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OCCURENCE OF DEOXYNIVALENOL IN WHEAT BRAN IN SERBIA DURING 2019-2020

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Abstract

Animal welfare continues to be an important issue in the whole world. Therefore the production of safe feed remains one of the most important tasks because the consumption of contaminated feeds by livestock has been associated with a variety of adverse health effects including feed refusal, reduced weight gain, diarrhoea and emesis. Wheat bran, a by-product of the dry milling of common wheat (*Triticum aestivum* L.) into flour, is one of the major agro-industrial by-products used in animal feeding. In addition, wheat bran can also been used in human diet as a source of fiber in the preparation of high-fiber pasta, noodles etc. 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. In this study, a total of 72 samples of wheat bran were collected from several producers from Serbia in the period 2019-2020. The samples were analysed for deoxynivalenol (DON) content by using an enzyme-linked immunosorbent assay Ridascreen® Fast DON SC. The occurrence of DON in the tested samples was 100%, with average concentration 2.79 mg kg^{-1} and maximum DON content of 9.12 mg kg^{-1}. These results suggest very high level of DON concentration in all tested samples. The limit of 8.00 mg kg^{-1} imposed by Serbian legislation for DON content in feed was exceeded in two studied samples. However, only fourteen of them (19%) would be suitable for human consumption, due to a much lower allowed limit (0.75 mg kg^{-1}), which raises a risk for consumers.

Key words: *Deoxynivalenol, 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). Although very rich in nutrients and popular among nutritionists who suggest a healthy diet, wheat bran is one of the major agro-industrial by-products used in animal feeding. Wheat bran, a by-product of the dry milling of common wheat (*Triticum aestivum* L.) into flour and it consists of the outer layers (cuticle, pericarp and seedcoat) combined with small amounts of starchy endosperm of the wheat kernel (www.feedipedia.org).

Animal welfare continues to be an important issue in the whole world. Therefore the production of safe feed remains one of the most important tasks because the consumption of contaminated feeds by livestock has been associated with a variety of adverse health effects including feed refusal (mainly by swine), reduced weight gain, diarrhea and emesis (Krska et al., 2001; Kuiper-Goodman, 2002).

Unfortunately, wheat like many other cereals is susceptible to fungal attack, therefore to possible mycotoxin contamination. Each year a large number of crops are affected by fungal invasion. Most of the infection is set in outer layers of the seed, which makes a wheat bran highly contaminated product. The most important agriculturally toxigenic fungi occurring in the moderate climatic zones of North America and Europe are *Fusarium* fungi (Kos et

al.2003). According to the literature, DON is the main *Fusarium* toxic secondary metabolite and its occurrence is also considered to be an indicator of the possible presence of other, more toxic, trichothecenes (Jajic et al., 2008). Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world (Lombaert, 2002). According to Serbian legislation maximum permitted level of DON in feed with a moisture content of 12 % in cereals and cereal products is 8000 μg kg^{-1} (Službeni glasnik RS, 27/2014). Significantly lower maximum permitted level is set for humans, 750 μg kg^{-1} (Službeni glasnik RS, 81/2019).

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 bran collected from Serbian producers in order to raise the awareness among consumer population.

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 June 2019 until May 2020, 72 samples of wheat bran were collected from 5 producers from Autonomous Province of Vojvodina, as a part of the food safety control. Before analysis, the samples were stored at 20-25 °C and protected from light.

Sample preparation

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 = 100 μg/kg, Recovery = 92%).

Results and Discussion

Out of the 72 samples analyzed, 100 % were contaminated with DON (Table 1). This level of occurrence of contamination is not surprising. In 2014 and 2017, 100 % of the samples were contaminated as well (Jaukovic et al., 2015) (Jaukovic et al., 2017). 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 and proves that the highest concentration of DON is always in bran. Trigo-Stockli et al. (1996) reported in the similar study that DON levels were the highest in the bran (3.4 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). However, the level of average DON concentration is significantly higher than in previous years (Table 1).

Last time this high concentrations were detected was in 2010 (Jajić et al., 2014).Among the 128 analyzed wheat samples from 2010, 100 (78.1%) samples were contaminated with DON. DON concentrations in positive samples ranged from 64 to 4808 µg/kg, with mean level of 779 µ/kg. In 16 of the examined wheat samples, the content of DON was above the maximum tolerable level (1250 µg/kg) adopted by the mentioned regulations.

Variations in DON concentration levels could occur due to a variety of factors such as wheat cultivars, the use of Good Agricultural Practices (GAPs), mold strain types, temperature, water activity, nutrient availability, and chemical agents applied (Bianchini et al., 2015). Jajić et al. (2014) pointed out that weather conditions during the plant growth, in particular in the flowering stage, have a major influence on the production of DON. According to the literature data, the optimal temperature for F. graminearum growth is 25 °C, at a water activity above 0.88. In addition, it is known that the incidence of FHB is strongly associated with moisture at the time of flowering (anthesis) and the timing of rainfall, rather than its amount (JECFA, 2001).Analysing samples from the 2010 harvest Jajić et al. (2014) definitely confirmed that the frequency of contamination is highly dependent on weather conditions. High humidity during May and June of 2010 contributed to the development of Fusarium already in the field, and thus the production of DON.

Table 1. Occurrence of DON in wheat bran in Serbia (2013-2020)

Year	No. of samples	No. of positive samples (%)	Average ± SD (µg kg^{-1})	Range (µg kg^{-1})
2013	14	5 (36)	93 ± 18.6	105-125
2014	28	28(100)	652 ± 130.4	343-1250
2015	12	10(83)	458 ± 91.6	120-1340
2016	15	15(100)	1074 ± 214.8	161-2790
2017	14	13(93)	831 ± 166.4	164-1950
2018	12	11(92)	586 ± 117.2	126-1740
2019	16	16(100)	2743 ± 548.6	378-9120
2020	56	56(100)	3068 ± 613.6	745-8510

*Source: Official control by Jugoinspekt Beograd

Conclusion

By analyzing the DON content in samples of wheat bran produced in Serbia in 2019 and 2020, it was found that the samples produced after the harvest in 2019 were 100% contaminated. Also, the average level of contamination in 2019 and 2020 is significantly higher than in previous years. Only 19% of the analysed samples would be suitable for human consumption and two of them even exceed the limits set for animal feed. This proves that natural occurrence of DON is strongly associated with moisture at the time of flowering (anthesis) and the timing of rainfall, rather than its amount. The obtained results for wheat bran confirmed that wheat should be continuously controlled to protect the population against the risk of mycotoxin contamination These results suggest a high percentage of contaminated samples, which raises a risk for consumers. 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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