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Preliminary Survey of Aflatoxins and Ochratoxin A in Dried Fruits from Iran

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Abstract Forty five dried fruits, 30 dried apricots and 15 prunes were tested for aflatoxins and ochratoxin A contamination utilizing immunoaffinity column clean up and high performance liquid chromatography (HPLC) with fluorescence detection. 30% and 3.33% of examined apricot samples and 13.33% and 20% of examined prunes samples contained aflatoxin B_1 and ochratoxin A more than 0.2 ng g^{-1}. The average recoveries were found to be 91.1% and 98.5% for aflatoxin B_1 and ochratoxin A, respectively, while the detection limit was 0.2 ng g^{-1} for both mycotoxins.

Keywords HPLC · Aflatoxin · Ochratoxin A · Dried fruits

This study will discuss the incidence and occurrence of mycotoxins in dried fruits. There have been many investigations of the occurrence of mycotoxins in these products. Dried fruits, particularly dried apricots (Zohri and Abdel-Gawad 1993; Apergi et al. 1998) and dried plums (prunes) (Zohri and Abdel-Gawad 1993; Engel 2000; Iamanaka et al. 2005; Apergi et al. 1998) have been of considerable interest. A wide variety of food is contaminated with mycotoxins and the contamination occurs throughout the food chain, both during the pre and postharvest periods. Processing steps such as drying, storage and transportation play an important part in food safety.

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Although ochratoxin A (OTA) and aflatoxin are the two most abundant mycotoxins to contaminate food, aflatoxins are mostly considered to be field contaminants while OTA is a drying and storage-related mycotoxin produced by Aspergillus and Penicillium fungi (Buchanan et al. 1975; Al-Anati and Petzinger 2006; Guler and Heperkan 2008). Have the capacity for producing OTA when the temperature, relative humidity and product moisture are favorable (Magnoli et al. 2004). Due to the fact that toxigenic species of *Aspergillus ochraceus*, *Penicillium verrucosum* and *Aspergillus carbonarius* can grow and produce OTA at moderate, low and high temperatures, respectively, widespread occurrence of OTA has been reported in a variety of different geographical regions and climates, and in several foods and beverages (Al-Anati and Petzinger 2006; Guler and Heperkan 2008).

Moreover, recent improvements in analytical methods have increased sensitivity and decreased detection limits using immunoaffinity columns cleanup and HPLC method. Using these techniques, researchers have discovered that OTA contamination is not solely a problem for improperly stored cereal products, as previously proposed. A diversity of foods, such as coffee beans, bread, beans, wine, grape juice and dried fruits have also been found to be contaminated (Senyuva et al. 2005; Guler and Heperkan 2008; Kabak 2008). Available toxicological data, evidence of occurrence in various food commodities established by sensitive analytical methods and growing health concerns have led many countries to establish OTA regulations in food and feed. The European Union has also established official regulations and guidelines for maximum levels of OTA and aflatoxins in foodstuffs such as cereals, coffee, dried fruits, wine and grape juices (European Commission 2006b). The aim of this type of regulation is to keep the level of contaminants in the toxicologically acceptable

range to protect public health. There is increasing interest among researchers in determining contamination levels of OTA and aflatoxins, and among regulatory agencies to establish new or replace old OTA limits for food commodities due to its widespread occurrence, increasing health risk concerns and development of new sensitive analytical methods.

Therefore, this study aimed to determine incidence levels of OTA and aflatoxins in Iranian dried fruits. This study also provides the number of dried fruits containing toxins above the EU limits and Iranian National Standard.

Materials and Methods

Thirty dried apricot and 15 dried prunes samples, each weighing 5 kg, were obtained locally from Mashhad (Khorasan, Iran). Samples were taken according to the alternative sampling plan for official control of mycotoxins in food (European Commission 2006a). Dried fruit Samples were blended with a high-speed blender to homogenize them, with the addition of water.

Methanol, acetonitrile, acetic acid and other chemical reagents were HPLC grade and supplied by Merck (Darmstadt, Germany). Aflatoxin and OTA powders were obtained from Sigma (St. Louis, USA). Immunoaffinity columns (Neogen Europe, Ltd, Scotland, UK) for the purification and preconcentration of aflatoxins and OTA prior to the quantitative analysis were used. Double distilled water was used in all the experiments. Stock standard solution of aflatoxins and OTA with concentrations of 100 μg mL^{-1} was prepared in methanol. Working standard solutions for aflatoxins and OTA were prepared daily by diluting the stock solution with water: methanol (6:4) and methanol: acetic acid (98:2), respectively. All of the working standard solutions were stored in darkness and refrigerator at 4°C.

HPLC analyses were performed on a HPLC system, Agilent Technologies SL 1200 Series (Waldbronn, Germany) composed of a binary pump equipped with 152 micro vacuum degasser, thermostated autosampler, column compartment and fluorescence detector and a Genesis RP C_{18} analytical column (250 × 4.6 mm, 4 μm) and a chromolith® performance RP C_{18} analytical column (100 × 4.6 mm), respectively for aflatoxins and OTA, respectively. The fluorescence detector for aflatoxin was operated at 365 and 445 nm and for OTA was operated at 333 and 443 nm for excitation and emission, respectively. ChemStation software was used for data management. For determination of aflatoxins and OTA concentration a UV–Visible spectrum of aflatoxins and OTA stock solution against solvent used for solution in reference cell (Fakoor Janati et al. 2011) was obtained using a Shimadzu UV-1700 Pharma spec. (Tokyo, Japan). Spectrophotometer equipped with a standard 10 mm path length spectrophotometer cell.

Linear isocratic elution chromatography have done using solvent system water: methanol: acetonitrile (6:2:2 v/v/v) for aflatoxins at 40°C and water: acetonitrile: acetic acid (99:99:1 v/v/v) for OTA at 30°C. For aflatoxins, the flow rate was kept constant at 1 mL min^{-1}. Online photochemical derivatization was performed using a commercially available system – UVE™ LCTech GmbH (Dorfen, Germany) – placed between the separation column and the fluorescence detector, which consisted of a 254 nm low pressure mercury lamp and a 1 mL knitted reaction coil fitted around the UV lamp. For OTA the flow rate was kept constant at 0.7 mL min^{-1} and 20 μL was injected.

For aflatoxins determination, 50 g apricot and prunes samples, either non-spiked or spiked with a known volume of an aflatoxins stock solution, was mixed with 40 mL of pure water, 160 mL methanol and 5 g of sodium chloride and blended (Waring 8011S, Torrington, CT, USA) at high speed for 5 min to obtain a homogeneous sample mix. After mixing, the slurry was filtered though filter paper (Whatman No. 4) and 20 mL of this diluted with 130 mL phosphate buffered saline (PBS) solution. This diluted solution was filtered through glass microfiber filter (Whatman, Inc., Clifton, NJ, USA) and 100 mL was passed through an immunoaffinity column. Aflatoxins were eluted from the column by passing 2 mL of HPLC grade methanol and then 2 mL of HPLC grade water and using gravity to collect the eluate into a glass vial at a flow rate of around 5 mL min^{-1}.

For OTA determination, 25 g apricot and prunes samples, either non-spiked or spiked with a known volume of an OTA stock solution, was mixed with 40 mL of pure water, 60 mL acetonitril and 0.3 g $NaHCO_3$ and blended (Waring 8011S, Torrington, CT) at high speed for 5 min to obtain a homogeneous sample mix. After mixing, the slurry was filtered though filter paper (Whatman No. 4) and 10 mL of this diluted was mixed with 40 mL phosphate buffered saline (PBS) solution. This diluted solution was filtered through glass microfiber filter (Whatman, Inc., Clifton, NJ, USA) and 40 mL was passed through an immunoaffinity column. OTA were eluted from the column by passing 1.5 mL of HPLC grade methanol: acetic acid (98:2 v/v) and then 1.5 mL of HPLC grade water and using gravity to collect the eluate into a glass vial at a flow rate of around 5 mL min^{-1}.

An external standard calibration curve was constructed using reference standard aflatoxins and OTA to quantify these mycotoxins content in all samples. Aflatoxins and OTA stock working solution containing 100 ng mL^{-1} was prepared and then diluted to appropriate concentration

ranges with methanol: water (4:6 v/v) and methanol:acetic acid (98:2 v/v) for aflatoxins and OTA, respectively for construction of calibration curve. Calibration curve was performed with seven different concentrations with square of correlation coefficient (r^2) $B_1 = 0.998$, $B_2 = 0.998$, $G_1 = 0.998$, $G_2 = 0.999$ and five different concentrations with square of correlation coefficient (r^2) OTA = 0.999. Calibration curves were derived by plotting concentrations as a function of peak area of each aflatoxin and OTA. Calibration curves exhibited good linear regression.

Accuracy was examined by the determination of the recoveries of the aflatoxins and OTA. The recovery study was performed by comparing the concentration in the apricot and prunes spiked samples to the respective non-extract standards (aflatoxins and OTA in solution). The recoveries of aflatoxins B_1, B_2, G_1, G_2 and OTA from samples spiked at 5 ng g^{-1} for AFB_1, AFG_1 and OTA, and 1 ng g^{-1} for AFB_2 and AFG_2 were quite good (Table 1). Relative standard deviations for within laboratory repeatability (RSDr, n = 6) range from 1.3 to 3.2.

This recovery range is within the guideline of acceptable recovery limits of AOAC and the Codex alimentarius. The AOAC guideline for the acceptable recovery at the 10 μg kg^{-1} level is 70%–125% and Codex acceptable recovery range is 70%–110% for a level of 10–100 μg kg^{-1} for a level of 10–100 μg kg^{-1}, and 60%–120% for a level of 1–10 μg kg^{-1} (Fakoor Janati et al. 2011).

Results and Discussion

The natural occurrence of OTA has been reported in dried apricots and dried plums (prunes) (Zohri and Abdel-Gawad 1993; Engel 2000; Iamanaka et al. 2005). Co-occurrence of OTA and citrinin in dry copra was reported from India. AFB1 has also been found in dried apricots and prunes (Apergi et al. 1998).

European regulations for certain mycotoxins in dried fruit have been published by the Commission of the European Communities. The maximum level for OTA in dried vine fruit (currants, raisins, sultanas) is 10 ng g^{-1} (European Commission 2006b). The maximum level for AFB_1 in dried fruit for human consumption or food ingredient is 2 ng g^{-1} and for total aflatoxins is 4 ng g^{-1} (European Commission 2006b); for dried fruit to be subjected to sorting or other physical treatment the maximum levels are 5 ng g^{-1} AFB_1 and 10 ng g^{-1} total aflatoxins.

The average recovery and the relative standard deviation (RSD) obtained from spiking the blank samples of dried fruits with level of OTA were 98.5% (RSD = 1.4%) for dried apricots, and 99.2% (RSD = 0.7%) for dried prunes. Also, spiking the blank samples of dried apricots with aflatoxins (B_1, B_2, G_1 and G_2) produced acceptable rates of recovery and RSD values ranging from 83.4% to 97.1% and 1.3%–4.1%, respectively and spiking the blank samples of dried prunes with aflatoxins (B_1, B_2, G_1 and G_2) produced acceptable rates of recovery and RSD values ranging from 85.4% to 96.8% and 2.3%–4.6%, respectively (Table 1). These results proved the suitability and the effectiveness of the utilized methods based on immunoaffinity column clean-up techniques to determine levels of both OTA and aflatoxin in dried fruits. Adjustment of the reported results based on the recovery experiment was performed on all samples. Although different extraction techniques were used, similar recovery rates for OTA have been reported by others: 80%–84% at 2, 5 and 10 ng g^{-1} (Guler and Heperkan, 2008), 82.4% at 10 ng g^{-1} (Zinedine et al. 2007) and from 80% to 85% at 1, 7.6 and 29.6 ng g^{-1} (Iamanaka et al. 2005) in dried fruits. However, Senyuva et al. (2005) found higher recovery rates (90%–98%) for dried fruits spiked with OTA at 5 and 10 ng g^{-1} concentration levels. An extensive inter laboratory study on currants, sultanas, raisins, figs, and mixed fruits using an immunoaffinity column cleanup method obtained lower recovery results, ranging from 69% to 74% with an RSD of 4.9% to 8.7% (Macdonald et al. 2003).

In this study 30 apricots and 15 prunes samples were analyzed to evaluate the concentration of aflatoxins B_1, B_2, G_1, G_2 and OTA with HPLC. As it is shown in Table 2, aflatoxins B_1, B_2, G_1 and OTA were detected in 30%, 3.33%, 3.33% and 3.33% of apricot samples with mean value 0.88, 0.32, 0.20 and 2.83 ng g^{-1}, respectively and aflatoxins B_1, G_1 and OTA were detected in 13.33%,

Table 1 Analytical data for the aflatoxins and OTA HPLC system

Compound	t_R (min)	Repeatability (%RSD, n = 6) Peak area	Limit of detection (ng g^{-1})	Limit of quantification (ng g^{-1})	Apricot recovery (%)	Prunes recovery (%)
AFB_1	14.75	1.3	0.2	0.6	91.1	92.9
AFB_2	12.04	2.4	0.1	0.3	97.1	96.7
AFG_1	10.72	1.5	0.2	0.9	85.2	86.3
AFG_2	8.89	3.2	0.1	0.3	83.4	85.4
OTA	5.49	1.4	0.2	0.5	98.5	99.2

Table 2 Mean value and range of apricot and prunes samples aflatoxins and OTA concentration (ng g^{-1})

Compound	Average (±SD)		Range	
	Apricot	Prunes	Apricot	Prunes
AFB_1	0.88 (±1.68)	0.70 (±0.66)	0.21–5.33	0.23–1.17
AFB_2	0.32	ND	0.32	ND
AFG_1	0.20	0.37	0.2	0.37
AFG_2	ND	ND	ND	ND
OTA	2.83	1.28 (±1.22)	2.83	0.22–2.62

ND not detected

3.33% and 20% of prunes samples with mean value 0.70, 0.37 and 1.28 ng g^{-1}, respectively. Aflatoxin G_2 was not detected in any of the apricot and prunes samples. All contaminated samples had a level of total aflatoxins, AFB_1 and OTA below the Iranian National Standard No. 5925. Based on Iranian National Standard No. 5925 the total aflatoxins, AFB_1 and OTA levels in apricot and prunes were 15, 5 and 10 ng g^{-1}, respectively (ISIRI Institute of Standards and Industrial Research of Iran 2002). Relevant data concerning the analytical system are summarized in Table 1. For aflatoxins B_1, B_2, G_1, G_2 and OTA, LOD were 0.2, 0.1, 0.2, 0.1 and 0.2 ng g^{-1} and LOQ were 0.6, 0.3, 0.9, 0.3 and 0.5 ng g^{-1}, respectively. For the repeatability measurement a standard solution containing 10 ng mL^{-1} of OTA was used.

Several studies in different countries and regions have established that dried fruits will support fungal growth and subsequent mycotoxin production. Our studies showed that from 30 apricot and 15 prunes samples, 7 apricot samples contained AFB_1 and 1 sample contained OTA with mean value 0.88 and 2.83 ng g^{-1}, respectively and 2 prunes samples contained AFB_1 and 3 sample contained OTA with mean value 0.70 and 1.28 ng g^{-1}, respectively. According to results obtained, the mean concentration of AFB_1 and OTA contamination of apricot and prunes in Mashhad, Iran, had a significant difference with the accepted limits by Iran regulations (5 and 10 ng g^{-1} for AFB_1 and OTA, respectively). So it seems that the present status of this mycotoxin is not at risk and don't be a serious problem for the public health. Therefore, it is need to routinely monitor this as a food quality control measure. The initial approach to control the occurrence of aflatoxins and OTA in apricot and prunes had been to control the contamination with aflatoxins and OTA in the field, however, is very difficult to control because it is influence primarily by climatic conditions such as relative humidity and temperature (Applebaum et al. 1982). However, it is reported that the highest concentrations of aflatoxins are associated with the post harvest growth of Aspergillus moulds on poorly stored stuffs (Jay 1992).

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