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Research Article

Presence of Mycotoxins and Heavy Metals in Organic Commercial Cereal-Based Foods Sold in Faisalabad Market

Yasir Abbas Shah, Muhammad Afzaal* and Umar Farooq

Institute of Home and Food Sciences, Government College University Faisalabad, Pakistan

***Corresponding Author:** Muhammad Afzaal, Institute of Home and Food Sciences, Government College University Faisalabad, Pakistan.

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Abstract

Epidemiological data indicates that mycotoxins and heavy metals can be harmful when ingested by humans and animals. The present study was conducted to report the presence of mycotoxins and heavy metals in organic commercial cereal-based products, available in the Faisalabad market. Forty-four samples of organic cereals products including wheat, barley, rice, oat and maize were examined for the presence of mycotoxins (aflatoxins), heavy metals (Pb, Cd) and trace elements (Cu, Zn, Ni). Results were induced and compared to the recommended levels. 23 (52.2%) of the collected samples were detected with the presence of aflatoxins in higher concentrations then allowed as according to the limits set by EU legislation for the presence of AFs. Wheat, oat, rice, barley and maize showed 61, 60, 54, 50 and 33% of the samples respectively contaminated with aflatoxins. 5 (11%) and 3 (6%) of the samples surpassed the allowed limit for Pb and Cd respectively. Trace elements were detected in higher amounts in some of the evaluated samples. Results of the present work specify the need of continuous monitoring of raw material and processed products regardless of them being organically grown or not, in order to minimize the risk of contamination in cereal-based foods.

Keywords: Heavy Metals; Mycotoxins; Organic Food; Cereals; Food Safety; ELISA

Introduction

The way in which a food is grown can have a greater effect on our health. Food must meet the quality and safety standards either it is organically grown or not. Consumers around the world are turning to organically grown foods in the expectation of having safer and healthier food products. Organic products are supposed as having lower levels of contaminants and more nutrients then conventional products [1,2]. As more farmers taking the organic route, it is important to assess the safety of these products. Cereals are the basic source of food for many people around the World therefore, health hazards and food safety problems related with them signify a serious concern. The main reason for the existence of a potential health hazard for the individual is the greater exposure than the daily dose that causes adverse effects [2]. Mycotoxins are generally the significant contaminants of various crops. In developing countries the aflatoxins are considered as the most significant mycotoxins due to these factors like occurrence, toxicity and economy. In developing countries like Pakistan, a part of the population is exposed to some level of aflatoxin intoxication.

Major factor in the production of mycotoxins and mould growth is inadequate or simply lacking of harvest and post-harvest technologies. Contamination of foods and feeds by mycotoxins highly depends on environmental factors that cause mould growth and toxin production. Generally, contaminated raw material and the uncontrolled conditions during processing are the main problems that cause high contamination levels of mycotoxins in food products. A variety of moulds continuously contaminate the cereals by producing various mycotoxins. Myco-toxins and particularly deoxynivalenol (DON) and afla-toxins (AFs), zearalenone (ZEA) and ochratoxin A (OTA) are the most significant contaminants of the cereals [3-5]. EU regulation established the limits for these mycotoxins in

all cereals and their products which are directly consumed by humans (EC 2006; 2007; 2010). There exist a controversy in the literature that compares the contamination levels of organically grown cereals with the conventionally grown ones. A study found that the levels of DON contents were similar in the cereals from two farming systems (organically and conventionally) [6]. The number of mycotoxins that can cause toxic effect on human and animal health are increasing constantly [7,8]. The consumption of mycotoxin-contaminated foods can cause toxic effects on human and animal health due to their teratogenicity, mutagenicity, nephrotoxicity, carcinogenicity, immunosuppression and so on.

On the other hand, heavy metals have toxic effects and considered severely harmful to the humans and animals. Various metals are identified as a cause of many diseases including Alzheimer's disease, nervous system and some types of cancer. Pb and Cd are considered carcinogens to humans and are linked with various diseases like cardiovascular, nervous system, kidney, as well as blood diseases [9]. They can cause adverse effects to humans even at low concentrations. Due to soil pollution in industrialized areas heavy metals can contaminate crops. Limits has established by the European Commission (EC) for Pb and Cd (EC 2006; 2008; 2015). According to EC (2015) the current maximum limit of Lead in cereals is 0.2 mg/kg and in processed cereal-based foods it is 0.05 mg/kg wet weight. Moreover, according to EC (2008) the maximum limit for Cadmium in cereals is 0.1 mg/kg excluding rice and wheat whereas in rice and wheat it is 0.2 mg/kg wet weight. A study reported the higher concentrations of Pb and Cd in organically grown cereals as compared to conventionally grown ones [6].

Copper, zinc and nickel are essential metals if consumed in allowed limits. They cause adverse effects to humans if ingested in higher concentrations. Many studies around the world showed comparison between organic and conventional crops in terms of risk to human health [10-12], in some studies reported results were opposite as compared to the hypothesis that conventional cereals have more contaminants then the organic ones. Faisalabad is an industrial and the 3rd most populous city in Pakistan. According to the recent IQ Air Visual's World Air Quality Report 2018, Faisalabad has been listed as the third most polluted city in the world.

In Faisalabad, there is no organized monitoring of pollutants and contaminants in organic cereals and their derived products. Data about incidence and levels of contamination of mycotoxins and heavy metals in food and feed are limited by several factors, including the resources to carry out surveys, the accessibility of laboratory facilities to perform analyses, the constancy and sensitivity of the analytical procedures used and the abilities of the analysts.

Present study demonstrates the presence of mycotoxins and heavy metals in organic cereals and their derived products available in Faisalabad market. Present data will be useful for the awareness of consumers about the health risks related with organic products.

Materials and Methods

Sampling plan

A total of 44 samples of cereals (wheat, rice, maize, oat, rye and barley) including their seeds and derived products were purchased from Faisalabad market. Specialized shops and superstores within the city area were chosen for samples collection. The obtained samples were seeds, flour, flakes and pasta which were derived from different cereals (Table 1). Collected samples were powdered with the help of mixture grander then wrapped in plastic bags individually and placed in refrigerator (< 10) till assessment. Product type, its origin, serial number, date of expiration and collected location was recorded for every sample.

Common Name	Botanical Name	No. samples
Wheat	*Triticum aestivum L.*	18
Rice	*Oryza sativa L.*	11
Maize	*ZeamaysL.*	6
Oat	*Avena Sativa L.*	5
Rye	*Secale cereale L.*	2
Barley	*Hordeum vulgare L.*	2
Total		44

Table 1: Sampling plan.

Determination of mycotoxins

To make inspection of different foodstuffs, accurate procedures of analysis are necessary. In this study all 44 collected samples were tested for the detection of aflatoxins using the screening method, and EIA - immunocompetition assay for screening detection of aflatoxin B1 and ochratoxin A. This process is based on the antigen-antibody reaction. This is a competitive enzymatic analysis, which was performed according to the commendations of assay producer.

The process of this method is based on competition of the toxin released from the analyzed sample with mycotoxin involved in the assay, being an essential part of the set with specific anti-mycotoxin antibodies. Chromogen substrate is added during the procedure of assay, which is converted from colorless into the colored form. After incubation, the reaction is stopped, followed by reading of the intensity of the obtained color with the help of ELISA reader. The measuring of the color intensity on ELISA reader was in the range of 450 nm. Color intensity is inversely proportional to the presence of mycotoxins in the sample. The values obtained were further processed according to the commendations of the assay producer.

Determination of heavy metals and trace elements

All the powdered samples for analysis were dried in an oven at 105 for 90 minutes. Dried samples were cooled to room temperature. All the glassware before used were soaked in 1% HNO_3 for 24h and then properly washed with distilled water. About 1g of the dried samples was weighted and placed in a porcelain crucible. Ashing process was done at 450 for 12 hours in a muffle furnace. The obtained ash was cooled to room temperature of 28 ± 1 then added to a solution containing 5 ml of 5M Nitric acid. Filtration of residue was done by using Whatman No. 41 filter paper, into a calibrated volumetric flask of 50 ml. The solution was made up to mark with DI water. The solution was analyzed for the concentrations of the heavy metals and trace elements like Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn) and Nickel (Ni) by using Atomic Absorption Spectrophotometer. After standardization procedures solution was aspirated into the Atomic Absorption Spectrophotometer, operational conditions were maintained in accordance with the instrument's specifications.

Results and Discussion

Presence of mycotoxins in organic cereals food samples

Assessment of 44 collected samples of organic cereals-based foods sold in Faisalabad market showed variation in results. Presence of aflatoxins was detected in 23 (52.2%) samples in higher concentrations then allowed as according to the limits set by EU legislation for the presence of AFs. Only 21 (47.7%) samples were totally free of aflatoxins. Result of the evaluation performed for the detection of aflatoxins is given in table 2.

61% of the wheat samples were found contaminated with aflatoxins. Oat, rice, barley and maize showed 60, 54, 50 and 33% of the samples respectively contaminated with aflatoxins. Although, analyzed samples of rye showed no presence of aflatoxins.

Cereal	Number of samples	Number of positive	% of the overall number of collected samples (44)
Wheat	18	11	25
Rice	11	6	13.6
Maize	6	2	4.5
Oat	5	3	6.8
Rye	2	/	/
Barley	2	1	2.2
Total	44	23	52.2

Table 2: Number and the percentage of samples positive to the presence of aflatoxins.

Organically grown crops may be more susceptible to contamination from fungi because in organically produced crops the use of synthetic fungicides is prohibited. A controversy in the literature exists related to the concentrations of the mycotoxins in cereals compared from organic farming to conventional farming. The values from the results may be affected by several factors like variety, year of growing and climate conditions. In addition, mycotoxins contamination is directly linked with inappropriate post-harvest storage conditions that increase mould growth and toxin production [2]. Other factors involved in contamination of food and feed by mycotoxins include inaccurate packaging, improper handling, transport and distribution of foodstuff.

Presence of heavy metals and trace elements in organic cereals food samples

Concentrations of heavy metals and trace elements in analyzed organic cereal samples varied widely. The allowed limit of Pb in cereals is 0.2 mg/kg (EC 2006). Concentrations of Pb in analyzed samples of cereals are expressed in table 3. Samples of Rye, maize and barley showed less than 0.130 mg/kg concentrations of Pb. Two rice and three wheat samples surpassed the allowed limit and mentioned as positive samples while the uppermost value for Pb among the samples of oat was 0.177 mg/kg. Industrial activities, contaminated soil, mining and automobiles are the main causes for the accumulation of heavy metals such as Pb [13,14]. Faisalabad is an industrial city of Pakistan and there is no organized monitoring of industrial waste that causes soil contamination.

Cereals	No of samples	No of positive samples	Minimum-maximum value for Pb (mg/kg)	Mean (Average) mg/kg
Wheat	18	3	0.102-0.245	0.151
Rice	11	2	0.142-0.239	0.170
Maize	6	/	0.109-0.129	0.118
Oat	5	/	0.113-0.177	0.139
Rye	2	/	0.091-0.118	0.104
Barley	2	/	0.107-0.123	0.115

Table 3: Presence of Lead (Pb) in organic cereals food samples.

The allowed limit for the Cd contents in cereals is (0.2 mg/kg) (EC 2006; 2015). Two sample of wheat showed higher concentrations of Cd then allowed while other samples of wheat showed less than 176 mg/kg. One maize samples surpassed the allowed limit for the presence of Cd, whereas the samples of barley, oat and rye showed less than 0.139 mg/kg values for the presence of Cd. A rice sample showed the highest concentrations of Cd 0.192 mg/kg while the other samples of rice showed less than 0.166 mg/kg. Various factors involved for the presence of Cd in higher concentrations in grains such as soil characteristics, weather circumstances and genotype [15]. Furthermore, higher values may be due to the contamination with Cd at some stages in processing [16]. The result of the analysis performed for the detection of cadmium (Cd) in organic cereals is given in table 4.

Cereals	No of samples	No of positive samples	Minimum-maximum value for Cd (mg/kg)	Mean (Average) (mg/kg)
Wheat	18	2	0.106-0.219	0.144
Rice	11	/	0.119-0.192	0.138
Maize	6	1	0.101-0.227	0.135
Oat	5	/	0.111-0.138	0.121
Rye	2	/	0.125-0.134	0.129
Barley	2	/	0.120-0.123	0.121

Table 4: Presence of Cadmium (Cd) in organic cereals food samples.

On the other hand trace elements in higher values cause adverse effects to humans. The results obtained after analysis of organic cereals food samples for the presence of trace elements is given in the table 4. The highest concentration of Cu 18.4 mg/kg was detected in a rice sample, whereas the lowest concentration 0.8 mg/kg was detected in a sample of rye. Values for Zn content among the samples ranged between 2.28 and 27 mg/kg. Rye sample showed lowest value for Zn and the highest value was observed in a wheat sample. A study showed that organic fertilizers had resulted in higher grain concentrations of Zn [17]. Ni content ranged between 0.17 to 15.2 mg/kg among the analyzed samples. Maximum value for Ni was observed in wheat sample whereas minimum value was detected in an oat sample (Table 5).

Cereals	No of samples	Minimum-maximum value for Cu (mg/kg)	Minimum-maximum value for Zn (mg/kg)	Minimum-maximum value for Ni (mg/kg)
Wheat	18	1.52-7.85 (4.24)	4.73-27.6 (10.4)	0.93-15.2 (7.54)
Rice	11	1.48-18.4 (6.49)	6.17-22.4 (11.6)	0.87-3.27 (1.93)
Maize	6	1.13-1.95 (1.38)	4.56-13.7 (7.28)	1.97-3.09 (2.54)
Oat	5	1.67-4.56 (2.58)	11.8-17.9 (3.10)	0.17-5.37 (2.22)
Rye	2	0.8-1.45 (1.125)	2.48-5.56 (4.02)	1.47-2.27 (1.87)
Barley	2	1.07-2.11 (1.59)	4.44-6.91 (5.67)	1.22-3.14 (2.18)

Table 5: Presence of trace elements Cu, Zn and Ni in organic cereals food samples.

Note* The mean values are shown in parentheses.

Final Considerations

In this study the obtained data do not support nor reject the hypothesis that conventional cereals have more contaminants than the organic ones. Evidently, the analyzed samples in the present study are not to be recommended to be used as baby foods. Cereals are the basic source of food for many people around the World, so health hazards and food safety problems related with them signify a serious concern. Results of the present study reveal that immediate action should be taken by the respective authorities to prevent any type of outbreak. Routine monitoring of raw materials and processed products is needed to minimize contaminants levels in cereal based food products. The result reported in the present study showed that organic cereals have lower levels of mycotoxins and heavy metals as compared to conventional ones reported in many studies around the world. This data will be helpful for the awareness of consumers about the health risks associated with organic products. Further research is needed to support the hypothesis that organic cereals are more safer than the conventional ones.

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