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RESEARCH ARTICLE

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Effect of debranning process on deoxynivalenol content in whole-wheat flours

Casiane Salete Tibola[1] | **Eliana Maria Guarienti**[1] | **Alvaro Renato Guerra Dias**[2] | **Marcio Nicolau**[1] | **Rafaela Julyana Barboza Devos**[3] | **Daniela Dalbosco Teixeira**[4]

[1]Embrapa Wheat, Passo Fundo, Brazil

[2]Department of Agroindustrial Science and Technology, Federal University of Pelotas, Capão do Leão, Brazil

[3]Food Engineering, University of Passo Fundo, Passo Fundo, Brazil

[4]Chemical Engineering, University of Passo Fundo, Passo Fundo, Brazil

Correspondence
Casiane Salete Tibola, Embrapa Wheat, Rodovia BR 285, Km 294 – C.P. 3081 – Passo Fundo, RS 99050-970, Brazil.
Email: casiane.tibola@embrapa.br

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Abstract

Background and objectives: Post-harvest mitigation strategies are essential to reduce mycotoxin contamination in wheat-based foods. The study aimed to evaluate the effect of the debranning process on the deoxynivalenol content in whole-wheat flour naturally contaminated by *Fusarium* spp. Thirty commercial wheat samples were used and were obtained in Southern Brazil Region in 2015 crop season.

Findings: The highest DON contamination level was found in the no-debranned samples and the outermost fraction (extraction rate >95%), which corresponds to the debranning time of 15 s. When all debranning times were included in the analysis, only the samples from Paraná State had the DON content significantly reduced after the debranning.

Conclusions: The debranning process reduced the DON content in whole-wheat flour with lower to moderate levels of contamination, corresponding to DON content of 1.032 and 1.174 μg/kg, obtained in Paraná and Rio Grande do Sul States, respectively.

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

KEYWORDS

ash content, debranning process, mycotoxins, whole-wheat flour

1 | INTRODUCTION

Most of the wheat growing area in Brazil is located in the southern region in the Paraná (50%) and Rio Grande do Sul (36%) States (CONAB, 2018). The current domestic production supplies half of the national demand of approximately 10.3 million tons annually (USDA, 2018).

Mycotoxins are poisonous compounds produced by certain species of fungi found in contaminated grains (Neme & Mohammed, 2017). In Southern Brazil, *Fusarium* head blight (FHB) is caused by the *Fusarium graminearum* species, which produces deoxynivalenol (DON) and zearalenone (ZON) mycotoxins (Del Ponte et al., 2015). DON is the most critical 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 in humans and animals (Vidal, Marín, Morales, Ramos, & Sanchis, 2014).

In the post-harvest stage, the cleaning, aeration, debranning, and milling processes influence the distribution of mycotoxins in wheat fractions. Mycotoxins tend to be concentrated in outer fractions (bran, flour shorts screenings, and middlings) and lower in inner fractions intended for human consumption—flour or semolina (Cheli, Pinotti, Rossi, & Dell'Orto, 2013). However, this mycotoxin distribution

pattern is highly variable in wheat milled fractions. The main factors for these conflicting results may be due to the type of mycotoxin (hydrophilic/hydrophobic), the genotype, the level and time of fungal contamination, and the post-harvest procedures (Cheli et al., 2013; Tibola, Fernandes, Guarienti, & Nicolau, 2015).

More recently, Khaneghah, Martins, Hertwig, Bertoldo, & Sant'Ana (2018) discussed the fate of DON throughout the processing of wheat, bread, and pasta and accessed data on the impact of specific steps of processing on DON contents in wheat-based products. The authors stated that it has been contradictory data, regarding the fate of DON during wheat and wheat-based products processing, due to factors as temperature, additives, processing time, and loaf size in addition to the occurrence of modified (masked) forms of DON.

Whole-wheat flour is a good source of dietary fiber and antioxidants, which can promote health benefits toward several chronic diseases usually associated with oxidative stress (Yu, 2008). However, the presence of natural and synthetic contaminants in the most external layers poses a risk for consumer safety and need to be taken into serious consideration (Zanoletti et al., 2017). The bran is the wheat fraction most heavily contaminated with DON, which represents a significant concern because this fraction is widely employed to produce feed for animals as well as raw material for whole food products (Khaneghah et al., 2018).

Debranning process, which consists of mild friction (kernel to kernel) and abrasion (kernel to a rough surface), can represent a valuable strategy to maximize the health benefits of cereal-based foods (Ciccoritti et al., 2017). The milling industry is increasingly recognizing debranning or pearling as a useful technology for improving the milling efficiency (Beta, Nam, Dexter, & Sapirstein, 2005; Meng, Xiao-hong, Xiu-li, & Shao-xia, 2017). The degree of debranning could be carefully modulated in order to separate the outermost fractions, which could be characterized by a higher content in contaminants and coarse fiber, from the intermediate fractions, which offer potentially high health benefits (Blandino et al., 2013; Giordano et al., 2017; Sovrani et al., 2012). Beta et al. (2005) stated that debranning is a useful technique to obtain wheat bran fractions enriched in phenolics and antioxidants compounds, thereby maximizing health benefits associated with wheat-based products.

Furthermore, Giordano and Blandino (2018) proposed debranning of wheat to reduce the contamination by arsenic, lead, and cadmium to avoid any potential risk for the health of consumers. Cheli et al. (2010) investigated the distribution of deoxynivalenol, cadmium, and lead in wheat milling fractions obtained by conventional milling and debranning before milling. In another experiment, Cheli et al. (2013) also explored the effect of debranning on wheat mycotoxin content. The authors reported that debranning efficiency is hugely variable, ranging from 15% to 78% of DON reduction, using both laboratory and industrial scales. Therefore, it is essential to explore the effects of debranning in the wheat mycotoxin reduction, especially in wheat lots with high DON contamination levels.

The objective of this study was to evaluate the effect of the debranning process on the deoxynivalenol content in whole-wheat flour naturally contaminated by *Fusarium* spp, to enhance the safety of whole-wheat products.

2 | METHODOLOGY

2.1 | Sampling characterization

Thirty commercial wheat samples, representing mainly bread wheat and domestic wheat, according to Brazilian wheat classes, were used in this work (Brasil, 2010). The wheat samples were naturally contaminated by *Fusarium* spp, and it was obtained in Southern Brazil Region, in 2015 crop season. The geographical origin of these cultivars was Paraná (19 samples), Rio Grande do Sul (nine samples), and Santa Catarina (two samples).

In Brazil, the wheat production areas were divided into four homogeneous regions that were defined based on the variables: rainfall, temperature, altitude, and description of grain yield. The southern region comprises the Region 1 (cold, humid, and high elevation); Region 2 (moderately hot, humid, and low elevation); and Region 3 (hot, moderately dry, and low elevation) (Munaro et al., 2014). The origins of the samples in this study were 40% from Region 1; 47% from Region 2; and 13% from Region 3.

The samples were selected based on the initial DON concentrations. It was selected only wheat samples with DON levels above 200 μg/kg. The DON content was analyzed by enzyme-linked immunosorbent assay (ELISA) kit (AgraQuant®).

2.2 | Debranning and milling procedures

The grains were polished using a laboratory Zaccaria rice machine (model PAZ-1-DTA), previously regulated for each treatment. The weight of samples fed to the polisher was approximately 50 g. The debranning process was monitored through time control. The treatments were no-debranned kernels and three consecutive debranning times 15 s, 30 s, and 60 s (Table 1). The samples were weighed before and after the debranning procedure. After each debranning passage, the equipment was thoroughly cleaned with dust aspiration and compressed air, to minimize contamination.

Intact grains and grains after each debranned treatment were milled to obtain the whole-wheat flour. The samples were milled in a Laboratory Mill 3,100 (Perten Instruments,

TABLE 1 Samples characterization and experimental conditions of the debranning process

State	Number of samples	Debranning time (s)	Starting kernels mass (g)[a]	Final kernels mass (g)[b]	Mean debranning level (%)[c]	Extraction rates
Rio Grande do Sul	9	15	102.2	98.2	3.9	96.1
		30	99.7	92.3	7.3	92.7
		60	101.1	87.5	13.5	86.5
Santa Catarina	2	15	97.5	93.2	4.4	95.6
		30	100.0	90.9	9.2	90.8
		60	97.5	81.9	16.1	83.9
Paraná	19	15	103.2	99.2	3.9	96.1
		30	103.7	96.6	6.8	93.2
		60	100.8	87.6	13.1	86.9

[a]Starting kernel mass = whole kernel mass.
[b]Final kernels mass = mass after the debranning process.
[c]Debranning level % = (Final kernel mass × 100)/Starting kernels mass.

Huddinge, Sweden). Wheat fractions were ground to pass through a 0.8-mm screen.

2.3 | Deoxynivalenol and technological quality analysis

2.3.1 | Deoxynivalenol analysis

The DON content was determined in whole-wheat flour using a commercial direct competitive enzyme-linked immunosorbent assay (ELISA) kit (AgraQuant®). The limit of detection (LOD) and the limit of quantification (LOQ) in this test were 200 μg/kg and 250–5,000 μg/kg, respectively. Extraction procedure, calibration, and reading were performed according to manufacturer's instructions. The method was approved by the AOAC Research Institute (Certificate Nº 110,701). The accuracy, sensitivity, and specificity of the ELISA method compared with a chromatographic method, for mycotoxin analysis, were determined in a previous study (Duffeck, Tibola, Guarienti, & Del Ponte, 2017).

2.3.2 | Moisture and protein content

The moisture and protein contents were determined by near-infrared reflectance spectroscopy (NIR instrument FOSS XDS—RCA, Hoganas, Sweden). The reference methods used for moisture and protein calibration development were, respectively, method 44-15.02 (AACC, 2010) and method 46-13.01 (AACC, 1999).

2.3.3 | Ash analysis

The whole-wheat flour was weighted (3 g) into crucibles, and then, the samples were placed in a muffle furnace at 900°C for two hours and a half. It was incinerated until light gray ash or constant weight was obtained. After cooling, the samples and ash contents were calculated on a dry basis (ICC, 1990).

2.3.4 | Color

The whole-wheat flour color was evaluated using colorimeter Minolta, model CR-410. Was used the CIEL*a*b* system, with reading angle of 10º and illuminant D65, following the manufacturer's instructions.

2.4 | Statistical analysis

All the analysis of wheat samples (no-debranned and debranned whole-wheat flour) were in triplicate.

Statistical analyses were performed using R Software 3.5.0 (R Development Core Team, 2018). The linear regression was selected to explain the relationship among debranning times and the deoxynivalenol, protein, ash, color in the whole-wheat flour. The Box–Cox power transformation was used to fix the normality and linearity issues, for deoxynivalenol and protein. For all tests, the statistical significance was set at $p < 0.05$.

3 | RESULTS

3.1 | DON content

The DON content of the whole-wheat flours from Southern Brazilian states is reported in Figure 1. The Box–Cox power transformation that provides the best result for deoxynivalenol was the lambda = 0.1613061. The highest DON contamination level was found in the no-debranned samples and the outermost fraction (extraction rate > 95%), which corresponds to the debranning time of 15 s.

The debranning times presented effect in the DON reduction and the debranning time of 15 s, which removed the outermost fraction (Table 1), where the most efficient in the reduction of the DON contamination (Figure 1). On average, the DON content decreased by 25%, 31%, and 31% in the debranning times of 15, 30, and 60 s, respectively, compared with no-debranned samples (Figure 1). Related to the States, the consecutive debranning times were effective in reducing DON levels in Paraná and Rio Grande do Sul, only until 30 s; after this, the debranning presented a stable behavior (Figure 2).

When the samples were separated by State of Origin, with all debranning times included (15, 30, and 60 s), a significant reduction in DON contamination ($p < 0.05$) was found only in the samples from Paraná State (Table 2). In the Santa Catarina State, the DON content decreased after each progressive debranning toward the inner layers, although it was not statistically significant (Table 2).

In Brazil, the upper limits of DON established for whole-wheat flour are 1.250 μg/kg (ANVISA, 2017). Considering this level, only the wheat samples from Paraná and Rio Grande do Sul States would meet the mycotoxin regulation, after the debranning process. The DON levels in the wheat samples from Santa Catarina remained above the permitted limit, even after the debranning time of 60 s.

3.2 | Protein content

Protein content reduced significantly in the debranned samples from Rio Grande do Sul and Paraná (Table 2). In the samples from Santa Catarina, no differences in protein levels were identified due to debranning process (Table 2). The protein content of whole-wheat flours reduced more pronounced after 30 s of debranning (Figure 1). The Box–Cox power transformation that provides the best result for protein was lambda = 0.4804147.

3.3 | Ash content

The ash content reduced significantly in the debranned fractions in all wheat samples evaluated (Table 2). The ash reduction was irrespective of State of Origin (Figure 3).

3.4 | Color a*, b*, and L*

Color *a** (red to green colors) and *b** (yellow to blue colors) presented the same pattern of significative reduction in all debranned samples (Table 2 and Figure 3). The parameter Color *L**, which measures the "lightness," with a score of 100 as white and 0 as black, increased significantly, after the debranning process, in all analyzed samples (Table 2 and Figure 3).

4 | DISCUSSION

The objective of this study was to evaluate the effect of the debranning process on the deoxynivalenol content in whole-wheat flour.

Overall, the highest DON contamination level in all wheat samples was found in the no-debranned samples and the outermost fraction (extraction rate > 95%), which corresponds to the debranning time of 15 s. According to Sovrani et al. (2012), deoxynivalenol contamination decreased from the external to the internal layers, 64% of total contamination of kernel was found in the 0%–5% and 5%–10% fractions.

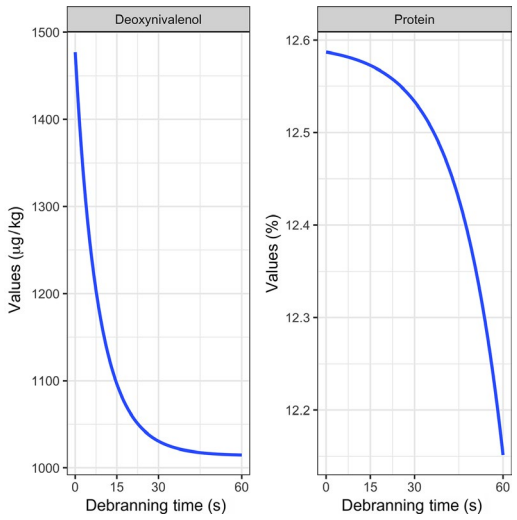

FIGURE 1 Adjusted curves for deoxynivalenol and protein levels of no-debranned and debranning times of 15, 30, and 60 s for whole-wheat flours [Color figure can be viewed at wileyonlinelibrary.com]

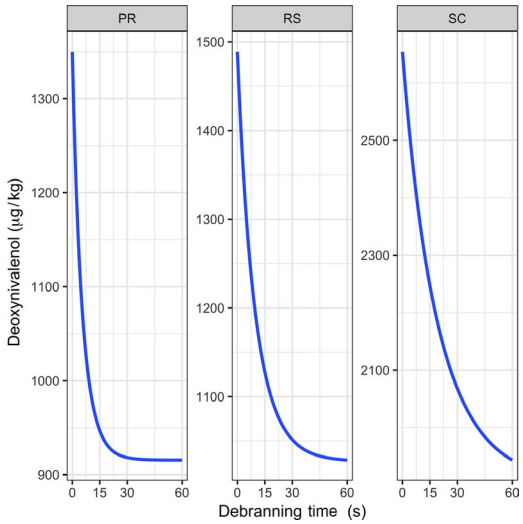

FIGURE 2 Adjusted curves for deoxynivalenol levels by State, of no-debranned and debranning times of 15, 30, and 60 s for whole-wheat flours [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Levels of significance of linear regression analyses performed for whole-wheat flours using data of wheat cultivars from Southern Brazil submitted to debranning process

State	Parameter	Intercept	Debranning time	*p*-value
Rio Grande do Sul	Ash	1.8117	**−0.0066**	0.0000
	Color_a	3.6212	**−0.0213**	0.0000
	Color_b	11.9234	**−0.0314**	0.0000
	Color_L	80.4711	**0.0783**	0.0000
	Deoxynivalenol	6.9479	−0.0049	0.1434
	Protein	12.3213	**−0.0077**	0.0354
Santa Catarina	Ash	1.7600	**−0.0070**	0.0000
	Color_a	3.2570	**−0.0213**	0.0000
	Color_b	11.7020	**−0.0375**	0.0000
	Color_L	80.4827	**0.0691**	0.0000
	Deoxynivalenol	7.7447	−0.0047	0.2455
	Protein	11.9350	−0.0088	0.4380
Paraná	Ash	1.8002	**−0.0059**	0.0000
	Color_a	3.7048	**−0.0217**	0.0000
	Color_b	12.0925	**−0.0334**	0.0000
	Color_L	80.0154	**0.0750**	0.0000
	Deoxynivalenol	6.8203	**−0.0060**	0.0085
	Protein	12.8911	**−0.0072**	0.0080

Note: Significant values were reported in bold style ($p < 0.05$). *p*-Value corresponds to the significance of debranning time factor.

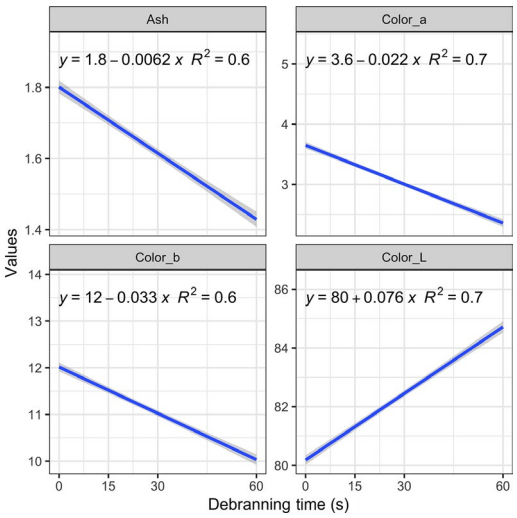

FIGURE 3 Linear models for ash content and colors parameters, with the coefficient of determination (R^2) and equations, in whole-wheat flours [Color figure can be viewed at wileyonlinelibrary.com]

In Santa Catarina and Rio Grande do Sul States, the DON content does not reduce significantly after the debranning process when all debranning times were included in the analysis (Table 2). Similarly, Cheli et al. (2010) reported that debranning reduced, although not significantly, the levels of DON in semolina when compared with conventional milling. These findings might be accounted for the heterogeneity of mycotoxin distribution in wheat grains (Cheli et al., 2013; Savi, Piacentini, Tibola, & Scussel, 2014). Edwards, Kharbikar, Dickin, MacDonald and Scudamore (2018) reported that repeated wetting and drying could cause movement of DON toward equilibrium across the milling fractions, whereas high levels of rainfall could cause reduction of DON in the bran fraction, resulting in a proportional increase within the white flour.

The mean DON content for Paraná, Rio Grande do Sul, and Santa Catarina States was 1.032, 1.174, and 2.228 μg/kg, respectively. Therefore, the debranning process was effective only in the lower to moderate levels of DON contamination. Different from our results, Ríos, Pinson-Gadais, Abecassis, Zakhia-Rozis, & Lullien-Pellerin (2009) concluded that debranning demonstrated as an efficient process, at least at the laboratory scale, to remove both *Fusarium* and DON irrespective of the initial level of mycotoxins in grains. Contrary to our findings, Aureli and D'Egidio (2007) detected for higher contaminated grain samples, a more pronounced reduction in the DON contamination of durum wheat semolina and flour (and of pasta produced from that) due to debranning before milling.

The debranning time of 60 s that removed 10%–16.1% (extraction rate between 83% and 87%), of the grain mass, was less effective in reducing DON content (31%) when compared with other debranning times (15 s and 30 s). The mean reduction was equivalent to the time of 30 s and very similar to the reduction obtained with 15 s of debranning (25%). Our

results for DON were similar to those reported by Sovrani et al. (2012). The authors stated that DON decreases moving from the external to the internal layers following a biphasic behavior, and a high reduction was observed in the first debranning steps and a slower decrease followed this.

The main weather characteristics in Southern Brazil for the 2015 spring season were described in Guarienti et al. (2017). In Rio Grande do Sul State, the wheat crops sown late were more intensely affected by the excess rainfall in the spring season. In Santa Catarina, was registered continued rainfall during most of October in 2015 season (Guarienti et al., 2017). The various number of days with excessive moisture associated with warmer temperatures at the heading stage of wheat crop development favored fungal diseases such as *Fusarium* spp (Tibola, Fernandes, & Guarienti, 2016).

Edwards et al. (2011) reported that DON concentration in flour fractions was equivalent to that of the milled wheat, with a lower concentration in outer layers (bran), in the wheat from a season characterized by high pre-harvest rainfall. This unexpected behavior resulted from the movement of DON to inner layers due to the DON highly solubility in water (Edwards et al., 2011). A more recent study indicated that DON is highly mobile within the grain and can migrate between grain structures and be leached from the grain pre-harvest resulting in varying distributions across milled fractions in post-harvest (Edwards et al., 2018). On the other hand, Tibola et al. (2015) reported that the higher levels of mycotoxin were more likely to occur due to the fungal growth in the inner grain layers, promoted by the ideal conditions for fungal infection, than mycotoxin diffusion among grain layers.

The debranning process does not remove the outer kernel tissues homogeneously, as abrasion affects especially the accessible parts of the kernels (De Brier et al., 2015). Wheat bran debranning fractions with different proportions of pericarp and aleurone can be produced (Hemdane et al., 2016). Further, also within the endosperm fractions, the extent of contamination with mycotoxins can vary. Usually, the outer layers (bran) of the wheat grain contain higher levels of *Fusarium* mycotoxins (Savi et al., 2016; Tibola et al., 2016, 2015). However, a correlation between the DON content and the ash content, as a marker for bran particles, was not found (Ríos et al., 2009; Schaarschmidt, & Fauhl-Hassek, 2018).

Overall, the debranning time of 15 s was the most effective in the DON reduction, approximately 500 µg/kg, when all samples were considered (Figure 1). According to Santos et al. (2013), the mean DON content in wheat from Paraná State was 1894.9 µg/kg. Savi et al. (2016) analyzed wheat samples from Southern Brazil and reported that the bran fraction had the highest mean concentration of DON (2,278 µg/kg), followed by milled wheat and finished flour (1895 µg/kg and 1,305 µg/kg). These data highlight how stable DON is to wheat and its products, reinforcing the importance of pre- and post-harvest strategies to diminish human exposure to DON.

Protein content reduced significantly with the consecutive debranning time in the samples from Rio Grande do Sul and Paraná States (Table 2). Figure 1 shows a reduction of the protein content with the increase in extraction rates. These results are mostly explained by the higher concentration of the protein in the outermost parts of the grain (pericarp and aleurone layers), which represent about 20% of the total proportion of proteins in the grain (Brouns, Hemery, Price, & Anson, 2012). The aleurone is relatively rich in proteins and minerals (Delcour & Hoseney, 2010; De Brier et al., 2015).

Similar results were reported by Azizi, Sayeddin, and Payghambardoost (2006) that studied eight rates of wheat flour extraction (from 70% to 93%). Another study conducted by Mueen-ud-Din, Rehman, Anjum, Nawaz, and Murtaza (2010), tested four different extraction rates (from 64% to 100%), also reported protein reduction.

According to Sarkar and Dexter (2016), the germ and bran proteins are non-gluten proteins that have better nutritional value than gluten proteins but are not beneficial to processing properties. Besides, bran contains most of the fiber in the kernel, which is an essential nutritional compound.

Similarly, to our results of protein in Santa Catarina State (Table 2), De Brier et al. (2015) did not report significant differences in protein content after pearling process.

In our study, moisture content was not significantly affected by debranning (ranging from 10.8% to 13.9%).

As expected, the ash content reduced significantly with the progressive removal of external layers of wheat grains by debranning (Table 2 and Figure 3). The highest concentration of minerals is located in the outermost layers of the grains—pericarp and aleurone layer (Belitz & Grosch, 1997). Other studies reported the same pattern (Ciccoritti et al., 2017; De Brier et al., 2015). Ash contents ranged from 3.3% to 4.2% in the flours obtained from debranned kernels and were thus lower than that of regular bran (6.1%) (De Brier et al., 2015).

Color a^* (red to green colors) and b^* (yellow to blue colors) presented the same pattern of significative reduction in all debranned samples (Table 2 and Figure 3). Otherwise, the parameter Color L^*, which measures the "lightness," increased significantly, after the debranning process, in all analyzed samples (Table 2 and Figure 3). This result was expected since a higher percentage of pigments that give color to the wheat are located in the outer layers of the grains. According to Lachman, Martinek, Kotíkov, Orsak, and Sulc (2017), anthocyanins are accumulated in the aleurone or pericarp layer and give blue, purple, or the combination of these colors, whereas flavonoids, such as yellow C-glycosides of flavones, flavonols, flavanonols, proanthocyanidins, and reddish-colored phlobaphenes are mainly concentrated in the outer layers of the grains.

Further studies involving a higher number of samples, especially with high DON contamination levels and different debranning conditions, would be important to explain the effects of the debranning process in the mycotoxin distribution in wheat products.

5 | CONCLUSIONS

The debranning process reduced the DON content in whole-wheat flour with lower to moderate levels of contamination, corresponding to DON content of 1.032 μg/kg and 1.174 μg/kg, obtained in Paraná and Rio Grande do Sul States, respectively. Debranning levels of 5% guaranteed low starch losses and, at the same time, noticeably reduced the DON contamination of the kernels. Therefore, the debranning process can be a complementary strategy to reduce DON contamination and to produce safer wheat-based products.

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ORCID

Casiane Salete Tibola https://orcid.org/0000-0003-2036-8163

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SUPPORTING INFORMATION

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