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Aflatoxin contamination level in Iran's pistachio nut during years 2009–2011

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ABSTRACT

An efficient monitoring system for sampling, analyzing and issuing the export certificates for pistachio consignments has been established in Iran in recent years. Accordingly, 3181 commercial raw pistachio nut lots were supplied for testing for European export certification since January 2009 till December 2011. Aflatoxin analysis was carried out by high-performance liquid chromatography with fluorescence detection after immunoaffinity column clean up with recoveries ranging from 77 to 99%. Amongst 8203 sub-samples analyzed, aflatoxin B_1 (AFB_1) was detected in 1921 cases (23.4%) with the mean and median values of 2.18 ± 13.1 ng/g and <LOD, respectively. Total aflatoxin (AFT) was detected in 1927 sub-samples (23.5%) with the mean and median values of 2.42 ± 14.7 ng/g and <LOD, respectively. AFB_1 level in 556 (6.78%) and 428 (5.22%) sub-samples was above the maximum tolerable levels set for AFB_1 in Iran (5 ng/g) and European Union (EU) (8 ng/g). The mean contamination levels of AFB_1 (2.18 ng/g) and AFT (2.42 ng/g) were lower than the maximum tolerable levels set in Iran and EU. The contamination levels of pistachio nut for export to EU were ~50% of those found in 2002–2003 indicating a satisfying improvement in hygienic conditions of pistachio cultivation, harvesting and post-harvesting practices in Iran.

1. Introduction

Pistachio nut is being produced in different regions of the world by various countries, among which Iran is the largest producer (Farzaneh et al, 2012; Zheng, 2011) most of which is exported to other countries. Traditionally, the European Union (EU) is one of the major destinations for Iran pistachio (Cheraghali & Yazdanpanah, 2010). During years 2009–2011, around 20,000 tons pistachio nut were exported to EU from Iran most majorly from Kerman and Rafsanjan located in the center of country (Iranian Pistachio Association website).

Contamination of agricultural products with mycotoxins including aflatoxins is one of the major challenges encountered by producers. Aflatoxins are polyketide secondary metabolites produced by some species of *Aspergillus* genus, particularly *Aspergillus parasiticus* and *Aspergillus flavus* (Cheraghali et al., 2007). Pistachio nuts are amongst the commodities with the highest risk of aflatoxin contamination (Pittet, 1998). The contamination is influenced by environmental factors such as temperature, humidity, and the extent of rainfall during cultivation, harvesting, and post-harvesting stages (Campbell, Molyneux, & Schatzki, 2003; Doster & Michailides, 1994; Emami, Suzangar, & Barnett, 1977). It is important to protect both consumers health and producers benefit through implementing a series of interventions in order to reduce the aflatoxin contamination to as low as practically feasible. Failure to do this, may create devastating economic consequences to producers, and also deprive consumers from a very valuable source of nutrient and pleasure. Many countries have established maximum tolerable levels on mycotoxins occurrence in foods due to the increasing awareness of their harmful carcinogenic, mutagenic and teratogenic effects on human and animals (Set & Erkmen, 2010). In Iran, the maximum acceptable levels for aflatoxin B_1 (AFB_1) and total aflatoxin (AFT) in pistachio nuts are 5 and 15 ng/g, respectively (ISIRI, 2002). In 1998, the Commission of the

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European Communities set the maximum level for AFB_1 in a range of commodities for human consumption at 2 ng/g (Moss, 2002). Recently, EU commission increased the maximum level in almonds, pistachios and apricot kernels intended for direct human consumption or use as an ingredient in foodstuffs to 8 ng/g and that for AFT from 4 to 10 ng/g (EC (European Commission), 2010a).

In 1997, based on the unacceptable levels of aflatoxin detected in pistachio consignments arriving in EU ports from Iran, special conditions were imposed on Iran pistachio entering the EU. Thereafter, extensive efforts were made to overcome the problem by Iranian authorities in collaboration with EU authorities based on a multi-approach intervention (Cheraghali & Yazdanpanah, 2010). A comprehensive survey on 3356 pistachio nut samples produced mostly in Kerman and Rafsanjan during March 2002–February 2003 indicated that 11.8% and 7.5% of samples had contamination levels higher than maximum tolerable limits set in Iran for AFB_1 and AFT (Cheraghali et al., 2007). Later, a brief study on 100 samples purchased from retail shops and local markets in Esfahan province of Iran from September to November 2007 revealed that 36 and 29% of pistachio nuts exceeded the maximum tolerable limit set for AFB_1 and AFT, respectively (Sarhang Pour, Rasti, Zighamian, & Daraei Garmakhani, 2010). These high contamination levels were most probably due to the extensive manipulation and exposure of pistachio nut in retail shops. Efforts were continued due to the enforcement and stricter control measures arising from Commission Decision 2006/504/EC (EC (European Commission), 2006a) on special conditions governing certain foodstuffs imported from certain countries (Ariño et al., 2009). Iranian health and agricultural authorities implemented strict regulations to manage the contamination by promoting good agricultural practices in the orchards and hazard analysis and critical control point principles in storage and processing plants (Farzaneh et al., 2012). Recently, Ariño et al. (2009) reported the occurrence of aflatoxins in pistachios available in northeast Spain from January to April 2007. They found that although all positive samples originated from Iran, no sample exceeded the maximum permitted level for aflatoxins in pistachio nuts set by EU.

Foodstuffs import/export checking and their monitoring on the borders and domestic markets are commonly carried out by governmental bodies. This is performed by the Ministry of Health in Iran as the official responsible for testing the pistachio nut exporting to EU. A network of Food Control Laboratories supervised by Ministry of Health accomplishes the task. The present paper reports the results of an extensive survey for aflatoxins occurrence carried out by Food Controls Labs to monitor the contamination of raw pistachio intended to export to EU in a period of three years from January 2009–December 2011.

2. Materials and methods

2.1. Sampling

A total of 3181 pistachio nut samples were collected by inspectors of Food Control Offices in Kerman and Rafsanjan since January 2009 till December 2011. The sampling was carried out exactly according to the sampling procedures established by EU. During January 2009–September 2010, samples were taken following the procedure described in Commission regulation (EC) No. 401/2006 (EC, 2006b) and then during October 2010–December 2011 according to the Commission regulation No. 178/2010 (EC, 2010b). The pistachio nut consignments, intended for export to EU, are usually about 25 tons. Accordingly, 100 incremental samples, each weighing 300 or 200 g were mixed together and divided into three or two 10 kg sub-samples. Preparation of sub-samples for analysis and further analytical experiments were then carried out in Toxicology Labs of Food Control Laboratories located in Rafsanjan and Kerman. A total of 1094, 1074 and 1013 samples divided in 3282, 2895 and 2026 sub-samples were analyzed in 2009, 2010 and 2011, respectively.

2.2. Sample preparation

A water slurry of pistachio nut samples was prepared to minimize the sub-sampling errors in aflatoxin analysis (Cheraghali et al., 2007). Therefore, 15 L water was added to each 10 kg sub-sample of pistachio nut, followed by mixing and grinding the mixture by using a slurry machine for 15 min. When ready, 125 g of slurry was taken as the test portion for analysis.

2.3. Chemicals and reagents

Aflatoxin B_1, B_2, G_1 and G_2 standards were procured from Sigma (MO, U.S.A). Methanol, acetonitrile (Caledon laboratories Ltd, Canada) and water were high-performance liquid chromatography (HPLC) grade. Sodium chloride, potassium bromide, nitric acid (Merck, Darmstadt, Germany) and phosphate-buffered saline [pH 7.4; 0.20 g KCl, 0.20 g KH_2PO_4, 1.16 g anhydrous Na_2HPO_4 (or 2.92 g Na_2HPO_4, $12H_2O$) and 8.0 g NaCl dissolved in 900 ml water and pH adjusted to 7.4 with 0.1 M HCl or 0.1 M NaOH and diluted to 1 L with water] were used in present research.

2.4. Standard solutions

After preparation of standard solutions of each aflatoxin, their concentration was determined by using an UV–Visible Spectrophotometer (Varian, CARY 100, USA) through AOAC Official method No. 971.22 (AOAC, 2006; chap. 49.2.03). These standards were used to prepare mixed standards for HPLC analysis. The working standard solution was prepared by diluting mixed standards, tertiary stock standard 40 ng/ml (AFB_1, $AFG_1 = 16$ ng/g; AFB_2, $AFG_2 = 4$ ng/g), with methanol and water.

2.5. Apparatus

Liquid chromatography (LC) analysis was performed using a reverse-phase HPLC system (Dionex, Sunnyvale, California LP, USA) equipped with a Gilson-Workstation (GX-271 Aspec Gilson, USA), vacuum degasser (Ultimate-3000, Dionex, Sunnyvale, California LP, USA), temperature-controlled oven (Ultimate-3000, Dionex, Sunnyvale, California LP, USA), and fluorescence detector (RF 2000; Dionex, Sunnyvale, California LP, USA). The Dionex LC column was C_{18}, 250 mm × 4.6 mm, 5 μm. Aflatest immunoaffinity columns (IAC) were purchased from Vicam (MA, USA).

2.6. Chromatographic conditions

Reversed-phase LC determination of aflatoxins was performed using the post-column bromination with KobraCell (Coring System, Gernsheim, Germany) with a flow rate of 0.8 ml/min and fluorescence detection at excitation wavelength 365 nm and emission wavelength 435 nm. The column temperature was adjusted to 36 °C. Retention times for AFG_2, AFG_1, AFB_2, and AFB_1 were 10, 11.63, 13.6, and 15.06 min, respectively. The isocratic mobile phase was water–acetonitrile–methanol solution and a ratio of 60:20:30 (v/v/v), containing 120 mg/L KBr and 350 μl HNO_3 4 M.

2.7. Extraction and clean up

Samples were analyzed for aflatoxins content based on the AOAC Official Method No. 999.07:2000 (AOAC, 2006; chap. 49.2.29)