ZEARALENONE OCCURRENCE IN CEREAL AND CEREAL-BASED FOODSTUFFS MARKETED IN TIMIS COUNTY

Petruta GALBENU[1], Lăcrămioara DAMIESCU[2], Alexandra TRIF[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 119, Calea Aradului, 300645 Timişoara, Romania
[2]Sanitary Veterinary and Food Safety Directorate Timiş, 4, Martir Caceu, 300585, Timişoara, Romania
E-mail: lavinia_galbenu@yahoo.com

Abstract: *Zearalenone (ZEA, F-2 toxin) is a known non-steroidal estrogenic secondary metabolite, mainly produced by F. graminearum and F. culmorum, which are common soil fungi, in temperate and warm regions. ZEA and its metabolites are frequently detected in cereal crops worldwide such as maize, barley, oats, wheat, rice, sorghum and also in breakfast cereals, bread and milk, thus, contaminating both the food and feed chain. ZEA is a resorcylic acid lactone and is well-known by its estrogenic activity in farm animals (pigs, cattle, sheep etc.) and occasionally is implicated in hiperestrogenic syndromes in humans. The present study aimed to screen for ZEA contamination different cereal and cereal-based foodstuffs purchased from local producers and supermarkets in Timi□ County, an area of western Romania. Hence, during a period of three years (2008-2010), a total of 125 commercially available foodstuffs, including small cereal grains (wheat, barley, maize), bread and related products (bakery, pastry), maize oil, breakfast cereals and other foods (biscuits, snacks), were investigated for ZEA presence and contamination levels using enzyme-linked immunosorbent assay (ELISA) commercially available kits. Although, the occurrence of ZEA varied during the three years of study from 6.25% in 2008 and 38.30% in 2009, to 53.33% in 2010, overall ZEA was detected in 37 samples, representing 29.6%, occurrence which was similar to other surveys. The values obtained ranged between 0.4 µg/kg found in barley, and 41.81 µg/kg found in wheat flour, and an overall mean and median value of 3.26 µg/kg and 1.82 µg/kg, respectively. However, none of the values exceeded the maximum allowed levels by the European Commission (EC) in the different commodities analyzed. Whereas most surveys focus on ZEA contamination of different crops (wheat, barley, maize etc.) this paper represents one of the first screenings on a variety of cereal and cereal-based foodstuffs marketed in an area of western Romania.*

Key words: *Zearalenone, occurrence, levels, foodstuffs, Timis County, Romania*

INTRODUCTION

It has been estimated by FAO (Food and Agriculture Organization) that worldwide approximately 25% of the crops get contaminated and are affected by molds and their secondary metabolites (mycotoxins), leading to considerable financial losses and impaired animal and human health. The exposure risk to human is either directly through foods of plant origin (cereal grains) or indirectly through foods of animal origin (kidney, liver, milk and eggs) (CAST, 1989; SCF, 2000).

Zearalenone (ZEA) or F-2 toxin as it was previously named, is a resorcylic acid lactone, (3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1H-2- benzoxacyclotetradecin-1,7(8H)-dione, $C_{18}H_{22}O_5$), and a known non-steroidal estrogenic secondary metabolite, mainly produced by *F. graminearum* and *F. culmorum*, which are common soil fungi in temperate and warm regions. The mode of action of ZEA and its derivatives involves displacement of estradiol from its uterine binding protein, revealing an estrogenic response (SCF, 2000; MARAGOS, 2010; ZINEDINE *et* al., 2007). ZEA is a stable compound both during storage/milling and the processing/cooking of food and it does not degrade at high temperatures (AZIZ et al., 1997; SCF, 2000; HELFERICH AND WINTER, 2001). Along with its metabolites, ZEA is frequently detected in cereal crops worldwide such as maize, barley, oats, wheat, rice, sorghum and also in breakfast cereals, bread and milk, thus, contaminating both the food and feed chain (BHAT et al., 2010; YAZAR and OMURTAG, 2008; CAST, 1989).

ZEA was discovered as the cause of a reproductive disorder in pigs known as vulvovaginitis (EDWARDS et al., 1987; MOSS, 2002), and is well-known by its estrogenic activity in other farm animals (cattle, sheep, etc.) and occasionally is implicated in hiperestrogenic syndromes in humans (GOLINSKY et al., 2010; HAGLER et al., 2001; MARAGOS, 2010). Moreover, α-Zearalanol (α-ZAL), also known as zeranol is used in some countries as a growth promoter in cattle due to its anabolic activity. Its use for increasing meat production in cattle is allowed in some countries, such as the USA, and forbidden in others, such as the countries of the European Community. Such differences in legislation in different parts of the world cause to difficulties in trade between such countries (CREPPY, 2002; MOSS, 2002). *In vivo* studies have showed that ZEA is rapidly metabolized in animals and humans. Free and conjugated forms of ZEA have been found in the milk of cows under experimental conditions. The fact that high concentrations of the toxin are required to produce such a response indicates that consumption of contaminated feed by dairy cows would not result in a risk to public health (WOOD, 1992).

A low acute toxicity after either oral or intraperitoneal administration in mice, rats and guinea pig (oral LD_{50} values of >4000 up to >20,000 mg/kg b.w.), was attributed to ZEA (KUIPER-GOODMAN et al., 1987; JECFA, 2000). Pigs and sheep appear to be more sensitive than rodents. In controlled studies with well- defined exposure to multiple doses, the NOEL in pigs was 40 μg/kg of b. w. per day on the basis of estrogenic effects in responsive tissues and reproductive performance (SCF, 2000). Based on this study, a provisional maximum tolerable daily intake (PMTDI) of 0.5 μg/kg of b. w. was established for ZEA (JECFA, 2000). In 1993, the International Agency for Research on Cancer (IARC) evaluated and classified ZEA together with other *Fusarium* toxins, in Group 3 (not classifiable as to their carcinogenicity to humans) (IARC, 1993; IARC, 1999).

The survey described in this paper was designed to obtain information on the incidence and levels of ZEA in cereal and cereal-based foodstuffs in Timiş County, an area of western Romania. Food commodities included small cereal grains (wheat and maize) intended for human use, bread and related products (bakery and pastry products), pasta, breakfast cereals and other foods (maize cans and wheat flour) purchased from local producers and supermarkets during a period of three years (2008-2010). In selected samples ZEA content was analyzed using ELISA kits and results were compared to the existing maximum levels allowed by EC and with the available literature data worldwide and from the neighboring countries. As most data focuses on ZEA's occurrence in small cereal grains and crops such as wheat, barley, maize, etc., this screening is one of the first studies reporting its levels in a variety of cereal and cereal-based foodstuffs marketed in an area of western Romania, Timiş County.

MATERIAL AND METHODS

During the period 2008-2010, a total of 125 cereal and cereal-based food samples purchased from the local producers and supermarkets from Timi County, an area in western Romania, were analyzed for ZEA presence and concentration using enzyme linked immunosorbent assay (ELISA) commercial kits. The samples analyzed in this study are represented by: unprocessed small cereal grains intended for human use (wheat - n=21, and maize - n=12); wheat flour (n=17); refined maize oil (n=3); bread (n=26) and related products (bakery - n=1, pastry - n=2); salted snacks (n=2); biscuits (n=2); breakfast cereals (n=16); maize cans (n=6); maize snacks (n=1); maize puffs (n=3); and cornflakes (n=13). Preparation and test method were conducted according to the instructions outlined in RIDASCREEN® Zearalenone (R-Biopharm AG., Darmstadt, Germany) in the mycotoxin laboratory of the Sanitary Veterinary and Food Safety Directorate. The optical density (OD) was measured photometrically by a SUNRISE™ ELISA microplate reader (TECAN, Ltd.) at a wavelength of λ = 450 nm and the results were interpreted using RIDAWIN software program. ELISA method was validated before the sample analysis.

RESULTS AND DISCUSSIONS

The results concerning ZEA contamination of small cereal grains intended for human consumption and cereal-based foods from Timiş County, during 2008-2010 years, are summarized in Table 1. As it can be seen in Table 1 and in Figure 1 also, the highest ZEA incidence occurred in 2010: from 30 analyzed samples, 16 were found positive for ZEA contamination, representing 53.33 %. From 30 samples analyzed, during 2009, only 18 were found positive (38.30 %), whereas in 2008 from 48 samples only 3 samples were positive (6.25 %). The prevalence of ZEA revealed in this study is in accordance with other screenings worldwide (JECFA, 2000; SCOOP TASK 2.3.10, 2003).

Table 1

ZEA occurrence in foodstuffs marketed in Timis County during 2008-2010 years

Year	Analyzed samples	Positive samples (%)	Values in positive samples (µg/kg)		
			Min. - Max.	Mean	Median
2008	48	3 (6.25)	1.82 - 2.72	2.23	2.13
2009	47	18 (38.30)	0.4 - 15.83	1.52	0.55
2010	30	16 (53.33)	0.41 - 41.81	5.41	3.16
Total	**125**	**37 (29.6)**	**0.4 - 41.81**	**3.26**	**1.82**

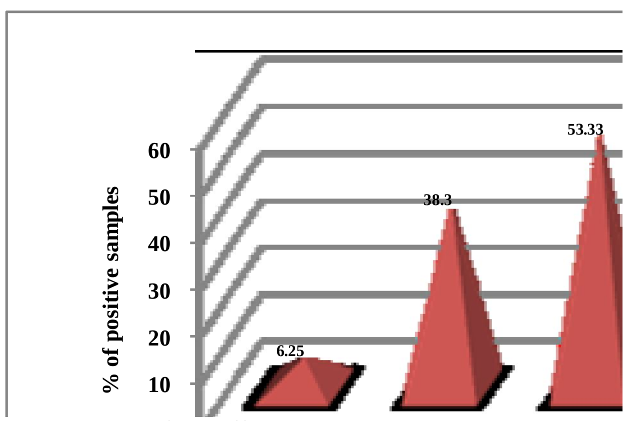

Figure 1: ZEA occurrence in foodstuffs marketed in Timis County during 2008-2010 years

The range of contamination levels varied between 1.82 - 2.72 µg/kg in 2008, 0.4 - 15.83 µg/kg in 2009 and 0.41 - 41.81 µg/kg in 2010. In 2009 was detected the minimum value of ZEA (0.4 µg/kg) when both mean and median levels were the lowest registered during the years of study (1.52 µg/kg and 0.55 µg/kg, respectively). The maximum value of ZEA was identified in 2010 (41.81 µg/kg), when both mean and median levels were the highest (5.41 µg/kg and 3.16 µg/kg, respectively).

During 2008-2010, the highest occurrence of ZEA (100 %) was found in barley, wheat bran, maize oil, bakery products, and maize snacks. With 50 % prevalence ZEA was identified in salted snacks and biscuits, and 33.33 % prevalence was found in maize, maize cans and maize puffs. In wheat flour and breakfast cereals ZEA was positive in 31.25 % of the analyzed samples, and in bread in 26.29 % of the analyzed samples. The lowest incidence was in the samples of cornflakes (15.38 %) and wheat (10 %). In this study the pastry products were not contaminated with ZEA (Table 2, Figure 2).

Table 2

ZEA occurrence in cereal and cereal-based foodstuffs marketed in Timis County during 2008-2010

Food type	Analyzed samples	Positive samples (%)	Values in positive samples (µg/kg)			
			Min. - Max.	Mean	Median	% of MAL*
Wheat	20	2 (10)	0.88 - 3.57	2.22	2.22	2.22
Barley	1	1 (100)	0.4	0.4	0.4	0.4
Maize	12	4 (33.33)	0.40 - 0.86	0.57	0.50	0.16
Wheat flour	16	5 (31.25)	0.41 – 41.81	9.09	13.70	12.13
Wheat bran	1	1 (100)	0.42	0.42	0.42	0.56
Refined maize oil	3	3 (100)	1.65 - 1.97	1.81	1.81	0.45
Bread	26	7 (26.29)	0.43 - 4.77	2.93	2.97	5.87
Bakery	1	1 (100)	0.46	0.46	0.46	0.93
Pastry	2	0 (0)	-	-	-	-
Salted snacks	2	1 (50)	2.49	2.49	2.49	4.97
Biscuits	2	1 (50)	2.76	2.76	2.76	5.51
Breakfast cereals	16	5 (31.25)	0.26 - 3.30	1.63	1.82	3.26
Maize cans	6	2 (33.33)	3.30 - 4.74	4.02	4.02	4.02
Maize snacks	1	1 (100)	0.71	0.71	0.71	0.71
Maize puffs	3	1 (33.33)	3.09	3.09	3.09	3.09
Cornflakes	13	2 (15.38)	0.54 - 15.83	8.19	8.19	8.19

***% of MAL (percent of the maximum admitted level) was calculated according to the COMMISSION REGULATION (EC) 1126/2007**

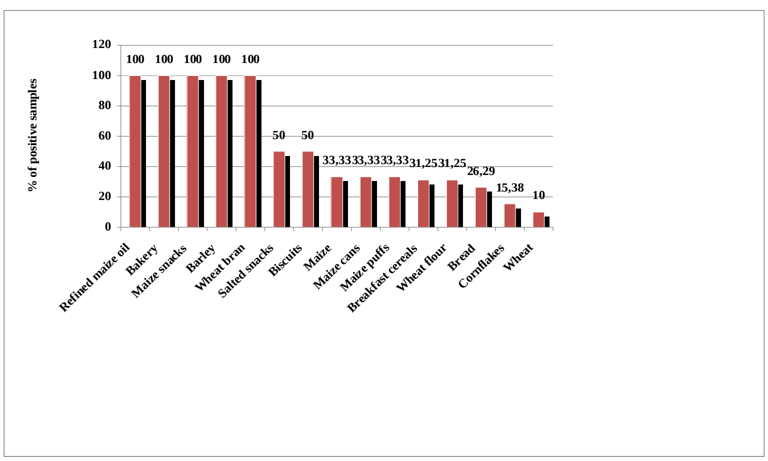

Figure 2: ZEA occurrence in cereal and cereal-based foodstuffs marketed in Timis County during 2008-2010

The highest mean and median values for ZEA contamination were found in wheat flour (9.09 µg/kg and 13.70 µg/kg, respectively), whereas the lowest mean and median levels were identified in barley (0.4 µg/kg). Low mean and median values were also found in wheat bran (0.42 µg/kg), bakery products (0.46 µg/kg) and maize (0.57 µg/kg mean and 0.50 µg/kg median) (Table 2).

Although ELISA methods are sensitive, rapid, and easy to use, none of the analyzed samples had ZEA concentrations above the maximum level adopted by the COMMISSION REGULATION (EC) 1126/2007 amending COMMISSION REGULATION (EC) 1881/2006. A percentage of the maximum allowed level (% of MAL) has been calculated. The lowest percentages of the maximum admitted levels were revealed in maize (0.16 % of 350 µg/kg), barley (0.4 % 100 µg/kg), refined maize oil (0.45 % of 400 µg/kg), wheat bran (0.56 % of 75 µg/kg), maize snakes (0.71 % of 100 µg/kg), and bakery (0.93 % of 50 µg/kg). The highest percentage was found in wheat flour with a MAL of 75 µg/kg (12.13 %), and close to this value were the results obtained in the samples of cornflakes 8.19 % of 100 µg/kg (Figure 3).

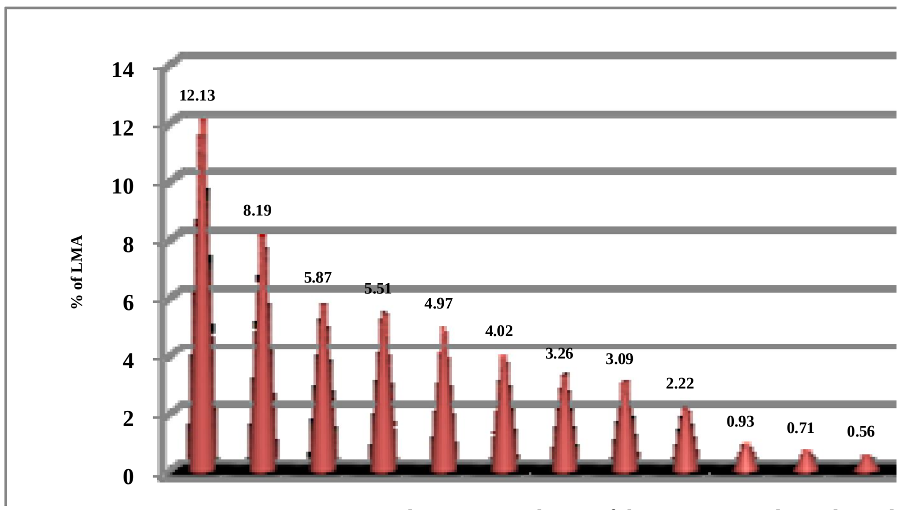

Figure 3: ZEA concentration in positive samples represented as % of the Maximum Admitted Level (MAL) in foodstuffs from Timiş County during 2008-2010

Comparing the results obtained in this study on the occurrence of ZEA in cereal and cereal-based foods to a wide study in the European Union (SCOOP TASK 2.3.10, 2003), the overall contamination with ZEA was similar: 29.9 % in this study and 32 % in the EU countries participating in the EU study on the prevalence of ZEA including data from 1996 to 2002. However, the values obtained in each analyzed commodity differed. In the unprocessed maize samples and maize based products group (cornflakes, maize snacks, etc.) both values and occurrence levels were higher in the EU study (79 % occurrence in maize and 50 % in maize based products). In cereal-based products group such as bread, pastry, biscuits, the occurrence of ZEA was higher in this study (66.67 %), but the values obtained were much lower (0.26 – 4.77 µg/kg) comparing to the EU study occurrence (11.4 %) and values (11 µg/kg – 232 µg/kg). Discrepancies between these data and the results of the present study may be attributed among others not only to the different origin of basing materials and differences in food production methods but also to varying limits of the methods used.

In the neighboring countries, in two recent studies on grains harvested in 2007 intended for human consumption, ZEA values were either below the MALs (MANOVA, MLADENOVA, 2009), or were under the detection limits (ŠKRBIĆ et al., 2011). Thus, in Bulgaria, MANOVA and MLADENOVA (2009), in a study on the incidence of ZEA and fumonisins in cereals (wheat, barley and maize - the 2007 harvest), obtained all ZEA values below the European regulations. ŠKRBIĆ et al. (2011), in a study in Serbia on the 2007 wheat harvest, from 54 samples analyzed, ZEA was below the detection limit.

In Hungary, during 1994-2002, in the Regional Laboratories of the Ministry of Agriculture and Rural Development were examined about 2300 samples of cereals for human consumption for ZEA conatamination. ZEA exceeded the MALs in about 0.5 % of the samples originating from the 1998 harvest and the average values were around 20 µg/kg. ZEA concentrations were even lower in wheat, flour and wheat meal samples in the period 200-2002 (VARGA *et al.*, 2004).

In 2010, in Romania, STROIA et al., published a study on the incidence of *Fusarium spp.* and its mycotoxins in cereals from western Romania. *Fusarium spp* were identified in all 56 cereal samples, in which *F.graminearum* and *F. culmorum* were the most frequent species. In this study, ZEA was detected in all analyzed maize samples (100 %), in 6 of the wheat samples (50%), 5 of the barley samples (50 %), and in 3 samples of oats samples (33.33 %). ZEA was surpassing the MALs in 22 maize samples.

CONCLUSIONS

The present study on ZEA occurrence in cereal and cereal-based foodstuffs from Timis County, revealed that during 2008-2010 in 125 analyzed samples ZEA was identified in 37 (26.9 %), occurrence which is in accordance with other similar studies. The highest occurrence of ZEA (100 %) was found in barley, wheat bran, maize oil, bakery products, and maize snacks. The lowest incidence was in the samples of cornflakes (15.38%) and wheat (10%).

The values obtained in this study ranged between 0.4 (barley) and 41.81 µg/kg (wheat flour), and the mean and median values were 3.26 µg/kg and 1.82 µg/kg, respectively. The low detection limits were also similar to other studies in the neighboring countries. In barley and wheat flour ZEA registered the lowest and highest mean and median level (0.4 µg/kg in barley and 9.09 µg/kg, and 13.70 µg/kg in wheat flour, respectively). In this study only the pastry products were not contaminated with ZEA.

Comparing the results obtained with the regulatory limits for the different foodstuffs presented in European Regulation 1126/2007, ZEA concentration was under the maximum admitted levels in all samples. ZEA obtained values ranged between 0.16 % (unprocessed maize) and 12.13 % (wheat flour) of MAL.

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