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Fusarium mycotoxins' occurrence in cereals harvested from Croatian fields

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ABSTRACT

Fusarium mycotoxins are frequent contaminants of cereals in this part of Europe. In this study, a total of 181 samples of maize, wheat, barley and oat were collected from different fields situated in six Croatian regions, and analysed using a validated ELISA method. Concentrations of deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FUM) and T-2 toxin (T-2) were determined. Methodology validation showed the mean recovery rates to range from 72.8% to 103.6%, with the intermediate precision of 70.699.4% and variation coefficients (CV) of 3.6–6.2% and 6.1–10.2%, respectively. Maize proved itself as the most contaminated cereal, with DON as the most represented *Fusarium* mycotoxin (52.5%) followed by ZEA (40.5%), FUM (37.5%) and T-2 toxin (33%). Mycotoxin concentrations higher than allowed were observed in 4 maize samples and a single wheat sample. Given the warm and dry study period, such a contamination might be associated with some factors other than climate conditions that could cause *Fusarium* mycotoxin formation. The obtained data pointed towards the necessity for a consistent control over these contaminants and the definition of their maximal allowed levels in different foodstuffs and feedstuffs.

1. Introduction

Cereals may be contaminated by mycotoxins because fungi which produce these toxins can grow on plants as pathogens or saprophytes during the cereal storage (Glenn, 2007). Mycotoxins of *Fusarium* species have been found to cause major damage, especially in cereals, and could frequently be associated with preharvest cereal contamination (Creppy, 2002; Geraldo, Tessmann, & Kemmelmeier, 2006; Tanaka et al., 1988). Their presence has traditionally been associated with temperate cereals, since these fungi require somewhat lower growth and mycotoxin production temperatures than aflatoxigenic *Aspergillus* species (Placinta, D'Mello, & Macdonald, 1999).

Major *Fusarium* mycotoxins that can occur in cereal grains and cereal-based products are deoxynivalenol (occurring mainly in wheat, maize, barley, oat and rye), T-2 and HT-2 toxins (oat, wheat, and barley), zearalenone (maize, wheat) and fumonisins (maize). Their biosynthesis can be affected by a number of factors including not only temperature, but also humidity, oxygen level, mechanical cereal damage and the presence of mould spores. The extent of their impact shall usually be dependent on climate conditions and widely varies across different world climate zones (Mateo, Mateo, & Jimenez, 2002; Sforza, Dall' Astra, & Marchelli, 2006).

As the consumption of *Fusarium*-contaminated products may cause the poisoning known as mycotoxicosis and induce teratogenic, carcinogenic, neurotoxic, estrogenic or immune-suppressive effects (Canady et al., 2001, pp. 419–555; Kabak, Dobson, & Var, 2006), contamination of food, feed and their ingredients can significantly jeopardise human and animal health (Bottalico, 1998; IARC, 1993; Sudakin, 2003). According to the results of risk assessment studies (SCOOP projects), cereals and cereal-based products are the main source of mycotoxin intake in EU population (Schothorst & van Egmond, 2004). The impact of mycotoxins on human and animal health depends not only on the concentration of an individual mycotoxin present in the grain, but also on its synergistic effects with other coexisting mycotoxins. These synergistic effects are the reason why fairly low concentrations of mycotoxins in food and feed can have serious consequences on human and animal health. Insofar, these effects have been insufficiently tested and require further research (CAST, 2003; Erber & Binder, 2004). Also, issues like fusariotoxin transfer within a human or an animal organism and the bio-significance of possible presence of toxic residua in animal products, have also remained unclear (Cavret & Lecoeur, 2006).

Due to modern laboratory methods and a growing interest in this field of research, more than 300 different mycotoxins have

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been differentiated thus far (Binder, Tan, Chin, Handl, & Richard, 2007). Analytical methods employed with the analysis of cereal and cereal-based products generally require toxin extraction from the matrix using an adequate extraction solvent, followed by a clean-up intended to eliminate the interferences and finally the detection and determination of a toxin using suitable analytical instruments. Chromatographic methods employed to this effect include high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV), diode array (DAD), fluorescence (FD) or mass spectrometry (MS) detectors, and gas-chromatography (GC) coupled with electron capture (ECD), flame ionization (FID) or MS detectors. Commercial immunometric assays such as enzyme-linked immunosorbent assays (ELISA) or membrane-based immunoassays are frequently used for screening purposes as well (Krska, Welzig, & Boudra, 2007; Schaafsma et al., 1998; Sforza, Dall'Astra, & Marchelli, 2006; Tanaka, Yamamoto, Hasegawa, Aoki, & Besling, 1990).

Previous studies on the occurrence of mycotoxins in maize, performed in Croatia, have shown the presence of mycotoxins in cereals and cereal-based products to be common and dependent on many factors, in particular climate conditions throughout the cereal growth period (Domijan et al., 2005; Pavičić, Brlek, & Nemanič, 1998; Pleadin et al., 2012a, 2012b; Sokolović & Šimpraga, 2006). As data on natural occurrence of *Fusarium* mycotoxins in Croatia are still insufficient to be compared with those coming from other European countries and/or those obtained worldwide, the aim of this study was to collect more data on the matter by virtue of determining deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FUM) and T-2 toxin (T-2) levels in different types of cereals (maize, wheat, barley and oat) harvested from different fields located in six Croatian regions.

2. Materials and methods

2.1. Samples

A total of 181 cereal samples (63 maize, 51 wheat, 34 barley and 33 oat samples) were collected within September 2011–December 2011 timeframe. Each cereal sample was collected from a different field situated in one of the six Croatian regions, as follows: Koprivničko-Križevačka County (Region 1 – R1), Požeško-Slavonska County (Region 2 – R2), Bjelovarsko-Bilogorska County (Region 3 – R3), Osječko-Baranjska County (Region 4 – R4), Sisačko-Moslavačka County (Region 5 – R5) and Zagrebačka County (Region 6 – R6). Sampling and sample preparation were completed in full line with ISO 6497:2002 and ISO 6498:1998, respectively. Prior to their analysis, the samples were not dried. Instead, the prepared test portions were ground into a fine powder having a particle size of 1.0 mm using an analytical mill (Cylotec 1093, Tecator, Sweden), and then stored at 4 °C.

2.2. Determination of mycotoxins

Mycotoxin (ZEA, FUM, T-2, and DON) concentrations were determined using competitive ELISA test kits as instructed by the kit manufacturer. Test kits (Ridascreen) were provided by R-Biopharm (Darmstadt, Germany). Each kit contains a microtiter plate with 96 wells coated with antibodies, standard solutions containing different concentrations of mycotoxins, an enzyme conjugate, an anti-antibody, the substrate & a chromogen solution (urea peroxide/tetramethylbenzidine), a stop solution, and washing & dilution buffers. The standards employed with the validation of analytical methods were provided by Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals used in the analysis were of analytical grade. ELISA tests were evaluated using a ChemWell auto-analyzer (Awareness Technology Inc. 2910, USA) with the absorbance being measured at 450 nm. In order to determine mycotoxin concentrations in the sample material, a standard curve was plotted based on the sample extract dilution factors. Final concentrations were calculated based on the average recoveries. Statistical analysis was performed using Statistica Ver. 10.0 software (StatSoft Inc. 1984–2011, USA), with a statistical significance set at 95% ($p = 0.05$).

2.3. Validation of methods

For each mycotoxin/applied method, the Limit-of-Detection (LOD) was calculated from the mean value yielded by ten blank maize sample analyses plus three standard deviations. The recovery rates were determined at three different levels (50, 100 and 200 μg/kg) by spiking the control maize samples with the standard in-house mycotoxin working solution (300 μg/L) correspondent to the assessed content levels (six replicates *per* concentration *per* day). As regards the determination of intermediate precision, the same steps were repeated on two other occasions within a three-month period by two different analysts and under the same analytical conditions. For all analyzed mycotoxins, validation parameters were determined using maize samples.

3. Results and discussion

Various studies performed worldwide have described numerous adverse effects of mycotoxins on human and animal health. Generally, cereals such as maize, barley, wheat and oat are widely used as nutrients, and are very often contaminated with several types of moulds and mycotoxins. Of particular concern is the co-occurrence of several *Fusarium* mycotoxins in a single grain or animal feed sample (Placinta et al., 1999). Of note, wheat, barley and maize account for almost two-thirds of the world cereal production (O'Donnell, Kistler, Tacke, & Casper, 2000).

Among mycotoxins, the fungi most frequently isolated from Croatian cereals are *Fusarium* species (Sokolović & Šimpraga, 2006); this could have significant safety implications on human and animal health and could cause significant economic losses. Studies conducted in previous years had indicated a constant presence of mycotoxins in cereals harvested in north-western Croatia, as well as the dependence of mycotoxin occurrence and concentrations on climate conditions witnessed in a particular period (Domijan et al., 2005; Mitak, Zadravec, Gojmerac, & Cvetnić, 2005; Pepeljnjak, Cvetnić, & Šegvić Klarić, 2008; Pleadin et al., 2012a, 2012b). The study conducted in 1997–2004 period indicated a high presence of *Fusarium* species in the analysed cereal samples (Pepeljnjak & Šegvić, 2004). Even 70% of the samples contained moulds of this genus, while *Fusarium graminearum*, *Fusarium sporotrichoides* and *Fusarium tricinctum* were found in 10% of the analyzed grain samples. Another study revealed *F. graminearum*, *F. sporotrichoides*, *Fusarium poae*, *Fusarium monoliforme* and *Fusarium trichintum* to be the most common in these parts (Pavičić et al., 1998). Based on the results of these and other studies conducted in Croatia in the last decades, it can be concluded that *Fusarium* fungi and the mycotoxins produced by them are common contaminants of cereals grown in this area.

A recent study performed in Croatia in 2010, narrowed its scope down to the identification of a particular mycotoxin in maize samples only, the maize thereby being the most frequently used cereal in human and animal nutrition (Pleadin et al., 2012a, 2012b), whereas this study aimed at establishing the occurrence of more than one *Fusarium* mycotoxin (DON, ZEA, FUM and T-2) in four different types of cereals (maize, wheat, barley and oat). Given that the earlier results have revealed a high correlation between ELISA