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Survey of aflatoxins and ochratoxin A in retail market chilies and chili sauce samples

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ABSTRACT

The aim of this study was to determine the levels of the aflatoxins (AFs) and ochratoxin A (OTA) in 312 samples of whole chili, chili powder, crushed chili and chili sauce samples from suburbs, open market and food restaurants of Punjab, Pakistan. The analysis was carried out using HPLC with fluorescence detector, after immunoaffinity column clean-up. The results have shown that 176 out of 312 (56.4%) samples were to be positive with AFs and 126 out of 312 (40.4%) sample of chilies were found to be contaminated with OTA. Total mean level of AFB_1 and total AFs in chilies were 12.50 ± 1.91, 15.16 ± 2.22 μg/kg, respectively. The total mean level of OTA in chilies was found 16.68 ± 2.58 μg/kg, ranged from LOD to 120.9 μg/kg. Sample 39.5, 26.3 and 32.7% of chilies were found containing level of AFB_1, total AFs and OTA, higher than the recommended limits for EU, respectively. The dietary levels of 3.26, 3.52, and 3.84 μg/kg were determined for AFB_1, total AFs and OTA in chilies. The incidence and levels of AFs and OTA in chilies are higher and could pose serious health hazards for consumers.

1. Introduction

Chili is very important and valuable cash crop of Pakistan and two species are commonly cultivated in Pakistan, i.e. *capsicum annuum* and *capsicum frutescens* (Iqbal, Paterson, Bhatti, Asi, Sheikh, & Bhatti, 2010). However, the production of chilies has decreased from 191, 800 tons in 2010–11 to 147,664 tons in 2013–14 (PARC, 2015). Generally, spices are produced in tropical and subtropical environmental conditions, where different factors like hot and humid climate, production and processing conditions with extended drying times, and often-inadequate resources, illiteracy and lack of awareness in farmers, may cause considerable compromises on the quality of food (Aydin, Erkan, Başkaya, & Ciftcioglu, 2007). Chilies have been reported as one of the most susceptible substrate for fungal attack and subsequently production of aflatoxins (AFs), ochratoxin A (OTA) and other mycotoxins (Marín, Colom, Sanchis, & Ramos, 2009).

AFs are the most toxic and most studied natural toxins, with aflatoxin B_1 (AFB_1) is the most carcinogenic, potent and teratogenic class (Iqbal, Bhatti, Asi, Bhatti, & Sheikh, 2011). AFs are mainly produced by fungi of the genus *Aspergillus* especially *A. flavus*, *A. parasiticus* and rarely by *A. nomius* and *A. tamari* (Iqbal, Asi, & Jinap, 2013). OTA is mainly produced in warm and tropical countries by *Aspergillus carbonarius* and *ochraceus* and in temperate climates by *Penicillium verrucosum* (Esteban, Abarca, Bragulat, & Cabañes, 2006; Iqbal et al. 2011b; Majeed, Iqbal, Asi, & Iqbal, 2013). The co-occurrence of various mycotoxins in chilies or red pepper could enhance the probability of synergistic interaction, which may amplify the risk to human health (Speijers & Speijers, 2004). European Union (EU) has established maximum permissible limits for AFB_1 and total AFs as 5 and 10 μg/kg, respectively (Commission Regulation EC No. 1881/2006), and 15 μg/kg for OTA in spices (Commission Regulation (EC) No 594/2012).

The contamination of chilies with AFs and OTA may take place during pre-harvest or post-harvest steps including drying, storage or during transportation (Iqbal, Asi, Zuber, Akhtar, & Saif, 2013). Considering high contamination of AFs and OTA in chilies, it is

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desirable to investigate the presence of AFs and OTA in chilies and chili sauce samples from suburbs, open market and food restaurants in major cities of Punjab, Pakistan and to compare the incidence levels of these toxins in chilies with EU permissible limits and to estimate the dietary intake levels for these toxins in chilies. The information of current study will be useful to check the current status and incidence levels of these toxins in chilies and to take further necessary actions.

2. Materials and methods

2.1. Sampling

A total of 312 samples of chilies of which 107 samples from suburbs (whole chili (n = 20), chili powder (n = 35), crushed chili (n = 30), chili sauce (n = 22), 97 samples from open market (whole chili (n = 25), chili powder (n = 30), crushed chili (n = 22), chili sauce (n = 20) and 108 samples from food restaurants (whole chili (n = 24), chili powder (n = 25), crushed chili (n = 31), chili sauce (n = 28) were purchased randomly from major cities (Lahore, Sheikhupura, Multan, Faisalabad, Gojra, Islamabad, Rawalpindi) of Punjab, Pakistan during November 2016 till December 2016. The food restaurants and retail markets (including fast food outlets) were selected from those situated in the most populated areas in the districts to the least populated one. The sample was not less than 500 g; however the chili sauce sample was at least of 200 ml. The samples were saved in plastic bags and stored in freezer at −4 °C, until further analysis.

2.2. Chemicals and reagents

AFs and OTA standards and tri-fluoro acetic acid (TFA) were purchased from Sigma Aldrich, Steinheim, Germany and immunoaffinity columns (IAC) (OchraTest™ and AflaTest) were purchased from VICAM, Watertown, MA, USA. Each standard of aflatoxin (AFB_1, AFG_1, AFB_2, AFG_2) contained 3 μg/ml toxin in 1 ml of benzene: acetonitrile (98:2 v/v). However, the concentration of ochratoxin A was 10 μg/ml in acetronitrile with 2 ml packing. Methanol and acetonitrile of HPLC grade were purchased from Merck, Darmstadt, Germany. Double-distilled water was used and all other chemicals and reagents used were at least of analytical grade.

2.3. Extraction of aflatoxins

Extraction and purification of AFs in chilies samples were carried out using our previously validated method (Iqbal et al., 2013a,b). Briefly, 25 (g/ml) sample was mixed with 3 g of sodium chloride and extracted with 150 ml of acetonitrile: water (86:14; v/v) by blending 8 min and then the extract was filtered through Whatman no. 5 filter papers. After filtration a portion of 10 ml filtrate with the addition of 70 μl acetic acid was passed through an IAC at a flow rate of 1.0 ml/min. AFs were eluted from the column by applying 1.5 ml of methanol and the extract was collected in a glass vial. Then, 5 ml aliquot was evaporated to dryness by N_2 stream at 40 °C in a glass centrifuge tube. Pre-column derivatization was done with the addition of 100 μl TFA to the residue or AFs standards to derivatize AFB_1 and AFG_1. The samples were left in dark place at room temperature for 20 min and then 400 μl mixture of acetonitrile: water (1:9, v/v) was added to the tube and 20 μl portion was subjected to HPLC analysis. The flow rate of isocratic mobile phase acetonitrile: methanol: water (20:20:60, v/v/v) was 1 ml/min. The samples were analyzed on reverse-phase HPLC (Shimadzu, Kyoto, Japan) with a Supelco C_{18} column (Discovery HS, Bellefonte, PA, USA) equipped with a fluorescence detector (RF- 530). Excitation and emission wavelengths were 360 and 440 nm, respectively.

2.4. Extraction of ochratoxin A

The extraction of OTA in chili and chili sauce samples were determined following our previously validated method (Iqbal et al., 2013a,b). Briefly, 25 g/ml of sample was added in 150 ml of acetonitrile: water (84:16, v/v), solution with the addition of 5 g of sodium chloride and blended with high speed for 5 min. After blending, the sample was extracted using Whatman no. 5 filter paper. After filtration, 50 ml of phosphate buffer saline (PBS) was mixed with 5 ml of the filtrate and filtered through a glass microfiber. A 10 ml of filtrate with the addition of 70 μl of acetic acid was passed through an OchraTest™ IAC. OTA was eluted from the column by passing 1.5 ml of methanol and collected in a vial. The eluate was evaporated until dryness at 40 °C under N_2 stream and the residues were re-dissolved in 1 ml of mobile phase i.e. acetonitrile: water: acetic acid (47/51/2, v/v/v) for HPLC analysis. The flow rate of mobile phase was 1 ml/min. Detection of OTA was carried out using fluorescence detector with excitation and emission wavelengths at 333 and 460 nm, respectively.

2.5. Statistical analysis

The results of AFs and OTA concentrations were statistically analyzed and presented as mean ± SD and coefficient of determination (R^2) was determined by simple linear regression/correlation analysis using SPSS software (IBM, PASW Statistics 22, USA).

3. Results and discussion

3.1. Quality control and quality assurance parameters

The analytical method was validated and evaluated using parameters i.e. linearity, selectivity, sensitivity, accuracy and precision. The selectivity of method was determined using known amounts of mycotoxins in blank uncontaminated chili powder samples. The fortified levels of AFB_1, AFG_1 and OTA were 2, 4 and 6 μg/kg and 1, 1.5 and 2 μg/kg levels for AFG_2 and AFB_2, respectively. The linearity of analytical method was determined by constructing a seven point standard curve for each mycotoxin. Standard curves for AFB_1, AFG_1 and OTA were constructed between a range of 1–140 μg/l and 0.5–20 μg/l for AFG_2 and AFB_2, respectively. All analytes have shown good linearity because the values of coefficient of determination (R^2) were ˃ 0.99 as presented in Table 1. The sensitivity of HPLC was determined in terms of limit of detection (LOD) using signal-to-noise (S/N) ration of 3:1. The LOD in present study was found same for specific toxin in different samples of chilies matrix. The method accuracy was assessed using known fortified levels in uncontaminated chili powder samples as presented in Table 1. All fortified samples have shown good recoveries, ranged from 85.8 to 92.4%, with relative standard deviation from 9.8 to 18.7%. Furthermore, the precision was evaluated in terms of repeatability and reproducibility using 3 different concentrations of each mycotoxin and analyzed them on same day with three replications. The method quality control parameters are in agreement with our previous study (Iqbal et al., 2013a,b). Fig. 1 shows the chromatograms of aflatoxins standards (a), natural occurrence of AFs in chili powder sample (b), standard chromatogram of OTA (c), presence of OTA in crushed chili sample (d).

3.2. Occurrence of aflatoxins and OTA in chilies

The distribution of AFB_1 and total AFs levels in whole chilies, chili powder, crushed chilies and chili sauce samples from suburbs,