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Occurrence of Deoxynivalenol (DON) in wheat flours in Guilan Province, northern Iran

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Abstract

Introduction and objective. Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect wheat, corn, rice, oats, barley and other grains in the field or during storage. Guilan, Golestan and Mazandaran areas are located in the North Iran with favorite conditions for *Fusarium* growth. DON affects animal and human health causing vomiting, acute temporary nausea, diarrhea, abdominal pain, headache, dizziness, and fever.
Materials and methods. In this study, a total of 96 wheat flour samples of different types were collected during summer 2013 from six flour mills in Northern Iran. All samples were analyzed for DON by enzyme-linked immunosorbent assay.
Results. DON was detected in 80 out of 96 wheat flour samples (83.33%), at levels ranging from 23 to1270 µg/kg. The maximum contamination level of DON (1270 µg/kg) was found in whole flour while the minimum value (23 µg/kg) was registered in debranned flour. The mean of contamination was 630.53 µg/kg.
Conclusions. According to results obtained, incidence and contamination levels of DON, seem to be a serious problem for public health. Therefore, cereal and cereal based foods should be controlled for the presence of toxins, storage conditions and moisture content, which is considered a major factor in the growth of the *Fusarium* fungi.

Key words

Deoxynivalenol, wheat flours, ELISA, *Fusarium graminearum*

INTRODUCTION

The major mycotoxins that can occur in cereal grains and cereal-based products are *Fusarium* (deoxynivalenol, T-2, nivalenol, fumonisins and zearalenone), *Aspergillus* or *Penicillium* (aflatoxins and ochratoxin A) mycotoxins [1]. *Fusarium* mycotoxins are the largest group of mycotoxins, which includes more than 140 known metabolites of fungi [2]. DON is a naturally-occurring mycotoxin mainly produced by *Fusarium graminearum* [3, 4]. The physico-chemical properties of DON are shown in Table 1 [2]. DON is reported to be a very stable compound, both during storage, milling, and the processing and cooking of food, and does not degrade at high temperatures; it also binds to the ribosomal peptidyltransferase site and inhibits protein and DNA synthesis; consequently, exposure results in decreased cell proliferation [5, 6]. The US Food and Drug Administration (FDA) has set advisory DON levels for wheat-based foods and feeds of no more than 1,000 µg/kg in finished human foods, 10,000 µg/kg in poultry and ruminant feed, and 5,000 µg/kg in other animal feeds [7]. Gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS), high-pressure liquid chromatography (HPLC), thin-layer chromatography (TLC), and enzyme linked immunosorbent assay (ELISA) are the most common techniques for detecting DON in cereals and cereal-based products. Therefore, the differences between these techniques may affect the results of different studies carried out by different investigators [4].

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Table 1. Physico-chemical properties of deoxynivalenol

Property	Information
Name	Deoxynivalenol (DON), vomitoxin
IUPAC name	12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8on
Molecular formula	$H_{15}O_{20}O_6$
Molar mass	296.32 g/mol
Physical state	Colourless fine needles
Boiling Point (°C)	543.9 ± 50.0 °C
Melting Point (°C)	151–153 °C
Flash Point (°C)	206.9 ± 2.5
Soluble in:	polar organic solvents (e.g., methanol, ethanol, acetonitrile, chloroform, ethyl acetate) and water

The areas of Guilan, Golestan and Mazandaran are located in north Iran, with favourable conditions for *Fusarium* growth. The presented research was conducted to determine the DON contamination of wheat flours in Guilan province.

MATERIALS AND METHOD

Preparation of samples. A total of 96 wheat flour samples of different types were collected during summer 2013 from 6 flour mills in the Guilan province of northern Iran. Water suspension of each sample was prepared by adding distilled water to 10 g of the wheat flour samples. The suspension was filtrated through No.1 Whatman filter paper, and 100 µl (per well) of this solution was used in the test.

ELISA test procedure. Before starting the test, the reagents were brought up to room temperature. 100 µl of each filtrated sample and DON standard solutions including 0, 3.7, 11.1, 33.3 and 100 ppb was dropped in each micro-titer well which were coated with capture antibodies directed against anti-DON antibodies. peroxidase conjugate DON was then added, and monoclonal anti-DON antibody and substrate (tetramethylbenzidine) added according to the manufacturer's recommendation (R-Bbiopharm kit, Germany). The optical absorbance of each well was read at 450 nm with ELISA-ReaderELISA reader; DA 3200ELISA reader; DA 3200ELISA reader; DA 3200. Absorbance percentages were taken to the calibration curve, performed with standards at different concentrations. Statistical analyses were performed using SPSS software.

RESULTS

A total of 96 wheat flour samples were analyzed with competitive ELISA. Of the 96 samples analyzed, 80 samples (83.33%) were found to be contaminated with DON (Tab. 2); the range of contamination was 23–1270 µg/kg. Mean DON level in positive samples – 630.53 µg/kg.

Table 2. Occurrence of DON in wheat flour samples in northern Iran

DON levels µg/kg	No. of samples	(%)	Mean ± SD (µg/kg)	Range (µg/kg)
Not Detected	16	16.66	-	-
<300	18	18.75	154.07±12.41	23–300
301–600	23	23.96	448.36±21.17	309.2–596.2
601–1000	26	27.08	777.23±27.88	604.5–998
≥ 1000	13	13.54	1142.46±33.80	1005.2–1270
Total	96	83.33	630.53±25.11	23–1270

Based on the different types of wheat flours (Debranned, Null, Star and Whole) in the flour mills, the maximum contamination level of DON (1,270 µg/kg) and the mean of 742.77 µg/kg was found in whole flour, while the minimum value (23 µg/kg) with a mean of 377.62 µg/kg was found in debranned flour (Tab. 3).

Table 3. Occurrence of DON in different types of wheat flour samples

Type of Flour	No. of samples	No. of positive samples	(%)	Mean ± SD (µg/kg)	Range (µg/kg)
Whole	24	24	100	742.77±27.25	205.5–1270
Star	24	20	83.33	673.28±26.06	148–1251.5
Null	24	19	79.17	525.26±22.92	50.5–1031.5
Debranned	24	17	70.83	377.62±19.43	23–1086.5
Total	96	80	83.33	581.23±24.11	23–1270

Based on the different types of flour mills in northern Iran, the flours of mill D with a contamination level of 93.75%, were the most contaminated product with toxin DON (mean contamination level – 717.45 µg/kg), and flour of mill C with a contamination level of 68.75%, mean – 503.55 µg/kg, had the least amount of toxin contamination. The flours of mills A, B, E and F also had contamination levels of 87.5%, 81.25%, 81.25% and 81.25%, respectively, with mean contamination levels of 696.23 µg/kg, 550.86 µg/kg, 515.56 µg/kg and 597.12 µg/kg, respectively (Tab. 4).

Table 4. Occurrence of DON, based on different types of flour mills

Type of flour mill	No. of samples.	No. of positive samples	(%)	Mean ± SD (µg/kg)	Range (µg/kg)
A	16	14	87.5	696.23±26.39	111–1193.3
B	16	13	81.25	550.86±23.47	50.5–1112.5
C	16	12	75	503.55±22.44	23–1053
D	16	15	93.75	717.45±26.79	105.4–1270
E	16	13	81.25	515.56±22.71	58.4–1251.5
F	16	13	81.25	597.12±24.44	63.2–1163
Total	96	80	83.33	596.81±24.43	23–1270

DISCUSSION

Mycotoxins are secondary metabolites produced by fungi, mainly by species from the genus *Fusarium*, *Penicillium* and *Aspergillus* [8]. There is increasing worldwide awareness of the serious consequences that undesirable levels of mycotoxins may have on human and animal supplies, such as mutagenic, carcinogenic, teratogenic and estrogenic effects [9]. Deoxynivalenol is the most commonly detected trichothecene in cereal grains and the one found at the highest concentrations [10]. Several surveys were performed in order to determine the DON levels in cereals and cereal-based products. In Serbia, of the 139 samples (76 maize, 16 wheat, 24 soybean, 19 sunflower and 4 barley) analyzed, 52 samples (37.41%) were found to be contaminated with DON; range of contamination – 0.04–2.46 µg/g [11]. Schollenberger *et al.* detected DON contamination in 59 (98%) of 60 wheat flour samples and a mean of 199 µg/kg in southwest Germany [12]. In a study by Mirabolfathy and Karami, DON was detected in 44.97% of wheat samples collected from the Golestan province of northern Iran, at levels from 18.53–192.81ng/g, with an average level of 40.99 ng/g. Also, 78.36% of Golestan›s barley samples were contaminated with DON, with an average level of 57.60 ng/g, and at levels from 15.19–280.6 ng/g [9]. In Poland, Kuzdralinski et al, found DON in 100% of beer samples, with a range of 6–70.2 mg/L [13]. In a study by Zhao et al, 40 soy sauce samples, including 35 domestic and 5 imported soy sauces, were investigated for the presence and contents of DON by using GC/MS; 97.5% of samples investigated were contaminated with DON (range 4.5–1245.6 µg/l, average value 141.5 µg/l) [14]. In Spain, Osnaya et al. found DON in 28% of bread samples with a mean of 42.5 µg/kg [15]. Omurtag and Beyoglu, analyzed a total of 50 beer samples in Turkey by HPLC, observing that none of them were contaminated by DON [16]. Karami et al. examined 60 corn samples collected in 2004–2005 from Golestan and Ardabil Provinces in Iran, and found that 76.6% of samples were contaminated with DON; the range of contamination was 54.4–518.4 ng/g. [17]. In Morocco, of the 81 samples of durum wheat analyzed, 11.1% were contaminated with DON, the range of contamination was 65–1,310 µg/kg [18]. Mishra et al, analyzed a total of 100 wheat, maize and barley samples in India by HPLC. DON was detected in 30% samples with a range of 0.01–4.73 mg/kg [19]. In Tunisia, Bensassi et al. found DON in 83% durum wheat samples with a range of 12.8–30.5 µg/g [20]. In a study by Setyabudi et al, 50 samples consisting of 24 maize kernels and 26 maize-based food products, obtained from a retail market in Yogyakarta, Indonesia, were analysed for DON using HPLC combined with ultraviolet detection after immunoaffinity column (IAC) clean-up process. All analyzed samples contained DON ranging between 47–348 µg/kg with median and means of 111 and 124.6 µg/kg, respectively [21].

CONCLUSIONS

In the presented study, a total of 96 samples of wheat flour were collected during the summer of 2013 from 6 flour mills in northern Iran. All samples were analyzed for DON by enzyme-linked immunosorbent assay. DON was detected in 83.33% of the samples tested, at levels ranging from 23–1,270 µg/kg. 13 samples (13.4%) failed to reach the desired level of the US Food and Drug Administration (FDA), defined as 1,000 µg/kg in finished foods for human consumption. According to the results obtained, the incidence and contamination levels of DON seem to be a serious problem for public health. Therefore, cereal and cereal based foods should be controlled for the presence of toxins, storage conditions and moisture content, which is considered a major factor in the growth of the *Fusarium* fungi.

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