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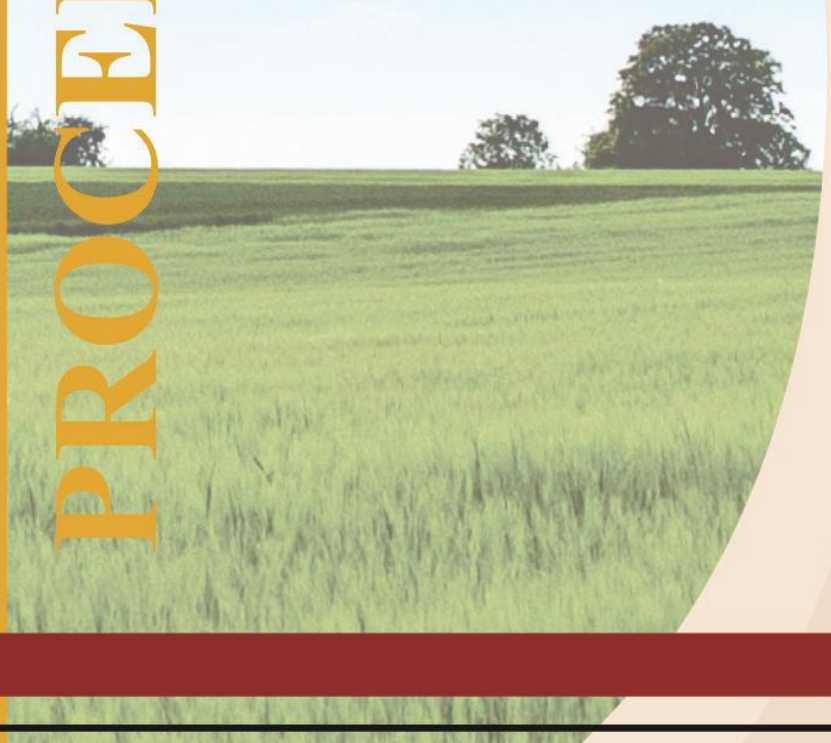

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PRESENCE OF DEOXYNIVALENOL IN BREAD IN SERBIA DURING 2018-2019

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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. Wheat flour and wheat flour-based products, such is bread take an essential place in Serbian diet which raises exposure level of total population in cases of higher contamination. The objective of this study was to evaluate the presence of DON in wheat flour bread. In this study, a total of 210 samples of wheat flour bread were collected in the period of 2018-2019. All samples were analyzed for DON by enzyme-linked immunosorbent assay. DON was detected in 47 out of 210 wheat flour bread samples (22.38%), at levels ranging from 81 to 214 μg/kg. The maximum contamination level of DON (214 μg/kg) in this study was found in wholemeal bread. These results suggest not very high percentage of contaminated samples. However, the level of contamination was higher in wholemeal bread than in white bread, which raises a risk for consumers of bread made of whole wheat flour.

Key words: Deoxynivalenol, bread, ELISA.

Introduction

Wheat bread is a staple food prepared by baking a dough of flour and water usually leavened with yeast, which is widely consumed around the world (Dewettinck *et al.,* 2008).

Unfortunately, wheat like many other cereals is susceptible to fungal attack and possible mycotoxin contamination, which affects wheat-based products, as well. The presence of mycotoxins is often associated with chronic or acute mycotoxicoses, therefore their occurrence in cereals is of great concern worldwide . Approximately 25% of cereals produced in the world are contaminated with mycotoxins (Charmley *et al.,* 1995).

A great variety of fungi can produce mycotoxins, however several *Fusarium* species are predominant pathogens on cereals in both temperate and semitropical areas and present 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-occuring 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).

Although, occurrence and prevention of DON has been intensively studied, there is a small number of studies conducted in Serbia on retention of DON after harvest and during processing. It is important to know survival rate of mycotoxins during processing in order to evaluate the risk that Fusarium mycotoxins might pose to the consumer. To achieve this it is necessary to study both the occurrence of the mycotoxins in primary agricultural crops such as wheat and the effect of processing and food manufacturing on their concentrations in the

retail products (Hazel and Patel, 2004). While considerable data are available to show the frequency and levels of Fusarium mycotoxins, particularly DON, occurring in wheat there is much less information on their transfer to wheat-based products such as bread, cakes, pastries and biscuits at the retail point (Scudamore *et al.,* 2009).

Wheat flour and wheat flour-based products, such are bread, pasta, pastry and cookies represent approximately 26% of Serbian market basket and hold and essential place in Serbian diet (Škrbić *et al.,* 2012).

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

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 September 2018 until April 2019, 210 samples of white bread and wholemeal bread were collected from 50 Serbian producers, as a part of the food safety control. Of total number of samples 150 were white bread, and 60 were wholemeal bread. Before analysis, the samples were stored at 4-6 °C and protected from light.

Sample preparation

Collected samples were prepared and analyzed in accredited laboratory for testing food and feed safety Jugoinspekt Beograd. All samples were thoroughly homogenized. Namely, 5 g of each sample of white bread and wholemeal bread was 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 is made photometrically at 450 nm. The absorbance is inversely proportional to the DON concentration in the sample. Multiskan FC microplate reader with absorbance range 0 - 6.000 A was used. Normal reading mode was used with reading speed t = 13 s. Using method was validated (LoD = 75 µg/kg, Recovery = 92%).

Statistical analysis

All obtained data were analyzed using SPSS 15.0 software (SPSS, IBM corporation, USA).

Results and Discussion

The results on occurrence of deoxynivalenol in white wheat flour bread, whole wheat flour bread are given in Table 1.

DON was detected in 18 out of 150 white bread samples (12.00%), at levels ranging from 78 to 176 µg/kg. The average and median values obtained for DON in white bread were 101 and 85 µg/kg, respectively. Of 60 samples of whole meal bread 29 was contaminated by DON (48.33%), at levels ranging from 118 to 214 µg/kg. The average and median values obtained for DON in wholemeal bread were 113 and 94 µg/kg, respectively. The maximum contamination level of DON (214 µg/kg) in this study was found in wholemeal bread. None of the white bread nor wholemeal bread samples exceeded the limit of 500 µg/kg set by Serbian regulative for allowed presence of DON in wheat flour bread („Sl. glasnik RS", br. 22/2018 - Official Gazette of the RS no. 22/2018). The obtained results are in compliance with the level of contamination of bread showed in a study report done by EFSA. (Scientific

report of EFSA, 2013). Higher levels of DON found in wholemeal bread are due to the fact that the distribution of DON is not uniform in the milling fractions (Abbas *et al.,* 1985). Abbas *et al.* (1985) found that the highest concentration 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) in the similar study, reported that DON levels were highest in the bran (3.4 mg/kg) and 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).

Table 1. Occurrence of deoxynivalenol (DON) in white bread and wholemeal bread

Commodity	DON (μg/kg)				
	No. of positives/total	Average value [a]	Median value	max value	Interval of concentration (contaminated samples)
white bread	18/150	124	122	176	81 - 176
wholemeal bread	29/60	169	189	214	88 - 214

a) Arithmetic mean. Values below the detection limit (75 μg/kg) are set to have concentration of half of detection limit

Conclusion

The presence of DON was detected in 47 out of 210 analyzed samples of white bread and wholemeal bread. Higher percent of contaminated samples was detected among wholemeal bread samples (48.33%) than in white bread samples (12.00%). The maximum contamination level of DON (214 μg/kg) in this study was found in wholemeal bread. All of the wheat flour bread samples are in compliance with Serbian regulative („Sl. glasnik RS", br. 22/2018 - Official Gazette of the RS no. 22/2018). These results suggest a high percentage of contaminated samples among wholemeal bread samples, which raises a risk for consumers. These data are also important for the realization of a 'Total Diet study' (TDS). The TDS can be a complementary tool to estimate the population dietary exposure to DON across the entire diet by analyzing main foods prepared 'as consumed'. A provisional tolerable daily intake (TDI) for DON was set in 2002 by the Scientific Committee for Food (SCF) at 1 μg/kg body weight (b.w.) per day.

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