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Deoxynivalenol in wheat and wheat products from a harvest affected by fusarium head blight

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Abstract

Fusarium head blight is an important disease occurring in wheat, caused mainly by the fungus *Fusarium graminearum*. In addition to direct damage to crops, reduced quality and yield losses, the infected grains can accumulate mycotoxins (toxic metabolites originating from prior fungal growth), especially deoxynivalenol (DON). Wheat crops harvested in 2014/2015 in southern Brazil were affected by high levels of Fusarium head blight. In this context, the aim of this study was evaluate the mycotoxicological quality of Brazilian wheat grains and wheat products (wheat flour and wheat bran for DON. DON contamination was evaluated in 1,504 wheat and wheat product samples produced in Brazil during 2014. It was determined by high performance liquid chromatograph fitted to a mass spectrometer (LC-MS / MS). The results showed that 1,000 (66.5%) out of the total samples tested were positive for DON. The mean level of sample contamination was 1047 $\mu g.kg^{-1}$, but only 242 samples (16.1%) had contamination levels above the maximum permissible levels (MPL) - the maximum content allowed by current Brazilian regulation. As of 2017, MPL will be stricter. Thus, research should be conducted on DON contamination of wheat and wheat products, since wheat is a raw material widely used in the food industry, and DON can cause serious harm to public health.

Keywords: Fusarium; mycotoxins; fungal diseases; scab; fusarium head blight.

Practical Application: The paper presents an overview of deoxynivalenol contamination in wheat and wheat products affected by Fusarium head blight. Contamination is compared with the maximum permissible limits set by current law in Brazil in order to warn consumers about the levels of this contaminant found in the study samples.

1 Introduction

As a result of its nutritional properties, wheat (*Triticum aestivum L.*) is one of the most frequently eaten cereals all over the world by both humans and animals (Vieira, 2006). Brazil is not self-sufficient in the production of this cereal; producing less than half of its consumption needs, and such production is subject to market fluctuations. Because of milder temperatures, the southern region of Brazil accounts for 94.6% of the national production of wheat. However, given the characteristics of the cropping system, the average grain yield in this region is not the highest in Brazil (Empresa Brasileira de Pesquisa Agropecuária, 2013). In addition, the quality of products after wheat processing is directly related to the quality of grains to be processed; therefore, careful cultivation, harvesting and storage of this cereal are crucial (Vieira, 2006).

In plantations, wheat can be contaminated by various diseases because of weather conditions, soil type and crop susceptibility. One of the best-known diseases that commonly affect this cereal is Fusarium head blight, triggered by infection of *Fusarium* fungi, which not only cause diseases in plants but also produce toxic substances known as mycotoxins through their secondary metabolism (Calori-Domingues et al., 2007).

Mycotoxins are naturally-occurring toxic compounds, produced by a variety of fungal species that grow on agricultural products, during their growth in the field and in storage, as well as in processed food and animal feed (Scussel, 2002). They cause significant economic impact because they reduce plant and animal productivity, and also toxicological impact, with clinical manifestations in both humans and animals (Santos, 2009).

Deoxynivalenol (DON) is a trichothecene mycotoxin, produced mainly by *Fusarium graminearum*, and it is one of the most frequently found in small cereal grains (Bando et al., 2007; Freire et al., 2007). Production of DON in wheat is considered a virulence factor of *F. graminearum* (Maier et al., 2006). DON is also known as vomitoxin, because it causes vomiting, especially when consumed by pigs. It is toxicologically relevant, and its synthesis is stimulated by high humidity (Miller, 1995; Beyer et al., 2005), with high incidence in winter cereals, such as wheat and wheat products consumed by humans (Miller, 1995; Mallmann et al., 2003; Calori-Domingues et al., 2007). The World

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Health Organization (WHO) considers DON as a neurotoxin with a teratogenic nature and immunosuppressive characteristics, and, as trichothecenes in general, it has been associated with chronic and fatal intoxication of humans and animals through consumption of contaminated food (Rotter et al., 1996).

Because the occurrence of fungal infection serves as a warning against mycotoxin contamination, it is worrying that wheat crops harvested in 2014/2015 in southern Brazil were affected by high levels of Fusarium head blight. In this context, the aim of this study was to evaluate the mycotoxicological quality of Brazilian wheat grains and wheat products (wheat flour and wheat bran) for DON contamination during the year of 2014.

2 Materials and methods

DON analyses were conducted on the premises of the Laboratory of Mycotoxicological Analyses (LAMIC), Federal University of Santa Maria - RS, Brazil.

2.1 Samples

A total of 1504 samples of wheat and wheat products (668 of wheat grain, 697 of wheat flour and 139 of wheat bran) were collected from southern Brazil between January and December 2014, and analyzed according they were acquired. The samples were being sold and/or used by cooperatives and agriculture companies.

2.2 Standards and solvents

The DON standard was purchased from Sigma (Sigma-Aldrich, Alcobendas, Spain). Acetonitrile and methanol were purchased from J. T. Baker (Deventer, The Netherlands). All solvents were LC grade. Water was purified with a Milli-Q purification system.

2.3 Preparation of the sample

The toxin was extracted according to the official method no. 986.17 from Association of Official Analytical Chemistry (1995), with some modifications. Three grams of the previously ground sample were extracted in vortex mixers with 24 ml of the solvent mixture containing methanol: water (70: 30, v/v). Sample cleanup was not performed in this method. The extracts of the samples were diluted in a combination of aqueous phase, water: ammonium acetate (995: 5, v/v) and organic phase (water: ammonium acetate: methanol (95: 990: 5, v/v/v), and 5 µL were injected into a liquid chromatography system.

2.4 Determination of DON

DON was determined by separation with high-performance liquid chromatography, and detected by sequential mass spectrometry (LC-MS/MS). The isotopes $_{13}C^{20}$-Don and $_{13}C^{20}$-Zea were used as internal standard. For quantification of mycotoxins, a seven-point calibration curve was prepared (200, 400, 600, 1000, 2000, 4000 and 8000 $\mu g.Kg^{-1}$). A validated method was used for sample preparation, extraction and dilution (LOD: 50 $\mu g.Kg^{-1}$; LOQ: 200 $\mu g.Kg^{-1}$). For the collected data, it was assumed that the calibration curve showed a linear behavior ($r^2>0.9$).

2.5 Statistical analysis

The average contamination of all samples and average contamination of positive samples, prevalence and percentage of samples above LMT of DON in wheat grain, wheat flour and wheat bran were calculated in each month of 2014. Tukey test at 5% significance was used to compare the average results of each month. The variables were calculated using Microsoft Excel software (Microsoft Excel 2016 MSO, Microsoft Corporation, Redmond, WA) and analyzed in the statistical program Statgraphics Centurion XV (Statgraphics Centurion 15.2.11, Manugistics Inc., Rockville, MD).

3 Results and discussion

Table 1 shows the results of the occurrence of DON in wheat and wheat products (flour and bran) in 2014. A total of 1000 samples (66.5%) were contaminated with DON, with mean levels of 1574.75 $\mu g.kg^{-1}$. Contamination was most common in November with 399 (90.9%) of positive samples and less frequent in April, with 38 (36.9%) positive samples.

A study by Calori-Domingues et al. (2007) evaluated the occurrence of DON in wheat grown in Brazil and imported from abroad. It was found that 94% of the Brazilian wheat and 88% of the imported wheat were contaminated with mean levels of 332 and 90 $\mu g.Kg^{-1}$, respectively. In addition, 4% of the Brazilian wheat samples analyzed had contamination levels above 1250 $\mu g.Kg^{-1}$, which is the maximum permissible level (MPL) in accordance with European Community law; however, this level is still allowed in Brazil. Although the extent of contamination was greater in that study, the mean levels presented by the authors were below those observed in the period evaluated in the present work, which included samples from crops containing high levels of Fusarium head blight (in the second semester).

Table 1. Occurrence of deoxynivalenol contamination in Brazilian samples of wheat grain, wheat flour and wheat bran harvested in 2014.

Sample	n	Deoxynivalenol (DON)					
		Mean ($\mu g.Kg^{-1}$)	Minimum ($\mu g.Kg^{-1}$)	Maximum ($\mu g.Kg^{-1}$)	Mean positive sample ($\mu g.Kg^{-1}$)	Prevalence (%)	% above LMT*
Wheat grain	668	710	0	11800	937	64.8b	12.1
Wheat flour	415	563	0	11400	693	47.3a	5.9
Wheat bran	139	478	0	5190	640	72.7b	2.2
Total	**1504**	**557**	**0**	**11800**	**817**	**56.5**	-

a-b: Means followed by different letters in the columns by Tukey test ($\rho<0.05$). * LMT= maximum tolerable limit set by Agência Nacional de Vigilância Sanitária (2011, 2013).

Wheat flour was the fraction showing the lower prevalence, while wheat grain and bran presented the highest prevalence of DON. A study by Nowicki et al. (1988) showed that the distribution of DON in ground wheat grain fractions is influenced by the degree of fungal penetration into the grain endosperm, and this susceptibility is dependent on plant variety. Those authors found that when penetration was low, higher levels of infection and DON were found in the grain surface and, consequently, low concentrations of DON were recovered in the flour.

A more recent study indicated that in the post-harvest stage, the cleaning, aeration, debranning and milling procedures have an influence in the distribution of mycotoxins in wheat fractions. In the milling process there is no mycotoxin reduction, although mycotoxin concentrations may be redistributed and concentrate according to the milling fractions. Mycotoxins tend to be concentrated in outer fractions intended for animal feed (bran, flour shorts screenings and middlings) and lower in inner fractions intended for human consumption (flour or semolina) (Cheli et al., 2013).

On the other hand, the not significant difference between the fractions contamination is not in accordance with findings reported in the literature (Nowicki et al., 1988; Samar et al., 2003; Lancova et al., 2008). However, it should be noted that the samples analyzed in this study were independent, and there is no interrelationship between the whole grain and the different fractions. In addition, knowing this survey deals with samples from two different seasons, involving different wheat cultivars yielded in 2014 and naturally showing different sensitivity to FHB, the variation in levels of contamination may have influenced the average results of DON and consequently the statistical results.

Twenty one percent (21% n = 145) of the analyzed wheat grain samples had contamination levels above 2000 $\mu g.kg^{-1}$ (the maximum permissible level for wheat grain for further processing by current Brazilian law (Agência Nacional de Vigilância Sanitária, 2011, 2013), and the highest level of contamination found for the samples was 11800 $\mu g.kg^{-1}$, confirming the low mycotoxicological quality of this cereal from the year 2014. Among the wheat flour samples, 10% (n = 71) were out of the limits currently allowed by Brazilian regulation.

According to the Agriculture Federation of Rio Grande do Sul (FARSUL), as a result of the legislation for mycotoxins (enforced in Brazil since 2011), if the allowed limit (1750-2000 $\mu g.Kg^{-1}$) is exceeded, wheat is not suitable for either human or animal consumption. It should be noted that new values will take effect as of 2017. The same organization also estimates that one million out of the 1.7 million tons produced in the last harvest in Rio Grande do Sul would be affected, and 700,000 tonnes of low quality wheat had been traded until December 9th, 2014 at low prices to international markets willing to absorb this production with low mycotoxicological quality (Batchold, 2015).

Figure 1 shows the mean of monthly concentration and the maximum level found in the evaluated samples of wheat and wheat products. The highest mean of DON contamination was in November, when the samples of the new wheat crop started to be introduced (2014/2015).

According to Companhia Nacional de Abastecimento (2015), in the 2014/2015 harvest in Rio Grande do Sul, the area sown with wheat was as big as 1.14 million hectares, which represents an increase of 9.8% over the previous harvest. However, the same government agency informed that during the growth of

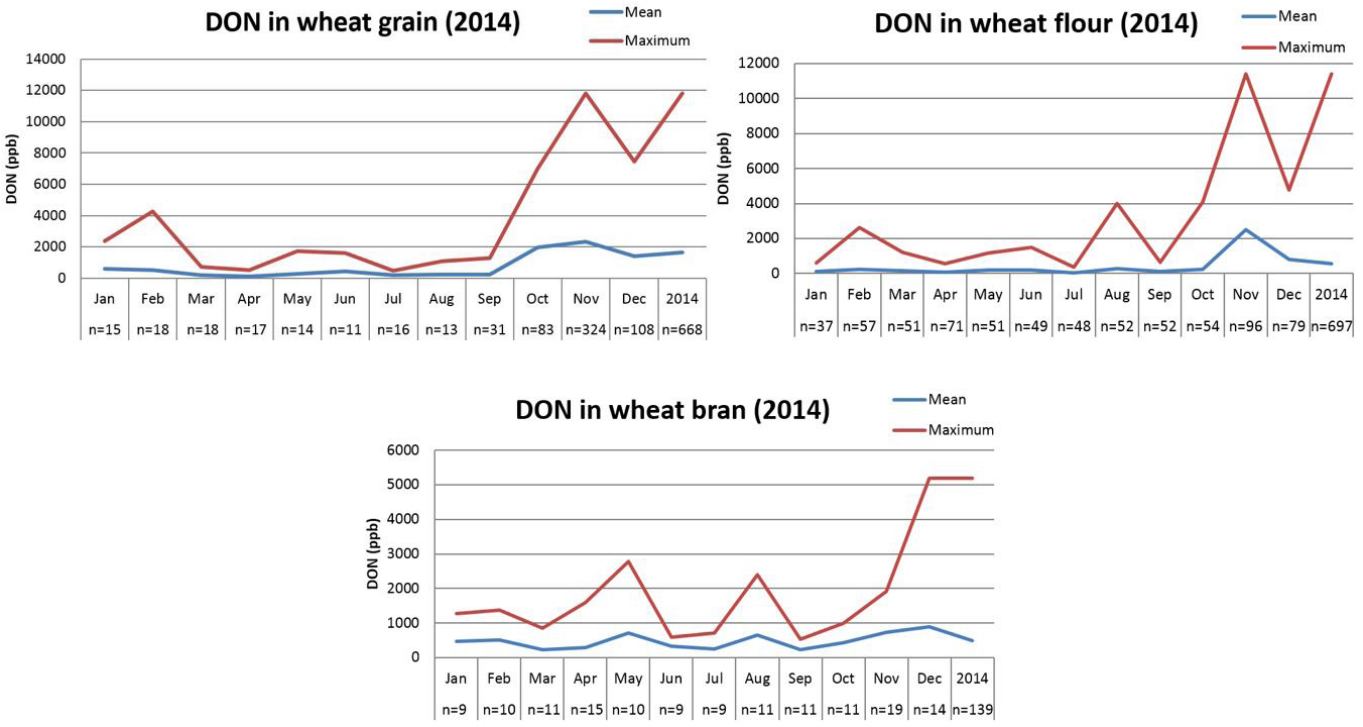

Figure 1. Mean results and monthly maximum concentrations of DON in wheat grain, wheat bran and wheat flour analyzed in 2014.

the crop, several adverse factors - frost, torrential rains, floods, lack of light, excessive heat, disease attacks in general, hail and lodging - influenced the end result.

Buerstmayr et al. (2009) reported that in Southern Brazil (latitude 28 S and 23 S), where wheat crops are located, the main limiting factor for wheat production is the high relative humidity, which favors the occurrence of outbreaks of fungal diseases, especially Fusarium Head Blight (FHB). This disease is influenced by warm temperatures and rainy days during the flowering time, causing losses in grain yield and reductions in baking and seed quality.

Within this framework, Rio Grande do Sul produced 1.5162 thousand tons in 2014, 52.3% less than the harvest in 2013, reflecting a decrease by 56.5% in productivity (Companhia Nacional de Abastecimento, 2015).

Several studies worldwide (Love & Seitz, 1987; Miller, 1995; Trigo-Stockli et al., 1995; Moschini & Fortugno, 1996; Osborne & Stein, 2007) have correlated periods of high humidity with high incidence of Fusarium head blight in wheat crops, which consequently tends to cause higher levels of DON in wheat and wheat products (Lori et al., 2003; Vanheule et al., 2014), as noted in our study in the final quarter of 2014.

4 Conclusions

Considering wheat as a raw material widely used in the food industry and the high levels of DON contamination observed in certain months of the year, especially in samples from the harvest presenting high incidence of Fusarium head blight, it is crucial to carry out appropriate mycotoxicological monitoring of the quality of wheat and wheat products traded to prevent the population exposition to levels above those allowed by regulation.

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