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Article

Aflatoxin Occurrence in Nuts Consumed in Arak, Iran

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Abstract: Aflatoxins are fungal toxins which may be present in food and due to their hazard effects to health, present a major concern for human and food industries. In the present study, the contamination of aflatoxin in nuts (fig, almond, hazelnut, walnut, pistachio and sunflower) consumed in Arak, Iran in 2012 was evaluated by means of ELISA method. 167 samples, consisted of fig (n=16), almond (n=14), hazelnut (n=20), walnut (n=25), pistachio (n=57), sunflower (n=35) were selected and analyzed for aflatoxin content. Results show that 59.9 % (100 of 167 samples nuts) of the samples were detected aflatoxin positive, the total average concentration of aflatoxin in all studied nuts is 1.12µg/kg. 1.2 % (2 of 167) of nuts samples with aflatoxins amount greater than EU limit (4µg/kg), and aflatoxins amounts in all studied samples are lower than legal limit of national standard of Iran (15µg/kg).

Key words: Aflatoxin, Nuts, Food safety, ELISA.

1. Introduction

Aflatoxins (AFs) as a subset of mycotoxins, consist of a large group of extremely toxic components which are produced by certain species of fungi, specifically by Aspergillus flavus and Aspergillus parasiticus (Fallah 2010). These fungi pollute wide range of agricultural products particularly cereal grains, during pre- and post-harvest stages (El Khoury, Atoui et al. 2011, Guan, Li et al. 2011). In fact, factors such as season, humidity, temperature and drought in field as well as storage conditions (i.e. temperature, relative humidity and duration) have critical roles in production of aflatoxins (EFSA 2004, Dashti, Al-Hamli et al. 2009, Kang'ethe and Lang'a 2010). These metabolites are highly carcinogenic, mutagenic and teratogenic components which pose a serious health and economic concern to humans. Hence, they should be monitored closely in potentially hazardous food (Elzupir and Elhussein 2010, Sadia, Jabbar et al. 2012).

Among four common aflatoxins available (aflatoxin B1, B2, G1 and G2), aflatoxin B1 (AfB1) is the most widespread and poisonous molecules and categorized as group 1 human carcinogen by IARC (Fallah 2010, El Khoury, Atoui et al. 2011, Guan, Li et al. 2011). Studies performed on hazelnuts and pistachios suggested that optimum temperature and relative humidity for AFs production are 25–30 ºC and 97–99%, respectively (Diener and Davis 1967, Şimşek, Arici et al. 2002). There are rules for AFs limitation in nuts in many countries. According to Codex Alimentarius and European Community legislations, permitted level of AFs concentration in nuts is fixed at 4 µg/kg (Commission 2001, EC 2006). The Institute of Standards and Industrial Research of Iran (ISIRI) put national legal limit to 15 µg/kg (ISIRI 2010). Taking into account that there is no information about AFs presence in nuts of Arak city form important part of human diet, we decided to determine the occurrence of AFs in nuts obtained from Arak, Iran.

2. Materials & Methods

2.1. Samples Collection

A total of 167 nuts samples, including fig (n=16), almond (n=14), hazelnut (n=20), walnut (n=25), pistachio (n=57) and sunflower (n=35) were randomly selected from local supermarket in Arak city in 2012. Samples were conditioned in sterile plastic container and kept at 4 ºC 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 was used, so that 75% was passed through a 20-mesh screen, then thoroughly mix the subsample portion. Weigh out 20 g of ground sample into a clean jar which can be tightly sealed. Then, 100 mL of 70/30 (v/v) methanol/water extraction solution was added and the jar was then sealed. Samples was extracted in a ratio of 1:5 (w:v) to extraction solution respectively and was vigorous shaken for 3 minutes. After sample preparation, the top layer of extract was filtered through a Whatman1 filter and the filtrate was collected.

2.4. Analysis of Total Aflatoxin

All reagents and kit components were at room temperature (18-30°C, 2 h) before used.

The following steps were done in order to complete the analysis:

Step 1: 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.

Step 2: 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.

Step 3: 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.

Step 4: 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.

Step 5: 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.

Step 6: 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.

Step 7: 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.

Step 8: 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.

Step 9: The strips with a micro well reader was read by using a 450 nm filter and a differential filter of 630nm. At the end, OD readings were recorded for each micro well.

2.5. Statistical Analysis

Data were analyzed by using Excel 2010 and then the results were reported as Mean± S.E.

3. Results & Discussion

In current study the total AFs of six types of Nuts which are commonly consumed by people in Arak were determined in the scale of µg/kg and the results are shown in table 1. It can be seen that 59.9% (100 of 167) of samples, the AFs concentrations were higher than Limit of Detection (LOD). The mean values of AFs in each type of nuts are presented in Figure 1.

Table 1: Aflatoxin mean values and ranges (µg/kg) in Nuts consumed in Arak, Iran

Product	**Number of samples**	**Mean± S.E**	**Range**
Fig	16	0.87±0.17	<LOD-2.5
Almond	14	0.81±0.24	<LOD-3.1
Hazelnut	20	0.73±0.23	<LOD-3.5
Walnut	25	0.86±0.18	<LOD-3.5
Pistachio	57	0.62± 0.11	<LOD-4.6
Sunflower	35	0.47±0.18	<LOD-4.7

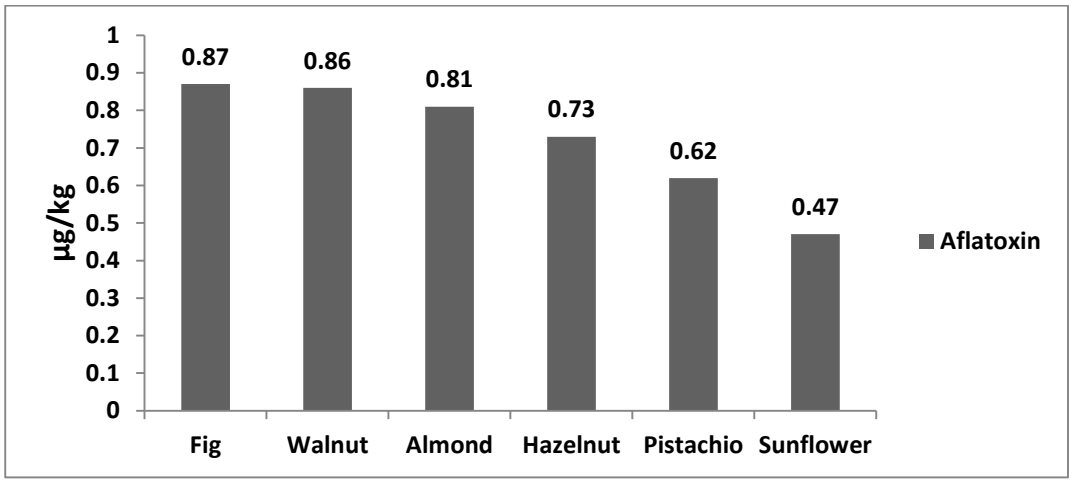

Figure 1: Mean AFs amount levels in each type of nut samples

From table 1, we can see that the amount of AFs found in various of nuts from high to low is as follows: fig, walnut, almond, hazelnut, pistachio and sunflower. Fig and walnut contain the highest amount, sunflower has the least. The average of AFs concentration in fig, walnut, almond, hazelnut, pistachio and sunflower were 0.87±0.17, 0.81±0.24, 0.73±0.23, 0.86±0.18, 0.62±0.11 and 0.47±0.18µg/kg, respectively. According to national standard of Iran, the limit of AFs content in nuts is 15µg/kg; and regarding Codex Alimentarius and European Community regulation, the limit is 4µg/kg. In our study, 1.2 % (2 out of 167 samples) of nuts samples the AFs amounts are over the EU limit (4µg/kg); and AFs amounts in all samples (100%) are either not detected or less than legal limit of national standard of Iran (15µg/kg). The results were different with Leong, Ismail et al. (2010), who reported AFs contamination in 16.3% of nuts samples exceeding EU action level (mean 17.2–350 µg/kg, Dini, Khazaeli et al. (2012) also pointed out a high contamination in 8203 raw pistachio samples produced in Iran, which AFs were detected in 1927 of samples (23.5%) with the mean value of 2.42 ± 14.7 ng/g and median value below LOD, respectively and all are lower than the EU limit. In similar following studies in Iran, AfM1 contamination in nuts samples were reported with 7.5% (Cheraghali, Yazdanpanah et al. 2007), and 29% (Pour, Rasti et al. 2010), both exceed EU limit.

Combination of high humidity and warm temperature during food storage accelerate fungi growth which resulted in increment of AFs in Food stuff. However, there is a little information about food storage in sampling regions. Traditional shop and retailers are more spread than industrial system, food storage improperly and predispose to mold growth and AFs accumulation. It is clear that proper training on agricultural practice for food storage to farmers, producers and sellers could play a major role in reduction of AFs content in agricultural products, especially nuts. It is recommended that proper storage should be taken to control and reduce contamination such as implementing a food control

systems (i.e. GAP and HACCP), educating farmers, manufacturers, distributors, retailers and warehouse owners.

4. Conclusion

From this study, it is clear that the amount of AFs in most of studied nuts were below EU limit. However, the presence of AFs remind the need for regular monitoring and a more stringent food safety management system (FSMS) in order to control the AFs to the lowest possible levels. It is also recommended that proper storage should be taken to control and reduce contamination such as implementing a food control systems (i.e. GAP and HACCP), educating farmers, manufacturers, distributors, retailers and warehouse owners.

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