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Incidence of aflatoxins contamination in dry fruits and edible nuts collected from Pakistan

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ABSTRACT

During 2012–2015, a total of 624 samples of 13 different types of dry fruits and edible nuts were collected from retail shops and local markets of Pakistan. The collected samples were then studied for the occurrence of total aflatoxins (AFs) after immunoaffinity cleanup followed by HPLC coupled with fluorescence detector and post-column derivatization unit (Kobra Cell™). The seasonal variation of AFs was studied with respect to average temperature and relative humidity in Pakistan. LOD (S/N; 3:1) and LOQ (S/N; 10:1) of the utilized method were in the range of 0.09–0.12 and 0.30–42 μg/kg, respectively. About 165 (26%) samples were found contaminated, ranging from 0.22 to 30.11 μg/kg with a mean equivalent to 0.85 ± 0.26 μg/kg. In 459 (74%) samples, the AFs contamination was found lower than detectable limit corresponding to0.12 μg/kg. In 99 (15%) samples, the contamination range of AFs was 1–4 μg/kg. However, 28 (4%) samples exceeded the maximum tolerated limit (MTL) of 4 μg/kg as imposed by EU. However, 6 (1%) samples were found beyond the MTL of20 μg/kg as regulated by USA. Highly contaminated samples were found during the months of July, August and September. Achieved data highlighted the requirement of continuous monitoring and further investigation of AFs contamination in dry fruits/edible nuts under the authority of a definite systematic surveillance program adapted as a food safety measure.

1. Introduction

Dry fruits and nuts are rich in essential amino acid, vitamins, minerals, dietary fibers and consumed by all age of the people. These are also used as medicines which provide energy and strength to long-term patients seeking out to recover (Emilio, 2010). Dry fruits are extensively cultivated all over the world including Pakistan.

Dry fruits and nuts are susceptible for pathogenic fungal attach during their cultivation, fruit growth, ripening, over ripening, handling, drying, storage and transportation. *Aspergillus*, *Fusarium*, *Penicillium* and *Alterneria* are the most common pathogenic fungi and produce toxigenic compounds known as mycotoxins (Zain, 2011). About more than 400 mycotoxins have already been discovered. However, aflatoxins (AFs) have become the most widely known mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* under favorable conditions (Khan, Asghar, Iqbal, Ahmed & Shamsuddin, 2014; Paterson & Lima, 2010; Paterson, 2007). Till to date, 20 AFs have been recognized. Among them only 4 AFs, the aflatoxins B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2), found naturally on a wide variety of agricultural commodities in significant levels (Asghar, Zahir, Rantilal, Ahmed, & Iqbal, 2016; Iqbal, Paterson, Bhatti, & Asi, 2010; Paterson, 2007; Zain, 2011). However, AFB_1 is the most toxic and has been widely reported in various agricultural commodities (Asghar, Khan, Iqbal, Ahmed, & Shamsuddin, 2014; Bircan & Koç, 2012). AFs has been

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reported as mutagenic, carcinogenic, hepatotoxic, immunosuppressive, neoplastic and classified by the International Agency for Research on Cancer (IARC) as Class 1 human carcinogens (IARC, 2002; Varga, Frisvad, & Samson, 2011). AFs could pose a potential risk to the human health because of aflatoxicosis and cancer. AFs are associated with many chronic diseases including immune suppression, digestive, blood and nerve defects, reproductive problems, anemia and jaundice (Ajani, Chakravarthy, Tanuja, & Pasha, 2014). However, the diseases verity depends on the mechanism of actions for each kind of AFs, the extent of exposure, age and intake of nutritional status.

Dry fruits and edible nuts could be infected with AFs contamination due to poor harvesting practices, improper storage, transportation and environmental condition. In addition, existing subtropical conditions in Pakistan, such as, high humidity, temperature and the nearness to sea also facilitate a vital role for the fungal growth and production of AFs. A number of monitoring programs and survey have been conducted in different countries to evaluate the a general pattern of the AFs level in different food commodities (Iqbal, Asi, Hanif, Zuber, & Jinap, 2015; Kabak, 2016). Due to severe toxicity concerned with AFs, various countries established guidelines for the acceptance level of AFs in dry fruits and nuts. For instance, the European Commission (Regulation no. 165/2010) has set maximum tolerated limits (MTL) 4 and 10 μg/kg for total AFs in dry fruits and nuts, respectively (Commission of the European Communities, 2010). According to the Food and Drug Administration and Food and Agriculture Organization of USA (2000), the MTL for total AFs in dry fruits and nuts is 20 μg/kg. Currently, Pakistan has not yet regularized MTL for mycotoxins in dry fruits and edible nuts. Thereby, followed EU and USA standards. However, Pakistan Standard Quality Control Authority (PSQCA) has been taken many steps to establish the MTL for mycotoxins in food/feed commodities for the protection of human and animal health.

On the basis of above mentioned fact, rapid detection and quantification of AFs in dry fruits and nuts is essential for assuring safety, quality and execution of hazard analysis and critical control points (HACCP). Consequently, current study was designed to find out the level of AFs in dry fruits and edible nuts which grown in Pakistan. The seasonal variation of AFs was also studied with respect to average temperature and relative humidity in Pakistan. Furthermore, the contamination levels were compared with reported levels in the national and international community.

2. Materials and methods

2.1. Sample collection

A total of 624 samples of 13 different types dry fruits and edible nuts including almond (n = 10), apricot (n = 65), dates (n = 170), raisins (n = 90), dried mulberry (n = 10), pistachio (n = 126), dried plum (n = 10), dried figs (Injeer) (n = 10), dried melon seeds (n = 5), walnuts (n = 8), pine nuts (n = 95), peanuts (n = 20) and fox nut (makakna) (n = 5) were collected from local markets and retailer shops of Pakistan during 2012–2015. It is well documented that AFs are found in the pocket form in high concentration and heterogeneously dispersed throughout the commodities. Hence, the sampling method was adapted according to the AOAC official method no. 977.16 (Latimer, 2012). In brief, about 0.5–1 kg of each sample was pulverized using mincer (Westmark, Germany) and then divided as a sub-sample in a final quantity of 100 g. An amount of 25 g of each representative and homogenous sub-sample were taken for the quantification of AFs contamination. The samples were preserved in air tight and opaque polyethylene bags at −20 °C in until analysis.

2.2. Chemical and reagents

Aflatoxin standard of B_1 (2 μg/mL; cat. # 002017), B_2 (0.5 μg/mL; cat.# 02018), G_1 (2 μg/mL; cat. # 002019) and G_2 (0.51 μg/mL; cat. # 002020) in acetonitrile were purchased from Biopure (Vienna, Austria). All standards were stored in freezer at −20 °C till further use. Analytical grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Potassium bromide and nitric acid were acquired from Sigma-Aldrich (St Louis, Missouri, USA). Phosphate-buffered saline (PBS) tablets (pH 7.4 + 0.2) were purchased from Oxide Ltd. (Hampshire, England). Highly purified water (18 MΩ cm) was obtained by processing de-ionized water (DI-H_2O) through a Purelab Ultra Option (ELGA, UK) system. All solvents and water were sonicated for 20 min using a Lucos Soniprobe, Lucas Dawe Ultrasonics bath (UK).

2.3. Instrumentation and apparatus

The chromatographic HPLC system consisted of a VWR-Hitachi (Darmstadt, Germany) system equipped with pump model no. L-2130, an auto-sampler model no. L-2200 and a fluorescence detector model no. L-2480. A column thermostat was from Jones-Chromatography (UK), a LiChroCART1 100 Å RP-18, (5 μm, 250 × 4.0 mm^2) column from Merck (Darmstadt Germany) and an electrochemical bromine derivatization cell (Kobra Cell™) were obtained from R-Biopharm (Glasgow, Scotland). Immunoaffinity AflaStar™ IACs (cat. # COIAC1001) was procured from Romer Labs. (Tulln, Austria).

2.4. Analysis of aflatoxin (AFs)

AFs were analyzed in dry fruits and edible nuts according to the method previously reported by Asghar, Aftab, et al. (2016); Asghar, Iqbal, et al. (2016) and Asghar, Zahir, et al. (2016) with some modifications. Briefly, whole analysis of AFs could be sub-divided in three steps as described below:

1. Sample extraction
2. Immunoaffinity column clean-up
3. Chromatographic analysis

2.4.1. Sample extraction

Twenty five g of each homogenized sample of dry fruits and edible nuts was weighted accurately and blended with 100 mL of methanol/water (80/20; v/v). The sample suspension was then blended at 5000 rpm using an explosion-proof blender (model no. 8018; Ebarch Corp., East Hampton, New York, USA) for 2min, filtered through Whatman filter paper no. 4 and collected in air-tight amber vials.

2.4.2. Immunoaffinity column clean-up

Sample clean-up was carried out using immunoaffinity columns (IACs). In brief, 2 mL of sample extract was mixed with 14 mL of PBS (pH 7.4). The diluted sample extract (16 mL) was applied to IACs at a flow rate of1–2 drop/s. IACs were washed with 20 mL of PBS buffer (pH 7.4) at a flow rate of about 5 mL/min and then dried bypassing air. AFs were then slowly eluted from the IACs with 1.5 mL of methanol followed by 1.5 mL of DI H_2O in amber vials.

2.4.3. Chromatographic analysis

AFs were quantified using an HPLC system and post column derivatization using Kobra Cell™. AFs were separated on a LiChroCART 100 A° RP-18 column. An aliquot of 99 μL of the AFs standard and sample was injected to the HPLC system through an