

Marko M. JAUKOVIĆ^{1*}, Veselinka M. ZEČEVIĆ²,
Slavica Ž. STANKOVIĆ³, Vesna S. KRNJAJA⁴

¹ Jugoinspekt Beograd ad, Teodora Drajzera 11, Belgrade 11000, Republic of Serbia

² John Naisbitt University, Faculty of Biofarming, Maršala Tita 39, Bačka Topola 24300, Republic of Serbia

³ Maize Research Institute Belgrade – Zemun Polje, Slobodana Bajića 1, Beograd 11185, Republic of Serbia

⁴ Institute for Animal Husbandry, Autoput 16, Belgrade 11080, Republic of Serbia

PRESENCE OF DEOXYNIVALENOL IN WHEAT MILLING PRODUCTS IN SERBIA DURING 2016–2017

ABSTRACT: Deoxynivalenol (DON) is one of several mycotoxins produced by certain *Fusarium* species that frequently infect wheat, corn, rice, oats, barley and other grains in the field or during storage. DON affects animal and human health causing vomiting, acute temporary nausea, diarrhea, abdominal pain, headache, dizziness and fever. The objective of this study was to evaluate the natural occurrence of deoxynivalenol (DON) in white wheat flour, whole wheat flour and wheat bran. In this study, a total of 75 white wheat flour, whole wheat flour and wheat bran samples were collected in the period of 2016–2017. All samples were analyzed for DON by enzyme-linked immunosorbent assay. DON was detected in 23 out of 45 white wheat flour samples (51.11%), at levels ranging from 99 µg/kg to 440 µg/kg. Out of 15 whole wheat flour samples, 14 were contaminated by DON (93.33%), at levels ranging from 98 µg/kg to 479 µg/kg. The maximum contamination level of DON (2,790 µg/kg) in this study was found in wheat bran. Presence of DON was detected in all 15 samples of wheat bran (100%). These results suggest a high percentage of contaminated samples, especially among wheat bran samples, which raises a risk for consumers of wheat bran and the need to monitor final products before consumption.

KEYWORDS: Deoxynivalenol, wheat flour, wheat bran, ELISA

INTRODUCTION

Wheat and wheat-based products are considered to be staple food for the majority of the world population (Škrbić *et al.* 2012). Unfortunately, wheat like many other cereals is susceptible to fungal attack, therefore to possible mycotoxin contamination. The occurrence of mycotoxins in cereals is of great concern worldwide, because their presence is often associated with chronic or acute mycotoxicoses. Approximately 25% of cereals produced in the world are contaminated with mycotoxins (Charmley *et al.* 1995).

* Corresponding author. E-mail: jaukovicmarko@gmail.com

A great variety of fungi can produce mycotoxins; however several *Fusarium* species, a widespread pathogens on cereals in both temperate and semitropical areas, are a major concern for all European cereal growing areas (Bottalico, 1998). The percentage of contamination on the worldwide level for some *Fusarium* toxins, such is DON, is considered to be much higher than 25% (Bullermann, 1996).

Deoxinivalenol (DON, vomitoxin) is a natural-occurring mycotoxin, type B-trichothecenes produced mainly by strains of *F. graminearum*, a food-borne fungi widely spread in crops. DON is considered to be one of the most important mycotoxins in wheat and wheat based products. It affects both animal and human health by causing gastro-intestinal problems followed by diarrhea and vomiting (Kushiro, 2008).

Approximately 600 million tons of wheat are produced per year and most of it is converted to wheat flour for human consumption (Kushiro, 2008). This makes DON contamination of wheat a great concern for human health. Wheat-based products, such is wheat flour, hold an essential place in Serbian diet, as well. Wheat flour and wheat flour-based products, such are bread, pasta, pastry and cookies represent approximately 26% of Serbian market basket (Škrbić *et al.* 2012). Although, occurrence and prevention of DON have been intensively studied, there are only a few of studies conducted in Serbia on retention of DON after harvest and during processing. The study on retention of DON during primary processing (milling) is important for the risk assessment and management for majority of world population (Kushiro, 2008).

The objective of this study was the examination and determination of the presence of DON in wheat flour and wheat bran collected from Serbian producers in order to determine the levels of contamination in different wheat milling products.

MATERIALS AND METHODS

Reagents and chemicals

RIDASCREEN FAST DON SC (R-Biopharm), a competitive enzyme immunoassay for quantitative analysis of DON in cereals, malt and feed was used according to manufacturer's instruction (RIDASCREEN FAST DON SC Art. No.: R5905). Distilled water was used for the extraction.

Collection of samples

From October 2016 until April 2017, 75 samples of white wheat flour, whole wheat flour and wheat bran were collected from 9 Serbian producers, as a part of the food safety control. Samples were collected in packs of 1 kg, according to European regulation on methods for sampling (EC 401/2006). Out of total number of samples, 45 were white wheat flour, type T 400 (25 samples) and T 500 (20 samples). 15 samples of whole wheat flour and 15 samples of wheat

bran were collected, as well. Ash content, calculated on dry matter for T 400 flour is up to 0.45%, for T 500 it ranges from 0.46 to 0.60%, for whole wheat flour it is up to 2.2%, while for wheat bran it is up to 7.0% (*Službeni glasnik Republike Srbije*, 68/16)*. Before analysis, the samples were stored at 4–6 °C and were protected from light.

Sample preparation

All samples were thoroughly mixed in order to homogenize. Namely, 5 g of each sample of white wheat flour, whole wheat flour and wheat bran were extracted by shaking with 100 mL of distilled water manually for 5 minutes. After shaking, sample extracts were filtered through Whatman No.1 filter. 50 µL of the filtrate was used for further analysis according to RIDASCREEN FAST DON SC manual.

Instrumental conditions

The measurement was performed photometrically at 450 nm. UT-2100C microplate reader with absorbance range 0–3,500 A was used. Continuous reading mode was used with reading speed $t < 5$ s. The absorbance is inversely proportional to the DON concentration in the sample. Using method was validated (LoD = 75 µg/kg, Recovery = 92%).

RESULTS AND DISCUSSION

The results on occurrence of DON in white wheat flour, whole wheat flour and wheat bran are given in Table 1.

Table 1. Occurrence of deoxynivalenol (DON) in white wheat flour, whole wheat flour and wheat bran. a) Arithmetic mean. Values below the detection limit (75 µg/kg) are set to have concentration of half of detection limit

Commodity	DON (µg/kg)				
	No. of positives/total	Average value*	Median value	max value	Interval of concentration (contaminated samples)
white wheat flour	23/45	142	81	440	99–440
whole wheat flour	14/15	307	354	479	98–479
wheat bran	15/15	1,074	961	2,790	161–2,790

* Official gazette of the Republic of Serbia, translator's comment

DON was detected in 23 out of 45 white wheat flour samples (51.11%), at levels ranging from 99 to 440 µg/kg. The average and median values obtained for DON in white wheat flour were 142 µg/kg and 81 µg/kg, respectively. Out of 15 whole wheat flour samples, 14 were contaminated by DON (93.33%), at levels ranging from 98 µg/kg to 479 µg/kg. The average and median values obtained for DON in whole wheat flour were 307 µg/kg and 354 µg/kg, respectively. None of the white wheat flour nor whole wheat flour samples exceeded the limit of 750 µg/kg set by Serbian regulative for allowed presence of DON in cereal flour (*Službeni glasnik Republike Srbije*, 29/2014, 37/2014 – isp. 39/2014, 72/2014, 80/2015, 84/2015, 35/2016 and 81/2016). The maximum contamination level of DON (2,790 µg/kg) in this study was found in wheat bran. Presence of DON was detected in all 15 samples of wheat bran (100%). The average and median values obtained for DON in wheat bran were 1,074 µg/kg and 961 µg/kg, respectively. Nine of the 15 contaminated samples of wheat bran exceeded the limit of 750 µg/kg set by Serbian regulative for allowed presence of DON in wheat bran intended for human consumption (*Službeni glasnik Republike Srbije*, br. 29/2014, 37/2014 – isp., 39/2014, 72/2014, 80/2015, 84/2015, 35/2016 and 81/2016). However, wheat bran is mostly used as an animal feed. The limit of 8000 µg/kg set by Serbian regulative for allowed presence of DON in wheat bran intended for animal feed was not exceeded in this case (*Službeni glasnik Republike Srbije*, 27/14).

The obtained results are in compliance with the conclusions of the study conducted by Abbas *et al.* (1985) which shows that the distribution of DON is not uniform in the milling fractions. They also found that the highest concentration of DON was in bran, followed by reduction flour and break flour, which proves that the invasion of fungus into the wheat is not uniform, as well. Trigo-Stockli *et al.* (1996) reported in the similar study that DON levels were the highest in the bran (3.4 mg/kg) and the lowest in the flour (1.5 mg/kg), as well. This could be due to the fact that after milling most of the concentration remains in outer layers (Tanaka *et al.* 1986). Others have also reported various concentrations of DON in different milling fractions. For example, Hart and Braselton (1983) reported concentrations of DON of 5.2 mg/kg and 4.5 mg/kg in bran and straight grade flour, respectively. The correlation of DON levels with ash concentration was also reported by Abbas *et al.* (1985). However, this still remains to be studied for application.

CONCLUSION

The presence of DON was detected in 52 out of 75 analyzed samples of white wheat flour, whole wheat flour and wheat bran. The highest percentage of contaminated samples was detected among wheat bran samples (100%), followed by whole wheat flour (93.33%) and white wheat flour (51.11%). The maximum contamination level of DON (2,790 µg/kg) in this study was found in wheat bran. All of the wheat flour samples are in compliance with Serbian regulative (*Službeni glasnik Republike Srbije* 29/2014, 37/2014 – isp. 39/2014,

72/2014, 80/2015, 84/2015, 35/2016 and 81/2016). All of the wheat bran samples are in compliance with Serbian regulative for allowed presence of DON in wheat bran intended for animal feed (*Službeni glasnik Republike Srbije*, 27/14). However, 60% of wheat bran samples could not be used for human consumption due to exceeding levels set by Serbian regulative for allowed presence of DON in wheat bran intended for human consumption (*Službeni glasnik Republike Srbije*, 29/2014, 37/2014 – isp. 39/2014, 72/2014, 80/2015, 84/2015, 35/2016 and 81/2016).

These results suggest a high percentage of contaminated samples, especially among wheat bran samples, which raises a risk for wheat bran consumers. Additionally, this study indicates the need for continuous monitoring of final wheat based products before consumption.

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ПОЈАВА ДЕОКСИНИВАЛЕНОЛА У МЛИНСКИМ ПРОИЗВОДИМА ОД ПШЕНИЦЕ У ПЕРИОДУ 2016–2017. У СРБИЈИ

Марко М. ЈАУКОВИЋ¹, Веселинка М. ЗЕЧЕВИЋ²,
Славица Ж. СТАНКОВИЋ³, Весна С. КРЊАЈА⁴

¹ Југоинспект Београд АД,
Теодора Драјзера 11, Београд 11000, Република Србија
² Универзитет „Дон Незбит“, Факултет за биофарминг,
Маршала Тита 39, Бачка Топола 24300, Република Србија
³ Институт за кукуруз „Земун Поље“,
Слободана Бајића 1, Земун Поље 11185, Република Србија
⁴ Институт за сточарство,
Аутопут 16, Београд 11080, Република Србија

РЕЗИМЕ: Деоксиниваленол (DON) један је од неколико микотоксина које продукују одређене врсте рода *Fusarium*, које често контаминирају пшеницу, кукуруз, пиринач, оvas, јечам и остале житарице, како у пољу, тако и током периода складиштења. DON утиче на здравље људи и животиња изазивајући повраћање, акутне мучнине, дијареју, абдоминални бол, главобоље, вртоглавице и грозницу. Циљ овог истраживања био је да се испита и процени природна појава деоксиниваленола (DON) у белом пшеничном брашну, интегралном пшеничном брашну и пшеничним мекињама. Укупно 75 узорак белог пшеничног брашна, интегралног пшеничног брашна и пшеничних мекиња узорковано је у периоду 2016–2017. Сви узорци су анализирани на присуство DON-а имуноензимским ELISA тестовима. Присуство DON-а је детектовано у 23 од 45 узорак белог пшеничног брашна (51,11%), у концентрационом опсегу од 99 до 440 µg/kg. Од 15 испитаних узорак интегралног пшеничног брашна 14 је било контаминирано (93,33%), а концентрација DON-а кретала се у опсегу од 98 до 479 µg/kg. Максимална концентрација DON-а (2.790 µg/kg) у овом истраживању забележена је код пшеничних мекиња. Присуство DON-а детектовано је у свих 15 испитиваних узорак пшеничних мекиња (100%). Ови резултати указују на висок проценат контаминираних узорак, поготово код узорак пшеничних мекиња, што представља ризик по потрошаче и изискује потребу за мониторингом финалних производа пре пуштања у промет.

КЉУЧНЕ РЕЧИ: деоксиниваленол, пшенично брашно, пшеничне мекиње, ELISA