T-2 Toxin Occurrence in Cereals and Cereal-Based Foods

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Abstract. T-2 toxin is a secondary metabolite mainly produced by fungi belonging to the genus Fusarium, principally by F. sporotrichioides, F. poae, F. equiseti and F. acuminatum which are common contaminants in staple foods of cereal origin such as oats, barley, rice, maize, wheat etc., and different by-products. T-2 toxin belongs to the closely related sesquiterpenoid family of trichothecenes and is a potent inhibitor of protein synthesis. Its main effects are usually observed in the immune system which has strong impact on the health of both humans and animals. Although is one of the most toxic type A trichothecene, T-2 toxin occurrence data is scarce and European Commission (EC) legal limits are not yet available. The objective of the present study was to monitor the occurrence of T-2 toxin in cereals and cereal-based foods marketed in an area of western Romania (Timis and Arad counties), using enzyme-linked immunosorbent assay (ELISA) kits. T-2 toxin was present in all analyzed samples (maize, wheat, corn flakes, breakfast cereals, biscuits), with values ranging between 0.8 g/kg and 23.4µg/kg and median value of 4.9. This study points out also the necessity of a continuous survey of cereals and cereal-based products for T-2 toxin presence and levels, and stresses the need for establishing legislative maximum admitted levels in foods.

Keywords: T-2 toxin, occurrence, cereals, foods, western Romania.

INTRODUCTION

T-2 toxin is a secondary metabolite mainly produced by fungi belonging to the genus Fusarium, principally by F. sporotrichioides, F. poae, F. equiseti and F. acuminatum. Fusarium fungi have been reported among the most prominent pathogens of various cereals such as oats, barley, rice, maize, wheat etc., and different by-products and the occurrence of mycotoxins in agricultural commodities has long been recognized as a potential hazard for human and animal health (Bhat et al., 2010; Foroud and Eudes, 2009; Glenn, 2007; Josephs et al., 2004; Klotzel et al., 2006; Kriska et al., 2001, 2007; Pascale et al., 2003).

Regarding its classification, T-2 toxin belongs to the closely related sesquiterpenoid family of trichothecene mycotoxins, which are divided into four groups (types A–D), according to their characteristic functional groups. Type A is represented by the HT-2 toxin and T-2 toxins, and type B is most frequently represented by deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, nivalenol, and fusarenon X (Foroud and Eudes, 2009; Hussein and Brasel, 2001; Kimura et al., 2007; Koch, 2004; Kriska et al., 2007). Trichothecenes are extremely stable, resistant to heat and ultraviolet light, while Type - A trichothecenes are of major concern as they are more toxic than the type B trichothecenes (Creppy, 2002; Kimura et al., 2007; Marijana et al., 2008).

Being considered the most toxic trichothecene, T-2 toxin is a potent inhibitor of protein synthesis and mitochondrial function both in vivo and in vitro, and shows immune
suppressive and cytotoxic effects (Kimura et al., 2007; Visconti et al., 1991; WHO 2002). Acute symptoms of human exposure to T-2 toxin and its metabolites result in nausea, vomiting, diarrhea, weight loss, necrosis, epidermal sloughing, skin pain, pruritus, redness and vesicles where as prostration, weakness, ataxia, collapse, reduced cardiac output, shock and death were reported as the results of severe poisoning (Hussein and Brasel, 2001; JECFA, 2001). In animals, these mycotoxins are held responsible for reduced feed uptake, vomiting, and immune suppression. In instances of chronic poisoning, they produce significant changes in the blood cell count and in immune function (Glenn, 2007; Eriksen and Pettersson, 2004; JECFA, 2001; SCF, 2001, 2002).

In order to make the safety assessment, the Scientific Committee for Food of the European Commission (SCF, 2001) based its study on the hematotoxicity and immunotoxicity of T-2 toxin in pigs in a short-term study (Rafai et al., 1995) and used a LOAEL (Lowest Observed Effect Level = 0.03 mg/kg bw/day), presumably close to the NOAEL (No Observed Effect Level) (Rafai et al., 1995; SCF, 2001); an extra uncertainty factor of 5 was included, giving an overall uncertainty factor of 500. Hence, the Committee established a temporary permissible limit of 0.06 µg/kg bw/day. The temporary TDI (t-TDI, Tolerable Daily Intake) was expressed for the sum of T-2 and HT-2 toxins due to i. T-2 toxin is rapidly metabolized to HT-2 toxin by the gut microflora of mammals, and ii. the acute toxicity of T-2 toxin and HT-2 toxin are within the same range. Thus, the t-TDI value was set to protect against the other chronic, subchronic and reproductive effects described in the literature and is in-line with the TDI derived for T-2 and HT-2 toxin by JECFA (2001). The Committee also recommended further research on the individual toxins to fill the data.

The limits for T-2 and HT-2 in food are scheduled for the near future (Edwards et al., 2009), but recent risk assessments on T-2 and HT-2 have not been done at the European level (van der Fels-Klerx 2010). This, mainly because of (to date) a lack of a clear overview of the information on their occurrence has not allowed the risk assessment required prior to deciding appropriate limits (van der Fels-Klerx, 2010). Based on the data presented above, the survey described in this paper was designed to obtain information on the occurrence and levels of T-2 toxin in several Romanian cereal and cereal-based foodstuffs using ELISA test kits for toxin detection.

**MATERIALS AND METHODS**

The research has been carried out during 2009, on a total of 22 cereals and cereal-based food samples purchased from the local producers and supermarkets from western Romania (Timis and Arad Counties). The samples analyzed in this study were represented by: unprocessed small cereal grains intended for human use (maize - n=7 and wheat - n=2); cornflakes (n=3), breakfast cereals (n=8) and biscuits (n=2). T-2 toxin presence and concentration was analyzed using enzyme linked immunosorbent assay (ELISA) commercial kits. Preparation and test method were conducted according to the instructions outlined in RIDASCREEN® T-2 toxin (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 and accredited.

**RESULTS AND DISCUSSIONS**

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The results concerning T-2 toxin contamination of small cereal grains intended for human consumption and cereal-based foods from western Romania, during 2009, are summarized in Tab.1.

Tab. 1

T-2 occurrence and values in the food samples analyzed from Timis and Arad Counties during the year 2009

<table>
<thead>
<tr>
<th>Food type</th>
<th>Analyzed samples</th>
<th>Positive samples</th>
<th>Values in positive samples (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nr.</td>
<td>Nr.</td>
<td>%</td>
</tr>
<tr>
<td>Maize</td>
<td>7</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Wheat</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>Biscuits</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Total of all analyzed samples</td>
<td>22</td>
<td>22</td>
<td>100</td>
</tr>
</tbody>
</table>

The analysis of Table 1 shows that the cereal samples were contaminated with T-2 toxin as follows:

- The T-2 toxin values obtained from the *maize samples*, in both counties were ranging between 1.1µg/kg and 8.4µg/kg, with an average value of 5.53µg/kg and median value of 6.3µg/kg.
- The *wheat samples* were contaminated with T-2 toxin in concentrations varying between 0.8µg/kg and 0.10 µg/kg, with an average and median value of 0.9µg/kg. These values were the lowest values registered in this study.

The cereal-based foods were also contaminated with T-2 toxin:

- The *corn flake samples* contained T-2 toxin with values ranging between 3.8 µg/kg and 6.8 µg/kg with an average 4.93µg/kg and median of 4.2µg/kg.
- In *breakfast cereal samples* T-2 toxin values ranged between 2.93µg/kg and 8.7µg/kg, with an average value of 5.50µg/kg and median value of 4.65µg/kg.
- In the *biscuits samples* T-2 values obtained ranged between 20µg/kg and 23.4µg/kg, with an average and median value of 21.7µg/kg.

In the top of the hierarchy of contamination levels represented by the median values of the samples analyzed in this study, are the biscuits samples, followed by maize, breakfast cereals, and corn flakes, whereas the lowest contamination level was obtained in the wheat samples (Fig.1).
The results obtained in this study were compared to those found in surveys in the European Union and to those pertaining to countries with a high occurrence data on these mycotoxins, and with similar climatic conditions. The analyzed data are somewhat difficult to compare due to the differences in sensitivity of the different methods used to detect T-2 toxin concentration.

A Scientific Cooperation (SCOOP, 2003) project on the occurrence of Fusarium toxins in food matrices in the European Union with data compiled from 1996 up to 2002 showed that 20% out of in total 3490 samples collected from eight countries were positive for T-2 (Schothorst and van Egmond, 2004). In the same study, T-2 toxin was present in 21% of the 1417 samples of wheat and wheat flour, with values ranging between 2-160 µg/kg. Samples were derived from seven countries including Denmark, Finland, France, Italy, Norway, Portugal and UK. The mean of all samples varied between 1.7 µg/kg (UK) and 90 µg/kg (Denmark). Data from a total number of 293 samples derived from three countries (Austria, France and Italy) showed the incidence of T-2 in corn was 28%, which ranged from 3 µg/kg (France) to 255 µg/kg (Austria).

In a Report submitted to the European Food Safety Authority (EFSA) by van der Fels-Klerx (2010) on the occurrence data of trichothecene mycotoxins T-2 toxin and HT-2 toxin in food and feed with emphasis on data from the year 2000 until 2009, the available data showed an increase in barley and oats contamination in 2004-2005 and some of the years to follow. In barley, the incidence of these toxins stabilized at about 80%. Maize and wheat seemed to be contaminated at moderate or low levels.

In a study made by Schollenberger et al. (1999) in southwest Germany during the first six months of 1998 on a total of 237 commercially available samples of cereal-based foods, T-2 toxin was found in 4% of the analyzed samples and a mean content of 14 µg/kg. T-2 toxin was detected in 6% of the breakfast cereals, with values ranging between 4-7 µg/kg, and mean of 6 µg/kg. In rice samples and other foods T-2 toxin was also present, whereas in other samples such as bread and related products, noodles, baby and infant foods T-2 toxin was absent. The maximum value obtained for T-2 toxin was of 39 µg/kg.

In Germany, during the years 2006 and 2007, was held joint research project on the improvement and validation methods of analysis for type A trichothecens, and occurrence of these mycotoxins in foods, comprising 2895 samples from retail products and T-2 incidence in these products was as low as 13%, and HT-2 was detected in 38% of all samples (Usleber, 2008). Biselli and Hummert (2005) reported the presence of T-2 toxin in nearly 40% of the
samples from analyses of 685 food samples of European origin, including different food product categories. The highest amounts were observed in maize (mean 0.8μg/kg and maximum value 8.4μg/kg), oats or oat-based products (mean 34μg/kg and maximum value 266μg/kg), respectively.

In a study on a total of 54 samples collected during the harvest of 2007 from the most important Serbian wheat-growing regions, T-2 toxin was not detected, and HT-2 contents were detected in 6% of the total number of samples (Škrbiš et al., 2011). Gottschalk et al. (2007) reported results of 70 oat samples (35 from conventional farming, 35 from organic farming) collected at mills and at wholesale stage from the Bavarian market of the production year 2005. Contamination of the oat flake samples with T-2 was 100%, with a mean of 6.4 μg/kg and a maximum value of 34 μg/kg.

Two years later, in Germany, Gottschalk et al. (2009) analyzed a total of 289 samples comprising wheat products (n=130), rye products (n=61), and oat products (n=98) collected from grain-milling factories and wholesale in Bavaria from the crop years 2005 and 2006. T-2 toxin was present in 85% of all wheat samples, 87% of all rye samples and 100% of all oats samples. Median levels were 0.11 μg/kg, 0.09 μg/kg, and 2.2 μg/kg, respectively. Highest levels were found in wheat bran (1.9 μg/kg), whole rye flour (0.8 μg/kg) and fine oat flakes (34 μg/kg).

CONCLUSIONS

Comparing the results obtained in this study with different surveys found in literature data, we conclude that even if the samples are limited to an area of western Romania, the T-2 toxin percentage of occurrence is in accordance with the increasing percentage discovered in the last years in Europe in foods (Gottschalk et al., 2009; van der Fels-Klerx, 2010).

Considering the values obtained, the cereal samples registered T-2 toxin contamination levels in wheat (mean = 0.9 μg/kg) and maize (maximum value = 8.4 μg/kg) samples, similar to the ones obtained in the literature data. The breakfast cereals samples were in range with the values obtained by Schollenberger et al. (1999) in southwest Germany.

The mean values of T-2 toxin concentration in this study was 6.44 μg/kg, whereas the median was slightly lower (4.9 μg/kg). The lowest value comprised in this study was found in the wheat samples (0.8μg/kg) and the highest in the biscuits samples (23.4 μg/kg). These values were also found in the literature data. Thus, in the reviewed literature, the values obtained ranged between 0.09 μg/kg (Gottschalk et al., 2009) and 266 μg/kg (Biselli and Hummert, 2005). This study points out also the necessity of a continuous survey of cereals and cereal-based products for T-2 toxin presence and levels, and stresses the need for establishing legislative maximum admitted levels in foods.

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