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Scientific Research

An Empirical Study on Aflatoxin Occurrence in Nuts Consumed in Tehran, Iran 2013

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Abstract

By definition, "aflaxions" refer to a group of chemically toxic fungal metabolites, which are generated by specific species of genus Aspergillus. The species flourish on some of raw foods. This research is an attempt to assess aflatoxin contamination in nuts (Fig, Almond, Hazelnut, Walnut, Pistachio and Sunflower) available in Tehran city market in 2013. The assessments were done using ELISA method. To this end, 200 samples including Fig (n = 30), Almond (n = 25), Hazelnut (n = 25), Walnut (n = 40), Pistachio (n = 40), Sunflower (n = 40) were collected and analyzed regarding contamination with aflatoxin. The result of the tests was positive for 96.5% (193 samples) with total average concentration of 1.68 µg/kg (0 - 6 µg/kg). Moreover, in 6% (12 samples) of the cases, contamination exceeded the EU limits (4 µg/kg), while 100% of the samples met the legal limits of National Standard of Iran (15 µg/kg). In spite of contamination of majority of the cases, none was at hazardous level as the condition for growth of mycotoxin fungus or antioxidant activity in the nut was not suitable in Tehran (humidity level).

Keywords

Aflatoxin; ELISA; Food Safety; Food Toxicology; Nuts

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1. Introduction

Mycotoxins are natural contaminants in food and feed. Aflatoxins (AFs) are classified as mycotoxins, which refer to a variety of seriously toxic components and the group of difuranocoumarins and classified in two broad groups according to their chemical structures; the difurocoumarocyclopentenone series (AFB, AFM and aflatoxicol) and the difurocoumarolactone series (AFG and AFB3). The aflatoxins fluoresce strongly in ultraviolet light (ca. 365 nm); B1 and B2 produce a blue fluorescence where as G1 and G2 produce green fluorescence. The toxins are generated by moulds of genera Aspergillus (specifically by *Aspergillus flavus* and *Aspergillus parasiticus*), *Penicillium* and *Fusarium* [1].

The agent is mainly found in warm and wet climates [2]. They are threat to agricultural products and cereal grain in particular. The products might be contaminated during pre-harvest, harvest, processing and handling stages [3] [4]. In this regard, season, humidity, daily variation, and storage term and temperaturemay help in providing preferred condition for growth of *A. flavus* and aflatoxin in variety of foods and feeds [5]-[7]. Potentially, the metabolites can be of serious danger to human health. They may induce immunotoxic, carcinogenic, mutagenic, teratogenic effects that can risk public health and economy. This means that there must be a close monitoring program on food products. Along with food safety, occupational safety is another concern [8]-[10]. The most prevalent and toxic molecule among the four commonly found AFs (aflatoxin B1, B2, G1 and G2) is AFB1 so that it is classified as group 1 human carcinogen by IARC [1] [3] [4]. Researches on nuts and hazelnuts and pistachios in particular have shown that the AFs expansion hits a maximum level at 25˚C - 35˚C and 97%-99% of humidity [11] [12]. Many regulations have been enacted by many countries to control AFs concentration in nuts. As stated by Codex Alimentaris and European Community legislation, the highest permitted concentration of AFs in nuts is 4 μg/kg [13] [14]. In Iran and according to The Institute of Standards and Industrial Research of Iran (ISIRI), this figure is 15 μg/kg [15]. The highest potential of contamination with AFs in nuts occurs at growth and storage stages. Given that there is no information regarding content of AFs in the nuts available in Tehran market and high consumption rate of the products, this study tries to determine presence of AFs in the nuts available in Tehran market.

2. Materials & Methods

2.1. Samples Collection

A total number of 200 nuts samples, integrated of Fig (n = 30), Almond (n = 25), Hazelnut (n = 25), Walnut (n = 40), Pistachio (n = 40) and Sunflower (n = 40) were randomly selected from local supermarket in Tehran city in 2013. Samples were conditioned in sterile plastic container and kept at 4 °C until analyses that were carried out in same day.

2.2. Chemicals and Reagents

All chemicals and Antigen ELISA Kit were obtained from Tecna (COKAQ, No.1148, Italy).

2.3. Sample Preparation

In order to obtain a representative sample, grind a Roomer Series II® Mill has been used, so that 75% has been passed through a 20-mesh screen, then thoroughly mix the subsample portion. Weigh out 20 g of ground sample into a clean jar that can be tightly sealed. Then, 100 mL of 70/30 (v/v) methanol/water extraction solution and seal jar added. Samples should be extracted in a ratio of 1:5 (w:v) to extraction solution respectively and there would be vigorous shake or blend for 3 minutes. After sample to settle, the top layer of extract filtered through a Whatman1 filter and the filtrate has been collected.

2.4. Analysis of Total Aflatoxin

All reagents and kit components were at room temperature (18˚C - 30˚C, about 2 h) before used. The following steps were done in order to complete the analysis: the appropriate number of blue/green-bordered Dilution Strips was placed in a micro well strip holder. One Dilution Well was required for each standard, (*i.e.* 0, 1.0, 2.0, 4.0, 10.0 and 20.0 ppb) or sample. An equal number of Antibody Coated Micro well strips were placed in a micro well strip holder, then, unused micro well strips were returned to the foil pouch with the desiccant packet and

reseal pouch with tape. The required amount of Conjugate was measured from the green-capped bottle (~240 µL/well or 2 mL/strip) and was placed in a separate container (e.g. reagent boat when using the 8-channel pipettor). In each blue/green-bordered Dilution Well, an 8-channel pipette was used to dispense 200 µL of Conjugate. In this stage, a single channel pipettes was utilized to dispense 100 µL of each standard or sample to the appropriate dilution well containing 200 µL of Conjugate. A fresh pipette tip was applied for each standard or sample. In this part, it was ensured the pipette tip was completely emptied. An 8-channel pipette with fresh tips was used for each 8-well strip and each well mixed by carefully pipetting it up and down 3 times and immediately transferred 100 µL of the contents from each Dilution Well into a corresponding Antibody Coated Micro wells. Incubation was done at room temperature for 15 minutes. To avoid well-to-well contamination, the plate was not agitated to mix. The contents of the micro wells strips were emptied into a waste container and washed by filling each micro well with distilled or deionized water, and then the water was pumped from the micro well strips. This step was repeated 4 times for a total of 5 washes. Care was taken to not dislodge the strips from the holder during the wash procedure. A piece of tape was placed on the edge of the holder to help keep strips in place. Several layers of absorbent paper towels were put down on a flat surface and micro well strips were tapped on towels to expel as much residual water as possible after the fifth wash, then the bottom of the micro wells was dried with a dry cloth or towel. The required amount of Substrate from the blue-capped bottle (~120 µL/well or 1 mL/strip) was measured and dispensed into a separate container (e.g. reagent boat for an 8-channel pipettor). 100 µL of the Substrate was pipetted into each micro well strip using an 8-channel pipettor and incubated at room temperature for 5minutes. The required amount of Stop Solution from the red-capped bottle (~120 µL/well or 1 mL/strip) was measured and dispensed into a separate container (e.g. reagent boat for an 8-channel pipettor). 100 µL of stop solution was pipetted into each micro well strip using an 8-channel pipettor. So, the color changed from blue to yellow. The strips with a micro well reader were read by using a 450 nm filter and a differential filter of 630nm. At the end, OD readings were recorded for each micro well.

3. Statistical Analysis

Statistical analyses were done in SAS (version 9) software. Regarding the results, $p < 0.05$ were considered as significant.

4. Results & Discussion

Six popular nuts were used in our assessment of AFs content and the results of the assessments are listed in **Table 1**. As shown by the results, 96.5% (193 samples) were found contaminated according to Limit of Detection (LOD). The highest levels of AFs were found in Fig, Walnut, Almond, Hazelnut, Pistachio, and Sunflower. Fig and Walnut were found as the two mostly contaminated nuts in the market and sunflower was the least contaminated product. On average, AFs concentration in Fig was 1.9 ± 1.1 µg/kg, 1.8 ± 1.5 in Walnut, 1.7 ± 1.2 in Almond, 1.6 ± 1.4 in Hazelnut, 1.5 ± 1.2 in Pistachio, and 1.3 ± 1.2 in sunflower. The acceptable concentration level of AFs according to national standard of Iran and Codex Alimentarius and European Community regulation are 15 µg/kg and 4 µg/kg respectively. In 6% of the samples, contamination level exceeded the EU limits and 100% of the samples met the limit of national standard of Iran regarding concentration of AFs in nuts. Statistical analysis of the difference between the content AFs in various nut samples are also listed in **Table 1**. Our results was al-

Table 1. Mean levels and ranges (µg/kg) for Aflatoxin in nuts consumed in Tehran, Iran.

Product	Number of samples	Mean ± S.E	Range
Fig	30	1.9 ± 1.1[a]	<LOD −3.4
Almond	25	1.7 ± 1.2[a]	<LOD −3.8
Hazelnut	25	1.6 ± 1.4[a]	<LOD −3.9
Walnut	40	1.8 ± 1.5[a]	<LOD −3.6
Pistachio	40	1.5 ± 1.2[a]	<LOD −5.8
Sunflower	40	1.3 ± 1.2[a]	<LOD −5.2

most consistent with [16] in which 16.3% of the samples were rejected according to EU action level (mean 17.2-350 µg/kg), while 26.5% of the nuts were found contaminated with AFs between 1.0 and 110 µg/kg [17]. Tolosa *et al.*, showed prevalence of contamination in nuts, shell, dried fruits, and dates equal with 50%, 80%, 35.7% and 83.3% respectively [18]. Another study in Isfahan, Iran showed that the total AF was above the maximum level in 9.8% of the pistachio nuts samples [19].

Another study by Fani *et al.* (2013) was carried out in Arak and found AFs contamination in 59.9% of the nuts so that total average concentration of the contamination was 1.12 µg/kg. They showed that 1.2% of the samples exceeded the EU limits [20]. In [21] high contamination of 1927 out of 3181 unprocessed pistachio samples (23.5%) in Iran was reported with mean and medial values of 2.42 ± 14.7 µg/g and <LOD, respectively; in addition, all the samples met the EU limits. Moreover, in [22] [23] nuts contamination of AFM1 crossed the EU limits (7.5%) and (29%) respectively. Warm condition with high humidity increases rate of growth of AFs in food stuff during storage. However there is low information about food storage in sampling regions, but regarding traditional shop and retailers is more spread than industrial system, food storage improperly and predispose to mould growth and AFs accumulation. Apparently, providing education for the farmers, producers, and distributers regarding agricultural methods and storage may lead to considerable reduction of AFs content in agricultural products and nuts in particular.

5. Conclusion

Although, concentration of AFs in majority of the samples under study meets EU limits, still it is recommended to improve storage procedure as a way to reduce contamination with AFs. In this regard, a food control system (*i.e.* GAP and HACCP), training courses for farmers, processing agents, distributers, retailers, and warehouse runners can be helpful. Moreover, AFs contaminations put emphasis on implementing regular monitoring and stricter food safety management system (FSMS).

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List of Abbreviations

ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
AFs	Aflatoxins
ISIRI	Institute of Standards and Industrial Research of Iran
LOD	Limit of Detection
GAP	Good Agricultural Practices
HACCP	Hazard Analysis and Critical Control Points
FSMS	Food Safety Management System