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Survey of mycotoxins in retail market cereals, derived products and evaluation of their dietary intake

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ABSTRACT

In current study a total 229 samples of cereal products, available in retail markets of main cities of Punjab, Pakistan were collected for the analysis of mycotoxins. The analysis was performed using reverse phase HPLC with fluorescence detector. The results have shown that 121 (53%) out of 229 samples of cereal products have found positive for aflatoxin B_1 (AFB_1) and total aflatoxins (AFs). Samples of 22 and 12% were found higher than the maximum level for AFB_1 and total AFs. The highest level of AFB_1 and total AFs was found in porridge samples i.e 3.90 ± 0.039 μg kg^{-1} and 5.60 ± 0.005 μg kg^{-1}, respectively. About 115 (50%) out of 229 samples of cereal products were found positive with ochratoxin A (OTA) and 26% samples were found to be higher than the maximum level of 3 μg kg^{-1}. About 130 (57%) samples of cereal were found contaminated with zearalenone (ZEN) and 31% of samples were found higher than the maximum level of 50 μg kg^{-1}. The highest mean level of 21.45 ± 1.90 μg kg^{-1} of ZEN was found in Suji (Semolina) samples. The estimated average exposure dose of AFB_1, OTA and ZEN based on our daily food consumption data were 3.50, 3.85 and 29 ng kg^{-1} bw day^{-1} found in some cereal samples, respectively. The calculated excess risk of liver cancer incidence by ingestion of cereals containing AFB_1 was 1.66 per 100,000 adults per year.

1. Introduction

Breakfast cereals and bread are main constituents in the human diet (Dewettinck et al., 2008; Mathebula, Mandiwana, & Panichev, 2017). To maintain good health, they provide essential macronutrients such as carbohydrate, protein and fat, as well as important minerals, vitamins, and micronutrients for optimal health (Collins et al., 2010). It is estimated that approximately 2592 million tonnes cereals have been produced with a consumption of 2567 million tonnes (FAO, 2017a). In world, Pakistan is ranked in the list of top 10 countries for the production of wheat, sugarcane, dates, cotton, mangoes and kinnow (oranges), and placed in 13th position in rice production. To meet the daily energy requirements, wheat is staple food but however, rice is also important food constituent of population (Iqbal, Rabbani, Asi, & Jinap, 2014a,b; Iqbal, Asi, & Jinap, 2013) and almost wheat provides 37 percent of daily caloric intake with 124 kg/year per capita consumption (FAO, 2013). The country has sub-tropical climatic conditions, such as high temperatures, flash floods, moisture, unseasonal rains and monsoons provide favorable conditions for fungal propagation (Iqbal et al., 2010). The issue becomes more serious because farmers use old conventional methods in farming and not obeying good harvesting and storage practices. Wheat, rice and other cereals crops are exposed to fungal attack in the field or during storage and this would result in the production of mycotoxins (Villa & Markaki, 2009).

Mycotoxins are classified as naturally occurring toxic secondary metabolites, produced by filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium* (Iqbal, Asi, Hanif, Zuber, & Jinap, 2016; Li et al., 2014). Currently about 450 groups of different types of

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mycotoxins are identified and characterized (Masood, Iqbal, Asi, & Malik, 2015). The most significant and important classes of mycotoxins based on their toxicity and economic losses are aflatoxins (AFs) ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEN) (Binder, Tan, Chin, Handl, & Richard, 2007). The International Agency for Research on Cancer (IARC) has placed aflatoxin B_1 (AFB_1), in most toxic group i.e. group 1 classification, due to its evidence of carcinogenic effects in humans. OTA has been included in IARC's Group 2B classification due to its potential carcinogen in humans (IARC, 1993). ZEN is a non-steroidal estrogenic mycotoxin, which has been involved in incidents of precocious pubertal changes and it is classified in group 3, not carcinogen to humans (IARC, 1993; IARC, 2002). The exposure to OTA both in animals and humans mainly effect on kidney. It is also associated with other toxic effects such as immunotoxicity, neurotoxicity, myelotoxicity, reproductive toxicity and teratogenicity (Cariddi et al., 2016).

These mycotoxins are carcinogenic, teratogenic, mutagenic, and immunosuppressive and their exposure can cause chronic and acute toxic effects on the consumers or even death. Therefore, the European Commission has set maximum levels i.e. 2 and 4 μg kg^{-1} for AFB_1 and total AFs in breakfast cereals, 3 μg kg^{-1} for OTA. The recommended limit established by EU is 50 μg kg^{-1} for ZEN in breakfast cereals and 100 μg kg^{-1} for maize-based products. The maximum level of AFB_1 for processed cereal-based foods and baby foods for infants and young children is 0.1 μg kg^{-1} (Commission Regulations (EC, 2006), 1881/2006; N1126/2007).

In our pervious study (Iqbal et al., 2014a,b), high concentration of AFs, OTA and ZEN was found in cereal products. Recently, high level of AFs and OTA was found in rice and rice products (Iqbal et al., 2016). In current survey, our main focus was to investigate the presence of main mycotoxins i.e. AFs, OTA and ZEN in wheat and wheat derived products and corn and corn derived products. Furthermore, the incidence and level will be compared with European Union permissible limit and dietary intake evaluation will carry out in cereal products. The results will be useful for farmers, traders, exporters and people working in government sector.

2. Materials and methods

2.1. Sampling

A total of 229 samples of cereal products including 18 samples of each wheat flour, corn flour, suji (semolina), each 13 samples of noddles, and seviyan (vermicelli), each 14 samples of Pratha (wheat oiled bread) and Rusk, 22 barley flour, 15 wheat bread, 16 corn bread, 12 Bakarkhani, 16 porridge, 18 cornflake, and 22 samples of instant cereal food were collected from the main cities of Punjab, Pakistan. The samples were collected from farmers, retail market and super stores during March to November 2016. The size of each sample was maintained at least of 1 kg each. The samples were collected in plastic bags and kept in an icebox. The samples were stored in a freezer at - 4 °C, until further analysis.

2.2. Regents and chemicals

The standards of AFs, OTA and ZEN and trifluoroacetic acid (TFA) used were of Sigma-Aldrich, Steinheim, Germany and immunoaffinity columns (IAC) for AFs AflaTest™, OTA OchraTest™ and ZEN ZearalaTest™ were purchased from VICAM, Watertown, MA, USA. Other solvents such as acetonitrile and methanol of HPLC grade were purchased from Merck, Darmstadt, Germany. All other chemicals and reagents used were at least of analytical grade.

2.3. Aflatoxins extraction and analytical conditions for HPLC

The extraction in cereal products for AFs was done according to our previously validated method (Iqbal et al., 2014a,b). After filtration, 20 mL of the filtrate was then passed through an AflaTest immunoaffinity column at 3–4 drops s^{-1} and washed with 10 mL of water twice at the same flow rate. Final elution was accomplished by passing 1.0 mL of HPLC grade methanol and collected in a vial. Finally, the residue was evaporated using nitrogen stream at 40 °C. After evaporation 100 μL of TFA was added to derivatize AFB_1 and AFG_1. Then, the samples were placed for 15 min in dark place at room temperature with caps were tightly on the vials. After that, 400 μL mixture of acetonitrile-water (1:9, v/v) was added to the vials. Finally, 20 μL portion of the solution was subjected to LC analysis. In current study the HPLC system used was a Shimadzu (LC-10A series, Kyoto, Japan) which is equipped with fluorescence detector (RF-530). The HPLC column was C18 Supelco Column (4.6 × 250 mm, 5 mm; Discovery, HS, Bellefonte, USA). The mobile phase used was acetonitrile–methanol–water (20:20:60, v/v/v) with a flow rate of 1.5 mL min^{-1}. The wavelength of fluorescence detector was set at 360 (excitation) and 440 nm (emission).

2.4. Ochratoxin a extraction and analytical conditions for HPLC

The extraction of OTA from cereal product were done followed our previous method (Iqbal et al., 2014a,b). After, filtration 100 μL of acetic acid was added in10 mL of filtrate and then passed through immunoaffinity columns OchraTest™. OTA was eluted from the column by passing 1.5 mL of methanol and collected in a vial. Then, the eluate was evaporated until dryness at 40 °C and residue was re-dissolved in 1 mL of mobile phase i.e. acetonitrile–water–acetic acid (47/51/2, v/v/v). The flow rate of mobile phase was 1 mL min^{-1}. Detection of OTA was carried out using fluorescence detector with excitation and emission wavelengths at 333 and 460 nm, respectively.

2.5. Zearalenone extraction and analytical conditions for HPLC

The extraction of ZEN from cereal products were carried out using method (Iqbal et al., 2014a,b). The samples are prepared and extracted as mentioned in earlier section and after dilution; the diluted extract was passed in immunoaffinity column (ZearalaTest™) at a flow rate of 3–4 drops s^{-1} following method Iqbal et al. (2014a,b) and Iqbal, et al. (2014). The excitation and emission wavelength of fluorescence detector was set at 274 and 450 nm, respectively.

2.6. Dietary intake evaluation

The estimated dietary intake of AFB_1 and OTA in cereal products were estimated following the method of Huong et al. (2016). The consumption of different cereals per person per day was estimated based on a food frequency questionnaire (February 2017) involving 500 individuals (Table 1), asking them of cereal products consuming per day. The survey/questionnaire did collect data on

Table 1
The data of individuals participated in questionnaire.

Individual Location	Gender	Number	Age	Average weight
Central Punjab	Male	125	19–26	65 ± 1.58
	Female	200	18–32	48 ± 2.01
South Punjab	Male	100	19–30	62 ± 1.57
	Female	75	19–32	49 ± 2.04
Total		500		56 ± 2.60