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Short communication

Natural occurrence of aflatoxins in dry fruits and edible nuts

Muhammad Masood [a], Shahzad Zafar Iqbal [b, c, *], Muhammad Rafique Asi [d], Noeen Malik [e]

[a] Department of Chemistry, Govt. Science College of Graduates, Samanabad, Faisalabad, Pakisthan
[b] Department of Applied Chemistry & Biochemistry, Government College University Faisalabad, 38000, Pakistan
[c] Food Safety Research Centre (FOSREC), Faculty of Food Science and Technology, University of Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[d] Food Toxicology Lab., Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Faisalabad 38950, Pakistan
[e] University Hospital, Clinic for Nuclear Medicine, Ulm, Germany

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ABSTRACT

A total 307 samples of dry fruits and edible nuts from Northern areas and Khyber Pakhtunkhwa, Pakistan were evaluated for the presence of aflatoxins (AFs). The samples were analysed using a reversed phase HPLC, equipped with fluorescence detector. Results have revealed that 132 out of 307 samples of dry fruits and nuts were found positive with aflatoxin B_1 (AFB_1) and total AFs. The highest mean level of total AFs i.e. 7.89 ± 0.99 μg/kg was found in peanuts without shell and lowest mean level (2.45 ± 0.11 μg/kg) was found in watermelon seeds without shell samples. Samples 75 (24%) were found contaminated with AFB_1, ranged from 8 to 10 μg/kg and 41 (13%) samples were found above the level of 10 μg/kg for total AFs. The high occurrence of AFs may cause health hazards for consumers and limit exports.

1. Introduction

Fruits are good sources of antioxidants, vitamins, minerals but low in fat contents. Pakistan has a rich heritage of fruit orchards, especially in the harvesting seasons, and dried fruits in the off-season. Due to their long shelf-life, dried fruits can provide a good alternate to fresh fruits; particularly in winter season (Waheed & Siddique, 2009). Studies have shown that nuts are the most susceptible commodity for fungal attack and consequently, the production of AFs (Iqbal, Asi, Zuber, Akram, & Batool, 2013). Fungal contamination can attack in the field, during harvest, transport or storage (Kader & Hussein, 2009).

Aflatoxins are a group of natural food toxins which are recognised as toxic, carcinogenic secondary metabolites mainly produced by certain strains of *Aspergillus flavus*, *Aspergillus paraciticus* and *Aspergillus nomius* (Iqbal, Mustafa, Asi, & Jinap, 2014). AFs are found as contaminants in various agricultural commodities including corn, peanut, cottonseed, Brazil nut, pistachio nut, fig, spices and copra (El-tawila, Neamatallah, & Serdar, 2013; Iqbal, Nisar, Asi, & Jinap, 2014). The International Agency for Research on Cancer (IARC) has classified AFB1 as a group I carcinogen which primarily affects the liver (IARC, 2002; Iqbal, Asi, & Jinap, 2014b).

In Pakistan the consumption of dry fruits and nuts is increased during winter season i.e. November till April. There are very limited reports for the presence of AFs contamination in dry fruits or edible nuts considering conducive environment for fungal proliferation. Therefore, the present study was designed to analyse the occurrence and levels of AFs contamination in these important commodities and disseminate the results to farmers, traders, local officers and law enforcement agencies.

2. Materials and methods

2.1. Sampling

A total of 307 samples of dry fruits and edible nuts were collected from Northern areas and Khyber Pakhtunkhwa province of Pakistan. The samples includes dried plums (21), dates (15), dried apricot (20), raisins (21), almonds (21), walnut with shell and without shell (20 each), peanuts with shell and without shell (20 each), dried figs (22), watermelon seeds (15), melon seeds (13), pistachio with shell and without shell (20 each), pine nuts (22) and cashew nuts (18) were collected randomly from different retail markets and local shops, during January 2013 to March 2013. The sample size was not less than 1 kg for each sample. The samples

* Corresponding author. Food Safety Research Centre (FOSREC), Faculty of Food Science and Technology, University of Putra Malaysia, 43400, Serdang, Selangor, Malaysia. Tel.: +60 12 686 121; fax: +60 3 8942 3552.
E-mail address: shahzad10542005@yahoo.com (S.Z. Iqbal).

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were ground with a grinding mill (Retsch ZM 200, Germany) and stored in a plastic bag in a refrigerator at 4 °C, until further analysis.

2.2. Regents and chemicals

The standards of AFs were purchased from Sigma Aldrich, Steinheim, Germany and the immunoaffinity columns (IAC) were obtained from VICAM, Watertown, MA, USA. The HPLC grade acetonitrile, methanol and analytical grade trifluoroacetic acid (TFA) were purchased from Merck, Darmstadt, Germany. Deionized water (Millipore, Bedford, MA, USA) was used in the study and all other chemicals and reagents were at least of analytical grade.

2.3. Extraction of aflatoxins

The sample preparation and analysis was done according to our previously validated method (Iqbal, Asi, & Jinap, 2014a).

2.4. HPLC conditions

The samples were analysed using HPLC (Shimadzu, Kyoto, Japan) in a reverse phase isocratic mode having Supelco C18 column (Discovery HS, Bellefonte, PA, USA) with a fluorescence detector (RF-530). The mobile phase (acetonitrile: methanol: water (20:20:60, v/v/v) was used at a flow rate of 1 ml/min. The temperature of column was maintained at 40 °C. Furthermore, the excitation and emission wavelengths were set at 360 and 440 nm, respectively. The method has shown good resolution and separation of aflatoxin standards (Fig. 1a) and natural occurrence of AFB_1 in date (Fig. 1b), plum (Fig. 1c) and apricot (Fig. 1d) samples.

2.5. Quality control parameters

The method was validated by using seven point calibration curves of analytes, to assess the linearity in a range of 1–80 μg/ml for AFB_1 and AFG_1, 0.5–12 μg/ml for AFB_2 and AFG_2. The values of coefficient of determination (R^2) for all analytes were found above 0.99. The sensitivity of the method was checked in terms of limit of detection (LOD) and limit of quantification (LOQ) i.e. signal-to noise (S/N) ratio of 3 and 10, respectively. The LOD of 0.04 μg/kg and LOQ 0.12 μg/kg was found for AFB_1 and AFG_1, and 0.07 and 0.21 μg/kg for AFB_2 and AFG_2, respectively. The selectivity was determined by spiking samples at levels of 1, 2, and 6 μg/kg for AFB_1 and AFG_1, 0.5, 1.5 and 3 μg/kg for AFB_2 and AFG_2. The recoveries of fortified samples in dry fruits and nuts were found in the range of 83–90% with RSD varies from 8 to 19%.

2.6. Statistical analysis

The data of AFs contamination in dry fruits and edible nuts was statistically analysed and presented as mean ± standard deviation (S.D.) by using SPSS software (IBM, PASW Statistics 19, USA). Furthermore, the value of R^2 was determined by using regression/correlation analysis.

3. Results and discussion

Results of 307 dried fruits and nuts samples analysed for the presence of AFs contamination are presented in (Table 1). Total 16 out of 20 samples of peanut (without shell) have found positive with AFs contamination and shown the highest level of total AFs 7.89 ± 0.99 μg/kg, ranging from LOD to 21.34 μg/kg. The lowest level

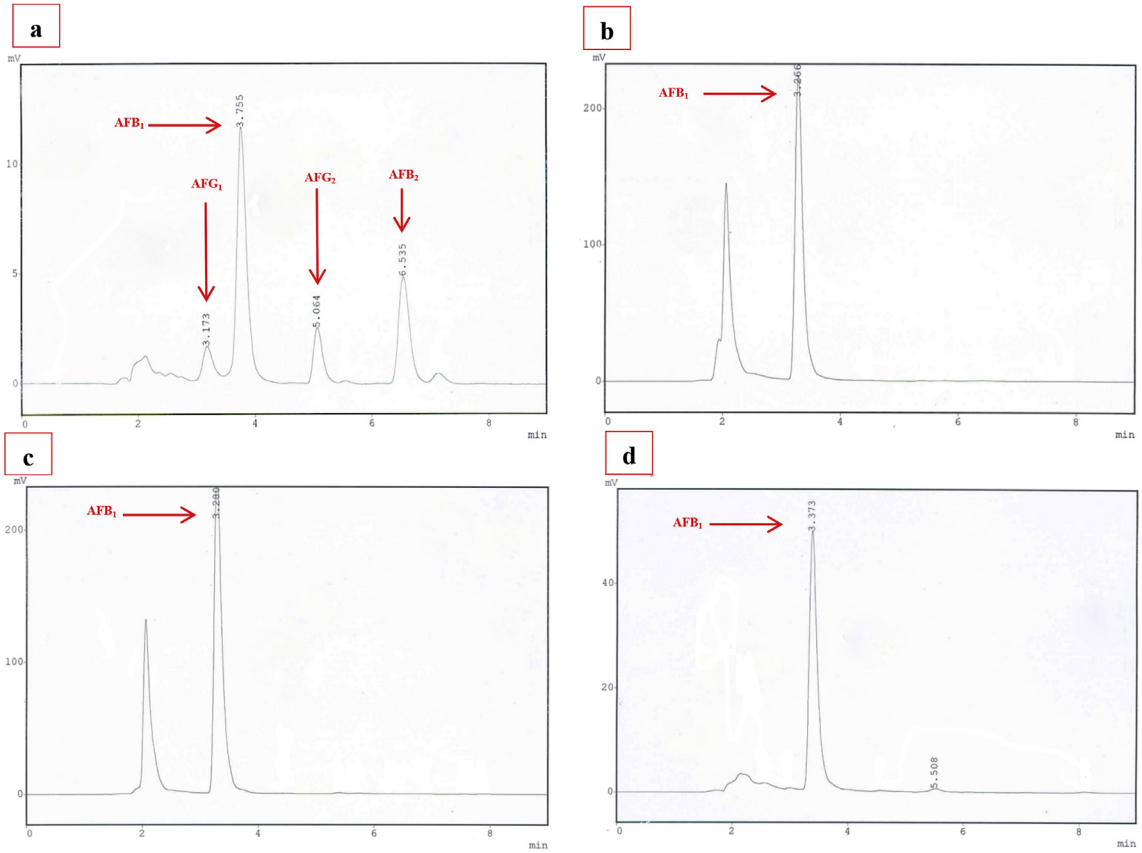

Fig. 1. Chromatograms showing the retention times of individual retention times of aflatoxins standards (a), natural occurrence of AFB_1 in date (b), in plum (c), and in apricot (d).