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RESEARCH

Wheat debranning: effects on mycotoxins, phenolic content, and antioxidant activity

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Abstract

The debranning process, at an industrial scale, was applied to grains of two wheat cultivars to determine its effect on *Fusarium* mycotoxin content and antioxidant activity. Grain samples from the BRS Marcante and BRS Reponte wheat cultivars, naturally contaminated by *Fusarium*, were used in the study. The dry wheat samples were processed on the polisher once or twice and evaluated by hardness index, chemical composition (moisture, protein, and ash), deoxynivalenol (DON) and zearalenone (ZON) levels, phenolic content, and antioxidant activity. In the BRS Marcante cultivar, the debranning process only slightly reduced the DON and ZON contents in whole-wheat flours compared with the previous cleaning treatment (no-debranned). In the BRS Reponte cultivar, the DON concentration decreased by 36% at a debranning ratio of 5%, obtained by polishing, compared with prior cleaning treatment (no-debranned). In addition, the polishing reduced the ZON level by 56% compared with the cleaned wheat. The debranning process did not reduce the antioxidant capacity. Therefore, debranning is a suitable technology to obtain safer and healthier food by minimizing the mycotoxin content and retaining antioxidant capacity.

Keywords Debranning · Deoxynivalenol · Zearalenone · Whole-wheat flour · Antioxidant activity

Introduction

Wheat is one of the essential staple grains in the world, and it is one of the most important food sources for humans (Cheli et al. 2017). *Fusarium* head blight (FHB) is a disease caused by the *Fusarium graminearum* species complex in Southern Brazil. The pathogen produces deoxynivalenol (DON) and zearalenone (ZON) mycotoxins (Del Ponte et al. 2015). DON disrupts normal cell function and is related to deleterious effects on human health, such as anorexia, weight loss, malnutrition, dysfunction endocrine, and immunological changes (Pestka 2010; Terzi et al. 2014). ZON causes hyperestrogenic syndrome and reproductive dysfunction (Iqbal et al. 2014). To prevent mycotoxicosis, the maximum limits of mycotoxins are regulated in many countries. International mycotoxins regulation differs among countries, with the acceptable level of DON ranging from 500 to 2000 ppb for foods intended for human consumption (Poroșnicu et al. 2023). In Brazil, the maximum DON limit allowed for unprocessed wheat is 2000 ppb, 1250 ppb for milled wheat, and 1000 ppb for flour (ANVISA 2021). The maximum limit for ZON is 400 ppb for wheat in grains, 200 ppb for milled wheat, and 100 ppb for wheat flour and other derivatives (ANVISA 2021).

According to Cheli et al. (2013), the highest concentration of toxins was found in the outer part of the kernel. The high DON concentration in the bran is of concern because it is used for animal feed, and the bran is also used for direct

Highlights
- The debranning process significantly reduced the DON contamination of the BRS Reponte wheat cultivar.
- The debranning process reduced the level of ZON in the BRS Reponte cultivar.
- The antioxidant capacity was preserved after the debranning process.

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human consumption due to their health benefits. The milling process removes the bran fraction layers, but it includes key ingredients, rich in bioactive compounds, for cereal-based foods with high antioxidant and nutritional potential (Ciccoritti et al. 2017).

Debranning is based on the sequential removal of the outer grain layers by abrasion and friction, until the desired level, which will depend on the raw materials and the final products (Dexter and Wood 1996). Debranning represents an alternative for using outer layers of kernels rich in natural bioactive compounds (i.e., natural antioxidants and fiber). These compounds potentially benefit human health and are removed during traditional milling (Delcour et al. 2012). Therefore, studies of the effects of debranning technology are important to define the distributions of healthy compounds that can then be preserved during milling and of contaminants (toxic trace elements) to prevent them from entering the food chain (Ficco et al. 2020).

There are many reports of debranning in wheat (Dexter and Wood 1996; De Brier et al. 2015; Martini et al. 2015; Giordano and Blandino 2018). However, there is limited information about applying the debranning process at an industrial scale and its impact on mycotoxin content. Zhao et al. (2019) reported the effect of light debranning in wheat decreased the deoxynivalenol (DON) level by 15.89% at a debranning ratio, percentage of removed bran from the wheat kernel, of 1.2% in the production line. Cheli et al. (2013) reported that the effect of debranning and the efficiency of mycotoxin removal are incredibly variable; the DON reduction ranged from 15 to 78%.

This work aimed to evaluate the effect of the industrial debranning process on *Fusarium* mycotoxins and antioxidant activity in whole-wheat flour from wheat grains harvested from two commercial wheat fields naturally infected by *Fusarium* head blight.

Material and methods

Wheat samples

Grain samples from the 2019 crop season of wheat cultivars BRS Marcante and BRS Reponte were obtained from commercial fields. The geographical origin of the wheat fields was Passo Fundo, located in the Rio Grande do Sul State (28° 13′ 30.2″ S 52° 24′ 31.9″ W). Wheat samples were likely to be naturally contaminated by *Fusarium graminearum*, based on former studies (Del Ponte et al. 2015).

From September to November of 2019, the average temperature was 16.3, 19.3, and 21.2 ºC, and there were 11, 13, and 12 days of rainfall, with total values of 55.8, 337.0, and 115.5 mm, respectively (INMET 2022). The humid and hot weather during the flowering and grain filling time was favorable to the FHB epidemic and consequent accumulation of mycotoxins in wheat.

The two wheat cultivars present different rheological properties. The cultivar BRS Marcante has high gluten strength ($W = > 220 \times 10\text{-}4$ J) and stability (13–20 min); BRS Reponte is characterized by intermediate gluten strength ($W = 160 \times 10\text{-}4$ J) and stability (6 min). Both cultivars are moderately resistant to *Fusarium* head blight (FHB). Moderately resistant cultivars show reduced symptoms compared to susceptible cultivars when exposed to the *Fusarium* disease, these categories are used based on their reaction to disease infection. The wheat samples were kept at 8 °C in the cold chamber before the analyses.

Cleaning process

Air-sieve separators (Kepler Webber, model: LC 160–1, Panambi/RS – Brazil) were used to primarily clean impurities from the samples, which differ from the wheat grain with geometric parameters and aerodynamic properties. The sieves' adjustments were as follows: 1st 6 mm round; 2nd 1.75 mm oblong; 3rd 5 mm round; and 4th 2 mm oblong. For BRS Marcante and BRS Reponte cultivars, the relative amount of discarded grain in the cleaning process was 11.6% and 8.9%, respectively.

Industrial debranning process

The wheat grains were debranned using a commercial polisher (Model Peeling Idugel Mod. PI). The polisher has a capacity of debranning of 5 tons per hour of wheat. The weight of the samples entering the polisher was approximately 500 kg. The dry wheat samples were processed through the polisher once (polished treatment) or two times (double polished treatment) (Fig. 1). In all the debranning treatments, the sampling procedure was to collect a sample each 5 min during the debranning process, thus resulting in four repetitions with 2 kg each. The samples and the residues were weighed before and after the debranning procedure. The discarded residues for the BRS Marcante were 1% in the polished and 2% in the double-polished treatment. Furthermore, for BRS Reponte, discarded residues were 8% in both treatments, polished and double-polished. The moisture content was recorded in each treatment (Portable Grain Moisture equipment AL-102 ECO—Agrologic). After each debranning passage, the equipment was thoroughly cleaned with a dust vacuum and compressed air to minimize cross-contamination.

Milling procedures and grain hardness index

The grain samples after each treatment were milled to obtain whole-wheat flour fraction. The samples were milled in a

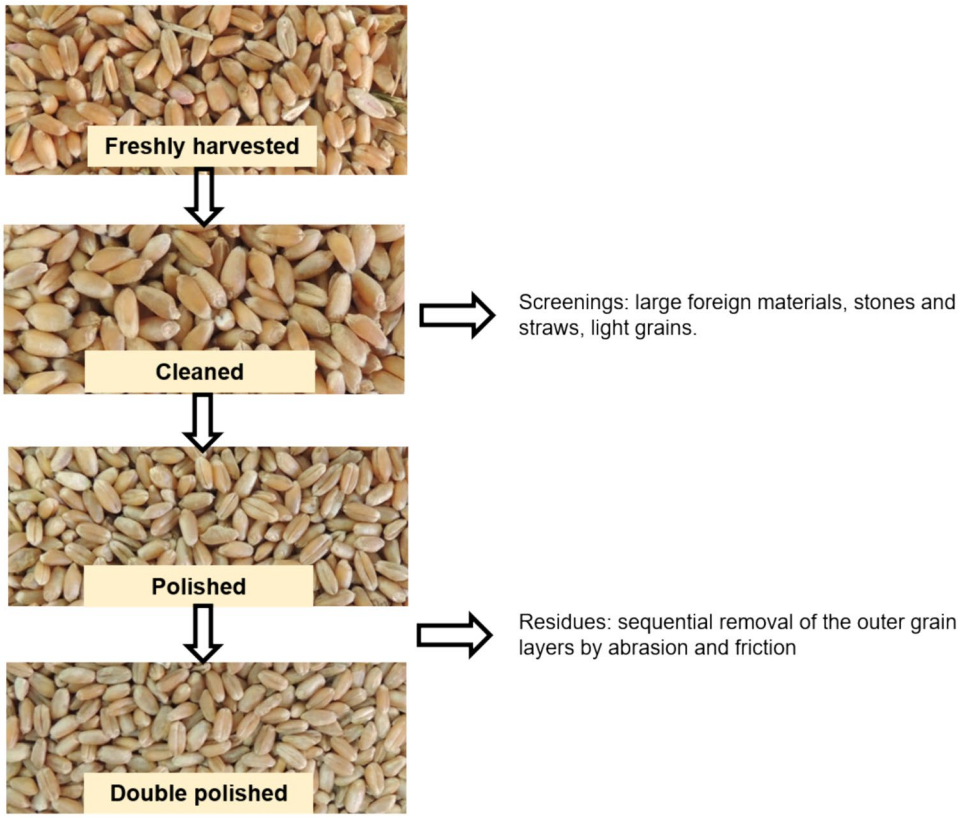

Fig. 1 Schematic treatments are freshly harvested, cleaned, and industrial debranning processes

Laboratory Mill 3100 (Perten Instruments, Huddinge, Sweden). The wheat fractions were grounded to pass through a 0.8-mm screen.

The wheat grain hardness index (GHI) was determined using NIR (NIR instrument FOSS XDS; RCA, Hoganas, Sweden) for the whole-wheat flour. The reference method used for the hardness calibration development was a single-kernel characterization system (SKCS), method 55–31.01 (AACC International 2010).

Moisture, protein, and ash contents

Moisture and protein contents were determined in 24 samples by 44–15.02 and 39–10.01 methods, respectively (AACC International 2010).

Ash content was determined according to the ICC Standard method 104/1 (ICC 1990) using a muffle furnace at 900 °C for 2.5 h. Protein and ash results were expressed as g/100 g.

Deoxynivalenol and zearalenone analysis

The wheat samples were grounded in ultra-centrifugal mill model ZM200 (Retsch) with a 1-mm sieve, weighed into a 50 mL Falcon-type tube, and 24 mL of extraction solvent containing methanol: ultrapure water (70:30, v/v) was added for mycotoxin extraction (Mallmann et al. 2021). After agitation in a vortex mixer for 20 min at 2500 rpm, the content of the tube was centrifuged at 2465 × g for 10 min (5804R, Eppendorf) and diluted in a vial-type flask.

DON and ZON contents were determined, in 24 samples, by ultra-high-performance liquid chromatography-tandem with triple quadrupole mass spectrometry (UHPLC-MS/MS) before and after the industrial debranning process. Briefly, the chromatographic separation was performed at 25 °C on a Gemini C18-column, 150 × 4.6 mm i.d. with 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, USA) (Oliveira et al. 2017). A binary gradient at a flow rate of 0.8 mL/min was performed with solvent A (water) and solvent B (acetonitrile), both modified with 0.5% (v/v) ammonium acetate (Sulyok et al. 2006). The analytical method was developed in-house and validated by the references ISO/IEC 17025: 2017 and Malachová et al. (2014). The accuracy of the technique used to measure DON and ZON was evaluated by recovery experiments (purified analytes in blank wheat) and a certified reference material (CRM), as detailed in Mallmann et al. (2017). The limits of quantification and recovery for DON and ZON were 200 µg.kg–1/80% and 20 µg.kg–1/85%, respectively.

Phenolic content and antioxidant activity

In the different treatments carried out on wheat, the free and bound phenolics were extracted according to the methods presented by Adom and Liu (2002) and Sosulski et al. (1982), respectively. Briefly, for the analysis of total phenolic compounds, approximately 2 g of the sample was subjected to extraction using 70% acetone, going through a homogenization and centrifugation processes, with a subsequent stage of concentration and redissolution of the extract (Alves et al. 2016). For the analysis of complexed phenolic compounds, the residue from the extraction of free phenolic compounds was used, which was subjected to basification, heat treatment, and acidification, with subsequent concentration and resuspension in 70% acetone (Alves et al. 2016). The extracts of the free and complexed phenolic compounds were used to measure the total phenolic content (TPC) and antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2 azino bis (3-ethylbenzene thiazoline-6-sulfonic acid) (ABTS) radicals (López-Perea et al. 2019).

Statistical analysis

A software R was used for statistical analysis and figure plotting (R Development Core Team 2018). The packages ggplot2 and ggpubr were used in this study. Analytical determinations conducted in triplicates for each cultivar were submitted to a one-way analysis of variance (ANOVA). A pairwise comparison of mean effects was applied for each cultivar, and those with $p \leq 0.05$ were considered significant.

For phenolic content and antioxidant activity, a comparison of the means was ascertained by Tukey's test to a 5% significance level using an analysis of the variance (ANOVA).

Results and discussion

Grain hardness index and chemical composition

For the parameter grain hardness index (GHI), the samples differed significantly ($p \leq 0.05$) after the debranning processes (polished and double-polished) in both cultivars (Table 1).

In our study, the moisture content ranged from 11.6 to 12.1% and was not significantly affected by debranning (Table 1). The ash content was significantly reduced with the progressive removal of the wheat grains' external layers by debranning (double-polished) in both cultivars (Table 1). Similarly, Ficco et al. (2020) reported that the ash levels for all wheat genotypes decreased progressively with increasing debranning time and after milling.

The protein level was not significantly affected by the debranning process, except for the cultivar BRS Marcante, which demonstrated a significant increase in protein level in the double-polished treatment (Table 1). De Brier et al. (2015) did not report any significant differences in the protein content after the debranning process in wheat.

Deoxynivalenol content

The DON contents of the whole-wheat flours from BRS Marcante and BRS Reponte cultivars are reported in Fig. 2.

In freshly harvested grains, obtained from wheat cultivars, DON was detected at higher levels (Fig. 2). After the cleaning process that eliminated light and shriveled grains (physical removal), DON contamination decreased by 28% and 34% for BRS Marcante and BRS Reponte cultivars, respectively, but did not differ significantly (Fig. 2). These

Table 1 Wheat sample characterization before and after debranning and milling processes. Each value represents the mean of three replicate test portions analyzed

Cultivars	Treatment	Grain hardness index	Moisture (%)	Ash (%)	Protein (%)
BRS Marcante	Freshly harvested	77.4	11.6	1.77	11.8
	Cleaned	77.9	11.6	1.74	11.8
	Polished	82.3*	11.8	1.70	11.8
	Double-polished	80.6*	11.7	1.66*	12.0*
BRS Reponte	Freshly harvested	72.0	11.8	1.66	13.2
	Cleaned	71.9	11.7	1.60	13.2
	Polished	73.8*	12.1	1.61	13.2
	Double-polished	72.9*	11.9	1.51*	13.4

*Significantly different by test f ($p \leq 0.05$)

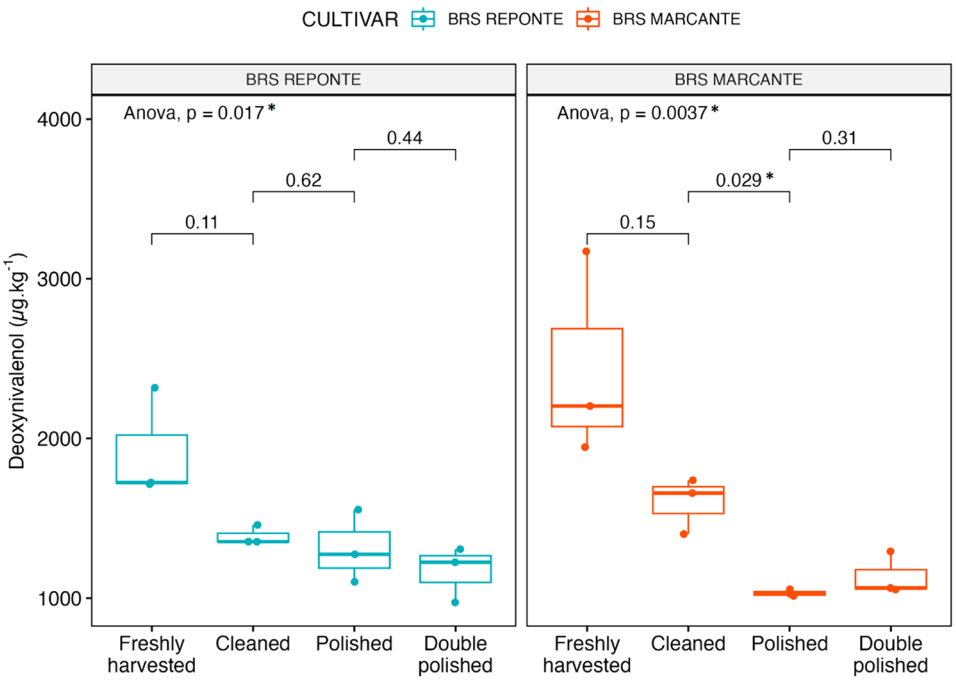

Fig. 2 Effect of the debranning process on deoxynivalenol (DON) levels in whole-wheat flours obtained from the BRS Marcante and BRS Reponte cultivars. *t.test p-values for pairwise group mean comparison and overall F-test p-value ($n = 24$)

findings might account for the heterogeneity of mycotoxin distribution in wheat grains and the time of the *Fusarium* infection (Cheli et al. 2013).

In the ANOVA analyses between every two subsequent treatments (pair comparison), the debranning process reduced the DON content significantly by 36% ($p = 0.029$) in the BRS Reponte cultivar when compared with the cleaning treatment (Fig. 2). The treatment polished was equivalent to the debranning level of 5%, with the removal of part of the aleurone of the wheat grains. Similarly, Ciccoritti et al. (2017) reported that debranning is an exciting technology that can provide debranned kernels, which can be used directly to produce improved whole products.

In the BRS Marcante cultivar, the debranning only slightly reduced the DON content in the treatments polished and double-polished, which were equivalent to the debranning levels of 1 and 2%, respectively, when compared with the cleaned treatment (Fig. 2). These differences among wheat cultivars might be due to variations in grain hardness and thickness of the branny layer (Katyal et al. 2019). Besides, the initial level of DON contamination in the BRS Reponte cultivar (2440.0 µg.kg^{-1}) was higher than in the BRS Marcante cultivar (1918.4 µg.kg^{-1}). Zhao et al. (2019) reported that the DON concentration in wheat decreased by 15.9% at a debranning ratio of 1.2% in the production line. Moreover, for the laboratory experiment, the maximum DON removal for grain and flour was 23.4% and 21.9%, respectively (Zhao et al. 2019). Finally, Ríos et al. (2009) showed that the debranning process in two wheat samples naturally contaminated with *Fusarium* was more efficient than milling alone in reducing the DON content, regardless of its initial levels.

The cleaning and debranning process can be associated during postharvest to alleviate DON content in wheat by-products. The cleaning process is useful in reducing DON content in wheat by eliminating the light and shriveled grains, especially in the crop seasons characterized by days with excessive moisture associated with warmer temperatures at the heading stage of wheat crop development (Tibola et al. 2016). Diversely, light debranning before milling is more useful when *Fusarium* infections happen later in the crop season, corresponding to the grain filling stage when the infected grains are similar to sound ones and the mycotoxins are located more externally in the wheat grains.

The progressive debranning of wheat grains did not reduce mycotoxin contamination. The treatments polished and double-polished did not present differences in the whole-wheat flours obtained from BRS Marcante and BRS Reponte cultivars. Our results for the DON content were similar to those reported by Sovrani et al. (2012). These authors stated that DON 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. The entire debranning with 25% tissue removal resulted only in a slightly higher DON loss of approximately 67% (Sovrani et al. 2012). Furthermore, Tibola et al. (2019) reported that the DON content decreased by 25%, 31%, and 31% in the debranning times of 15, 30, and 60 s, respectively, compared to the no-debranned samples. The distribution profile of mycotoxins in the ground wheat fractions is variable. It

depends on mycotoxin, chemical traits, genotypes, fungal contamination levels, and grinding methodology (Cheli et al. 2010, 2013).

Wheat sorting, cleaning, debranning, and milling influence mycotoxin distribution in wheat products entering the food chain (Cheli et al. 2017). In our study, the DON level obtained in the residues from debranning was 12,743 μg.kg^{-1} and 18,931 μg.kg^{-1}, for BRS Marcante and BRS Reponte cultivars, respectively. Similarly, the DON content in the residues from double-polished was 18,575 μg.kg^{-1} and 21,508 μg.kg^{-1}, for BRS Marcante and BRS Reponte cultivars, respectively.

In Brazil, the DON upper limits established for whole-wheat flour in 2021 were 1250 μg.kg^{-1} (ANVISA 2021). Considering this level, the wheat samples from the cultivars BRS Marcante and BRS Reponte reduced the mycotoxin contamination to levels near the permitted limit after cleaning and debranning processes.

Zearalenone content

Cleaning and debranning processes significantly reduced the ZON contamination in the whole-wheat flours from the BRS Reponte cultivar compared with freshly harvested (Fig. 3). The BRS Reponte cultivar showed a ZON level statistically higher than the cultivar BRS Marcante in all treatments (Fig. 3).

For the cultivar BRS Marcante, the effect of debranning in reducing ZON content was not statistically significant (Fig. 3). Otherwise, the debranning process reduced the level of ZON by 56% when compared with the cleaned sample for the BRS Reponte cultivar (102.6 μg.kg^{-1}) (Fig. 3). Another reduction in ZON levels (43%) was observed by double-polished, but it was not statistically significant (Fig. 3). Considering both these debranning treatments, the discarded residue was 8%. The ZON level obtained in the residues from debranning was 346 and 2494 μg.kg^{-1}, for BRS Marcante and BRS Reponte cultivars, respectively.

The second pass on the polisher equipment had no significant effect on the ZON contamination in both cultivars (Fig. 3). In the treatment double-polished, ZON content found in the residue was 498 and 1721 μg.kg^{-1}, for BRS Marcante and BRS Reponte cultivars, respectively.

Phenolic compounds and antioxidant activity

BRS Reponte and BRS Marcante cultivars, the debranning process reduced the total phenolic content in both free and bound extracts (Table 2). For BRS Reponte and BRS Marcante cultivars, the double-polished treatment showed a 14% and 7.6% reduction in free phenolics, respectively, concerning cleaned wheat. In our study, the free and bound phenolic contents were similar. Otherwise, Zhang et al. (2018) concluded that the contribution of bound phenolic acids to the total phenolic content and antioxidant activity was significantly higher than that of free phenolic acids. The BRS Marcante cultivar presented higher levels of total phenolic

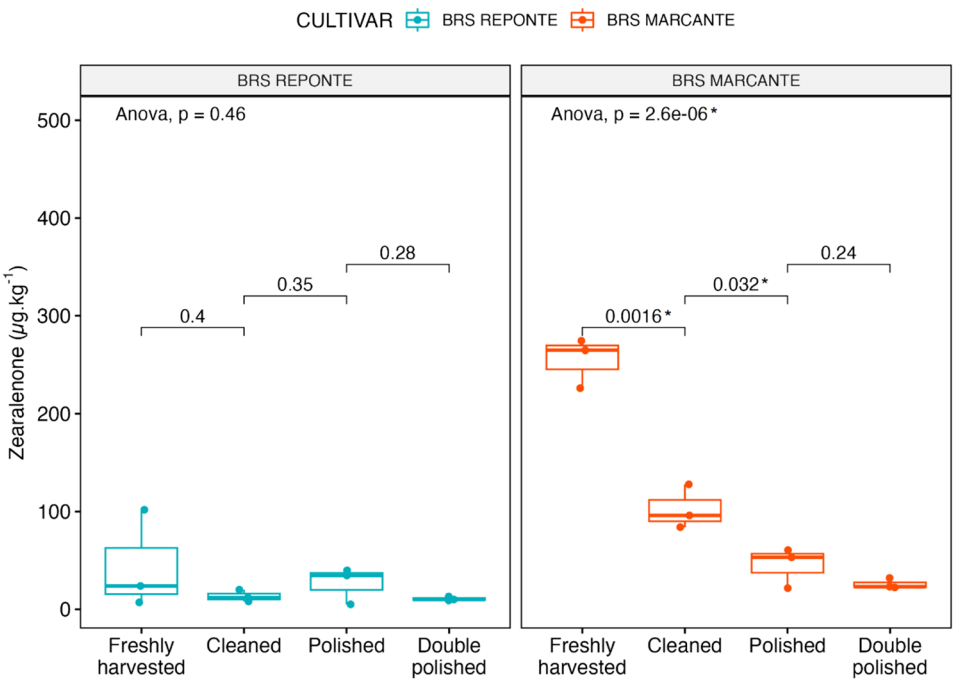

Fig. 3 Effect of the debranning process on zearalenone (ZON) levels in whole-wheat flours obtained from the BRS Marcante and BRS Reponte cultivars. *t.test p-values for pairwise group means comparison and overall F-test p-value ($n = 24$)

Table 2 Free and bound phenolic contents and antioxidant activity by the DPPH and ABTS methods of the whole-wheat flour subjected to the debranning process. Each value represents the mean of three replicate test portions analyzed

Cultivar	Treatment	Total phenolic compounds			Antioxidant activity			
		Free	Bound		DPPH assay		ABTS assay	
		(mg GAE. kg^{-1}d.w)	(mg GAE.kg^{-1}d.w)		Free (µmol TE.g^{-1})	Bound (µmol TE.g^{-1})	Free (µmol TE.g^{-1})	Bound (µmol TE.g^{-1})
BRS Reponte	Freshly harvested	48.6 ± 0.9^{d}	47.3 ± 0.5^{d}		28.5 ± 0.3^{a}	38.4 ± 8.9^{a}	33.9 ± 0.1^{a}	30.9 ± 0.2^{bc}
	Cleaned	45.7 ± 0.9^{d}	46.5 ± 0.6^{d}		29.0 ± 0.2^{a}	44.6 ± 0.3^{a}	34.5 ± 0.4^{a}	31.3 ± 0.6^{abc}
	Polished	42.2 ± 0.3^{e}	45.4 ± 1.0^{de}		29.3 ± 0.4^{a}	45.4 ± 0.7^{a}	35.4 ± 0.7^{a}	32.5 ± 0.2^{a}
	Double-polished	39.3 ± 1.0^{e}	42.9 ± 0.2^{e}		29.2 ± 0.0^{a}	44.6 ± 0.4^{a}	35.2 ± 0.3^{a}	32.5 ± 0.3^{a}
BRS Marcante	Freshly harvested	70.2 ± 0.7^{a}	70.6 ± 1.0^{a}		28.5 ± 0.6^{a}	44.8 ± 0.1^{a}	35.1 ± 0.6^{a}	30.1 ± 0.3^{c}
	Cleaned	68.1 ± 0.3^{ab}	69.0 ± 1.7^{ab}		28.8 ± 0.6^{a}	44.5 ± 0.9^{a}	35.0 ± 0.8^{a}	30.1 ± 1.0^{c}
	Polished	65.2 ± 1.5^{bc}	67.0 ± 1.2^{bc}		29.1 ± 0.6^{a}	44.8 ± 0.7^{a}	34.8 ± 0.6^{a}	31.2 ± 0.5^{abc}
	Double-polished	62.9 ± 1.2^{c}	65.1 ± 1.1^{c}		29.2 ± 0.4^{a}	44.8 ± 0.8^{a}	35.4 ± 0.6^{a}	31.8 ± 0.4^{ab}

Means followed by the same letters in the column do not differ, by the Tukey test, at 5% probability

GAE gallic acid equivalent, *TE* Trolox equivalent

compounds in all treatments than the BRS Reponte cultivar (Table 2).

The results for antioxidant activity referring to the DPPH test for the BRS Reponte and BRS Marcante cultivars indicated that debranning treatments did not influence the free and bound phenolic contents compared to the cleaned sample (Table 2). A similar pattern was observed in the ABTS assay, where the total antioxidant capacity did not reduce in the debranned whole-wheat flour when compared with previous treatments (Table 2).

The bound phenolics were more abundant than the free form, following Adom and Liu (2002), who reported that most phenolic compounds in grains exist in bound form (75% in oats and wheat). Bound phenolics, such as phenolic acids and flavonoids, display strong bioactivities, including anticancer, anti-inflammation, and cardiovascular disease ameliorating properties (Shahidi and Yeo 2016).

There were no significant differences in free and bound phenolics among the cultivars. According to Giordano et al. (2017) regardless of the cultivar considered, the bran fraction resulted in a total antioxidant activity 10 and threefold higher than the refined flour and wholegrain flour, respectively.

The future direction of the research should investigate in more detail the degree of the debranning process and the percentage of grain tissue removal to preserve healthy compounds and reduce mycotoxin content. Moreover, more samples from different cultivars, with contrasting levels of mycotoxin content, should be included to improve the evaluation of the debranning process and identify appropriated wheat fractions to make superior end-use wheat products.

Conclusion

The debranning process has the potential to be an effective post-harvest strategy to minimize mycotoxin contamination in wheat products. The debranning process was effective in reducing DON contamination in the BRS Reponte cultivar (initial level of 2440.0 µg.kg^{-1}), contributing to obtaining safer whole-wheat products. Furthermore, for BRS Reponte and BRS Marcante wheat cultivars, the debranning process did not reduce the antioxidant activity. Our results are relevant for the proper definition, optimization, and scale-up of in-line debranning at an industrial scale to reduce mycotoxin contamination and produce safer and healthier wheat-based food products.

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Author contribution Dr. CST was responsible for conceiving the idea, carrying out the work, and writing the MS, while Dr. LE obtained the samples for the experiment, Dr. JMCF carried out the statistical

analysis and corrected the manuscript, Dr. DS conducted the industrial debranning experiment, and Drs. ERZ and ARGD carried out antioxidant experiments. All the authors revised the manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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