ANALYSIS OF DEOXYNIVALENOL IN CEREALS AND FOODS DERIVED FROM THEM, AT THE LEVEL OF IASI COUNTY

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Abstract

The paper presents information on deoxynivalenol contamination of cereals and food products derived from them, in Iasi County, in the 2015-2019 study interval. Data are presented on the incidence of deoxynivalenol, which has a negative influence on food safety, with a negative impact on the consumer's health. The study was conducted to assess the level of contamination of cereals and food products derived from them in Iasi County and to adopt European Commission regulations on maximum levels of mycotoxins in various raw materials and products for human consumption.The method used for the study is a direct competitive enzyme-linked immunosorbent assay (ELISA), which allows to obtain exact concentrations for the studied mycotoxin, expressed in µg / kg. The obtained results were within the maximum limits allowed by the current specific legislation.

Key words: mycotoxins, deoxynivalenol, cereals, cereal products, ELISA

Contamination with fungi and mycotoxins of food and feed is a major risk to consumer safety. The appearance of mycotoxins in food and feed can lead to acute intoxications, to high levels of contamination; it is necessary to mention that mycotoxins are toxic even in extremely low concentrations and have cumulative, immunosuppressive and immunotoxic effects in the human body.

Molds that can appear on cereals and products derived from them are mostly toxigenic, so they require special attention. Apart from the fact that their toxins act strongly, even in small quantities, there is the problem of the synergism of these toxins once they reach the higher organisms. It will not matter much how many µg / kg of a toxin have been found in an environment, but how many types of toxins that potentiate their effects (even in smaller quantities) are in it. Mycotoxin-induced conditions are called mycotoxicosis. (Suteanu E. *et al*, 1995)

Trichothecenes are a group of mycotoxins having the same basic structure. All these substances contain an epoxy group placed at C12-C13, which is responsible for their toxic activity, being classified into two major groups: macrocyclic and non-macrocyclic.

Macrocyclic trichothechenes are subdivided into types C and D. Type C (crotocin, baccharin) has an additional epoxy group at positions C7-C8 or C9-C10. Type D (satratoxin, roridine) contains a macrocyclic ring between C4-C15.

Non-macrocyclic trichothecenes are subdivided into types A and B, where type A appears to be more toxic than type B. The toxicity is due to the various radicals on the hydro- and lipophilic chains.

Type A includes: T-2 toxin, HT-2 toxin, diacetoxycirpenol (DAS), etc.

Type B includes: deoxynivalenol (DON), 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol (NIV), fusarenone-X (FUX identical to 4-acetylnivalenol) etc.

Trichothecenes - type B are mainly synthesized by *Fusarium culmorum* and *Fusarium graminearum*. Although *Fusarium* species (*F. roseum, F. graminearum, F. solani, F. oxysporium*) are not of major biotechnological importance, we must remember that they appear on cereals, fruits, vegetables, and their mycotoxins are particularly dangerous. The toxicity of trichothecans is due to their ability to inhibit protein synthesis (Sesan Tatiana Eugenia *et al*, 2009).

Deoxynivalenol (DON, vomitoxin) is a mycotoxin produced by many species of the genus *Fusarium (F. culmorum, F. graminearum, F. roseum, F. sporotrichioides and F. sambucinum*). The incidence of deoxynivalenol is associated with *Fusarium graminearum* (teleomorph *Gibberella zeae*) and *Fusarium culmorum* (teleomorph

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unknown), both fungi being important plant pathogens, found in cereals and other crops. It is synthesized and found on plant material and its presence depends a lot on temperature (6-24°C) and other climatic conditions favorable to its development (extremely humid environment). (Toth B. *et al*, 2006).

Deoxynivalenol causes general weakening of organisms, necrosis (gangrene) in various tissues (gastrointestinal wall, bone marrow, lymphatic tissue), changes blood parameters and attacks the immune system. In fact, the Scientific Committee on Food of the European Commission (EFSA) suggests that the toxicity of deoxynivalenol is due to the general effect on organisms and in particular the harmful effect on the immune system. Another problem lies in the synergistic effect of deoxynivalenol with other trichothechenes, from which it can practically not be separated, because they are synthesized simultaneously.

Fungal infection and deoxynivalenol production, simultaneously in the field, depend on weather conditions, being favored by low temperatures and high humidity. Contamination is most severe in fields where crop rotation is not applied (corn follows corn or corn follows wheat), especially if the previous crop has been infected. These fungi have two distinct growth cycles, with mold growing during the day when the temperature is high (21-25ºC), while toxins are produced during the night when temperatures drop. Although deoxynivalenol is among the least toxic trichothechenes, it is the most commonly detected worldwide, and its occurrence is considered to be an indicator of the possible presence of other and more toxic trichothechenes (Alexander N.J. *et al*, 2006).

In humans, DON can cause bleeding, septic arthritis, endophthalmitis, osteomyelitis, cystitis and brain abscesses, with an invasive or disseminated outbreak.

Cereals and cereal by-products are a major part of the human and animal diet. It has been estimated that up to 25% of the world's crops can be contaminated with mycotoxins. The relevance of mycotoxins to human / animal health has led the European Community to introduce maximum permitted limits in food and feed. In Romania, deoxynivalenol is present in wheat and triticale, in particular. Romanian wheat contains on average between 100-500 µg/kg mycotoxins (1.0-5.0 ppm), ie 10-50 times above the maximum limit allowed by the NOEL norms (NO EFFECT LEVEL - the limit up to which DON can be supported without negative effects) (Berca M., 2011). The tolerable daily dose (DZT) of deoxynivalenol is 1 µg/kg body weight.

The fungal and mycotoxin surveillance program for food and feed should be applied equally to farms producing plant substrates, grain reception bases, compound feed processing plants, livestock farms, and food manufacturing units. The placing on the market of food containing a contaminant in an amount considered unacceptable from the point of view of human health is prohibited. In addition, the level of the contaminant must be kept as low as possible by the use of good practices at all levels of production, processing, preparation, treatment, packaging, storage, transport or marketing of foodstuffs.

MATERIAL AND METHOD

The determinations were performed in the period 2015-2019, from samples of cereals and products derived from cereals, which were taken randomly, according to an objective sampling strategy, both from grain storage units and from retail units. of food products (supermarkets), from Iasi county.

Sampling was performed based on a pre-established working procedure, in accordance with Annex no. 1 of the EC Regulation no. 401/2006 which establishes the sampling modalities and the analysis methods for the official control of the mycotoxin content in food (*figure 1*).

Mycotoxins are unevenly distributed in a batch; therefore, all necessary measures are taken to ensure that the sample taken is representative of the batch. Therefore, it is necessary to take a large number of elementary samples from various places in the lot, according to the legislation (random sampling = several elementary samples by joining which the aggregate sample is formed). The incremental samples shall be taken from different places in the lot so that the aggregate sample is representative of the lot. The area intended for sampling or storage of those products must not expose the product to any risk of contamination or degradation. In the case of mycotoxins, it is essential that the sampling action be carried out correctly because it is impossible to establish a subsequent measure based on the analysis of a non-representative sample.

The global sample must be thoroughly mixed but must not be ground before splitting the sample into laboratory samples. The aggregate sample shall be clearly labeled and sealed at the place of sampling. It must reach the laboratory as soon as possible, sealed and stored in an opaque bag / container (because mycoxins are destroyed under the influence of ultraviolet rays or natural light).

In the case of samples taken during the storage-marketing stage, these being pre-packaged in packages ready for sale, the product

is not removed from the original packaging until it is to be analyzed in the laboratory.

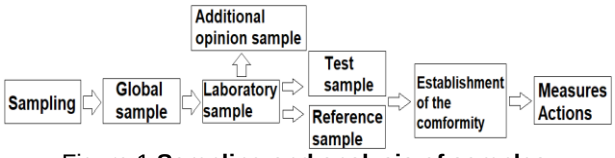

Figure 1 **Sampling and analysis of samples**

The samples that arrive at the laboratory for analysis are each divided into three distinct samples: the test sample, the additional opinion sample and the reference sample. All these are obtained only in the laboratory, being separated from the previously homogenized laboratory samples.

The sample for additional opinion may be kept by the laboratory or operator. If the sample for additional opinion is kept in the laboratory, the storage time is:

-until the issuance of the analysis bulletin, in case of compliant products;

-1 year when the evidence is non-compliant.

The reference sample is kept in the laboratory for the following period:

-until the release of BA in the case of compliant products:

-1 year when the evidence is non-compliant.

The establishment of the conformity of the samples is made on the basis of the results obtained following the laboratory analysis, to which the correction for recovery and the degree of uncertainty of the measurement are applied, in accordance with the provisions of Reg. CE 401/2006.

Acceptance of lots:

-The test is compliant if the value obtained after performing the laboratory analysis is less than or equal to that provided in EC Regulation no. 1881/2006 with the subsequent completions and modifications.

-The test is non-compliant if the value obtained after performing the laboratory analysis is higher than the one provided in the EC Regulation no. 1881/2006 establishing maximum levels for certain contaminants in foodstuffs.

-The lot is declared accepted if the analyzed sample is compliant.

During the study period (2015-2019) a total number of 118 samples were taken and analyzed consisting of cereals (wheat, corn) and products derived from cereals (wheat flour, corn, bread, pasta, biscuits). The product samples taken for the quantitative determination of the deoxynivalenol content were analyzed based on the specific RIDASCREEN DON determination kit, and the ELISA photometer was used to interpret the results.

Sample preparation: cereals, cereal products - extraction, filtration.

Detection limit: cereals, bakery products - 18.5 ppm.

Reproducibility: 85-100%.

Principle of the test: the test is based on the antigen-antibody reaction. The plate wells are labeled with anti-DON antibodies. Add standards or samples, enzyme conjugate and anti-DON antibodies. Free deoxynivalenol and the enzyme conjugate compete for antibody binding sites (competitive enzyme-linked immunosorbent assay). At the same time, deoxynivalenol antibodies are bound to immobilized antibodies. The unbound enzyme conjugate is removed by the washing step. Add the substrate-chromogen mixture to the wells and leave to incubate; the bound enzyme conjugate will convert the colorless chromogen into a blue substance. Adding the stop reagent will cause the color to change from blue to yellow. The measurement is made spectrophotometrically at 450 nm (optional wavelength> 600 nm). The absorbance is inversely proportional to the concentration of deoxynivalenol in the sample.

Equipment used: microELISA photometer (450nm), mill, shaker, graduated cylinder: 100ml, 1l, funnel and filter paper Whatman nr. 1, graduated pipettes, micropipettes: 50µl, 100µl, 1000µl.

Reagents: distilled water.

Samples preparation:

Samples should be kept in a cool place, protected from light. The representative sample must be ground and thoroughly homogenized before starting the extraction procedure.

Working procedure:

-weigh 5g of ground sample and transfer to a container with a lid over which 25ml of distilled water is added. The sample size can be increased if necessary, but the volume of distilled water must be adapted accordingly (for example: 25 g of sample in 125 ml of distilled water or 50 g of sample in 250 ml of distilled water);

- shake vigorously for 3 minutes (manually or with a stirrer);

-filter the extract using Whatman filter paper no.1;

-50µl of extract per well is used, in the test.

Protocol:

- Before starting work, bring all reagents to room temperature.

-Insert a required number of wells for standards and test samples into the holder.

-Add 50l of standard sample.

-Add 50 µl of enzyme conjugate to each well.

-Add 50µl of antibody solution to each well.

-Mix manually by rotating the plate and incubate for 30 minutes at room temperature (20-25°C).

-Pour the liquid and beat it vigorously face down on an absorbent paper to remove traces of liquid.

-Add 250µl wash buffer and remove the liquid again. Repeat the washing step 2 times.

-Add 100µl (2 drops) of substrate / chromogen in each well. Mix by hand, rotating the plate and incubate for 15 minutes. at room temperature (20-25°C).

The average value of the absorbents obtained for standards and samples are divided by the absorbance value of the first standard and multiplied by 100. The zero standard is equal to 100% and the absorbent values are expressed as a percentage:

$$\frac{abs.\ standard}{absorbanta} \times 100 = \%\ abs.\ zero\ standard.$$

The values calculated for the standards are entered in a coordinate system on semi-logarithmic millimeter paper with respect to the deoxynivalenol concentration expressed in μg/kg. The deoxynivalenol concentration corresponding to the absorbance of each sample can be read using the calibration curve.

To obtain the mycotoxin concentration in the sample in μg/kg, the concentration read from the calibration curve must be multiplied by the corresponding dilution factor (for cereals and cereal products its value is 5).

RESULTS AND DISCUSSIONS

Deoxynivalenol is one of the most common fusariotoxins that contaminates feed and food, resulting in food poisoning in animals and humans.

EC Regulation no. 1881/2006 establishes the maximum allowed levels for some contaminants in food products. According to this Regulation, the maximum permitted level of deoxynivalenol in foodstuffs is:

-500 μg / kg for bread and bakery products;

-750 μg / kg for wheat flour, pasta, cereal flakes and breakfast cereals;

-1250 μg / kg for wheat grain;

-1750 μg / kg for grain corn.

In *table 1* are presented the analytical results of the analyzed samples which registered positive values, obtained in the study interval 2015-2019, for the characterization of deoxynivalenol from cereals and food products derived from them.

Table 1

Analytical results of the analyzed samples

Nr. crt.	The sample analyzed	Total number of samples analyzed	No. of negatives samples	No. of positives samples	The value of the positive sample analysis (μg/kg)	LMA (μg/kg) according to Reg. CE 1881/2006
1.	biscuits	2	1	sample no. 1	43.8	**500**
	breakfast cereals	5	1	sample no. 1	25.0	**750**
				sample no. 2	26.3	
				sample no. 3	27.0	
				sample no. 4	57.0	
2.	pretzel	3	0	sample no. 1	31.15	**500**
				sample no. 2	128.0	
				sample no. 3	230.71	
3.	wheat flour	12	3	sample no. 1	21.0	**750**
				sample no. 2	22.0	
				sample no. 3	23.0	
				sample no. 4	82.0	
				sample no. 5	82.0	
				sample no. 6	136.0	
				sample no. 7	242.32	
				sample no. 8	347.35	
				sample no. 9	491.0	
4	cereal flakes	6	2	sample no. 1	39.0	**750**
				sample no. 2	60.0	
				sample no. 3	70.0	
				sample no. 4	70.0	
5.	wheat grains	17	16	sample no. 1	636.0	**1250**
6.	white bread	26	13	sample no. 1	25.0	**500**
				sample no. 2	25.0	
				sample no. 3	26.2	
				sample no. 4	38.0	
				sample no. 5	43.83	
				sample no. 6	51.0	
				sample no. 7	54.1	
				sample no. 8	99.0	
				sample no. 9	124.0	
				sample no. 10	140.0	

				sample no. 11	161.24	
				sample no. 12	226.36	
				sample no. 13	295.0	
7.	pasta	21	1	sample no. 1	26.0	**750**
				sample no. 2	26.0	
				sample no. 3	30.0	
				sample no. 4	31.2	
				sample no. 5	37.77	
				sample no. 6	38.72	
				sample no. 7	40.0	
				sample no. 8	47.0	
				sample no. 9	57.9	
				sample no. 10	68.0	
				sample no. 11	68.0	
				sample no. 12	68.0	
				sample no. 13	74.0	
				sample no. 14	94.0	
				sample no. 15	152.0	
				sample no. 16	206.69	
				sample no. 17	213.0	
				sample no. 18	294.94	
				sample no. 19	318.0	
				sample no. 20	424.0	
8.	corn grains	26	15	sample no. 1	41.0	**1750**
				sample no. 2	69.0	
				sample no. 3	114.0	
				sample no. 4	157.92	
				sample no. 5	170.49	
				sample no. 6	252.27	
				sample no. 7	324.2	
				sample no. 8	324.2	
				sample no. 9	324.2	
				sample no. 10	324.2	
				sample no. 11	446.96	
Total samples		**118**	**52**	**66**		

CONCLUSIONS

Mycotoxin contamination is favored by the humid continental temperate climate specific to our country and implicitly to Iasi County, this aspect having negative effects on the productivity and quality of crops.

In the studied period (2015-2019) a total number of 118 samples were analyzed, of which 52 samples representing a percentage of 44% did not register values for the deoxynivalenol parameter. A number of 66 samples, representing approx. 56% of all those studied presented positive values for this mycotoxin, which is a considerable percentage. None of the samples analyzed for the determination of deoxynivalenol did not exceed the maximum permitted limit provided by the legislation in force (EC Reg. 1881/2006).

The general conclusion is that, based on the results of the analyses performed for the selected products taken from the entire food chain, there is a potential contamination with deoxynivalenol. Apart from the particular regional circumstances, the general situation is characterized by widespread contamination at low levels, leading to risks to human health related to chronic exposure. At the same time, the synergistic toxicity of mycotoxins encountered simultaneously, especially in cereals, should not be ignored.

The maximum levels for mycotoxins, permitted by the legislation in force, shall be set at a level which takes into account human exposure in relation to the tolerable dose of the toxin in question and which can reasonably be achieved by observing good production practices and hygiene in all the stages of production, storage, processing and distribution of agricultural products and food derived from them. This approach ensures that food business operators apply all possible measures to prevent / limit as far as possible mycotoxin contamination in order to protect public health.

Deoxynivalenol is the most common contaminant of cereals and cereal products, its presence being a potential indicator of contamination with other mycotoxins. Increased attention should be paid to all sources of deoxynivalenol contamination in order to minimize its presence in food, in order to remove the subsequent negative impact on human health.

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