per capita nut consumption in,quoted as saying by Eranico.

11. ISIRI. Foods-Nuts Sampling for Aflatoxins. National Standard No. 13534. Institute of Standards and Industrial Research of Iran. Karaj, Iran. 2010.

12. FSA. Sampling Advice: Mycotoxins in Foodstuffs. 2007: 24.

13. ISIRI. Food & Feed-Measurment of Aflatoxins B and G by HPLC Method. Iranian National Standard No. 6872. Institute of Standards and Industrial Research of Iran. Karaj, Iran. 2011.

14. Mazaheri M. Determination of Aflatoxins in Imported Rice to Iran. *Food Chem Toxicol.* 2009; 47(8): 2064-6.

15. Hedayati MT, Mahdavi Omran S, Soleymani A, Taghizadeh Armaki M. Aflatoxins in Food Products in Iran: A Review of the Literature. *Jundishapur J Microbiol.* 2016; 9(7): 1-8.

16. Cheraghali AM, Yazdanpanah H, Doraki N, Abouhossain G, Hassibi M, Aliabadi S, et al. Incidence of Aflatoxins in Iran Pistachio Nuts. *Food Chem Toxicol [Internet].* 2007; 45(5): 812-6. Available from: http://www.sciencedirect.com/science/article/pii/S0278691506003188.

17. Dini A, Khazaeli P, Roohbakhsh A, Madadlou A, Pourenamdari M. Aflatoxin Contamination Level in Iran′ s Pistachio Nut During Years 2009 e 2011. 2013; 30: 2009-13.

18. Reza SSM, Masoud A, Ali T, Faranak G, Mahboob N. Determination of Aflatoxins in Nuts of Tabriz Confectionaries by ELISA and HPLC Methods. *Adv Pharm Bull.* 2012; 2(1): 123-6.

19. Ismail A, Riaz M, Akhtar S, Yoo SH, Park S, Abid M, et al. Seasonal Variation of Aflatoxin B_1 Content in Dairy Feed. *J Anim Feed Sci [Internet].* 2017; 26(1): 33-7. Available from: http://dx.doi.org/10.22358/jafs/69008/2017.

20. Rustom IYS. Aflatoxin in Food and Feed: Occurrence, Legislation and Inactivation by Physical Methods. *Food Chem [Internet].* 1997; 59(1): 57-67. Available from: http://www.sciencedirect.com/science/article/pii/S0308814696000969.

21. Leong YH, Ismail N, Latif AA, Ahmad R. Aflatoxin Occurrence in Nuts and Commercial Nutty Products in Malaysia. *Food Control [Internet].* 2010; 21(3): 334-8. Available from: http://dx.doi.org/10.1016/j.foodcont.2009.06.002.

22. Weatheronline. Iran Weather [Internet]. 2019. Available from: https://www.weatheronline.co.uk/reports/climate/Iran.htm.