Deoxynivalenol and T2 toxin content in wheat and bread from different Transilvania Region

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Abstract
The toxins produced by Fusarium fungi that contaminate cereals are a serious concern. The most important and broad family of fusariotoxins are trichothechenes, which comprise several components divided into 4 groups, with types A and B being the most significant. In order to determine the level of DON and T2 toxin, we collected a number of 25 samples, of which 10 were bread and 15 wheat, all of them were collected from the Transylvania region. In wheat samples, DON was identified in all 15 samples analyzed, with values between 33-2225 µg / kg, with an average value of 672.6 µg / kg and with a median of 372 µg / kg. Of these, 4 samples exceeded the maximum limit imposed by European legislation of 1250 µg / kg for DON, the maximum value being 2225 µg / kg. In the bread samples, the presence of DON was found in 8 out of 10 samples analyzed (80%) with values not exceeding the limits of European legislation of 500 µg / kg, between 0-321 µg / kg. Regarding the T2 toxin content, it was identified in only 2 of the samples analyzed (8%), a wheat sample and a bread sample, with values of 7 µg / kg and 5 µg / kg, these values not exceeding European standards. The results obtained by us show a high DON contamination at the level of wheat grains harvested from different counties of Transylvania, some of the values not respecting the European standards, but with low values recorded in bread samples. Regarding T2 mycotoxin, this was detected only in 2 samples, with low values and for this reason do not consist a high risk for human health.
Keywords*: wheat, bread, deoxynivalenol, T_2 toxin*

Introduction

There are a number of mycotoxins that belong to the trichothecene chemical compound family. Most of these (T2 toxin, deoxynivalenol) are derived from one or more species of *Fusarium*.

One of the most studied trichothecenes, T2 toxin, apparently binds to cell membrane receptors, decreases both RNA and DNA production, and interferes with protein synthesis by blocking the initiation of translation. The chemical effects of this toxicosis are epithelial necrosis – both dermal and mucosal - severe enteritis, vomiting in some animals, coagulopathy, hematopoietic depression and leukopenia with secondary septic status.

According to the FAO, an allowable limit of 1 µg / kg / body weight and 0.06 µg / kg / body weight was set for T2 toxin and HT2 toxin. It should be noted that these data are valid only for European regions, so that more information and analytical methods are needed for other parts of the world (Van der Westhuizen et al, 2002). Following a study in the Netherlands, a concentration limit of 129 µg / kg DON was proposed in wheat-based products for children as the maximum dose limit. The researchers also pointed out that between September 1998 and January 2000, due to increased concentrations of DON in wheat, the dietary intake of DON has exceeded the permitted level, which leads to the negative effects on health.

Despite data on lack of consumption in some countries and little information on children and baby food, the researchers pointed out that among cereals, maize has the highest level of trichothecene contamination, and wheat and wheat products (bread and pasta) is the major source of contamination.

Materials and methods

For the determinations we collected a number of 25 samples, of which 10 samples of bread and 15 samples of wheat. All analyzed samples were purchased from Transylvania region.

Sampling and preparation were performed according to the standardized methodology. As for the wheat, it was stored either in bulk or in sacks. For sampling the bulk wheat samples, the following method was used: an imaginary division of the lot into an approximately equal number of parts was made. A certain number of parts were randomly selected; corresponding to the number of partial or elementary samples, at least one sample was taken from each part. The next step was to mix and homogenize the partial (elementary) samples, thus obtaining the general sample, and from this, using the reduction, by the method of quarters, an average sample was obtained, with a mass of about 100 grams for laboratory analysis. The samples were packed in new nylon bags, followed by labeling and delivery to the laboratory for recordings and the actual examinations.

For laboratory tests we used the RIDASCREEN®FAST Mycotoxin test, a competitive enzyme-linked immunosorbent assay, based on the antigen-antibody reaction, for the quantitative determination of mycotoxins in bread and cereals. An ELISA photometer was for data interpretation.

The samples were kept in a cool place, protected from light. For T2 toxin determination, were weighed 5 g of ground sample into a vessel and dissolve in 25 ml of methanol / distilled water (70/30), then mix using the magnetic stirrer for 10 minutes. The extract was filtered with a filter paper. 50 µl of the filtered sample was diluted with 300 µl of buffer for diluting the sample 1: 7 (1 + 6), dilution factor 35. From the mixture obtained, 50 µl per well was used. A required number of wells for standards and samples were inserted into the support and then 50 µl of standard or sample was added. 50 µl of conjugate was added to each well followed by 50 µl of anti-toxin T2 antibody for each well. It was stirred by rotating the plate and left in the incubator for 1 hour at room temperature in the dark. After the time expired, the liquid was discarded, tapping the plate face down on absorbent paper to remove traces of liquid, and 250 µl of distilled water was added, discarding the liquid again. This washing step was repeated twice according to the protocol requirement. Further, 50 µl of substrate and 50 µl of chromogen were added to each well and mixed by rotating the plate, then incubated for 30 minutes at room temperature (20-25 ° C) in the dark. 100 µl of stop solution was added to each well, stirring gently by rotating the plate and measured at 450 nm from the air blank. It is important that the measurement does not exceed 10 minutes after the addition of the stop solution. The minimum limit of detection is 0.1 µg / kg (ppb). According to the implementation of the test, the detection limit is 3.5 µg / kg (ppb) T2 toxin in the grain or hayfield sample.

For DON determination, were weighed 5 g of ground sample into a pot with a lid and added 25 ml of distilled water to each of them and stirred vigorously for 3 minutes. The extract was filtered with a Whatman filter paper no. 1. A dilution was made for 100 µl of filtrate. From the mixture obtained, 50 µl per well was used. A required number of wells for standards and samples were inserted into the support and then 50 µl of standard or sample was added. 50 µl of enzymatic conjugate was added to each well followed by 50 µl of antibody to each well. It was mixed by hand by gently rotating the plate and left to incubate for 30 minutes at room temperature (20-25 ° C) after which the liquid was discarded and beaten vigorously face down on an absorbent paper to remove traces of liquid. 250 µl of distilled water was added and the liquid was discarded again. The washing step was repeated twice. 100 µl (2 drops) of substrate / chromogen was added to each well. Mixed by rotating the plate and incubated for 15 minutes at room temperature (20-25 ° C). 100 µl of stop solution was added to each well. It was mixed gently by rotating the plate and measured at 450nm from the air blank.

Results and discussions

In the wheat samples, DON was identified in all 15 samples analyzed (100%), with an average value of 672.6 µg / kg and with a median value of 372 µg / kg (table 1). Regarding the bread samples, out of the total of the 10 analyzed samples, the presence of DON was found in 8 samples (80%), average of 106.2 µg / kg, median of 82 µg/kg (table 1).

Out of the total of the 15 wheat samples, a number of 4 samples exceeded the values imposed by the European legislation of 1250 µg / kg for DON, the registered values being of 1530, 1650, 1485, respectively 2225 µg / kg.

Regarding the T2 toxin content, it was identified in only 2 of the analyzed samples (8%), respectively a wheat sample and a bread sample, with values of 7 µg/kg and 5 µg/kg, respectively. These values did not exceeding European standards (EC Reg. No. 1881/2006 and No. 1126/2007).

Table 1.

DON values found in wheat and bread samples

Sample type	Number of analyzed samples	Detected mycotoxin	Number of pozitive samples	Range	Average	Median
				µg/kg	µg/kg	µg/kg
Wheat	15	DON	15	33-2225	672.6	372
Bread	10	DON	8	0-321	106.2	82

Compared to other countries, following a study on bread and pasta in Spain in 2010, the occurrence of mycotoxins in bread was 28.0% and 2.6% for deoxynivalenol and T2 toxin, whereas in pasta, the occurrence for both toxins was higher, ranging from 9.3% to 62.7%. The average content of DON (42.5 µg / kg) in bread was lower than the content of mycotoxin T2 (68.37% µg / kg), while in pasta the content of DON (137.1% µg / kg) was higher (Gonzáles-Osnaya, 2011). A study from 2012 shows the presence of DON in wheat in Parana, Brazil. The occurrence of this mycotoxin was evaluated in a study conducted on 113 wheat samples from the northern and central / southwestern regions of the state of Parana, during the growing seasons of 2008-2009. DON was detected in 66.4% of samples at levels ranging from 206.3 to 4732.3µg / kg, many of the samples exceeding the estimated daily dose of 1,250µg / kg (Sifuentes dos Santos, 2013).

The presence of 7 major mycotoxins in wheat flour, purchased from supermarkets in Novi Sad, a Serbian city in the capital of Vojvodina, was determined in a study from May 2012. DON was the predominant toxin in all samples analyzed, followed by zearalenone (ZON), and T2 toxin, with a frequency of 33.3% and 26.7%, respectively. All samples complied with European / Serbian legislation, except for one sample that exceeded the maximum permitted DON level of 750µg / kg. However, DON doses were assessed to be close to the tolerated daily dose level for adults (Biljana, 2012).

Conclusions

The results obtained by us show a high DON contamination at the level of wheat grains harvested from different counties of Transylvania, with some values that exceed European standards, but with low values recorded in bread samples. Regarding T2 mycotoxin, this was detected only in 2 samples, with low values and for this reason do not consist a high risk for human health.

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