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STUDY REFERRING TO THE APPEARANCE OF CONTAMINATION WITH DEOXYNIVALENOL IN GRAINS, GRAIN FLOUR AND BAKERY PRODUCTS ON THE ROMANIAN MARKET

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Abstract

The increase of the agricultural surfaces cultivated with cereals that came to represent ~ 50% of the total areas destined to the agricultural activity, as well as the last years' climatic changes mainly associated with the global heating which caused the increase of the temperature and humidity during the period of cereal harvest (in particular of wheat) required that monitoring of deoxynivalenol contamination would be a permanent concern in order to ensure the health of both, the human and animal population. In this study, the quantitative determination of deoxynivalenol contamination was pursued on a number of 584 samples, represented by unprocessed cereals, cereal flour, bread and bakery products, breakfast cereals and pasta. In the analysed samples, by the immuno-enzymatic technique and / or high performance chromatographic liquid, there were no registered values higher than the maximum level allowed for this parameter provided in Reg. EC no. 1881/2006 with the subsequent modifications and completions, but it was visible (even a doubling) of the deoxynivalenol contamination of the samples analysed in the year under test during which there were more abundant precipitation and increased temperatures in the wheat harvesting period. Following the study, it was found that the analysed samples correspond to the legislative requirements for the deoxynivalenol contaminant, and this parameter is permanently monitored in order to comply with the incident legislation.

Key words: *deoxynivalenol, food safety, contamination.*

INTRODUCTION

Cereals, cereal flour and bakery products are a staple food and represent about 45% of the world's energy source, which is why about half of the planet's arable area is cultivated with cereals. By their nature, cereals and products derived from them (cereal flour, breakfast cereals, flours, breads, bakery products) have a high risk of contamination with mycotoxins (deoxynivalenol, aflatoxin, ochratoxin A, etc.). The present study will address the evolution of deoxynivalenol contamination in the same geographical areas from our country but during different time periods (two consecutive years).

Contaminants are represented by any substance that is not intentionally added to food, which are present in them as a result of their production, manufacture, processing, preparation, treatment, packaging, packaging, transport or handling or as a result of environmental contamination. Foreign matters, such as insect fragments, animal hair, etc. are not included in this definition. (Codex STAN 193/2005).

The Codex Alimentarius definition of a contaminant implicitly includes natural toxins, including toxic metabolites of certain fungi that are not intentionally added to food and feed, respectively mycotoxins (Codex STAN 193/2005).

Mycotoxins

Mycotoxins are metabolism products of several mold species, the most commonly involved species being those of the genus *Aspergillus, Penicillium* and *Fusarium* which can develop on different nutritional substrates and under varied climatic conditions.

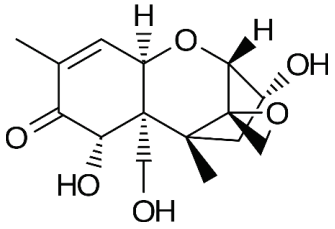
Mycotoxins may develop during cultivation, transport, storage or at other times during production. The end result is that they are found in many foods (especially those based on cereals). (Goran & Crivineanu, 2016).

The appearance of mycotoxins is influenced by numerous biological, environmental and harvesting factors. The most important biological factors are even represented by the plans susceptible to contamination with different mold species. Environmental factors need to be looked at considering two aspects, as well in the field, when we are talking about temperature, humidity, different biological vectors, as storage, when we talk especially about maintaining the products in optimum temperature and humidity conditions that prevent the occurrence of mycotoxin contamination. Harvesting factors are the represented by the harvesting method and they are very important for the harvesting period. As it is known, mycotoxin contamination varies from year to year, depending on the climate and other environmental factors. For example, contamination with deoxynivalenol is associated with rainy years and high temperature during the harvesting period which is subsequently reflected in increased contamination not only of cereals, but also of products obtained from them (flour, breakfast cereals, pasta, bread and bakery products).

Deoxynivalenol

Deoxynivalenol, also known as vomitoxin due to its effect on the body, is a type B trichothecene and it occurs mainly in cereal grains represented by wheat, barley, oats, rye and maize and rarely in rice, sorghum and triticale, being the most common contaminant of cereals and cereal products (Goran & Crivineanu, 2016).

The chemical formula of deoxynivalenol $C_{15}H_{20}O6$ is shown below:



Deoxynivalenol is a metabolism product, especially of the following species of molds: *Fusarium graminearum* and *Fusarium culmorum.*

Deoxynivalenol has been implicated in mycotoxicosis incidents in both, humans and farm animals, with the following clinical signs being most commonly described: decreased appetite, vomiting, intestinal transit disorders associated with immunodeficiency.

The regulations regarding the maximum allowed level of contaminants are represented at national level by observing the European legislation in force, respectively Reg. EC no. 1881/2006 with the subsequent modifications and completions (Table 1) and globally by the Codex Alimentarius regulations, respectively Codex STAN 193-1995.

Table 1. Maximum allowed level of Deoxynivalenol according to Reg. EC No. 1881/2006

Product type	Maximum allowed level µg/kg
Unprocessed cereals other than durum wheat, oats and corn	1250
Raw wheat and unprocessed oats	1750
Raw maize	1750
Cereals intended for direct human consumption, cereal flour, pasta	750
Bread (including bakery products), breakfast cereals	500
Dishes made from processed cereals and baby foods for infants and young children	200

Codex STAN 193-1995 has not yet set the maximum permitted level for deoxynivalenol contamination.

MATERIALS AND METHODS

In this study, it has been observed the quantitative determination of contamination with deoxynivalenol in cereals and different products derived from cereals for samples taken and analysed in an accredited laboratory.

During the period subjected to the study, number of 584 samples represented by the matrices defined in Reg. Ec 1881/2006.

At the reception of the samples the conformity of the sample, from the point of view of the quantity, with the batch from which it was taken, according to Reg. EC no. 401/2006.

The obtained results were compared with the maximum level allowed by Reg. EC no. 1881/2006 and expressed corrected for recovery and after that, reported depending on the extended uncertainty obtained after the validation of the analysis method.

The methods of analysis that were used were the immuno-enzymatic method and the liquid chromatographic method with DAD detector.

The minimum requirements that must be done for the analysis methods are the following:

- to allow the determination of mycotoxins at a level lower than the legal limit;
- the methods must be at least validated within the laboratory;
- the current trend is that all working methods have to be evaluated for accreditation.

The Immuno-enzymatic method (ELISA Enzyme linked Immunosorbent Assay)

It represents the method of detecting mycotoxin using an antigen-antibody complex. conjugated to an enzyme.

Benefits

It is a method of screening with high sensitivity that allows you to obtain, in a relatively short time, results for many samples and it does not involve a difficult stage of sample processing.

Disadvantages

The samples can be easily contaminated, false positive or false negative results can occur. Any possible result that does not comply requires a re-examination of the sample by a confirmation method (HPLC).

High performance liquid chromatography (HPLC)

High performance liquid chromatography has as a general principle the separation of the mixture of compounds by passing it through a stationary phase represented by a chromate-graphic column dedicated to the compound of interest, passing through the detector and issuing a characteristic signal transposed by a specific signal called 'peak chromatography'.

In order to use this technique to quantify deoxynivalenol contamination, the sample preparation stage is required by using the immunoaffinity columns which involve the passage of the extract by an immunoaffinity column coated with specific anti-deoxyniva-lenol antibodies, the stage in which antibody antigen complexes take place.

Benefits

This method has a high selectivity, being used as a confirmation method.

Disadvantages

Compared to the immune-enzymatic technique, it presents higher costs and specialized personal.

RESULTS AND DISCUSSIONS

The cereals and derived products are monitored from the point of view of contamination with deoxynivalenol at national level by applying the provisions of the Order of the President of ANSVSA no. 35/2016 with subsequent modifications and completions.

During the period under study, a number of 584 samples analysed during two years (2016 and 2017) were analysed, the samples came from the counties of Argeș, Botoșani, Brașov, Brăila, Bucharest, Călărași, Constanța, Dâmbovița, Dolj, Galați, Giurgiu, Gorj, Ialomița, Ilfov, Mureș, Olt, Prahova, Teleorman and Timiș. Because of the fact that Braila and Mureș counties are not found with samples analysed during two consecutive years, they will be eliminated from the statistical calculation (Table 2).

Table 2. Total number of samples analysed in the study

Year	No. of samples analysed	No. of samples analysed by ELISA	No. of samples analysed by HPLC
Year 1 - 2016	264	243	21
Year 2 - 2017	320	201	119

From the samples analysed by the two techniques it is noted that most of the samples were analysed by the immune-enzymatic technique, which has a higher sensitivity but cannot be used as confirmatory methods, therefore the results obtained by the HPLC method are generally expressed as undetectable because the limit of quantification of the method is much higher (157µg / kg by HPLC compared to 26.6 µg / kg by ELISA).

The centralized data, as a way of expressing ELISA vs HPLC results, are expressed in Tables 3 and 4.

Table 3. Expression of HPLC results Not detectable vs. Numerical values

Year	No. of samples analysed by HPLC	Results expressed as undetectable	Results expressed <limit of quantification of the method	Results with values
Year 1	21	21	-	-
Year 2	119	111	5 results <157µg/kg	3

Table 4. Expression of ELISA results Not detectable vs. Numerical values

Year	No. of samples analysed by ELISA	Results expressed as undetectable	Results expressed <limit of quantification of the method	Results with values
Year 1	243	163	4 results <20.6 µg/kg	76
Year 2	201	51	1 result <20.6 µg/kg	195

Following the statistical calculation, it is observed that in the second year (2017), the number of samples contaminated with deoxynivalenol increased, so that the number of samples at which values were recorded, but without exceeding the admitted level, doubled. In this way, in the first year (2016) values were registered at a percentage of 28.78% of the analysed samples, and in the following year, at a percentage of 61.87%, correlated with the meteorological conditions from year 2 of study.

In the first year of study (2016), a number of 112 unprocessed cereal samples were analysed, in 51 samples there were recorded values ranging from 21.52 µg / kg to 721.88 µg / kg in a barley sample, with an average contamination of 92.73 µg / kg.

In the second year of study (2017) a number of 152 cereal samples were analysed, a number of 85 samples were recorded values that ranged from 21.05 µg/kg to a maximum of 868 µg/kg in one sample wheat, with an average deoxynivalenol contamination of 226.24 µg/kg (Figure 1).

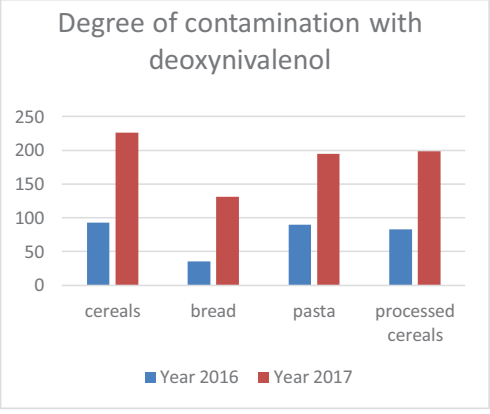


Figure 1. Graphic representation: contamination with deoxynivalenol of the different samples analysed

In 2016, a number of 86 bread samples, bakery products including biscuits and different products were analysed, in 12 samples there were values ranging from 23.31 µg / kg to 57.87 µg / kg in a bread sample, with an average contamination of 35.11 µg / kg. In the year 2017, a number of 97 bread samples were analysed, bakery products including biscuits and different products for a total of 36 samples were recorded values that ranged from 24.16 µg / kg to a maximum of 354.41 µg / kg in a bread sample, with a mean deoxynivalenol contamination of 131.49 µg / kg (Figure 1).

In the first year of study, there were analysed a number of 24 samples of processed products, especially pasta, in 4 samples there were values ranging from 28.23 µg / kg to 173.55 µg / kg in a sample of pasta, with an average contamination of 89.63 µg / kg.

In the second year of study, there were analysed a number of 32 samples processed products, especially pasta, at a number of 14 samples were recorded values ranging from 44 µg / kg to a maximum of 372.78 µg / kg at a sample of pasta, with an average deoxynivalenol contamination of 194.53 µg/kg (Figure 1).

In 2016, a number of 42 processed cereal samples were analysed – especially flour and breakfast cereals, at a number of 10 samples were recorded values ranging from 31.56 µg / kg up to a maximum of 172.37 µg / kg in a flour sample, with an average deoxynivalenol contamination of 82.84 µg / kg (Figure 1).

In 2017, a number of 39 samples represented by processed cereals, especially flour and breakfast cereals were analysed, at a number of 22 samples were recorded values that ranged from 59.79 µg/kg to a maximum of 453.64 µg/kg in a breakfast cereal sample, with an average deoxynivalenol contamination of 198.79 µg/kg (Figure 1).

CONCLUSIONS

From the point of view of deoxynivalenol contamination, all samples corresponded to the analysed parameter reported in Reg. What no. 1881/2006 with subsequent modifications and completions.

In 2017, there was not only a 100% increase in the number of samples analysed, but also an

increase in the level of deoxynivalenol contamination, as well as a doubling of the average level of contamination, mainly due to the weather conditions of the respective period.
There was noticed a correlation between the level of contamination in unprocessed cereals and the level quantified in the derived products, finding a decrease in the level of contamination following the different processing stages (milling, heat treatment, etc.) being directly proportional to the level of contamination.
Monitoring the level of contamination with deoxynivalenol should be continued taking into account the levels identified by deoxynivalenol, which in some cases are close to the maximum regulated level for taking the necessary legal measures.

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*** Commission *Regulation* (*EC*) No *1881/2006* of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

*** COMMISSION REGULATION (EC) No *401/2006 of 23 February 2006* laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs

*** Codex STAN 193-1995 *Codex General Standard For Contaminants And Toxins In Food And Feed.*