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

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Abstract: *Fumonisins (FUM) are a group of mycotoxins synthesized by different species of the genus Fusarium, mainly Fusarium verticillioides and Fusarium proliferatum, which known to contaminate maize and maize-based foods and feeds all over the world. The fumonisins belong to the most toxic fungal metabolites. In human and animals they have been associated with equine leucoencefalomalacia (ELEM), porcine pulmonary edema (PPE) and esophageal cancer (EC) in different countries. The goal of this study was to determine the presence and contamination level of one of the most toxic and frequently detected fumonisin, fumonisin* B_1 *(*FB_1*), in cereal and cereal-based foods purchased from Timis County, an area of western Romania, during a period of two years (2009-2010).* FB_1 *concentration was determined using enzyme-linked immunosorbent assay (ELISA) kits. To reach the aim of this study there were investigated 29 samples of foodstuffs mainly of maize origin such as unprocessed maize, maize cans, maize puffs, cornflakes, and maize snacks, but also unprocessed wheat and breakfast cereals.* FB_1 *was identified in eight samples (27.59 %) belonging to the maize and maize-based products categories. The* FB_1 *values obtained in this study were ranging between 30 µg/kg (cornflakes) and 92.42 µg/kg (corn puffs).* FB_1 *was not detected in the unprocessed wheat and breakfast cereal samples. The mean and median values of all commodities analyzed ranged between 30.5 µg/kg (cornflakes) and 92.42 µg/kg (corn puffs). Overall, the mean and median values registered were of 67.94 µg/kg and 78 µg/kg, respectively. However, none of the values exceeded the maximum allowed levels by the European Commission (EC) in the different commodities analyzed. The results obtained where compared to the occurrence data found in the literature. Whereas most surveys focus on FUM contamination of different crops this paper represents one of the first screening on a variety of cereal and cereal-based foodstuffs mostly of maize origin marketed in an area of western Romania.*

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

INTRODUCTION

In the current Romanian temperate climatic conditions, *Fusarium* fungi are significant in the cereal food chain, being capable of reducing crop yields and contaminating grain with mycotoxins (CREPPY, 2002). Fumonisins are a group of mycotoxins synthesized by different species of the genus *Fusarium* but the most significant producers are *Fusarium verticillioides* (former *Fusarium moniliforme*) and *Fusarium proliferatum*, one the most prevalent fungi associated with corn (*Zea mays L.*) and other plants (CAWOOD et al., 1991, ARIÑO et al., 2007; IARC, 1993). The natural contamination of maize and maize-based foods and feeds has been reported from countries all over the world. Among the cereals, maize as a staple food in many countries is mainly infected with field fungi of the genus *Fusarium* (section *Liseola*) (CREPPY, 2002; HELFERICH and WINTER, 2001; MARASAS, 2001).

Until now, several fumonisins have been isolated and characterized, but only FB_1, FB_2 and FB_3 are the major ones produced in naturally contaminated foods (SORIANO, 2004; YAZAR and ORMUTAG, 2008). FB1 and FB2 are diesters of tricarballylic acid and polyhydric alcohols, and as they have similar structure to sphingosine, they can interfere with sphingosine metabolism, blocking the biosynthesis of complex sphingolipids and ceramides. FB_1 is the most frequently detected, which toxicity has been reported (CAWOOD et al., 1991; SCF, 2000) showing different toxicological effects in humans and animals (MARASAS, 2001). FB1 has been pointed as a natural cause of leukoencephalomalacia in horses (ELEM), pulmonary edema and hepatic syndrome in swine, poor performance in poultry, alteration in hepatic and immune function in cattle (and esophagic cancer in humans (SORIANO, 2004; SORIANO *et al.*, 2005; STOCKMANN-JUVALA and SAVOLAINEN, 2008). FB_1 is a cancer promoter but a poor cancer initiator. Thus, FB1 was classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC, 2002).

The Scientific Committee for Food of the European Commission (SCF, 2003) has evaluated the FUM and established a provisional maximum tolerable daily intake (PMTDI) of 2 lg/kg b. w./day for the total of fumonisins B_1, B_2 and B_3, alone or in combination on the basis of the NOEL of 0.2 mg/kg of body weight per day and a safety factor of 100.

Bearing in mind that these mycotoxins can be very stable to food processing (MOLINIÉ et al., 2005) and can be present in final products, and that the Rapid Alert System for Food and Feed of the European Union (RASSF, 2006) has reported several alert notifications in conventional and organic corn-based commodities, FUM represent a cause of concern for consumers and regulatory authorities (SCHIBAMOTO and BJELDANES, 2009; VISCONTI, 2001).

In this context, the purpose of this study was to obtain data on the presence and contamination levels of FB_1 in cereal and cereal-based products, mainly of maize origin destined to human consumption. Thus, during 2009-2010 a total number of 29 samples were analyzed for FB_1 identification using enzyme-linked immunosorbent assay (ELISA) kits. Food commodities included small cereal grains (wheat and maize), breakfast cereals, maize cans and maize puffs purchased from local producers and supermarkets. The results obtained here were compared to the existing maximum levels allowed by EC and with the available literature data. While most data focuses on FUM occurrence in small cereal grains and crops, this screening is one of the first reporting its levels in different cereal and cereal-based foodstuffs marketed in an area of western Romania, Timiş County.

MATERIAL AND METHODS

For reaching the aim of this study, during the period 2009-2010, a total of 29 cereal and cereal-based food samples, mainly of maize origin were analyzed for FB1 presence and concentration using enzyme-linked immunosorbent assay (ELISA) commercial kits. The commodities analyzed were purchased from the local producers and supermarkets from Timi County (an area in western Romania) and were represented by: unprocessed small cereal grains intended for human use (wheat - n=1 and maize - n=11); breakfast cereals (n=2); maize cans (n=6); maize puffs (n=4); cornflakes (n=4) and maize snacks (n=1). Preparation and test method were conducted according to the instructions outlined in RIDASCREEN® Fumonisin (R-Biopharm GmbH, 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. The method was validated before the sample analysis.

RESULTS AND DISCUSSIONS

The results regarding FB_1 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 also in Figure 1, the highest FB_1

incidence occurred in 2010: from 11 samples analyzed, 4 were found positive for FB1 contamination, representing 36.36 %, whereas in 2009, from 18 samples analyzed, 4 were found positive (22.22 %). The prevalence of FUM revealed in this study is low in comparison with other screenings worldwide (CREPPY et al., 2002; SCOOP TASK 2.3.10, 2003).

Table 1

FB_1 occurrence in foodstuffs marketed in Timis County during 2009 and 2010 years

Year	Analyzed samples	Positive samples (%)	Values in positive samples (µg/kg)		
			Min. - Max.	Mean	Median
2009	18	4 (22.22)	30 - 88	61.75	64.5
2010	11	4 (36.36)	31 – 92.42	74.14	86.57
Total	**29**	**8 (27.59)**	**30 – 92.42**	**67.94**	**78**

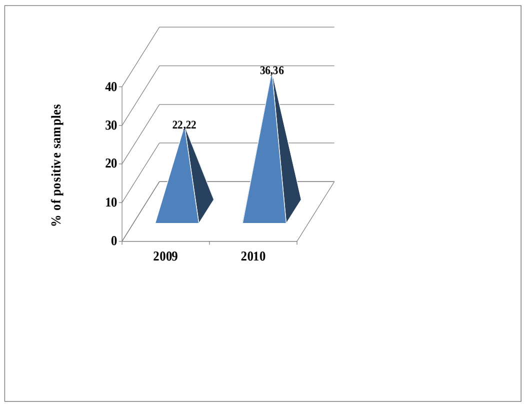

Figure 1: FB_1 occurrence in foodstuffs marketed in Timis County during 2009 and 2010 years

The range of contamination levels varied between 30 µg/kg and 88 µg/kg in 2009, and between almost the same limits in 2010: 31 µg/kg and 92.42 µg/kg. In 2009 was detected the minimum value of FB1 from this study of 30 µg/kg while highest value registered during the years of study was in 2010, 92.42 µg/kg. Both mean and median levels were similar during the two years of study: 61.75 µg/kg and 64.5 µg/kg, respectively in 2009, and 74.14 µg/kg and 86.57 µg/kg, respectively in 2010. Overall, the mean and median values varied very little (67.94 µg/kg and 78 µg/kg, respectively).

During 2009-2010, the highest occurrence of FB1 was found in cornflakes (50 %), followed by maize (36.36 %), maize puffs (25 %), and maize cans (16.67 %). The highest mean and median values for FB1 contamination were found in maize puffs (92.42 µg/kg), whereas the lowest mean and median levels were identified in cornflakes (30 µg/kg). In this study FB1 was not detected in maize snacks, wheat and breakfast cereals (Table 2, Figure 2).

Although enzyme immunoassay methods are sensitive, rapid, and easy to use, none of the samples analyzed had FB1 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 highest percentage of the MAL was revealed in maize puffs with 9.24 % of the 1000 µg/kg MAL, followed closely by maize cans with 9.11 % of the same 1000 µg/kg MAL while the lowest percentage of the MAL was calculated in maize with 1.87 % of the 4000 µg/kg MAL.

No contamination level with FB1 was observed in maize snakes, wheat, and breakfast cereals (Figure 3).

Table 2

FB1 occurrence in cereal and cereal-based foodstuffs marketed in Timis County during 2009-2010

Food type	**Analyzed samples**	**Positive samples (%)**	**Values in positive samples (µg/kg)**			
			Min. - Max.	**Mean**	**Median**	**% of MAL***
Maize	11	4 (36.36)	55 - 88	74.75	78	1.87
Maize cans	6	1 (16.67)	91.14	91.14	91.14	9.11
Maize puffs	4	1 (25)	92.42	92.42	92.42	9.24
Cornflakes	4	2 (50)	30 - 31	30.5	30.5	3.81
Maize snacks	1	0	-	-	-	-
Wheat	1	0	-	-	-	-
Breakfast cereals	2	0	-	-	-	-

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

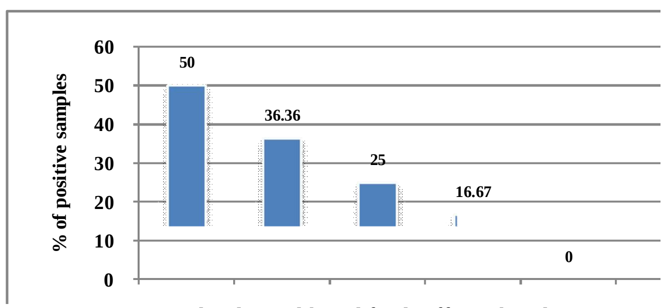

Figure 2: FB1 occurrence in cereal and cereal-based foodstuffs marketed in Timis County during 2009-2010

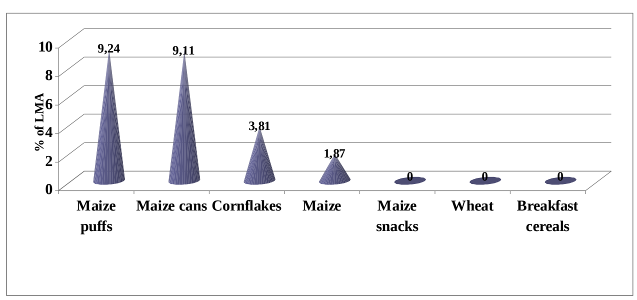

Figure 3: FB1 concentration in positive samples represented as percentage of the Maximum Admitted Level (MAL) in foodstuffs from Timiş County during 2009-2010

Comparing the results obtained in this study on the occurrence of FB1 in cereal and cereal-based foods during 2009-2010 to a wide study in the European Union (SCOOP TASK 2.3.10, 2003), the overall contamination with FB1 was much lower in this study (27.59 %) than in the EU one (46 %) which included data from 1996 to 2002. Moreover, the values obtained in each commodity analyzed differed also. In the unprocessed maize samples both values and occurrence levels were much lower in this study (55 µg/kg – 88 µg/kg and 36.36%, respectively) than in the European one (5 µg/kg – 10200 µg/kg, and 66%, respectively). In cornflakes the values obtained in this study were lower but prevalence was higher: 30 - 31 µg/kg, 50% compared to 2 – 1092 µg/kg and 45 %. The wheat samples were not contaminated with FB1 in this study, whereas in the European study the prevalence was 79 % and values ranged between 10 µg/kg and 736 µg/kg.

In Bulgaria, MANOVA and MLADENOVA (2009) in a study on the incidence of ZEA and fumonisins in cereals - the 2007 harvest), in the tested maize samples, obtained FUM levels ranging between 849 µg/kg and 4050 µg/kg with only one negative sample and one exceeding the MAL, while the mean value was 1150 µg/kg. All these values are much higher than the ones obtained in the present study: 30 µg/kg -92.42 µg/kg and mean value of 67.94 µg/kg.

In Hungary, during 1997, in the Regional Laboratories of the Ministry of Agriculture and Rural Development various samples of maize and maize flour intended for human use were analyzed for FUM contamination. The FUM were present in 67 % of the analyzed samples with values ranging between 16 and 58 µg/kg (VARGA et al., 2004), which are close to the results obtained in the present study.

In 2010, in Romania, STROIA et al. published a study on the incidence of *Fusarium spp* and its mycotoxins in cereals from western Romania. FUM were identified in 44 % of the maize samples with values ranging between 7.3 and 76.6 µg/kg, and no wheat sample was positive for these mycotoxins. These results are also similar to the results obtained in this study.

CONCLUSIONS

The present study on FB1 occurrence in cereal and cereal-based foodstuffs, mainly of maize origin, from Timis County, an area in western Romania, revealed that during 2009-2010 in 29 analyzed samples FB1 was identified in 8 (27.59 %), occurrence which is lower than the data presented in the European study. The highest occurrence of FB1 (50 %) was found in cornflakes whereas the lowest incidence was in the samples of maize cans (16.67 %).

The values obtained in this study ranged between 30 µg/kg (cornflakes) and 92.42 µg/kg (maize puffs), and the mean and median values were 67.94 µg/kg and 78 µg/kg, respectively. The low detected levels were similar to the ones revealed in the studies performed by VARGA et al., (2004) in Hungary and STROIA et al., (2010) in western Romania. Maize snacks, wheat and breakfast cereals were not contaminated with FB1.

Comparing the results obtained with the regulatory limits for the different foodstuffs presented in European Regulation 1126/2007, FB1 concentration was under the maximum admitted levels in all samples. FB1 percentages of MALs ranged between 1.87 % (maize) and 9.11 % (maize cans) of MAL.

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