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RESEARCH ARTICLE

Effect of breadmaking process on mycotoxin content in white and whole wheat breads

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Abstract

Background and objectives: *Fusarium graminearum* may reduce the wheat flour yield, technological quality, and breadmaking performance. The objective was to evaluate the effect of breadmaking on the mycotoxin content in white and whole wheat breads to obtain information about the safety of wheat products. The wheat samples were contaminated via the addition of Fusarium-damaged kernels to produce different levels of deoxynivalenol (DON), ranging from <500 to >5,000 μg/kg.

Findings: Because of the breadmaking process, a significant reduction in the DON concentration was observed in both the white and whole wheat breads at the upper DON levels (>2,000 μg/kg) compared with the flours. The DON reduction was 49% and 39%, respectively, in whole and white breads, compared with the original flours. ZON was only detected in the two higher levels of DON in the whole wheat flour (mean 33.1 μg/kg) and in the two whole wheat breads (mean 42.4 μg/kg).

Conclusions: The breadmaking process can be a complementary strategy to reduce the mycotoxin content in wheat products.

Significance and novelty: These results are important for wheat supply chain to meet the legislation requirements and to produce safer foods.

KEYWORDS

deoxynivalenol, food safety, mycotoxin contamination, wheat

1 | INTRODUCTION

Wheat is the main source of nutrients for the world population. Most wheat is converted into wheat flour for human consumption, that is, bakery products, such as bread, pasta, noodles, and cakes (Pacin, Bovier, Cano, Taglieri, & Pezzani, 2010; Šramkova, Gregová, & Šturdík, 2009). Wheat bread provides more nutrients to the world community than any other single food source (Peña, 2002).

Most of the wheat growing area in Brazil is located in the Southern region in the Paraná (56%) and Rio Grande do Sul (33%) states. The current domestic production is unable to supply the national demand of approximately 10.3 million tons annually (USDA, 2017). In Southern Brazil, Fusarium head blight (FHB) is caused by the *Fusarium* graminearum species complex, which produces deoxynivalenol (DON) and zearalenone (ZON) mycotoxins (Del Ponte et al., 2015). DON is the most important mycotoxin because of its widespread occurrence and high concentration in wheat grains. DON disrupts normal cell function, which results in protein synthesis inhibition and affects cell signaling, differentiation, and proliferation (Terzi, Tumino, Stanca, & Morcia, 2014; Vidal, Marín, Morales, Ramos, & Sanchis, 2014). ZON causes a hyperestrogenic syndrome

and reproductive dysfunction (Iqbal, Asi, Jinap, & Rashid, 2014).

Ingestion of contaminated food is a major route for exposure to many contaminants, including mycotoxins (Monaci, Garbetta, De Angelis, Visconti, & Minervini, 2015). Exposition studies have shown the high level of exposure of humans to DON, and high percentages of the population exceed the tolerable daily intake, which is mostly attributed to the consumption of contaminated, wheat-derived products (Vidal, Sanchis, Ramos, & Marín, 2016). In Brazil, Savi et al. (2016) reported that 17% of whole wheat bread and 10% of salted cracker products were contaminated with DON at mean levels of 437 and 624 μg/kg, respectively. In another study, cracker-type biscuit samples from Southern Brazil were analyzed, and 78% of the samples had DON levels ranging from 377 to 5,295 μg/kg, and 22% were contaminated at levels higher than those allowed by regulation (Souza, Caldas, Primel, & Furlong, 2015).

The thermostability of some mycotoxins allows them to withstand most food processes (Vidal, Sanchis, Ramos, & Marín, 2015). Deoxynivalenol is stable at 120°C and moderately stable at 180°C, and it is damaged at 210°C after 40 min (Kamimura, 1989). Zearalenone is a chemically stable compound with a melting point of 164–165°C (EFSA, 2004).

Determining the effects of processing on mycotoxin levels is crucial to estimate consumer exposure. The main factors that affect the fate of mycotoxins during food processing are the food matrix, pH, moisture content, processing temperature, natural or spiked contamination, and concentration of the toxin (Samar, Neira, Resnik, & Pacin, 2001; Wu & Wang, 2015; Zhang & Wang, 2014). Specifically, the mycotoxin stability in the baking process is based on the temperature, time, dough moisture content, type, and quality of the flour and recipe (Milani & Maleki, 2014).

The baking quality (parameters/baking ingredients) and DON stability have been extensively investigated, but the results are conflicting. The inconsistencies may exist because of the following factors: different scales (laboratorial or industrial), the size of the baked products, the use of enzymes, and the baking time and temperature (Vidal et al., 2016).

Zhang and Wang (2014) reported that the DON levels approximately doubled when mixed and fermented doughs were processed into Chinese steamed bread. Another study found that the DON levels in baked bread were significantly higher than those in flour and increased 1.63- to 1.95-fold (Zhang & Wang, 2015). The breadmaking process resulted in low, insignificant reduction rates compared with the initial levels ($p > 0.05$) for DON in both common bread and bread with the addition of 15% wheat germ (Giménez, Blesa, Herrera, & Ariño, 2014). Cakes baked at 160, 180, and 200°C had a significantly lower DON concentration compared with the concentration at the beginning of the process ($p < 0.05$), but the reduction achieved at 140°C was not significant (Vidal et al., 2015). Bergamini et al. (2010) reported a significant reduction in DON levels in bread compared with wheat flour.

The objective of this study was to evaluate the effect of breadmaking on the mycotoxin content in white and whole wheat breads made with *Fusarium* contaminated wheat flour to obtain information about the safety of wheat products.

2 | MATERIALS AND METHODS

2.1 | Sampling characterization

Wheat samples of 6,000 g each, harvested in 2013 crop season from Southern Brazil, were used in this study. Different ratios of wheat kernels naturally contaminated with Fusarium mycotoxins, and sound kernels were used in this work. The amount of DON content was selected using trial error basis and confirmed by ELISA. The wheat sample was divided in four subsamples that were contaminated with different amounts of FHB wheat symptomatic grains. The final deoxynivalenol content in the subsamples was (a) <500, (b) 500–1,999, (c) 2,000–4,999, and (d) >5,000 μg/kg. Detailed information about the contamination process is described in Tibola, Fernandes, Guarienti, and Nicolau (2015).

In all subsamples, moisture and protein contents were determined by Near Infrared Reflectance Spectroscopy (NIR instrument FOSS XDS – RCA, Hoganas, Sweden). The protein content ranged from 12.3% to 13.5% and moisture content ranged from 14.6% to 15.8%. The yield of flour obtained from each subsample in the milling process was 43.9%, 42.0%, 42.6%, and 40.1%, respectively, from level 1 to level 4 of initial DON contamination.

Two milling processes were used to obtain the wheat flour: first, a Laboratory Mill 3100 (Perten Instruments, Huddinge, Sweden) was used to obtain the whole grain flour used to bake the whole wheat bread. The Quadrumat Senior Brabender experimental mill (Brabender, Duisburg, Germany) was used to obtain the white wheat flour for traditional white bread processing.

2.2 | Baking procedures

Breads were baked without fat using the straight-dough breadmaking process based on the bread method used in breeding programs (IRAM, 1996). The ingredients were (Oro, Miranda, Tatsch, & De Francisco, 2012) wheat

flour (100%), sucrose (1.7%), instant active dry yeast (4%), sodium chloride (0.7%), and deionized water (in a sufficient amount to stabilize a 14% moisture content). The ingredients were mixed in one operation for 6 min, until their complete hydration and optimum dough development, in a Brabender farinograph mixer (300 g). The dough was kneaded, manually sheeted (Atlas 150 & Pastabike, Marcato S.P.A., Italy), divided into 100 g dough pieces, with three replicates, and placed into a rectangular mold (4 x 8 x 3 cm). The dough was then fermented for 40 min in a proofing cabinet (Gelopar, Brazil) at 30°C with a 100% relative humidity. Subsequently, the dough was baked in an electric oven (Fischer, Brazil) for 35 min at 180°C.

After cooling (at least 1 hr at room temperature), the bread was weighed. The mean bread loaf weight was 85.63 and 85.86 g for white and whole wheat breads, respectively.

The breads were dried in an oven at 50°C for 24 hrs, milled in a laboratory blender, and mixed before 60 g samples were taken for the mycotoxin analysis. The crushed dry bread was homogenized prior to the mycotoxin analysis. The collected set of samples was sent to a reference laboratory (Samitec, Santa Maria/RS) for mycotoxin quantification.

2.3 | Mycotoxin analysis

The mycotoxin content was determined in flour and in bread on an "as is" basis, that is, the concentration determined without any correction for moisture content.

The DON and ZON contents in the breads were determined using ultra high-performance liquid chromatography in tandem with triple quadrupole mass spectrometry (UHPLC-MS/MS). The extraction, clarification, and derivatization methodologies were fully automatized and developed and validated in house using the method described by Varga et al. (2012). The laboratory obtained the following limits of quantification and recovery: for DON 200 μg/kg and 80%, respectively; for ZON, 20 μg/kg and 85%, respectively. The relative standard deviation (RSD) for DON and ZON was 4% and 4.75%, respectively. The DON-acetylated derivatives (15-ADON and 3-ADON) and nivalenol were not detected in any of the whole wheat and white flours.

2.4 | Statistical analysis

Statistical analyses were performed using R Software 3.3.3 (R Development Core Team, 2017). The independent t test was used to determine the differences in the DON and ZON contents, in each contaminated subsample. The significance was set at $p < 0.05$.

3 | RESULTS

3.1 | Deoxynivalenol

After the breadmaking process, a reduction in the DON content was observed at the higher contamination levels (>2,000 μg/kg) in the white and whole wheat breads compared with the original flours (Table 1).

Overall, in the whole wheat bread, the DON reduction was 49% compared with the original flour. Similarly, in white bread, the DON content decreased significantly in the two higher contamination levels ranging from 2,000 to >5,000 μg/kg. The overall DON reduction in white breads was 39%, when compared to white flours.

When the four contamination levels were individually analyzed, the DON content in the whole wheat bread was lower than that in the whole wheat flour for the levels ranging from 500–1,999 μg/kg to higher than 5,000 μg/kg.

3.2 | Zearalenone

The zearalenone (ZON) content slightly increased in the breads compared with the original content in the flours, but the difference was not statistically significant. ZON was only detected in the two higher levels of the whole wheat flour (mean 33.1 μg/kg and standard deviation 4.2 μg/kg) and the two whole wheat breads (mean 42.4 μg/kg and standard deviation 15.1 μg/kg) (Figure 1). In the white flour fraction, ZON was not detected in any of the analyzed subsamples.

4 | DISCUSSION

Deoxynivalenol is one of the most frequent wheat contaminants worldwide, and it poses a risk to human and animal health due to its wide range of adverse effects (Vidal et al., 2016). Therefore, studying the factors that affect DON degradation and contribute to the food safety is important. Flour is the fraction that contributes the most to the daily intake of DON in Southern Brazil, representing 89.6% of the provisional maximum tolerable daily intake (Savi et al., 2016). According to Santos et al. (2013), the average intake of the inhabitants of Londrina City in northern Paraná State (Brazil) was 0.79 μg/kg body weight for bread and 0.35 μg/kg body weight for pasta. The total estimated daily intake of DON was 1.13 μg/kg, which is above the provisional tolerable daily maximum intake of 1 μg/kg body weight, established by FAO/WHO Expert Committee (JECFA, 2001).

In our study, the DON content was reduced in both breads made from whole wheat and white flours for the higher levels of DON contamination (>2,000 μg/kg). In the

TABLE 1 Concentration of DON in the original flours and in the white and whole wheat breads

Levels of DON contamination in wheat grains (µg/kg)	Mean whole wheat flour (µg/kg)	Standard deviation (µg/kg)	Mean whole wheat bread (µg/kg)	Standard deviation (µg/kg)	Mean white flour (µg/kg)	Standard deviation (µg/kg)	Mean white bread (µg/kg)	Standard deviation (µg/kg)
<500	498.75	29.31	293.00[a]	-	265.25	46.96	ND	-
500–1,999	746.50	70.71	293.00*	132.51	495.75	36.11	305.00[a]	-
2,000–4,999	2,747.50	140.80	1,072.33*	422.55	2,157.50	79.32	297.33*	25.89
>5,000	5,985.00	260.58	2,593.33*	1,071.56	5,360.00	331.36	1,486.67*	77.67

Notes. [a]Single value because the other samples were ND (not detected), that is, below the limit of quantification (<200 µg/kg).
*Significantly different value in columns ($p < 0.05$).

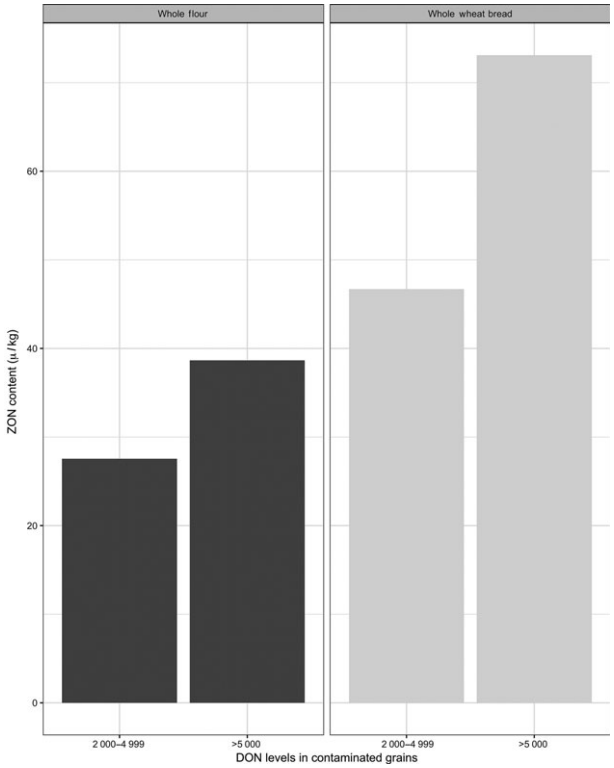

FIGURE 1 Zearalenone levels in whole wheat flour and whole wheat bread with different DON gradients

lower DON contamination levels (<500 and 500–1,999 µg/kg), a reduction in the DON content was observed, and some breads had levels below 200 µg/kg (not detected). Although these results are desirable for wheat product safety, it was not possible to compare the data statistically.

The main factors involved in diminishing DON content during bread making process are temperature, time, loaf size, ingredients, and experimental scale (industrial/laboratorial) (Vidal et al., 2015, 2016). In our study, the DON reduction during the baking process (180°C for 35 min) could be explained by the small size of loaves (100 g), which favored temperature distribution through the inner layers and consequently affected the DON stability. Kostelanska et al. (2011) reported DON reduction of 13%, during the baking process (240 °C for 14 min), of breads with 80 g each. Noticeably, the DON reduction occurred in the bread crust that was exposed to higher temperatures. There were no changes in the DON content in crumbs when the baking temperature did not exceed 85°C. Similarly, Vidal et al. (2014) reported that DON degradation took place exclusively in the crust, with loaf size of 260 g. Such degradation was not enough to detect a significant decrease in DON content in the whole sample. In contrast, De Angelis, Monaci, Pascale, and Visconti (2013) reported that DON levels markedly increased upon baking at 200°C for 55 min, with breads of approximately 500 g weight.

Our results were in accordance with previous studies (Milani & Maleki, 2014; Pacin et al., 2010; Scudamore, Hazel, Patel, & Scriven, 2009; Vidal et al., 2015). The mean reduction in DON between flour and products was 42.3% for French bread and 58.3% for Vienna bread (Pacin et al., 2010). Milani and Maleki (2014) reported that baking regular bread, cookies, and biscuits provided a variable DON reduction, from 24% to 71% in bread and 35% in cookies and biscuits. The mean concentrations of DON in white bread and wholemeal bread were reduced by about 35% and 39%, respectively, than the concentration in the flour (Scudamore et al., 2009). In the baking process, the relative DON reduction after 40 min varied from 29% at 140°C to 81% at 200°C (Vidal et al., 2015).

In the whole wheat bread, the observed relative reduction was 41% for the lowest level (<500 µg/kg) and 57% for the highest contamination level (>5,000 µg/kg). In the white breads, the highest DON reduction (86%) was obtained for the level ranging from 2,000 to 4,999 µg/kg. Our results differed from the study conducted by Vidal et al. (2016). They concluded that the initial DON concentration affected the DON reduction, when the initial DON concentration was higher, a greater reduction was obtained.

The levels of DON in the whole wheat bread were higher compared with the white bread. This difference was not due to the effect of the baking process and was derived

instead from the different DON levels in the original flours. Similarly, Vidal et al. (2014) concluded that the DON concentration during breadmaking was not significantly affected by the bran content of the bread. Scudamore (2008) described a DON reduction of approximately 50% from the flour to the white bread due to the dilution effect from the increased moisture content. For whole meal bread, the DON concentration ranged from 28 to 71 μg/kg compared to whole flour 58–125 μg/kg. The author stated that based on the DON levels required by European legislation, the relative reduction in the DON content via processing wheat into bread (from 1,250 to 500 μg/kg) would allow white bread but not whole wheat bread to meet the DON level requirements.

In Brazil, the upper limits of DON established for whole wheat and white flour are 1,250 and 1,000 μg/kg, respectively (ANVISA, 2017). The maximum levels allowed for final bakery products are the same as that for the white flour. The Brazilian regulations also limits the ZON levels in cereals and by-products; in 2017, the upper levels for whole wheat and white flour were set at 200 and 100 μg/kg, respectively (ANVISA, 2017). Considering these levels, only the flours and breads with the two lower levels of DON contamination in our study would meet the mycotoxin regulation.

In line with previous studies, we did not find that the breadmaking process had any effect on the ZON content. Cano-Sancho, Sanchis, Ramos, and Marín (2013) did not see an effect on the ZON content after baking (at 200°C for 20 min) or fermentation with *Saccharomyces cerevisiae*.

Further studies involving a higher number of samples, different baking conditions, and refined analysis will be important to explain the factors that affect the mycotoxin degradation in wheat products.

5 | CONCLUSIONS

This study focused on evaluating the effects of the breadmaking process on wheat mycotoxin content. The breadmaking process reduced the DON content in breads compared to the original whole wheat and white flours. Therefore, the baking process can be a complementary strategy to reduce dietary exposure to DON. Otherwise, ZON reduction is unlikely to occur in the breadmaking process.

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