FUSARIUM TOXINS IN DIFFERENT FLOURS COLLECTED ON THE CROATIAN MARKET OVER SEASONS 2002/2004

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ABSTRACT

The occurrence of deoxynivaloenol (DON), T-2 toxin and fumonisin B_1 was investigated in different wheat flour types, graham flour and corn flour. A total of 36 samples of different flours were collected from 2002 to 2004 from the retail market in Croatia. The contents of DON and fumonisin B_1 were determined by high performance liquid cromatography and that of T-2 toxin by gas chromatography/mass spectrometry. T-2 toxin and fumonisin B_1 were not detected in any sample. DON was detected with an incidence rate of 77.78% over all samples for all years. The contamination level varied from year to year and from sample to sample and was in the range from 0 to 0.53 mg kg^{-1}. The EC is discussing the setting of limits for DON, such as 0.5 mg kg^{-1} for cereal products for direct consumption, and 0.75 mg kg^{-1} for raw cereals. Croatia has set a limit for DON of 2 mg kg^{-1} for cereals and rice, and 4 mg kg^{-1} for combination of fumonisins B_1, B_2 and B_3 for corn, corn flour, corn products and rice. Croatia is still lacking legislation for T-2 toxin for human consumption.

Key words: flour, *Fusarium* toxins, deoxynivalenol, T-2, fumonisin B_1

INTRODUCTION

Cereals are often subject to mold contaminations and *Fusarium* species are the most common pathogens of small grain cereals and maize worldwide. *Fusarium* head blight of wheat and *Fusarium* ear rot of maize are diseases causing considerable yield (reduced number and test weight of infected grains) and quality losses (destruction of cell walls, starch granules and storage proteins) [3]. *Fusarium* infection is influenced by environmental conditions, especially if temperature, rainfall and relative humidity are high during heading and flowering periods [2]. *Fusarium* growth often implies production of mycotoxins (e.g. trichotecenes, fumonisins, zearalenone, moniliformin, etc.) that poses a threat to human or animal health [6]. It has been estimated that 25% of world's crops are contaminated by mycotoxins and this percentage is even higher for deoxynivalenol (DON) and fumonisin B_1 [5]. *Fusarium moniliforme*, prevalent fungus on maize corn in Croatia [3], and *Fusarium proliferatum* are the most common fumonisin B_1 producing fungi. *Fusarium* species reported as major producer of T-2 toxin is *Fusarium sporotrichioides*, new identified *Fusarium* species in Croatia. The most important producers of DON are *Fusarium graminearum* and *Fusarium culmorum* [4]. DON is the most prevalent of the *Fusarium* toxins [8]. The toxicity of trichotecenes

(DON, T-2 toxin, etc.) is largely due to their ability to inhibit protein synthesis. Toxic effects of DON include: oral lesion, growth retardation (animals), nausea, vomiting, gastrointestinal upset, dizziness, diarrhoea, headache (acute poisoning in humans) [1]. Several countries have established tolerance levels for DON in cereals, which are in an order of magnitude of 1000 μg kg^{-1}. The European Commission (EC) is discussing the setting of limits such as 500 μg kg^{-1} for cereal products for direct consumption, and 750 μg kg^{-1} for raw cereals. To date EC has recommended but not legislated maximum levels for combinations of fumonisin B_1 and B_2 which range from 2000 μg kg^{-1} for unprocessed corn to 100 μg kg^{-1} for infant food, and is lacking legislation for T-2. Croatia has set a limit for DON of 2 mg kg^{-1} for cereals and rice, and 4 mg kg^{-1} for combination of fumonisins B_1, B_2 and B_3 for corn, corn flour, corn products and rice. Croatia is currently lacking legislation for T-2. The aim of this study was to obtain data on the occurrence of Fusarium toxins in different flours available on the Croatian retail market. The Fusarium toxins covered included the trichotecens (DON and T-2 toxin) and fumonisin B_1.

MATERIALS AND METHODS

A total of 36 flour samples were collected from the retail market between 2002 and 2004. The samples originated from crops grown in different parts of Croatia. Collected samples were of 1 kg and sub samples were taken to perform mycotoxin analysis: 25 g for DON and fumonisin B_1 analysis and 50 g for T-1 analysis. The type and number of samples from each harvest year are given in Tab. 1.

DON content of flour samples was determined by immunoaffinity column (DONPREP column; R-BIOPHARM RHONE LTD) cleanup followed by high-performance liquid chromatography (IAC-HPLC). 25 g of sample was extracted with 200 ml of distilled water and 8 g PEG on a shaker for 2 hours. The extract was filtered through a filter paper. 8 ml of filtrate (equivalent to 1 g sample portion) passed through a DONPREP column. Passing 10 ml of distilled water washed the column. DON was eluted by passing 2 ml of LC grade methanol, and the elute evaporated in a vacuum centrifuge (type RC10.10, Juan, France). The evaporated sample was dissolved in 200 μl of mobile phase and 20 μl was injected. DON was separated on a reversed-phase, C_{18} column (LiChrospher RP 18, 150x4 mm, 5 μm) using acetonitrile-water (80 ml + 920 ml) as eluent. The flow rate and temperature of the eluent were 1 ml/min and 40 °C. A Hewlett-Packard 1050 quaternary pump was used for the analysis. DON was detected with a Hewlett-Packard 1050 Diode Array Detector at 218 nm. The recovery was 92% with 4.5% deviation. The detection limit was 0.025 mg kg^{-1} DON.

T-2 toxin analysis was preformed by GC-MSD. 50 g of flour sample was extracted with 100 ml methanol-water (800 ml + 200 ml). Extract was filtered through the filter paper. 10 ml of filtrate was collected and mixed with 40 ml distilled water. The diluted extract was filtered through a glass microfibre filter and the filtrate was collected. 10 ml of diluted extract passed through the T-2 immunoaffinity column (T-2 test columns, VICAM), followed by 10 ml of distilled water. T-2 was eluted with 2 ml of methanol and the elute evaporated in a vacuum centrifuge. 100 μl TMSI reagent (trimethylsilylimidazole, SUPELCO) was added to the evaporated residue. The mixture was shaken with a test tube mixer and transferred to an oven for 1 hour at 60 °C;

1 ml of hexane and 1 ml of water were added to the cooled mixture after the reaction. The sample was mixed by swirling and the upper hexane layer was then separated and 1 µl was injected to the gas chromatograph using the splitless mode. A Perkin-Elmer gas chromatograph Auto System XL with Turbo Mass Spectrometer (GC-MSD) was used for the analysis. Operating conditions were as follows: 20 m x 0.1 mm capillary column, film thickness: 0.1 µm (PE-5MS capillary column); helium carrier gas 0.2 ml/min; injector and source temperature 280 °C and 200 °C; temperature programme: 50 °C (3 min); 50-150 °C (40 °C/min); 150-250 °C (5 °C/min); 250 °C (10 min). The MSD was operated in the electron impact-selected ion monitoring (EI-SIM) mode with the ioning voltage set at 70 eV. The ions monitored for the detection of T-2 toxin were as follows: 350/436 and 256/346 (internal standard 19-nortestosterone). The recovery was 82% with 3.2% deviation. The detection limit was 0.025 mg/kg T-2 toxin.

For the determination of fumonisin B_1 content by HPLC, the flour samples were processed as described previously by Shephard et al. [10]. 25 g of sample was extracted with 100 ml of methanol-water (3+1). 10 ml of filtered extract was purified on a SAX SPE column (Varian). The evaporated residue was dissolved in 200 µl of methanol. The derivative form with o-phthaldialdehyde (OPA) and mercaptoethanol was separated on a 150 x 4 mm LiChrospher RP 18 column, using acetonitrile-water-acetic acid (50+50+1) as eluent. A Hewlett-Packard 1050 type quaternary pump was used for the analysis (flow rate: 1 ml/min; eluent temperature: 40 °C). HP 1046 type fluorescence detector (excitation: 335 nm; emission: 440 nm) was used for the detection. The average recovery was 82% with 2.5% deviation. The detection limit was 0.05 mg kg^{-1} fumonisin B_1.

All samples were analysed in duplicate.

RESULTS AND DISCUSSION

T-2 toxin and fumonisin B_1 were not detected in any sample.

The results for occurrence of DON in flour samples of wheat and corn are given in Tab. 1.

DON was detected with an incidence rate of 77.78% over all samples for all years. High incidence rate of DON compared to other two mycotoxins is in agreement with observations of DON being predominant of *Fusarium* mycotoxins [8, 9]. The contamination level varied from year to year and from sample to sample and was in the range from 0 to 0.53 mg kg^{-1}. Only one sample exceeded the EC recommended maximum limit of 0.5 mg kg^{-1} for cereal products for direct consumption. The high frequency of DON in tested flours indicates that the original wheat and corn samples were contaminated at least to the same level. This is consistent with the previous findings concerning *F. graminearum* and *F. culmorum* distribution in wheat over seasons 2001/2003 in Slavonia [6]. The mean *F. graminearum* and *F. culmorum* infection levels for wheat in this survey were 10.2% and 1.5% respectively. Percentages above 10% are considered high infection levels [7]. Due to a low number of samples (the investigation was preliminary) the impact of crop year and flour type on DON content was not determined.

Table 1: Occurrence of DON in flour samples of wheat and corn collected from 2002 to 2004

Type of flour	Harvest year	DON mg/kg			
		Number of positives	Mean	Median	Maximum
Wheat					
Type 400	2004	2/2	0.08	0.08	0.12
Type 500	2002	4/5	0.175	0.085	0.487
	2003	4/5	0.132	0.14	0.22
	2004	1/2	0.018	0.018	0.037
Type 850	2002	3/5	0.101	0.122	0.24
	2003	5/5	0.24	0.390	0.53
	2004	1/2	0.024	0.024	0.049
Graham	2003	2/2	0.32	0.32	0.38
	2004	1/2	0.098	0.098	0.196
Corn	2003	2/2	0.2	0.2	0.25
	2004	3/4	0.212	0.22	0.41

CONCLUSION

In this study the occurrence of DON, T-2 toxin and fumonisin B_1 was investigated in different flours collected on the Croatian market over seasons 2002/2004. Fusarium toxins pose safety concerns for cereal grain intended for human consumption. High incidence rate of DON (77.78%) in tested samples emphasize the need for regular screening for DON in cereal products and the need for monitoring activities to ensure the safety of cereal grain products.

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FUSARIUM TOKSINI U RAZLIČITIM BRAŠNIMA PRIKUPLJENIM NA HRVATSKOM TRŽIŠTU U RAZDOBLJU 2002/2004

SAŽETAK

Istraživana je prisutnost deoksinivalenola (DON), T-2 toksina i fumonizina B_1 u različitim tipovima pšeničnog brašna, graham brašna i kukuruznog brašna. Ukupno 36 različitih uzoraka brašna prikupljeno je u razdoblju od 2002. do 2004. godine. Uzorci su uzimani direktno s polica maloprodajnih mjesta u Hrvatskoj. DON i fumonizin B_1 određivani su metodama HPLC, dok je T-2 toksin određivan metodom plinske kromatografije/masene spektrometrije. T-2 toksin i fumonizin B_1 nisu detektirani niti u jednom uzorku. DON je detektiran u 77,78% svih uzoraka. Stupanj kontaminacije DON-om varirao je ovisno o godini žetve i vrsti uzorka brašna i kretao se od 0 do 0,53 mg kg^{-1}. Iako još uvijek nisu točno određene dozvoljene koncentracije ovih mikotoksina u proizvodima za ljudsku prehranu, EU za DON predlaže koncentraciju od 0,5 mg kg^{-1} za proizvode od žitarica za direktnu prehranu, odnosno 0,75 mg kg^{-1} za sirove žitarice. Kao najvišu dopuštenu količinu DON-a u žitaricama i riži Hrvatsko zakonodavstvo propisuje količinu od 2 mg kg^{-1}, dok je najviša dopuštena količina kombinacije fumonizina B_1, B_2 i B_3 u kukuruzu, kukuruznom brašnu, proizvodima od kukuruza i riži 4 mg kg^{-1}. Hrvatsko zakonodavstvo još uvijek nije donijelo odgovarajuću regulativu za T-2 toksin u proizvodima za ljudsku prehranu.

Ključne riječi: brašno, *Fusarium* toksini, deoksinivalenol, T-2, fumonizin B_1