PRESENCE OF DEOXYNIVALENOL IN MAIZE OF VOJVODINA

ABSTRACT: By applying previously established optimal conditions for the determination of deoxynivalenol (DON) by liquid chromatography with DAD detector, in this work, its content was determined in maize samples collected during the past 3 years (2004—2006) from different locations in Vojvodina. Analyzing 103 maize samples in total, the presence of deoxynivalenol was established in 42.7% of the samples. Only 3 samples of maize contained DON in concentrations that exceeded the maximum permitted level (1 mg/g) legislated in most countries.

KEY WORDS: deoxynivalenol, liquid chromatography, maize

INTRODUCTION

DON is the most widely spread mycotoxin from the trichotecene group, produced by fungi from the genera Fusarium, most frequently Fusarium graminearum and Fusarium culmorum. The most sensitive to the presence of these fungi are wheat and maize, a disease of wheat caused by this fungus is known as Fusarium head blight, while of corn, it is known as Gibberella ear rot (JECFA, 2001). Infection of these cereals causes a decrease in the grain size and the protein content in the grain, and has harmful effect on germination. The final result is a decrease in the yield and the feed quality. Its presence in feed is manifested by rejection of feed, vomiting, diarrhea and finally, the weight loss in livestock (Kuiper-Goedman, 2002). The most sensitive to the presence of deoxynivalenol are pigs, hence already at concentrations of 1 mg/kg in feed a certain percentage of these animals refuse food. That is exactly why this deoxynivalenol doses is the maximum permitted dose in feed intended for this animal species in most countries worldwide.

The maximum permitted level of deoxynivalenol in feed for milk cows is also 1 mg/kg, while the level considered to be permitted for cattle and sheep is...
5 mg/kg, although these species can tolerate levels above 10 mg/kg. The least sensitive species to the presence of this mycotoxin in feed is poultry, although the maximum permitted level of deoxynivalenol in this case is 5 mg/kg (Canadian Grain Commission, 1999).

The maximum permitted levels for this mycotoxin, however, have not been yet legally regulated in our country, either in foodstuffs or in feed.

Most frequently used for quantitative determination of DON are chromatographic methods, *i.e.* liquid chromatography (LC) with or without derivatization of DON, as well as gas chromatography with almost compulsory derivatization, and somewhat less commonly used thin layer chromatography (TLC), immunochemical method and others (JECFA, 2001; Krska, 2001; Lombaert, 2002; Jajić, 2004). All quantitative methods for deoxynivalenol determination (except immunochemical) require clean-up of crude sample extract by solid phase extraction (SPE). To that purpose, columns with different sorbents are used: activated charcoal, alumina and celite (Epley et al., 1986), florisil (Sano et al., 1987), silica-gel, ion exchange resins, as well as different combinations of the above sorbents (Lauren and Greenhalgh, 1987). Lately, the most frequently used are multifunctional, so called MycoSep columns (Weingartner et al., 1997; Mateo et al., 2001) and to some extent less often immunoaffinity columns filled with antibodies specific for an individual mycotoxin (Cahill et al., 1999).

In the study that encompassed the available data from the entire world, performed by JECFA (2001), deoxynivalenol was found as a frequent contaminant of cereal grains such as wheat (11444 samples, 57% contaminated), maize (5349 samples, 40% contaminated), oats (834 samples, 68% contaminated), barley (1662 samples, 59% contaminated), rye (295 samples, 49% contaminated), and rice (154 samples, 27% contaminated).

The aim of this work was to determine the DON content in maize samples collected in Vojvodina by applying previously established optimal conditions for DON determination by liquid chromatography with DAD detector (Abramović et al., 2005). In addition, this paper attempted to compare the data with those found in relevant literature about the incidence of this mycotoxin in countries of our region.

**MATERIAL AND METHODS**

*Materials*

All solvents used for DON extraction from corn samples, as well as for the mobile phase preparation were of LC grade. All chemicals used in the investigation were of reagent grade. Solutions were prepared in deionized water, except when stated otherwise.

*Deoxynivalenol calibrant solutions.* Deoxynivalenol (Biopure, Tulln, Austria) was purchased as an analytical standard. Calibrant solution was prepared in ethyl acetate-methanol (19:1, v/v) at the concentration of 85.05 μg/cm³ from crystalline substance, according to AOAC method 986.17. Stock solution con-
taining DON at 17.01 μg/cm³ was prepared by measuring 2.00 cm³ calibrant solution of DON into a 10 cm³ volumetric flask, and diluting to volume with ethyl acetate-methanol (19:1, v/v). Working calibrant solutions were prepared by evaporation of the appropriate volume of the stock solution and dilution with the appropriate volume of methanol. Standard solutions were stored at 4°C.

**Sample and preparation.** Maize samples were collected during past 3 years (2004—2005) from different locations in Vojvodina. Immediately after the sampling, each sample was prepared by grinding in a laboratory mill. After that, the sample was homogenized by mixing. Sample prepared in such a way was packed in plastic bags and stored in a freezer at −20°C until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

**Apparatus**

The equipment consisted of an LC system — HP1090 Liquid Chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a DAD detector (Hewlett Packard, Palo Alto, CA, USA) and a column Hypersil ODS (100 x 4.6 mm i.d., particle size 5 μm, Agilent Technologies, USA).

Activated charcoal-alumina-Celite-cation exchange resin (CACC) column. The column was prepared in the following way: a plug of glass wool was inserted into the tapered end of a glass tube (9 cm x 1.5 cm i.d.); then 0.1 g of Celite (545, Merck, Darmstadt), 1.5 g of activated charcoal (Darco G-60, Sigma-Aldrich, Steinheim), alumina (70—230 mesh, Merck, Darmstadt), and Celite mixture (7:5:3) were added, loosely packed, and tapped to level. 2 g of cation exchange resin (0.3—0.9 mm, Kemika, Zagreb), prewashed with 10 cm³ of methanol, was added and lightly compacted above activated charcoal-alumina-Celite by pushing down a second glass wool plug.

The following equipment was used to perform the analyses: magnetic stirrer (MM-530, Tehtnica Železniki, Yugoslavia), sample evaporator (Rotavapor-R, Buchl, Switzerland), 1.5 μm microfiber filters (110 mm i.d., Vicam, Watertown, MA, USA), pipettes of different volumes (Eppendorf, Hamburg, Germany), 5B Advantec filter paper (0.13 mg/circle, 125 mm i.d., Toyo Roshi Kaisha, Ltd., Japan), and single position pump stand (Vicam, Watertown, MA, USA).

**Procedure**

**Principle.** DON was extracted from maize with the mixture of acetonitrile (ACN) — water. After filtration, the crude extract was cleaned-up on CACC column. The cleaned-up extract was evaporated up to dryness, residue redissolved in methanol and analyzed by liquid chromatography with DAD detection.

**Extraction and clean-up.** 25.0 g of the sample were extracted with 100 cm³ of ACN-water (84:16, v/v) and shaken on a magnetic stirrer for 60 minutes. After filtration through Advantec filter paper, 6.0 cm³ of the extract were applied to the prepared column. The column was then washed with 5 cm³ of the solvent mixture comprising of ACN-water (84:16, v/v) at about 0.6 cm³/
The cleaned-up extract was evaporated to dryness, dissolved in 3 cm$^3$ of ethyl acetate and quantitatively transferred to an evaporation vessel by triple washing with 1.5 cm$^3$ ethyl acetate. The eluate was evaporated up to dryness.

**Liquid chromatography.** The purified, evaporated residue was redissolved in 300 ml methanol, and a 15 ml aliquot of the solution was injected into the LC system at following chromatographic conditions: mobile phase, a mixture of solvents ACN-water (16:84, v/v), $\lambda = 220$ nm, flow rate 0.6 cm$^3$/min. Calibration curves used for quantitative determination were constructed on the basis of the area under the DON chromatographic peaks, using the working standard solutions.

### RESULTS AND DISCUSSION

Samples of maize, collected over the period 2004—2006, in Vojvodina were analyzed and the results are presented in Table 1. As it can be seen, the DON content was above the LOQ in 50% maize of samples collected in 2004. The number of samples from the 2005 harvest was much higher (76) and the percentage of DON positive samples was 42.1%. During 2006, 17 samples were collected and analyzed, and 41.2% was positive. Only 3 samples of maize contained DON in concentrations that exceeded the maximum permitted level (1 µg/g) legislated in most countries.

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of samples</th>
<th>No. of positive samples (%)</th>
<th>Concentration in samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average (mg kg$^{-1}$)</td>
</tr>
<tr>
<td>2004</td>
<td>10</td>
<td>50.0</td>
<td>0.54</td>
</tr>
<tr>
<td>2005</td>
<td>76</td>
<td>42.1</td>
<td>0.36</td>
</tr>
<tr>
<td>2006</td>
<td>17</td>
<td>41.2</td>
<td>0.26</td>
</tr>
</tbody>
</table>

In case of maize, the infection of the ear most frequently takes place through the tip of the ear, when the fungi penetrate through the silk in the phase of maize flowering (Sutton, 1982). Exceptionally humid weather in the period from silking to ripening, enables ear contamination (Vigier et al., 1997). The ear is the most sensitive to contamination at the beginning of silking, while this sensitivity lowers with silk aging (Reid et al., 1992; Reid and Hamilton 1996). The silking period in the climatic region of Vojvodina and Serbia takes place within about 60 days from the moment of plant sprouting (during the month of July and the first half of August).

According to the reports of the Republic Hydrometeorological Service of Serbia (2004; 2005; 2006) the average monthly spring and summer temperatures were somewhat higher (2004), i.e. around the average value (2005) in comparison to the appropriate long-term average value (1971—2000). With respect to humidity, the year 2004 was somewhat more humid than the appropriate long-term average (1971—2000), while 2005 can be classified as “highly humid” because of frequent rains, especially in the period July-August, which
is, as it has already been said, the critical period for the development of fungi in maize. In July 2006, the average daily temperature values were higher, with deficit rainfall, while in August, it was colder and more humid than the appropriate long-term average (1971—2000), especially in the south of Vojvodina and central Serbia.

Aforementioned data infers that favourable conditions for the growth of *F. graminearum* and subsequent DON toxin production might have occurred, especially in 2005. However, the similar results, regarding the contamination with DON during 2004 and 2005, and in some cases, even higher contamination of the 2004 harvest (although the climatic conditions were somewhat less favourable for the fungal growth), are most likely due to the fact that the samples were analyzed one year after storage in barns, which enabled further mycotoxin production.

In the study, performed by JECFA (2001), deoxynivalenol was a frequent contaminant of maize (5349 samples, 40% contaminated) in the concentration range 3—3700 μg kg⁻¹. The European study on the occurrence of *Fusarium* toxins (EC, 2003) revealed that 57% of the samples of cereals from 11 countries (11022 samples) were positive for DON. A high frequency of DON was found in maize (89%).

In the period between 1991 and 1998, Rafai et al. (2000) investigated maize (760) for the presence and concentration of DON in Hungarian cereals. The incidence rate of DON in maize was 10.8%.

Curtui et al. (1998) analyzed samples of maize (30), collected in 1997 after the harvest in western Romania, by enzyme immunoassays. Frequency of DON contamination was 46% (median value 890 μmg kg⁻¹ and maximum concentration 160,000 μmg kg⁻¹). Climatic conditions prevailing in the summer months of 1997 were characterized by heavy rainfall before harvest.

As it can be seen, in respect to the obtained results of the incidence rate of DON in analyzed samples, it can be said that they mostly fluctuate. Namely, differing from the results for Hungarian maize samples (Rafai et al., 2000), in which low incidence rate of DON (10.8%) was observed, in Romanian and our maize samples it is significantly higher (46 and 42.7%, respectively). Similar results, 40%, were presented in JECFA (2001), while the EC (2003) reports a significantly higher contamination of maize, even 89%.

Such differences in contamination by DON in maize of the region are most likely due to several factors. Data are not from the same years and impact of the climatic factors, as it can be seen, is considerable. Some authors like Rafai et al., (2000), did not provide data about the climatic conditions during the period of their research (1991—1998). Also, the limit of detection or limit of quantitation of the analytical method used for DON determination also influence the incidence rate of DON in maize. We believe that different agrotechnical conditions among the countries of region are not important, because land treatments and growing crops are performed in similar manner. This is particularly the case if comparisons are made in respect to the results presented in the studies of JECFA (2001) and EC (2003).

In conclusion to the occurrence of the range of DON in maize in Vojvodina and Serbia, on the basis of the foregoing discussion of our findings, with
reference to data in relevant literature, it can be said that although the incidence rate of DON in Vojvodina-grown maize is occasionally considerable, the position of the country is not worse than the average of the surrounding countries. Besides, the concentration range of DON is low or medium, while the concentration was higher than the maximum level adopted by EC only in three cases. By regulating the maximum permitted level of DON in feed and food in Serbia, as well as by establishing monitoring programs the risk for the consumer could be minimized.

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ПРИСУСТВО ДЕОКСИНИВАЛЕНОЛА У КУКУРУЗУ VOJVODINE

Игор М. Јајић1, Биљана Ф. Абрамовић2, Верица Б. Јурић1, Саша З. Крстовић1
1 Пољопривредни факултет, Департман за сточарство, Трг Доситеја Обрадовића 8, 21000 Нови Сад, Србија
2 Природно-математички факултет, Департман за хемију, Трг Доситеја Обрадовића 3, 21000 Нови Сад, Србија

Резиме

Примењивањем претходно утврђене оптималне услове за одређивање деоксиниваленола тачном хроматографијом са DAD детектором, у овом раду је одређен његов садржај у узорцима кукуруза који су током протекле 3 године (2004—2006) сакупљени са различитих локалитета у Војводини. Анализом укупно 103 узорка кукуруза утврђена је присутност деоксиниваленола у 42,7% уз констатацију да је свега 3 узорка имало садржај изнад 1 µg/g, количине која је максимално дозвољена у већини земаља.