Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XIX, Issue 2- 2020, pag. 116 - 121

STUDY ON OCHRATOXIN A AND ZEARALENONE CONTENT IN CORN GRAINS FROM DIFFERENT AREAS OF BACAU COUNTY

***Sonia AMARIEI[1] , Alina MIHALCEA[1]**

[1]*Faculty of Food Engineering, "Stefan cel Mare" University, Suceava, Romania,* sonia@usm.ro, alina.filip@fia.usv.ro
*Corresponding author
Received 15th April 2020, accepted 28th June 2020

Abstract: *The purpose of the study is to evaluate the contamination with mycotoxins of the corn cultures in the county of Bacau, in correlation with the geographical and agro-climatic conditions of this area. Romania is one of the largest corn producers from the European Union and harvested almost 10746.4 thousand of tons in 2016, 14326.1 thousand of tons in 2017, 18353 thousand of tons in 2018, 16990 thousand of tons in 2019, over 1.5 million tons going into exports in countries both from the EU and outside. The main mycotoxins identified in the corn cultures in the county of Bacau are: Aflatoxins, Deoxynivalenol, Zearalenone, Toxin T2 and HT2, Fumonisins. The study aimed to determine the quantity of ochratoxin and zearalenone from corn kernels harvested in the county of Bacau, The identified mycotoxins do not exceed the maximum limits according to Reg. (CE) 1881/2006, which regulates the maximum limits for certain contaminants in food products.*

Keywords*: mycotoxins, corn, ochratoxin, zearalenone*

1. Introduction

Molds are biological agents that cause significant damage to cereal crops. The fungi that produce mycotoxins are divided into 2 groups: those that invade before harvest, called field fungi, and those that appear after harvest, called deposit fungi. Cereal contamination occurs in the field under the influence of agro-climatic conditions that are favorable to fungal attack and mycotoxin production. The appearance of extreme meteorological phenomena (high temperature, drought, heavy rains), non-compliance with agricultural technologies, negatively affects the quantity and quality of crops, favoring the appearance of fungi and the production of mycotoxins. *Ochratoxins* are part of the group of nephrotoxic mycotoxins and are produced by fungi belonging to the group of *Penicillium* and *Aspergillus*. Ochratoxin A is a phenylalanine derivative resulting from the formal condensation of the amino group of L-phenylalanine with the carboxy group of (3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-2-benzopyran-7-carboxylic acid (ochratoxin alpha).The main toxic components isolated in *A. ochraceus* strain extracts were Ochratoxin A and Ochratoxin B [1]. The optimal temperature for ochratoxin production on natural environments (corn, wheat, rice) varies between 20 and 28°C, and the optimum humidity of 18-19% [1].

Zearalenone is a resorcyllic acid lactone, with a chemical structure similar to steroid hormones, produced by fungi of the type *Fusarium* (*F. roseum, F. tricinctum, F. oxysporum, F. moniliforme, F. culmorum, F. graminearum*) [1]. Zearalenone contamination is closely related to

substrate moisture, drying and product storage techniques. Balzer et al. (1976) showed that the humidity of corn in 1974 was very high, it was strongly invaded by *Gibberella zeae* (*F. graminearum*), zearalenone being found in more than 50% of the analyzed samples [1]. The study verifies the corn crops in Bacau County regarding the content of Ochratoxin A and Zearalenone. 9 collection points were established for the investigation of the two mycotoxins: Onesti, Bacau, Racaciuni, Damienesti, Calugareni, Sascut, Buhusi, Margineni, Orbeni.

The ELISA test is a commonly used immunoassay method to rapidly monitor mycotoxins. It is commonly used by agri-food laboratories [2, 3, 4]. There are commercially available kits for all regulated mycotoxins; they contain microtiter ELISA plates that have well-defined applicability, analytical range and validation criteria [3, 5, 6]. There are several commonly available ELISA formats, but the predominant form is the competitive one [7]. The competitive format is characterized by the fact that the signal intensity is inversely correlated with the antigen concentration in the sample [8, 9]. The classic competitive format is to immobilize the antigen standard on the plate surface. Then, an incubation of the antibodies directed against the target mycotoxin with the sample takes place. The antigens in the sample will compete with those immobilized for binding to these antibodies. After the washing step, the analyte-bound antibodies are washed [8]. Direct detection uses a primary antibody labeled with an enzyme that reacts with the antigen, while a secondary antibody labeled with the enzyme is used for indirect detection of the primary antibody [9]. In the competitive inhibition format, competition occurs between unlabeled antigens in samples and enzyme-labeled antigens (enzyme conjugate) for binding to an antibody directed against the target mycotoxin. In this format, the plate can be coated with capture antibodies with affinity for the analyte or for a primary antibody [8, 10]. For both types of competitive assays it is similar to add a suitable substrate which is allowed to incubate so that the enzyme which has conjugated to the antibody or antigen (classical or inhibitory format, respectively) acts and produces changes in a given parameter [10, 11, 9]. The last step of all assays is to add a stop solution that causes the reaction between the enzyme and the substrate to stop. The signal strength weakens as the antigen concentration of the sample increases, as a higher amount of analyte results in either fewer enzyme-labeled antibodies bound to the antigen adsorbed on the plate (classical format) or fewer enzyme-labeled antigens bound to the antibody on the test plate [11, 8, 9].

The advantages of ELISA include, in addition to the specificity of antibody-antigen binding, a relatively low detection limit (LOD), a high sample yield with low sample volume, minimal cleaning procedures and ease of application [3, 5].

2. Matherials and methods

2.1 Materials

Bacău County is located in the eastern part of the country, occupying an area of 6621 km, about 2.8% of Romania's area. The climatic regime of Bacău County is an example of a gradual transition from the pronounced continental climate in the east to the moderate one in the west. Characteristic for Bacău County is the insular distribution of temperatures, conditioned by the specificity of the relief steps. The pedogenetic conditions led to the formation of various soils, on the territory of Bacau county, the brown and brown argiloiluvial soils predominated, which had humus content of 1-5%. They

ensure good average fertility for agricultural land.
Cereals are a group of cultivated plants belonging to the Poaceae family that includes: wheat, corn, rye, barley, rice, sorghum, millet, oats [12]. One of the most important grains is corn (*Zea mays*). It is a plant with a high stem, broad leaves, monoecious asexual flowers arranged separately: the male ones in terminal panicles, the female ones on a thick spadix. It will form corn cobs after fruiting [13].

2.2. Reagents

ELISA kits produced by ProGnosis Biotech Greece were used to determine Ochratoxin A and Zearalenone, which comply with the specifications of EN ISO 14675: 2003. Bio-Shield Ochratoxin A, B2448 / B2496, Bio-Shield ZON, B2748 / B2796 are immunoassay methods that determine Ochratoxin A, Zearalenone in grains, ears, grains and other products including food for animals. The ELISA kit contains all the reagents needed for the immunoassay method.
Other equipment used: Fritsch Pulverisette 14 rapid laboratory mill, Kern precision balance, PLT 2000-3DM, used for weighing (with a precision of 10^{-3} g) of the samples to be studied, Elisa Line consisting of: Biomerieux Tecan A-5082 washer Washer 430, Biomerieux Shaker 50X incubator, Biomerieux Reader 230S reader, used for homogenization, incubation, sample reading.

2.3. Methods

The Elisa test produced by ProGnosis Biotech is based on the principles of the linked enzyme immunoassay test. The wells of the microtiter strips are coated with mycotoxin-specific antibodies. The toxins are extracted from the ground sample with 70% methanol. Mycotoxin standards or samples and the conjugate (detection solution) are added to the lined wells. The conjugate binds to the binding sites of the lined antibodies that are not already occupied by the mycotoxin in standards or samples. Any unbound conjugate in the detection solution is removed in a wash step. A chromogenic substrate is added to the wells, which leads to the progressive development of a blue-colored complex with antibody detection. The development of the color is then stopped by the addition of acid, which turns yellow in the resulting final product. The measurement is made photometrically at 450 nm and the intensity of the colored complex produced is indirectly proportional to the mycotoxin concentration present in the samples and standards.

3. Results

3.1. Ochratoxina A

Table 1 presents the results of the tests performed for the identification and dosing of ochratoxin in the maize grains from the mentioned areas.

Undetectable values (<LOD) were recorded in all 10 samples taken to investigate the presence of Ochratoxin A. Each analysis was performed in triplicate.

3.2. ZEARALENONE

Zearalenone contamination is low in field grains and increases in storage conditions with humidity greater than 30% –40%. [14]. A tolerable daily intake for Zearalenone of 0.25 g/kg b.w./day was established by the European Food Safety Authority. [15] Currently, the limits for ZEN in cereals vary between countries and range from 50 to 1000 pg/kg [16].

Table1.

Ochratoxin A in corn grains from different areas of Bacau County

Samples	Place of sampling	LOD (µg/kg)	LOQ (µg/kg)	The result (µg/kg)	Maximum allowed limit (µg/kg)
1	Bacau	1.054	1.755	<1.054(undetectable)	5
2	Onesti	1.054	1.755	<1.054(undetectable)	5
3	Racaciuni	1.054	1.755	<1.054(undetectable)	5
4	Damienesti, Village Calugareni	1.054	1.755	<1.054(undetectable)	5
5	Damienesti	1.054	1.755	<1.054(undetectable)	5
6	Buhusi	1.054	1.755	<1.054(undetectable)	5
7	Margineni	1.054	1.755	<1.054(undetectable)	5
8	Sascut	1.054	1.755	<1.054(undetectable)	5
9	Sascut	1.054	1.755	<1.054(undetectable)	5
10	Orbeni	1.054	1.755	<1.054(undetectable)	5

LOD- limit of detection (the lowest quantity of a substance that can be distinguished from the absence of that substance with a stated confidence level
LOQ- limit of quantification (the limit at which the difference between two distinct values can be reasonably discerned)

The maximum limits allowed for ZEN should be in the range of 100-200 µg/kg in processed cereals, 75 µg/kg for processed cereals, 20 µg/kg in processed cereal foods and 50 µg/kg in cereal snacks according to the laws in EU [17]. Risk assessments were performed based on exposure to ZEN given in France, Germany, Finland, China and India. In only a few cases, it has been found that the possible ZEN contribution exceeds the TDI and almost all studies have agreed that the majority of the population does not exceed the TDI value given by the EU [18].

Table 2.

Zearalenone in corn grains from different areas of Bacau county

Sample no.	Place of sampling	LOD (µg/kg)	LOQ (µg/kg)	The result (µg/kg)	Maximum allowed limit (µg/kg)
1	Bacau	2.061	2.457	<2.061(undetectable)	350
2	Onesti	2.061	2.457	<2.061(undetectable)	350
3	Racaciuni	2.061	2.457	2.68	350
4	Damienesti, Village Calugareni	2.061	2.457	2.68	350
5	Damienesti	2.061	2.457	3.8	350
6	Buhusi	2.061	2.457	2.68	350
7	Margineni	2.061	2.457	<2.457(unquantifiable)	350
8	Sascut	2.061	2.457	<2.457(unquantifiable)	350
9	Sascut	2.061	2.457	14.13	350
10	Orbeni	2.061	2.457	<2.457(unquantifiable)	350

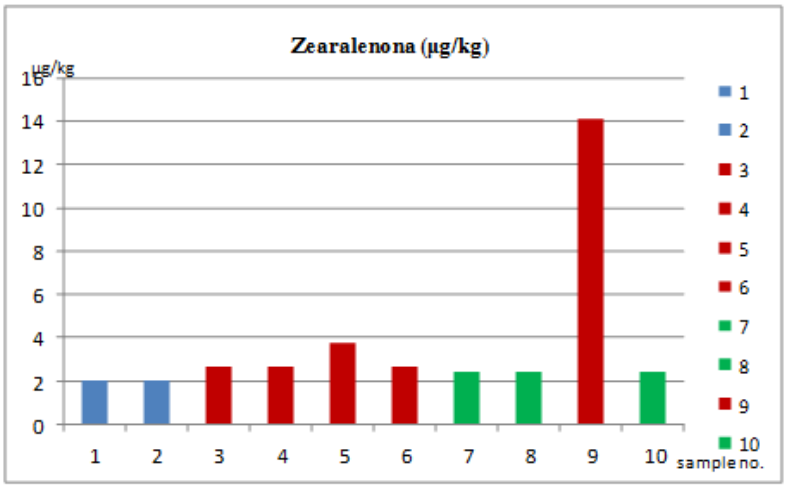

Fig.1. Zearalenone content in the tested samples

Analyzing the results (Fig. 1), we observe that 5 samples have undetectable (<LOD, represented by blue) and non-quantifiable (<LOQ, represented by green) values, 5 samples have numerical values (represented by red): 2.68 µg / kg for the sample collected from Racaciuni, 2.68 and 3.8 µg / kg for the sample collected from Damienesti, 2.68 µg / kg for the sample collected from Buhusi, 14.13 µg / kg for the sample collected from Sascut.

Zearalenone was present in 50% of the samples, in 50% of the analyzed samples and it was below the limit of detection, quantification (Fig.2).

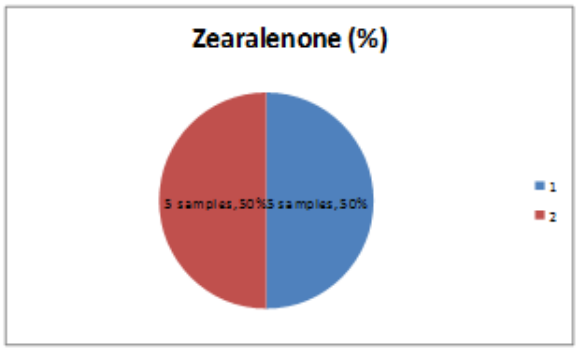

Fig.2. Share of samples in which Zearalenone was identified

4. Conclusions

The presence of both ochratoxins and zearalenones was found in the analyzed corn samples. The determined quantities were far below the limit allowed by the legislation. In 50% of the analyzed samples, these mycotoxins were below the limit of detection, quantification.

Considering the accentuated climatic changes, the pluviometric regime of the area and the fact that the analyzed raw material is a basic one in animal feeding, but also the basis for a large number of food products, the systematic analysis of the presence of mycotoxins and the factors that favor their appearance is absolutely necessary.

5. References

[1] COMAN I., Popescu O., Mycotoxins and mycotoxicosis, *Ceres*, 82-99, (1985).
https://www.madr.ro/culturi-de-camp/cereale/porumb.html
https://www.agro.basf.ro/ro/stiri/fermier-in-romania/cultura-porumb-rotatie-semanat-ingrijiretratamente-recoltare.html
https://www.prognosis-biotech.com/
[2] PEREIRA, V.L.; FERNANDES, J.O.; CUNHA, S.C. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. *Trends Food Sci. Technol*. 36, 96–136 (2014**)**
[3] XIE, L.; CHEN, M.; YING, Y. Development of Methods for Determination of Aflatoxins. *Crit. Rev. Food Sci. Nutr*. 56, 2642–2664 (**2016)**
[4] DZUMAN, Z.; ZACHARIASOVA, M.; LACINA, O.; VEPRIKOVA, Z.; SLAVIKOVA, P.; HAJSLOVA, J. A rugged high-throughput analytical approach for the determination and quantification of multiple mycotoxins in complex feed matrices. *Talanta* 121, 263–272 (**2014)**
[5] VENKATARAMANA, M.; CHANDRANAYAKA, S.; PRAKASH, H.S.; NIRANJANA, S.R. Mycotoxins Relevant to Biowarfare and Their Detection. *Toxinology* 1–22 (**2014)**
[6] ANFOSSI, L.; GIOVANNOLI, C.; BAGGIANI, C. Mycotoxin detection. *Curr. Opin. Biotechnol*. 37, 120–126 (**2016)**
[7] SANTOS PEREIRA, C.; C. CUNHA, S.; FERNANDES, J.O. Prevalent Mycotoxins in Animal Feed: Occurrence and Analytical Methods. *Toxins* 11, 290 (**2019)**
[8] ROBINSON, R.; PELLENZ, S. An Introduction to ELISA (Part 2). Available online: https://www.antibodies-online.com/resources/17/1464/an-introduction-to-elisa-part-2/.
[9] Bio-Rad Laboratories ELISA Basics Guide; Bio-Rad Laboratories: Kidlington, UK, 2017; pp. 1–40.
[10] Thermo Fisher Scientific Overview of ELISA. Available online: https://www.thermofisher.com/pt/en/home/life-science/protein-biology/protein-biology-learningcenter/protein-biology-resource-library/pierceprotein-methods/overview-elisa.html#2
[11] R-Biopharm AG. Good ELISA Practice Manual; *R-Biopharm AG: Darmstadt, Germany*, (2016)
[12] L. OPRICA, Food biochemistry, *Ed. Tehnopress*, 251-261, (2011)
[13] SAVA D, Botanical atlas, *North's Star*, 165-167, (2018)
[14] TOLA, M.; KEBEDE, B. Occurrence, importance and control of mycotoxins: A review. *Cogent Food Agric*. 2, 1–12 (**2016**)
[15] ÜNÜSAN, N. Systematic review of mycotoxins in food and feeds in Turkey. *Food Control 97*, 1–14 (**2019**)
[16] ZINEDINE, A.; SORIANO, J.M.; MOLTÓ, J.C.; MAÑES, J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol*. 45, 1–18 (**2007**)
[17] European Food Safety Authority. Scientific Opinion on the risks for human and animal health related to the presence of modeified forms of certain mycotoxins in food and feed. *EFSA J*. 9, 2197 (**2011)**,
[18] RAI, A.; DAS, M.; TRIPATHI, A. Occurrence and toxicity of a fusarium mycotoxin, zearalenone. *Crit. Rev. Food Sci. Nutr.* 59, 1–20 (**2019)**