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Total aflatoxin contamination of maize produced in different regions of Qazvin-Iran

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Abstract

Aflatoxins are hepatotoxic metabolites and found as contaminants in various agricultural commodities such as maize, rice, sorghum, wheat, oats and various spices. In this study, Aflatoxin total (AFT) contamination of maize were evaluated by high performance liquid chromatography (HPLC). A total of 54 samples of maize were randomly collected from maize farm of different city of Qazvin, Iran. All maize samples were contaminated with AFT. the average concentrations of AFT: All corn samples studied in Qazvin province was 2.79 ± 0.17 ng/g. In addition, the eight number (14.81%) of maize (21.42% of Boeen Zahra samples, 7.14% of Takestan samples and 28.57% of Qazvin samples), contamination rate were higher than of maximum tolerat level (MLT, 4 ng/g). Effective controlling of all food and foodstuffs which are vulnerable to AF contamination is necessary to prevent its effects.

Introduction

Mycotoxins are secondary metabolites of molds specially produced by two closely related fungi, *Aspergillus flavus* and *Aspergillus parasiticus.* They are distributed worldwide and infect a number of crops (Hussein and Brasel, 2001; Razavilar, 2003; Speijers, 2004). The risk of contamination by mycotoxins is an important food safety concern for grains and other field crops (Bhat and Vasanthi, 2003; CAST, 2003; Bryden, 2007). Aflatoxins (AFs) (hepatotoxic metabolites of molds) are found as contaminants in various agricultural commodities such as maize, rice, sorghum, wheat, oats and various spices. The four major AFs that occur in crops are B1, B2, G1, and G2 (Zheng *et al*., 2006).

These toxins are produced by fungi during production, storage and food processing. According to FDA although it is an unavoidable foodstuff contamination but it could be minimized by supervising systems (Williams *et al.*, 2004). The knowledge that AFs effect on humans and animal health has led many countries to establish a MTL on AF levels allowed in food and feed in the last decades to safeguard the health of humans, as well as the economical interests of producers and traders. The European Union has a maximum level of 2 ng/g for AFB1 and 4 ng/g for AFT in crops. However, the Iranian standard institute has a maximum level of 5 ng/g for AFB1 and 15 ng/g for AFT in crops. Maize is an important cereal as a source of food and feed. It is one of the major crops grown in different regions of Iran with production of approximately two million tons per year. Maize is one of the five main cereals in an Iranian food basket and also it's used as a component of animal feed, and may have an advantage like as slower fermentation and also had more starch that will be available for intestinal digestion and absorption as glucose, it made maize as a good source of feed for cattle. Due to the significant health risks associated with the presence of AFs in foods specially agriculture products, it is important to establish a data collection on the occurrence of these toxins in cereal as valuable food (FAO/WHO, 2002).

Maize and Its products are known to be prone to contamination by fungi that produce secondary metabolites such as AFs (Groopman and Donahue, 1988). The aim of this study was to screen the content of AFT in maize produced in different region in Qazvin as a leader for this agricultural product in Iran.

Material and Methods

Sample collection

Fifty four samples were collected randomly from maize farms in different region of Qazvin province,

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Iran from May 2010 to February 2011. Then, all the samples were transferred to Toxicology Labs in Food Quality Control Laboratory, Qazvin, Iran.

Reagents and apparatus

All reagents (potassium chloride, phosphoric acid, hydrochloric acid) and solvents (methanol, acetonitrile, propanol-2-oil, n-hexane, chloroform) used were of high performance liquid chromatography (HPLC) grade. AF standards were purchased from Sigma Chemical Company, USA. Aflatest immunoaffinity columns (IAC) were purchased from VICAM Company, Watertown, MA, USA. Apparatus characteristics were WATERS 1525 binary HPLC pump, and 2475 Multy λ fluorescence detector. HPLC column (C18, 250 x 4.6 mm: 4 µm) was purchased from Waters, USA.

Extraction and clean up

Samples were analyzed using a HPLC following AOAC (1995) with some modifications. Samples were extracted with methanol: water: n-hexane (240:60:100, v/v/v). The mixture was shaken for 30 min on a mechanical shaker. The solution was left to sediment and filtered through a WHATMAN Filter No.1. After filtration, the extract was diluted with water and filtered through the glass micro fiber filter. Aflatest was used for samples to clean up. First, 10 ml phosphate buffer saline (PBS) was passed through the IAC. Then, 75 ml of the filtrate was passed through the IAC at a flow rate of 1 ml/min. The column was washed with water and dried using vacuum. Finally, AF was eluted with methanol using the following procedure. First, 0.5 ml methanol was applied to the column which passed through by gravity. After 1 min, the second portion of 0.75 ml methanol was applied and collected. The Aflatest was diluted with water and analyzed using HPLC.

AFT standard

After preparation of standard solutions for AFT, its concentration was determined using a UV spectrophotometer. This standard was used to prepare mixed working standard for HPLC analysis (Stroka *et al*., 2000).

Recovery and limit of detection (LOD)

The effectiveness of the extraction procedure was confirmed by sample fortification. The recovery of extraction method was determined by fifty grams of milled maize fortified with a solution of AFT in methanol at 5 µg/ml. The AFT fortification solution was prepared in methanol and used for quantification of analyte recovered after extraction. The sample was fortified with 0.25 ml of this solution in order to have 5 mg/g of AFT in the maize, which is the maximum permitted limit in cereals by National standards of Iran. LOD was 0.32 ng/g for AFT, respectively.

Analysis of AFT using HPLC

AFT was quantified by reverse-phase HPLC and 2475 Multy λ fluorescence detector with post column derivatization (PCD) involving bromination. The waters HPLC system was applied with a Kobra cell and the addition of bromide to the mobile phase. After dilution of AF eluate with water, 100 µl was injected into the HPLC. Mobile phase was water: methanol: acetonitrile (600:300: 200, v/v/v) and 350 µl of nitric acid 4 M and 120 mg of potassium bromide with a flow rate of 1 ml/min. The fluorescence detector was operated at an excitation wavelength of 365 nm and emission wavelength of 435 nm. The calibration curve for AFT was used to check for the linearity and quantification of AFT in maize samples.

Results and Discussion

In this study, 54 maize samples were analyzed twice for the levels of AFT by HPLC. The all sample contaminated with AFT, The mean concentrations of AFT in maize samples of the all region Qazvin was 2.79 ± 0.17ng/g. The eight samples (14.81%) that showed AF contamination rate of higher than MLT (4 ng/g), (Table 1). According to National Standard of Iran (No: 6872), permitted rate of the AFT in cereal is 15 ng/g, so that AFT content was lower than of the acceptable limit. The highest mean concentration of AFT (3.03 ± 0.55 ng/g) was observed in maize samples from Abyek city and followed by maize samples from Qazvin city (2.96 ± 0.39 ng/g).

Contamination of foods and agricultural products with AFs is a public health concern because of the ability of AFs to cause human and animal diseases (Mutungi *et al*., 2008). Among 54 samples analyzed, 4 samples (8.90%) were not contaminated with AFT (<LOD). A high proportion of samples (91.10%) showed positive for AFT contamination (Table 1). Mean concentration of the AFT in the samples was 2.79 ± 0.17ng/g. However, there was no sample with AFT above MTL of 15 ng/g assigned by Institute of Standard and Industrial Research of Iran (ISIRI 2002). Maximum level of the AFT in maize samples was 5.68 ng/g. Mean levels of the AFT in maize samples were differences between different regions of Qazvine. The highest mean level AFT detected was in maize samples collected in Boeen Zahra and the lowest levels observed in Abyek city.

The history of food which is contaminated

Table 1. Mean value and range of AFT concentration in maize samples

City	Sample size (N)	AFT		
		Means ±SE	Min-Max	*Exceed legal limit n
Abyek	14	2.24±0.33	<0.3-3.77	0
Boeen Zahra	14	3.03±0.55	<0.35.68	3(21.42%)
Takestan	14	2.85±0.22	1.95-4.93	1(7.14%)
Qazvin	14	2.96±0.39	<0.3-4.78	4(28.57%)
Totall	54	2.79±0.17	<0.3-4.93	8(14.81%)

European Commission (EC) limit for AF Total in nut is 4ng/g.

with AFs in Iran dates back to 1967, when Tabibi and Salehian (1967) reported the outbreak of food poisoning caused by bread which had become molded by *A. flavus* in the winter of 1967. Most common mycotoxin contaminations of agricultural products and feed have extensively been studied in Iran, in this regard, the amount of AFs levels in rice samples imported to Bushehr Iran were analysis by HPLC (Mohammadi *et al*., 2012). Among 152 samples analyzed, 75% showed levels of AFB1 contamination. However, there was no sample with AFB1 above MTL in pistachio nut (5 ng/g) assigned by Institute of Standard and Industrial Research of Iran (ISIRI, 2002).

A survey of zearalenon (ZEA) was performed on the 72 samples of rice, bread, puffed corn snack and wheat flour collected from the Tehran retail market. All samples had contamination levels lower than the maximum tolerated level of ZEA in foods in Iran. The mean intake of ZEA from all samples was much lower than the tolerable daily intake estimated by JECFA (Yazdanpanah *et al*., 2012).

Determination of the AFs contamination levels of nuts 142 used by the confectionery in Tabriz by ELISA and HPLC method showed 13 cases (9.28%) contamination rate of higher than 15 ppb were observed. AFB1 was the highest detected AFs (Siahi Shadbad *et al*., 2012). Another survey conducted by Mazaheri (2009) to determine the levels of AFs shows that among 71 rice samples, AFB1 was detected in 59 samples (83% of the total). The mean of AFB1 was 1.89 ng/g for all samples. Total AF was detected in 83% of samples. The mean of AFT was 2.09 ng/g. AFB1 levels in two samples (2.8%) were above the MTL of AFB1 in Iran (5 ng/g). Karami-Osboo *et al.* (2010) examined AFB1 contaminations in 373 maize samples collected during 2006-2008 at harvest stage, from different agro climatic regions of the major maize production area of Iran , based on results, AFB1 was detected in 146 samples (43.6%), in which only 22.5% were contaminated to higher than MRL level.

Harvested maize in Iran is chiefly used for domestic animal feed and there are many published data about AFM1 in Iranian milk products (Rahimi *et al*., 2009). Which was well documented tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and production of AFs (Bhat and Vasanthi, 2003). In this study, Boeen Zahra maize products had the highest means AFT contamination in comparing to other region of the Qazvine province (Table 1). Also, the range of AFT levels (<0.3-5.683 ng/g) in this city was significantly different from other part Qazvin's maize products. Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth and increase the risk of AF contamination (Bhat and Vasanthi, 2003). In this study, the amount of the AFT in damaged grain was so high. Damaged grain by birds and insects are more prone to fungal invasion and the most vulnerable time for fungal penetration during injures to attain maximum AF concentration at harvest is approximately 20 days after full silk (Wiatrak *et al*., 2005).

In conclusion, the levels of the AFT in maize samples or its metabolite in dairy products, indirectly, which are consumed in Iran are high and seem to pose a risk to public health. Although only a few samples of maize, AFT levels in most maize samples were below the ISIRI and internationally acceptable limits for human consumption. But, detection of small quantities of AFs in most of the samples warrants further investigations, since intake of the maize is very high in Iran. So finding a practical strategy to reduce the risk of AF contamination of food and feed is vital.

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