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Investigation of the deoxynivalenol and ochratoxin A levels by high-performance liquid chromatography of cereals sold in the markets in Türkiye

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Abstract

The current research was conducted to determine the deoxynivalenol (DON) and ochratoxin A (OTA) levels of 24 pieces of wheat flour, 24 pieces of rice, 24 pieces of corn flour, and 24 pieces of whole wheat flour obtained from the markets in Istanbul by HPLC. DON was detected in 4 of the 96 grain cereal samples at levels between 0.94 and 1.16 µg/kg (LOQ, 0.92 µg/kg). Besides, from the analyzed samples, DON was detected in 3 pieces of ashura wheat, 2 pieces of rice, and 5 pieces of corn flour between LOD and LOQ. The highest DON level was found in the corn flour sample (1.16 µg/kg), which was sold unpacked. OTA was detected in 11 of the samples (0.87-6.97 µg/kg) consisting of 3 corn flours (1.51-2.23 µg/kg) and 8 whole wheat flours (0.87-6.97 µg/kg). The highest OTA level was found in the packaged whole wheat flour sample (6.97 µg/kg) bought from the market. Since DON and OTA exposure can be observed frequently in cereals and may increase to possible risky levels, further work should be done to determine the precautions necessary to minimize the risks of contamination.

Keywords: mycotoxins; deoxynivalenol; ochratoxin A; cereal; HPLC.

Practical Application: Detection of mycotoxin levels in these food products is the first step required to reduce the exposure and mitigate the health risks to consumers.

1 Introduction

Cereals, such as corn, wheat, and barley, are consumed by most people in the world as a primary source of energy and food. Contamination of crops, especially cereals, by molds during the pre-harvest and post-harvest stages can lead to the production of secondary toxic metabolites known as mycotoxins (Silva et al., 2022; Lima et al., 2022; Khorshidi et al., 2022). The main mycotoxins such as are aflatoxins, deoxynivalenol, trichothecenes, fumonisins, zearalenone, and ochratoxin A are produced by *Aspergillus*, *Penicillium*, *Alternaria*, *Claviceps* and *Fusarium* species (Udovicki et al., 2018). Among them, deoxynivalenol (DON), also called vomitoxin (type B trichothecene), is one of the most common mycotoxins associated with cereals such as corn, wheat, and barley (Machado et al., 2017; Medina et al., 2019). DON is mainly produced by *Fusarium* species such as *F. graminearum* and *F. culmorum*. DON was first isolated from moldy barley in Japan (1972) and was found to be identical to the emetic factor found in corn in the USA (Richard et al., 1993).

Another common type of mycotoxin is ochratoxins. Ochratoxins (OTA, OTB, and OTC) are secondary metabolites synthesized from various species of *Penicillium* and *Aspergillus* genera, especially *P. verrucosum*, *A. ochracecus,* and *A. carbonarius* (European Food Safety Authority, 2006). Ochratoxins were first identified from fungal cultures in South Africa (1965) and have three derivatives such as OTA, OTB, and OTC (Van der Merwe et al., 1965).

It has been reported that 25-50% of the crops harvested in the world each year are contaminated with mycotoxins (Ricciardi et al., 2013). Reactions during food processing have shown that DON and OTA can occur in cereals (Wang et al., 2022). The main factors that increase mold growth and mycotoxin biosynthesis in stored grains are known as high grain moisture (16-30%), high grain temperature (25-32 °C), and high air relative humidity (80-100%) (Shanahan et al., 2003). The optimum temperature for the formation of DON varies between 26-30 °C (Milani, 2013). While some *Aspergillus* species produce ochratoxin under high humidity and temperature conditions, some *Penicillium* species can produce ochratoxin even at temperatures as low as 5 °C (Gupta et al., 2018). The optimum temperature for OTA formation is specified as 25-30, 20-25, and 10-20 °C for *A. ochraceus*, *A. niger,* and *A. carbonarius*, respectively (Bui-Klimke & Wu, 2015). It has also been reported that a temperature rise of 2-3 °C has been caused to mold growth or insect infestation (Neme & Mohammed, 2017). Moreover, it is known that the most effective method of reducing mycotoxin levels in foods involves sorting and cleaning, such as the physical removal of rotten parts or grains. (Bullerman & Bianchini, 2007). Various factors such as

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the chemical structure of mycotoxins, temperature conditions, processing time, humidity during processing, or lack of moisture can affect mycotoxin formation during specific processing steps (Lancova et al., 2008). Among *Fusarium* toxins, DON is the most common and important indicator of Fusarium Head Blight formation in wheat (EFSA Panel on Contaminants in the Food Chain, 2013). There is insufficient toxicological data on chronic exposure to DON in humans; however, the FAO/WHO Expert Committee on Food Additives (JECFA) reported in 2010 that the maximum tolerable daily intake for DON was 1 µg/kg per day (Knutsen et al., 2017). Regulatory authorities around the world have set maximum levels for both unprocessed and processed grains and cereals to protect consumers from exposure to high DON levels. For example, both the European Union (EU) and Turkish Food Codex have set the maximum levels of DON at 1250 µg/kg for unprocessed cereals other than wheat, oats, and corn, 1750 µg/kg for unprocessed wheat, oats, and corn, 750 µg/kg in cereals, 500 µg/kg for cereal flour, bran, germ, pasta, bread, pastries, cereal snacks, and breakfast cereals, and 200 µg/kg for cereal-based foods and infant formulas (Commission of the European Communities, 2006). On the other hand, the FDA (Food and Drug Administration, U.S.A.) has set the maximum levels for DON at 10 mg/kg for animal feeds, 1000 µg/kg for wheat products (flour, bran, and germ), 10 mg/kg for cereals and cereal-based products, and 30 mg/kg for distillers and brewer grains (U.S. Food and Drug Administration, 2010).

Regarding OTA and other mycotoxins, it was first decided in 1993 in the EU Council Regulation (EEC) No. 315/93' that maximum tolerance limits should be established for specific contaminants to protect the public health (Council Regulation, 1993). The limits for cereals (5 µg/kg), processed grain-based foods (3 µg/kg), and dried vine fruits (10 µg/kg) were previously determined regarding OTA in the EU Commission Regulation No. 1881/2006 published in 2006, including the limits for supplementary foods for infants and small child and special medical purpose dietary supplements for infants (0.5 µg/kg), as well as roasted coffee beans and ground coffee (5 µg/kg), coffee extract or soluble coffee (10 µg/kg). Both the EU and Turkish Food Codex have determined the maximum limit values of OTA in cereals as 5 µg/kg in unprocessed cereals, 3 µg/kg in all products derived from unprocessed cereals (including cereals and processed cereal products for direct human consumption), 0.5 µg/kg in special medical-purpose diet foods for infants and supplementary foods for infants and small children (Commission of the European Communities, 2006).

Mycotoxins are harmful to human health due to their chronic effects such as carcinogenic, teratogenic, immunotoxic, nephrotoxic, and estrogenic effects (Zain, 2011). Toxicological studies and *in vitro* and *in vivo* studies regarding trichothecenes have shown various effects (Lu et al., 2013). Some of the toxic effects related to trichothecenes are mitochondrial dysfunction, protein inhibition, immunosuppression, cytotoxicity, and changes in blood cell count (Cano-Sancho et al., 2011). Trichothecene has also been associated with skin and mucous membrane disorders, loss of appetite, vomiting, abdominal pain, hemorrhagic disorders, and cardiovascular dysfunction (Etzel, 2006). Since DON, OTA, and other mycotoxin species are found in trace amounts in foods, it is of great importance to detect these compounds with reliable, accurate, and fast analysis methods. Thin-layer chromatography (TLC), HPLC, liquid chromatography-mass spectrometry (LC-MS), and enzyme-linked immunosorbent (ELISA) methods are used in mycotoxin analysis (Berthiller et al., 2007). The HPLC method is the most common method to determine and diagnose DON, OTA, and other mycotoxins in food and feedstuffs. Importantly, HPLC analysis provides accurate, reliable, sensitive (low detection and measurement limit), and high precision results (Girelli & Mattei, 2005).

It is nearly impossible to avoid mycotoxin exposure in cereals. However, the detection of mycotoxin prevalence and levels in these food products is required to reduce the mycotoxin levels as determined by the various countries and prevent the health risks to the consumers. The present study aims to determine the DON and OTA levels of cereals sold on the market in Türkiye by HPLC with a UV and fluorescence detector.

2 Materials and methods

2.1 Sampling

In this study, 50% of the cereals that make up our samples were procured from the markets (branded products) in İstanbul and 50% from the street markets, collected between September and October in 2020 in Türkiye. The total number of samples was 96, but 24 of these samples were ashura wheat, 24 of them were rice, 24 of them were corn flour, and 24 of them were whole wheat flour. The production place and sample types for DON and OTA are presented in Tables 1-8.

2.2 Chemicals

OTA, DON standard, and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade solvents were used in the extraction and mobile phase. Millipore Milli Q-RG water was used for the preparation of standards.

2.3 DON analysis

DON content was determined according to a method described by Omurtag & Beyoğlu (2003). DON stock standard solution was prepared in acetonitrile at a concentration of 1 µg/µL. The stock standard solution was then used to prepare a 0.5 ng/µL working standard solution in methanol/water (20:80 v/v). The prepared stock solutions were stored at -20 °C.

Cartridge preparation for DON

First, a filter paper (Whatman No. 4) was placed in a 6 mL empty SPE column by cutting it to completely cover the bottom. Then, 0.35 g of aluminum oxide, 0.25 g of celite, and 0.4 g of activated charcoal, which were weighed on a precision scale, were placed on the filter. Care was taken to create these layers without mixing them. Filter paper (Whatman No. 4) was placed on the last layer of activated charcoal.

For OTA, the immunoaffinity columns (IAC) containing OTA-sensitive antibodies were obtained from OchraStar Romer Labs. The IAC was stored at 4-8 °C, but it was allowed to come to room temperature before performing the analysis.

Table 1. DON analysis results by HPLC of Ashura wheat samples.

	Sample Number	Production Place	DON (µg/kg)		Sample Number	Production Place	DON (µg/kg)
Off-the-shelf	1[a]	Konya	ND	Sold in the Market	5[P]	OU	> LOQ
	10[a]	Konya	< LOQ		14[P]	OU	< LOQ
	16[a]	Konya	< LOQ		19[P]	OU	< LOQ
	23[a]	Malatya	< LOQ		27[P]	Sakarya	ND
	31[a]	Konya	ND		34[P]	OU	ND
	42[a]	Malatya	ND		46[P]	Ankara	ND
	55[a]	Konya	ND		51[P]	OU	ND
	62[a]	Konya	ND		59[P]	OU	ND
	66[a]	Konya	ND		70[P]	OU	ND
	76[a]	Konya	< LOQ		80[P]	OU	ND
	89[a]	Konya	ND		87[P]	Ankara	ND
	91[a]	Konya	ND		93[P]	Ankara	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 2. DON analysis results by HPLC of rice samples.

	Sample Number	Production Place	DON (µg/kg)		Sample Number	Production Place	DON (µg/kg)
Off-the-shelf	2[a]	Çorum	**> LOQ**	Sold in the Market	6[P]	Edirne	< LOQ
	11[a]	Çorum	< LOQ		15[P]	Trakya	< LOQ
	17[a]	Samsun	< LOQ		20[P]	Bolu	< LOQ
	24[a]	Bolu	< LOQ		28[P]	OU	ND
	32[a]	Çorum	ND		35[P]	Çorum	ND
	43[a]	Bolu	ND		37[P]	Çorum	ND
	56[a]	Bolu	ND		47[P]	Samsun	< LOQ
	63[a]	Çorum	ND		52[P]	OU	ND
	67[a]	Çorum	ND		60[P]	Bolu	ND
	77[a]	Çorum	ND		71[P]	Çorum	ND
	90[a]	Bolu	ND		81[P]	Bolu	ND
	92[a]	Bolu	ND		94[P]	OU	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 3. DON analysis results by HPLC of corn flour samples.

	Sample Number	Production Place	DON (µg/kg)		Sample Number	Production Place	DON (µg/kg)
Off-the-shelf	3[a]	Giresun	< LOQ	Sold in the Market	7[P]	OU	**> LOQ**
	4[a]	Ordu	**> LOQ**		12[P]	OU	< LOQ
	13[a]	Rize	< LOQ		21[P]	OU	< LOQ
	18[a]	Giresun	< LOQ		29[P]	OU	ND
	25[a]	Rize	< LOQ		36[P]	OU	ND
	33[a]	Rize	ND		48[P]	Eskişehir	ND
	41[a]	Giresun	ND		53[P]	OU	ND
	44[a]	Trabzon	ND		61[P]	OU	ND
	57[a]	Ordu	ND		72[P]	OU	ND
	64[a]	Trabzon	ND		82[P]	OU	ND
	68[a]	G.antep	ND		88[P]	Ankara	ND
	78[a]	Mersin	ND		95[P]	OU	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 4. DON analysis results by HPLC of whole wheat flour samples.

	Sample Number	Production Place	DON (µg/kg)		Sample Number	Production Place	DON (µg/kg)
Off-the-shelf	26[a]	Malatya	ND	Sold in the Market	8[P]	OU	< LOQ
	38[a]	Antalya	ND		9[P]	Kırşehir	ND
	39[a]	OU	ND		22[P]	OU	< LOQ
	40[a]	Muğla	ND		30[P]	Tekirdağ	ND
	45[a]	Muğla	ND		49[P]	Aydın	ND
	50[a]	OU	ND		54[P]	Konya	ND
	58[a]	Konya	ND		65[P]	Konya	ND
	69[a]	G.antep	ND		73[P]	OU	ND
	74[a]	İzmir	ND		83[P]	Tekirdağ	ND
	75[a]	OU	ND		85[P]	Konya	ND
	79[a]	OU	ND		86[P]	OU	ND
	84[a]	OU	ND		96[P]	Tekirdağ	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 5. OTA analysis results by HPLC of Ashura wheat samples.

	Sample Number	Production Place	OTA (µg/kg)		Sample Number	Production Place	OTA (µg/kg)
Off-the-shelf	1[a]	Konya	ND	Sold in the Market	5[P]	OU	ND
	10[a]	Konya	ND		14[P]	OU	ND
	16[a]	Konya	ND		19[P]	OU	ND
	23[a]	Malatya	ND		27[P]	Sakarya	ND
	31[a]	Konya	ND		34[P]	OU	ND
	42[a]	Malatya	ND		46[P]	Ankara	ND
	55[a]	Konya	ND		51[P]	OU	ND
	62[a]	Konya	ND		59[P]	OU	ND
	66[a]	Konya	ND		70[P]	OU	ND
	76[a]	Konya	ND		80[P]	OU	ND
	89[a]	Konya	ND		87[P]	Ankara	ND
	91[a]	Konya	ND		93[P]	Ankara	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 6. OTA analysis results by HPLC of rice samples.

	Sample Number	Production Place	OTA (µg/kg)		Sample Number	Production Place	OTA (µg/kg)
Off-the-shelf	2[a]	Çorum	ND	Sold in the Market	6[P]	Edirne	ND
	11[a]	Çorum	ND		15[P]	Trakya	ND
	17[a]	Samsun	ND		20[P]	Bolu	ND
	24[a]	Bolu	ND		28[P]	OU	ND
	32[a]	Çorum	ND		35[P]	Çorum	ND
	43[a]	Bolu	ND		37[P]	Çorum	ND
	56[a]	Bolu	ND		47[P]	Samsun	ND
	63[a]	Çorum	ND		52[P]	OU	ND
	67[a]	Çorum	ND		60[P]	Bolu	ND
	77[a]	Çorum	ND		71[P]	Çorum	ND
	90[a]	Bolu	ND		81[P]	Bolu	ND
	92[a]	Bolu	ND		94[P]	OU	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 7. OTA analysis results by HPLC of corn flour samples.

	Sample Number	Production Place	OTA (µg/kg)		Sample Number	Production Place	OTA (µg/kg)
Off-the-shelf	3[a]	Giresun	ND	Sold in the Market	7[P]	OU	ND
	4[a]	Ordu	ND		12[P]	OU	ND
	13[a]	Rize	ND		21[P]	OU	ND
	18[a]	Giresun	ND		29[P]	OU	ND
	25[a]	Rize	ND		36[P]	OU	ND
	33[a]	Rize	ND		48[P]	Eskişehir	**1.51 ± 0.03**
	41[a]	Giresun	ND		53[P]	OU	**2.23 ± 0.03**
	44[a]	Trabzon	ND		61[P]	OU	**1.50 ± 0.03**
	57[a]	Ordu	ND		72[P]	OU	ND
	64[a]	Trabzon	ND		82[P]	OU	ND
	68[a]	G.antep	ND		88[P]	Ankara	ND
	78[a]	Mersin	ND		95[P]	OU	ND

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Table 8. OTA analysis results by HPLC of whole wheat flour samples.

	Sample Number	Production Place	OTA (µg/kg)		Sample Number	Production Place	OTA (µg/kg)
Off-the-shelf	26[a]	Malatya	**1.31 ± 0.03**	Sold in the Market	8[P]	OU	**1.79 ± 0.05**
	38[a]	Antalya	ND		9[P]	Kırşehir	**6.97 ± 0.21**
	39[a]	OU	ND		22[P]	OU	ND
	40[a]	Muğla	ND		30[P]	Tekirdağ	ND
	45[a]	Muğla	**1.48 ± 0.03**		49[P]	Aydın	ND
	50[a]	OU	ND		54[P]	Konya	**0.92 ± 0.03**
	58[a]	Konya	ND		65[P]	Konya	ND
	69[a]	G.antep	ND		73[P]	OU	**4.20 ± 0.05**
	74[a]	İzmir	ND		83[P]	Tekirdağ	ND
	75[a]	OU	ND		85[P]	Konya	ND
	79[a]	OU	ND		86[P]	OU	ND
	84[a]	OU	**0.87 ± 0.03**		96[P]	Tekirdağ	**0.84 ± 0.03**

[a]Bulk product for sale; [P]Packaged product from the market; OU: origin unknown; ND: not detected.

Sample preparation for DON

First, 50 g of sample was weighed and transferred to a Waring blender. Next, 250 mL of acetonitrile/water (21:4, v/v) solution was added and mixed for 3 minutes, and then, left for 30 min. Then, it was filtered into a 500 mL volumetric flask using Whatman No. 4 filter paper. After that, it was passed through a pre-washed SPE column. The SPE column was pre-washed with 10 mL of acetonitrile/water (21:4, v/v) solution. Then, 5 mL of the filtrate was passed through the SPE column. Finally, DON was eluted with 10 mL of acetonitrile/water (21:4, v/v) solution. The eluent was held in a round bottom flask and evaporated in a vacuum evaporator at 60 °C.

2.4 Sample preparation for OTA

OTA content was determined according to a method described by Solfrizzo et al. (1998). To prepare OTA standard stock solutions (2 µg/mL), 2 mL of OTA standard solution containing 10 µg/mL of OTA dissolved in acetonitrile was added to a 10 mL flask and made up to 10 mL with methanol.

First, 50 g of sample was weighed and transferred to a Waring blender. Next, 200 mL of acetonitrile/pure water (6:4, v/v) solution was added and mixed for 3 min. It was filtered in a 250 mL flask using Whatman No. 4 filter paper. Then, 10 mL of filtrate and 40 mL of phosphate-buffered saline (PBS) were added into a 100-mL beaker. The IACs were used for the purification of OTA. The IAC was kept in the refrigerator until the analysis, but they are allowed to come to room temperature before analysis begins. The IACs were placed in a Chromabond vacuum manifold, and then, 25 mL of the diluted filtrate was passed at a constant rate through the IACs at a flow rate of 3 mL/min. After that, the column was washed by passing 10 mL of PBS and 10 mL of ultrapure water at a constant rate of 2 drops/second from the IAC and dried by applying low vacuum for 5-10 seconds. OTA that attached to the antibodies in the IAC was eluted by backflushing with 0.5 mL of methanol (two times), and it elute

was collected into a glass tube. Finally, 1 mL of ultrapure water was passed through the IAC, and the total eluate was collected in the same glass tube.

2.5 HPLC procedure for DON

The purified DON samples were dissolved in 1.0 mL of 20% methanol-water and filtered with a Millipore Millex HV filter (0.45 µm), Hydrophilic Duropore (PVDF), and then, injected into the HPLC system. Injection volume was 50 or 100 µL. Linearity was observed between 2 and 40 ng for DON using five calibration levels in duplicate.

The HPLC system was a combination of a variable wavelength UV-visible detector tuned at 220 nm (Agilent 1100) and a Model 600 pumped multi-solvent delivery system (Waters Corporation, USA). The injector was a Rheodyne 7725 sample injector with 20 loop accessories. The injection volume was 100 µL for each sample. A reverse-phase symmetry C18 column (4.6 x 250 mm, 100 Å, 5 µL particle size, part number HX56005269, Merck, Germany) was used for the separation of DON. The Agilent ChemStation program was used for the data analysis.

The mobile phase was prepared by mixing with methanol and water (70:30 v/v). The mobile phase was filtered through a Millex HV Millipore filter (0.45 µm) and degassed with a Waters in-line degasser. The flow rate was 0.7 mL/min. The graph speed was 0.5 cm/min and the oven temperature was set at 25 °C.

2.6 HPLC procedure for OTA

The purified OTA sample was filtered through a 2-mL syringe-tipped filter (0.45 µm). Linearity was observed between 0.4 and 60 µg/kg for the Ochratoxin A standard using five calibration levels in duplicate.

For HPLC, a Shimadzu LC Shimadzu-Nexera-I LC-2040C system with a Shimadzu RF-20A fluorescence detector (Shimadzu Corporation, Kyoto, Japan) was used. The mobile phase was prepared by mixing acetonitrile/water/acetic acid (47:51:2, (v/v/v)). An Inertsil ODS-3 (4.6 x 250 mm, 5 µm) column was used for the separation of OTA. The excitation and emission wavelengths were 333 and 443 nm, respectively. The flow rate was 1 mL/min. The column oven temperature was 40 °C.

2.7 Method validation

For the performance evaluation of the method used for DON and OTA analysis; In addition to the linearity range and correlation coefficients (R^2) data of the calibration curves, LOD, LOQ, and recovery were made. A calibration curve was created by calculating the areas of the peaks prepared at five different concentrations and injected into the HPLC device.

3 Results and discussion

In this study, we determined the amount of mycotoxins DON and OTA in ashura wheat, rice, corn flour, and whole wheat flour, which are produced and consumed in significant quantities in Türkiye. The findings of the study are shown in the following Tables 1-8.

The limit of detection (LOD) and limit of quantification (LOQ) for DON were determined as 0.28 and 0.92 µg/kg, respectively. The recovery of the DON analysis was calculated as a result of the recovery by adding DON intermediate standard solution (5 µg/kg) to a blank sample that was found not to contain deoxynivalenol and was found to be 96%. The correlation coefficient of the calibration curve (R^2) was 0.9998. The 0.10 µg/kg OTA standard solution was spiked to blank sample and recovery was found as 96.75%. The LOD and LOQ values of the OTA analysis were calculated as 0.03 µg/kg, 0.10 µg/kg, and the correlation coefficient of this calibration curve (R^2) = 1 was found as a result of the calculations.

As shown in Tables 1-4, DON was detected in 4 of the 96 grain cereal samples at levels between 0.94 and 1.16 µg/kg (LOQ, 0.92 µg/kg). Besides, from the analyzed samples, DON was determined in 3 pieces of ashura wheat, 2 pieces of rice, and 5 pieces of corn flour between LOD and LOQ. The highest DON level was found in the corn flour sample (1.16 µg/kg), which was sold unpacked. In the study conducted in 2002 on DON exposure in cereals in Türkiye, 68 kinds of cereal and 15 pulses samples were examined and DON was detected in 8.82% of the grain samples and the highest DON level was found in the corn flour sample (2.67 µg/g) bought from the market, as in our study (Omurtag & Beyoğlu, 2003). In the study conducted by Bakırcı in 2014, the DON levels were investigated in a total of 381 grain-based samples by HPLC. In that study, DON was detected in the range of 132.4-9589.4 µg/kg in 13 of the 144 samples (Bakırcı et al., 2014). In another study carried out by Türkeşsiz and Bostan in 2020, 112 different flour samples were collected from various bakeries and markets in Istanbul, and the DON level was detected between 0.06-70.04 µg/kg in 41 of the 112 samples (LOD-LOQ, 2.22-7.40 µg/kg) (Türkeşsiz & Bostan, 2020). In that study, DON level was found below the acceptable limits determined by Turkish food codex in all samples. In another study, the levels of DON were examined in 240 cereals and cereal-based samples collected from various regions of Türkiye, and DON was detected in 13 wheat (58-1092 µg/kg), 2 corn (313-331 µg/kg), 3 barley (138-973 µg/kg), 7 paddy (136-256 µg/kg), 3 wheat flour (92-151 µg/kg), 2 biscuits (31.2-71.3 µg/kg), and only 1 pasta sample (49.3 µg/kg) (LOD-LOQ, 46.9-72.3 µg/kg) (Golge & Kabak, 2020). It was reported in that study, DON was the most prevalent mycotoxin in wheat compared to other mycotoxins. The DON levels in 106 cereals and cereal-based samples were examined in Qatar and detected in 4% out of the samples (86.43-182.94 µg/kg) (Abdulkadar et al., 2004). In a study conducted in South Africa, DON was detected in 16 of the 18 corn samples at an average level of 294 µg/kg (Shephard et al., 2010). In a study examining corn samples in Italy between 2006 and 2007, DON was detected at the levels of 197-3980 µg/kg in all 47 samples. DON levels reaching 14 µg/kg were detected in 89% of the 36 corn samples in a study from 2007 (Golge & Kabak, 2020). DON levels of 206-4732 µg/kg were detected in 75 of the 113 wheat samples obtained from 2008-2009 in Parana State, Brazil (Santos et al., 2013). Blesa et al. (2014) investigated 80 durum wheat grains for DON in 2014. In that study, DON was determined at the levels of 121-1480 µg/kg in 4 of the 80 samples (Blesa et al., 2014). In the study conducted with samples of 31 unprocessed wheat and

35 grains of white wheat flour, harvested in 2014 in Romania, the DON levels were determined at 110-1787 µg/kg in 8 wheat samples and 190 µg/kg in 1 wheat flour sample (Stanciu et al., 2017). As a result of our work and similar studies carried out around the world, it can be concluded that DON exposure is common in cereals, especially in corn flour. In 2016, the joint press release made by the International Agency for Research on Cancer and the World Health Organization called for action against widespread mycotoxin contamination in developing countries: "Worldwide, more than 160 million children under the age of five are stunted. Improving mycotoxin control can have far-reaching health benefits. The time has come to mobilize existing knowledge and technology to control mycotoxin food contamination in low-income countries" (International Agency for Research on Cancer, 2016, p. 242). Therefore, studies in cereals and cereal-derived foods on a regular basis are required to assess the risk of DON exposure in developing countries, and especially in children.

From Tables 5-8, the OTA was detected in 11 of the 96 samples (0.87-6.97 µg/kg) (LOD-LOQ = 0.03-0.10 µg/kg). From the results, OTA was detected in 3 corn flours (1.51-2.23 µg/kg) and 8 whole wheat flours (0.87-6.97 µg/kg). The highest OTA level was found in the packaged whole wheat flour sample (6.97 µg/kg) purchased from the market. In a study, 34 wheat flour, 14 whole wheat flour, and 10 corn flour sample were taken from various markets and bakeries in Bursa province in Türkiye and OTA levels were determined at 6.89 ± 0.46 µg/kg in wheat flour, 9.3 ± 1.33 µg/kg in whole wheat flour and 6.93 ± 1.10 µg/kg in corn flour (Cengiz et al., 2007). In another OTA study, with 60 wheat flour, 24 corn flour, and 16 rice flour, OTA was detected at 0.11-0.92 µg/kg in 27% of the wheat flour, 0.06-0.59 µg/kg in 42% of the corn flour, and 0.06-0.21 µg/kg in 19% of the rice flour (LOD-LOQ, 1-20 ng/mL) (Kara et al., 2015). In a study conducted in Türkiye in 2000, OTA levels were investigated in 100 food samples such as wheat, corn, and corn flour. OTA was detected in 1 ashura wheat (0.27 µg/kg), 2 grains of corn (1.79-9.84 µg/kg) samples (Kargözlü & Karapinar, 2000). In another study, OTA levels in 811 plant-based samples were examined, including cereals and cereal-based products, and it was determined that 37.5% of the cereals and cereal-based samples contained an average of 0.77 µg/kg of OTA (Cengiz et al., 2007). The level of OTA was investigated in 106 cereals and cereal-based samples taken from the markets in Qatar; OTA was detected in 11% of the samples (0.18-6.81 µg/kg) (Jiao et al., 1994). In another study conducted with samples from 60 cereals and cereal-based markets, average OTA levels of 1.08, 0.42, and 0.17 µg/kg were found in corn, wheat, and barley, respectively (LOD-LOQ, 0.01-0.02 µg/kg) (Zinedine et al., 2006). In 83 organic and non-organic rice, wheat, barley, rye, corn, and oat samples obtained from Spain and Portugal, it was determined that 22% of the samples contained OTA at the levels of 0.2-27.10 µg/kg (Juan et al., 2008). Kumar et al. (2012) investigated the OTA levels in 50 wheat samples in India. It was determined that 29 of the samples contained OTA between 1.36-21.17 µg/kg and 13 samples exceeded the limit set by the EU (5 µg/kg) (LOD-LOQ, 3.3-10 ng/mL) (Kumar at al., 2012). In a study conducted in Poland in 2019, OTA was detected in 13 of the 113 wheat flours (0.7-5.8 µg/kg) and in 3 of the 45 corn flours (0.7-1.6 µg/kg) (Lee & Ryu, 2017). Different studies on cereals show the frequency of OTA exposure in whole wheat flour and corn flour, as in our study. Therefore, more frequent studies of cereals and cereal-derived foods are needed to assess the risk of OTA exposure.

4 Conclusion

Today, cereal and cereal products are consumed more especially in low-income countries because they are both cheap and easy to access. However, contamination of crops, especially cereals, by molds during the pre-harvest and post-harvest stages can lead to the production of secondary toxic metabolites known as mycotoxins. It is demonstrated in many clinical studies, mycotoxins are harmful to human health due to their chronic effects such as carcinogenic, teratogenic, immunotoxic, nephrotoxic, and estrogenic effects. As a result of our work and similar studies carried out around the world, it can be concluded that DON exposure is common in cereals, especially in corn flour. In our study, the level of DON was higher in 4 of the 25 detected samples than LOQ, and OTA was higher in 6 of the 11 detected corn and wheat flour samples. Since DON and OTA exposure can be observed frequently in cereals and may increase to possible risky levels, further work should be done to determine the precautions necessary to minimize the risks of contamination.

Conflict of interest

The authors declare no conflict of interest.

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References

Abdulkadar, A. H. W., Al-Ali, A. A., Al-Kildi, A. M., & Al-Jedah, J. H. (2004). Mycotoxins in food products available in Qatar. *Food Control*, 15(7), 543-548. http://dx.doi.org/10.1016/j.foodcont.2003.08.008.

Bakırcı, G. T., Aflatoksin, T. V. T. Ü., Okratoksin, A., & Zearalenon, F. V. D. M. Y. (2014). Incelenmesi [determination of aflatoxin, ochratoxin A, zearalenone, fumonisin and deoxynivalenol myctoxins in cereals and cereal based products]. *Akademik Gıda*, 12, 46-56.

Berthiller, F., Sulyok, M., Krska, R., & Schuhmacher, R. (2007). Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals. *International Journal of Food Microbiology*, 119(1-2), 33-37. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.022. PMid:17761332.

Blesa, J., Moltó, J. C., Akhdari, S., Mañes, J., & Zinedine, A. (2014). Simultaneous determination of Fusarium mycotoxins in wheat grain from Morocco by liquid chromatography coupled to triple quadrupole mass spectrometry. *Food Control*, 46, 1-5. http://dx.doi.org/10.1016/j.foodcont.2014.04.019.

Bui-Klimke, T. R., & Wu, F. (2015). Ochratoxin A and human health risk: a review of the evidence. *Critical Reviews in Food Science and Nutrition*, 55(13), 1860-1869. http://dx.doi.org/10.1080/10408398.2012.724480. PMid:24874522.

Bullerman, L. B., & Bianchini, A. (2007). Stability of mycotoxins during food processing. *International Journal of Food Microbiology*, 119(1-2), 140-146. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.035. PMid:17804104.

Cano-Sancho, G., Valle-Algarra, F. M., Jiménez, M., Burdaspal, P., Legarda, T. M., Ramos, A. J., Sanchis, V., & Marín, S. (2011). Presence of trichothecenes and co-occurrence in cereal-based food from Catalonia (Spain). *Food Control*, 22(3-4), 490-495. http://dx.doi.org/10.1016/j.foodcont.2010.09.033.

Cengiz, M., Uzunoğlu, İ., & Sonal, S. (2007). Ochratoxin A levels in different types of bread and flour (farklı tip ekmek ve unlarda okaratoksin A düzeyleri). *Uludag Üniversitesi Veteriner Fakültesi Dergisi*, 26(1-2), 7-10.

Commission of the European Communities. (2006). *Commission Regulation (EC) no 1881/2006 of 19 December 2006: setting maximum levels for certain contaminants in foodstuffs*. Brussels: Official Journal of the European Communities.

Council Regulation – EEC. (1993). Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. *Official Journal L*, 37(13/02), 0001-0003.

EFSA Panel on Contaminants in the Food Chain. (2013). Statement on the risks for public health related to a possible increase of the maximum level of deoxynivalenol for certain semi-processed cereal products. *EFSA Journal*, 11(12), 3490.

Etzel, R. A. (2006). What the primary care pediatrician should know about syndromes associated with exposures to mycotoxins. *Current Problems in Pediatric and Adolescent Health Care*, 36(8), 282-305. PMID: 16935759.

European Food Safety Authority – EFSA. (2006). Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ochratoxin A in food. *EFSA Journal*, 4(6), 365. http://dx.doi.org/10.2903/j.efsa.2006.365.

Girelli, A. M., & Mattei, E. (2005). Application of immobilized enzyme reactor in on-line high performance liquid chromatography: a review. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 819(1), 3-16. http://dx.doi.org/10.1016/j.jchromb.2005.01.031. PMid:15797515.

Golge, O., & Kabak, B. (2020). Occurrence of deoxynivalenol and zearalenone in cereals and cereal products from Turkey. *Food Control*, 110, 106982. http://dx.doi.org/10.1016/j.foodcont.2019.106982.

Gupta, R. C., Srivastava, A., & Lall, R. (2018). Ochratoxins and citrinin. In R. C. Gupta (Ed.), *Veterinary toxicology: basic and clinical principles* (3rd ed.). London: Academic Press. http://dx.doi.org/10.1016/B978-0-12-811410-0.00072-6.

International Agency for Research on Cancer. (2016). *New IARC report urges action against widespread mycotoxin contamination in developing countries*. Lyon: IARC. Press release no 242.

Jiao, Y., Blaas, W., Rühl, C., & Weber, R. (1994). Ochratoxin A in foodstuffs (vegetables, cereals, cereal products and beer). *Deutsche Lebensmittel-Rundschau*, 90(10), 318-321.

Juan, C., Moltó, J. C., Lino, C. M., & Mañes, J. (2008). Determination of ochratoxin A in organic and non-organic cereals and cereal products from Spain and Portugal. *Food Chemistry*, 107(1), 525-530. http://dx.doi.org/10.1016/j.foodchem.2007.08.019.

Kara, G. N., Ozbey, F., & Kabak, B. (2015). Co-occurrence of aflatoxins and ochratoxin A in cereal flours commercialised in Turkey. *Food Control*, 54, 275-281. http://dx.doi.org/10.1016/j.foodcont.2015.02.014.

Kargözlü, N., & Karapinar, M. (2000). Fungal Contamination and the Incidence of ochratoxin-A in some cerealsand products. *Turkish Journal of Biology*, 24(3), 561-572.

Khorshidi, M., Heshmati, A., Hadian, Z., Smaoui, S., & Mousavi Khaneghah, A. (2022). The occurrence of aflatoxin M_1 in doogh, kefir, and kashk in Hamadan, Iran. *Food Science and Technology*, 42, e42022. https://doi.org/10.1590/fst.42022.

Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Nebbia, C. S., Oswald, I. P., Petersen, A., Rose, M., Roudot, A. C., Schwerdtle, T., Vleminckx, C., Vollmer, G., Wallace, H., Saeger, S., Eriksen, G. S., Farmer, P., Fremy, J. M., Gong, Y. Y., Meyer, K., Naegeli, H., Parent-Massin, D., Rietjens, I., van Egmond, H., Altieri, A., Eskola, M., Gergelova, P., Bordajandi, L. R., Benkova, B., Dörr, B., Gkrillas, A., Gustavsson, N., van Manen, M., & Edler, L. (2017). Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA Journal*, 15(9), e04718. PMid:32625635.

Kumar, R., Ansari, K. M., Saxena, N., Dwivedi, P. D., Jain, S. K., & Das, M. (2012). Detection of ochratoxin A in wheat samples in different regions of India. *Food Control*, 26(1), 63-67. http://dx.doi.org/10.1016/j.foodcont.2012.01.004.

Lancova, K., Hajslova, J., Kostelanska, M., Kohoutkova, J., Nedelnik, J., Moravcova, H., & Vanova, M. (2008). Fate of trichothecene mycotoxins during the processing: milling and baking. *Food Additives and Contaminants*, 25(5), 650-659. http://dx.doi.org/10.1080/02652030701660536. PMid:18473219.

Lee, H. J., & Ryu, D. (2017). Worldwide occurrence of mycotoxins in cereals and cereal-derived food products: public health perspectives of their co-occurrence. *Journal of Agricultural and Food Chemistry*, 65(33), 7034-7051. PMID: 27976878.

Lima, C. M. G., Costa, H. R. D., Pagnossa, J. P., Rollemberg, N. C., Silva, J. F., Dalla Nora, F. M., Batiha, G. E.-S., & Verruck, S. (2022). Influence of grains postharvest conditions on mycotoxins occurrence in milk and dairy products. *Food Science and Technology*, 42, e16421. http://dx.doi.org/10.1590/fst.16421.

Lu, H., Leal, M. R., Míguez, M. P., & Fernández-Franzón, M. (2013). Detection of seven trichothecene mycotoxins in infant cereal foods by QuEChERS extraction and liquid chromatography coupled to tandem mass spectrometry. *Reviews in Toxicology*, 30(2), 203-208.

Machado, L. V., Mallmann, C. A., Mallmann, A. O., Coelho, R. D., & Copetti, M. V. (2017). Deoxynivalenol in wheat and wheat products from a harvest affected by fusarium head blight. *Food Science and Technology*, 37(1), 8-12. http://dx.doi.org/10.1590/1678-457x.05915.

Medina, B. G., Sartori, A. V., Moraes, M. H. P., Cardoso, M. H. W. M., & Jacob, S. C. (2019). Validation and application of an analytical method for the determination of mycotoxins in crackers by UPLC-MS/MS. *Food Science and Technology*, 39(3), 583-591. http://dx.doi.org/10.1590/fst.33717.

Milani, J. M. (2013). Ecological conditions affecting mycotoxin production in cereals: a review. *Veterinarni Medicina*, 58(8), 405-411. http://dx.doi.org/10.17221/6979-VETMED.

Neme, K., & Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. *Food Control*, 78, 412-425. http://dx.doi.org/10.1016/j.foodcont.2017.03.012.

Omurtag, G. Z., & Beyoğlu, D. (2003). Occurrence of deoxynivalenol (vomitoxin) in processed cereals and pulses in Turkey. *Food Additives and Contaminants*, 20(4), 405-409. http://dx.doi.org/10.1080/0265203031000082512. PMid:12775484.

Ricciardi, C., Castagna, R., Ferrante, I., Frascella, F., Marasso, S. L., Ricci, A., Canavese, G., Lorè, A., Prelle, A., Gullino, M. L., & Spadaro, D. (2013). Development of a microcantilever-based immunosensing

method for mycotoxin detection. *Biosensors & Bioelectronics*, 40(1), 233-239. http://dx.doi.org/10.1016/j.bios.2012.07.029. PMid:22878081.

Richard, J. L., Bennett, G. A., Ross, P. F., & Nelson, P. E. (1993). Analysis of naturally occurring mycotoxins in feedstuffs and food. *Journal of Animal Science*, 71(9), 2563-2574. http://dx.doi.org/10.2527/1993.7192563x. PMid:8407669.

Santos, J. S., Souza, T. M., Ono, E. Y. S., Hashimoto, E. H., Bassoi, M. C., Miranda, M. Z., Itano, E. N., Kawamura, O., & Hirooka, E. Y. (2013). Natural occurrence of deoxynivalenol in wheat from Paraná State, Brazil and estimated daily intake by wheat products. *Food Chemistry*, 138(1), 90-95. http://dx.doi.org/10.1016/j.foodchem.2012.09.100. PMid:23265460.

Shanahan, J. F., Brown, W. M. Jr., & Blunt, T. D. (2003). *Aflatoxins* (Doctoral dissertation). Colorado State University, Fort Collins.

Shephard, G. S., van der Westhuizen, L., Katerere, D. R., Herbst, M., & Pineiro, M. (2010). Preliminary exposure assessment of deoxynivalenol and patulin in South Africa. *Mycotoxin Research*, 26(3), 181-185. http://dx.doi.org/10.1007/s12550-010-0052-9. PMid:23605382.

Silva, J. V. B., Oliveira, C. A. F., & Ramalho, L. N. Z. (2022). An overview of mycotoxins, their pathogenic effects, foods where they are found and their diagnostic biomarkers. *Food Science and Technology*, 42, e48520. http://dx.doi.org/10.1590/fst.48520.

Solfrizzo, M., Avantaggiato, G., & Visconti, A. (1998). Use of various clean-up procedures for the analysis of ochratoxin A in cereals. *Journal of Chromatography. A*, 815(1), 67-73. http://dx.doi.org/10.1016/S0021-9673(98)00271-4. PMid:9718708.

Stanciu, O., Juan, C., Miere, D., Loghin, F., & Mañes, J. (2017). Occurrence and co-occurrence of Fusarium mycotoxins in wheat grains and wheat flour from Romania. *Food Control*, 73, 147-155. http://dx.doi.org/10.1016/j.foodcont.2016.07.042.

Türkeşsiz, K., & Bostan, K. (2020). İstanbul'daki tahıl unlarında aflatoksin, deoksinivalenol ve zearalanon düzeyleri. *Türk Doğa ve Fen Dergisi*, 9(1), 131-136. http://dx.doi.org/10.46810/tdfd.724695.

U.S. Food and Drug Administration – FDA. (2010). *Advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed*. Silver Spring: FDA.

Udovicki, B., Audenaert, K., Saeger, S., & Rajkovic, A. (2018). Overview on the mycotoxins incidence in Serbia in the period 2004–2016. *Toxins*, 10(7), 279. http://dx.doi.org/10.3390/toxins10070279. PMid:29976881.

Van der Merwe, K. J., Steyn, P. S., Fourie, L., Scott, D. B., & Theron, J. J. (1965). Ochratoxin A, a toxic metabolite produced by Aspergillus ochraceus Wilh. *Nature*, 205, 1112-1113. http://dx.doi.org/10.1038/2051112a0. PMid:5833211.

Wang, J., Gu, Y., Zhang, Y., Chen, S., Li, L., Liao, Z., Shan, X., He, L., & Chen, J. (2022). Toxigenic potential analysis and fumigation treatment of three Fusarium spp. strains isolated from Fusarium head blight of wheat. *Food Science and Technology*, 42, e53822. http://dx.doi.org/10.1590/fst.53822.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2), 129-144. http://dx.doi.org/10.1016/j.jscs.2010.06.006.

Zinedine, A., Brera, C., Elakhdari, S., Catano, C., Debegnach, F., Angelini, S., Santis, B., Faid, M., Benlemlih, M., Minardi, V., & Miraglia, M. (2006). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, 17(11), 868-874. http://dx.doi.org/10.1016/j.foodcont.2005.06.001.