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Foodomics in wheat flour reveals phenolic profile of different genotypes and technological qualities

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ABSTRACT

Phenolic compounds (PC) strongly contribute to the beneficial health effects of wheat, but their interactions can affect the quality of end-use wheat products. Free and bound PC were comprehensively characterized in 14 wheat flours (*Triticum aestivum*) from different Brazilian genotypes and technological qualities by using a metabolomics approach (UPLC-ESI-QTOF-MS^E) combined with classical characterizations: colorimetry, ash, protein, starch and total phenolic content (TPC). Globally, 43 PC were identified: 33 (bound, 28 (free) and 15 in all flours, regardless of extract. Ferulic acid isomers were the most abundant PC, representing 25–50% of ion abundance depending on genotype. Campeiro, Sossego and Topázio genotypes showed a distinguished profile, with the highest total relative abundance of PC. TPC was significantly higher in flours with higher gluten strength (66.5–58.0 mg GAE/100 g flour). The ratio free-to-bound of PC averaged 1.15 between the flours of different technological qualities. Although PCA highlighted specific PC related to technological qualities, the genotype effect was very pronounced. This study correlates the phenolic profile and technological quality of wheat flours and provides the most recent data on the secondary metabolites profile, especially PC in refined flour, attesting to its significant nutritional importance due to its large consumption in refined forms.

1. Introduction

Wheat (*Triticum* spp.) is the second most cultivated cereal in the world. In 2020, world wheat production reached 764 million tonnes, an increase of 4.9% over the last five years (USDA, 2020). In 2020, Brazil produced 6.23 million tonnes of common wheat from a cultivated area of 2.4 million hectares, but imported 6.6 million tonnes (CONAB, 2021) due to its high per capita consumption of 57 kg per year (ABITRIGO, 2018). Wheat is considered the most suitable raw material for bread and pasta due to its viscoelasticity and protein quality. Brazilian production and consumption are almost exclusively restricted to common wheat (*Triticum aestivum*) while durum wheat (*T. durum*) use remains limited; as a result the rheological properties define the technological quality of flour and thus the industrial end-use of wheat.

Furthermore, wheat research has intensified over the last years due

to its bioactive compounds and wide range of reputed beneficial effects on human health. Epidemiological studies have shown that the consumption of whole wheat and grain-based products is associated with reducing chronic non-communicable diseases. The health benefits of cereals have fostered research on the phytochemical composition, mainly, the phenolic compounds (PC), of the different varieties and species of wheat (Dinelli et al., 2009; Fardet, 2010).

Indeed, PC are ubiquitous compounds; they are secondary metabolites synthesized during plant development in response to stress conditions and are among the most abundant bioactive compounds on Earth (Saltveit, 2017). The largest proportion of PC is found in wheat's outer layers - aleurone, testa and pericarp - and exert a wide range of bioactivities, but their beneficial effects are generally attributed to their antioxidant activity (Shewry & Hey, 2015). PC can be found in three forms in wheat: soluble free, soluble conjugated (e.g., with mono and

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polysaccharides), and insoluble bound to cell wall components, such as arabinoxylan fibers, or macronutrients, such as proteins. The most abundant form found in wheat grains is the insoluble bound (77%), followed by the soluble conjugated (22%) and the soluble free form (<0.5–1%) (Acosta-Estrada et al., 2014). Phenol-protein interactions can alter these molecules physicochemical properties, solubility, availability and digestibility (Ozdal et al., 2013). Due to these chemical interactions, PC can impact the quality of end-use products. Sharma et al. (2016) showed that wheat flours classified as “poor” had higher levels of PC and higher expression of enzymes related to their synthesis pathways when compared to “good” wheat flour.

Phenolic acids and flavonoids are the most important class and ferulic acid is the dominant phenolic acid in wheat (>90%). However, agroclimatic parameters, such as location and growing conditions, and processing, such as wheat dry fractionation processes, strongly impact the PC profile (Hemery et al., 2007; Wang et al., 2013). This impact is evident in the milling process to obtain refined wheat flour; whole flours (100% extraction rate) presented the highest levels of PC (Wang et al., 2013). However, the consumption of refined white flour is six times higher than wholemeal flour. Despite this, information about refined flour’s phenolic profile and content remains limited (Sharma et al., 2020; Shewry & Hey, 2015).

Due to the great diversity of PC and their isomers in cereals, especially in wheat grains, it is essential to employ advanced analytical techniques for reliable identification (Santos et al., 2019). The metabolomic approach is useful since it combines different techniques, presenting high sensitivity, selectivity, and resolution. Similarly, the concept of “Foodomics” employs omics tools to understand and map the chemical compounds, as well as to characterize food contaminants (Herrero et al., 2012).

In this work, omics tools were applied to characterize the PC profile of wheat flour from different Brazilian genotypes classified into three different technological qualities: low, medium and superior. Thus, a total of 14 refined wheat flours were investigated, representing 12.5% of cultivars currently produced in Brazil, with different technological qualities and indicative end-use commercial classes (e.g., pasta, bread, or biscuit production). A modern non-targeted method based on a multiplexed MS-MS acquisition with simultaneous application of low (precursor ions) and high collision energy (MS^E) (fragment ions) was used to relatively identify and quantify the PC of wheat samples. Total phenolic, ash, protein, and starch contents and colorimetric properties were also determined, and some correlations were established.

2. Materials and methods

2.1. Chemicals

The following reference standards, as well as MS-grade acetonitrile and methanol, were purchased from Sigma-Aldrich (St. Louis, MO, USA): vanillic acid, *p*-coumaric acid, catechin, caffeic acid, ellagic acid, *trans*-ferulic acid, kaempferol, myricetin, pyrogallol, flavanone, quercetin, gallic acid, epicatechin, 4-hydroxybenzylalcohol, 4-hydroxybenzaldehyde acid, 4-hydroxybenzoic acid, 4-hydroxybenzoic acid, 4-phenylacetic acid, sinapic acid, benzoic acid, quercetin-3-O-glucoside, 3,4-dihydroxy phenylacetic acid, epigallocatechin, epicatechingallate, chlorogenic acid, 2,5-dihydroxybenzoic acid, 4-methoxycinnamic acid, 2-hydroxycinnamic acid, 3-hydroxy-4-methoxycinnamic acid, *trans*-cinnamic acid, 3-methoxycinnamic acid, and L-(–)-3-phenylacetic acid. Formic acid was purchased from Fluka (Switzerland). Ultrapure water was obtained through the Barnstead™ Smart2Pure™ (Thermo Fisher Scientific, USA) purification system. Other unmarked reagents were of analytical grade.

2.2. Plant material

OR Melhoramento de Sementes and Biotrigo Genética (Passo Fundo,

Brazil) kindly provided common winter wheat flours (*Triticum aestivum*) from 14 different Brazilian genotypes (Campeiro, ORS Vintecinco, ORS1401, ORS1402, Marfim, Jadeite 11, Ametista, ORS Vinteseite, Topázio, Noble, Iguaçú, Sintonia, Sossego and Alpaca) and different technological qualities according to the gluten rheological properties (Supplementary table 1). All samples were cultivated in normal agro-nomical conditions at the same location in Coxilha (RS, Brazil) and were ground with the experimental grinder “Moinho Experimental VG 2000i” (Vitti Molinos, Santa Catarina, Brazil) at the classical flour extraction rate (~50–55%). The flours were kept at –20 °C for three months before use.

2.3. Moisture, ash, protein and starch contents and colorimetric parameters

The moisture, ash and protein content of wheat flours were determined according to the AACCI methods (44-15.02 and 08.01.01, respectively) (AACCI, 2000) through a micro-Kjeldahl with a conversion factor of 5.7 (AOAC, 1984). The total starch content was determined using the Megazyme assay (K-TSTA 07/11) (AOAC Method 996.11, AACCI Method 76-13). The colorimetric parameters were determined in triplicate by reflectance colorimeter (CM-5, Konica Minolta, Japan) using the CIELAB color model, hue angle and chroma (C*) as parameters.

2.4. Extraction of free and bound phenolic compounds

PC from wheat flour was extracted in triplicate (Santos et al., 2019). An amount of 70 mg of sample and 50 mg of Celite were weighed, manually macerated and then extracted with 80% ethanol. Samples were stirred (200 rpm, 10 min, 25 °C) and centrifuged (5000×g, 10 min, 25 °C). The extraction was repeated twice, and the collected supernatants were combined. The extracts were dried in an evaporator centrifuge (Speed Vac Concentrator, Thermo Scientific, USA). The pellets were resuspended with NaOH (4M) and submerged in an ultrasonic bath (42 kHz, 90 min, 40 °C). After the alkaline hydrolysis, acid hydrolysis was performed with concentrated HCl (~pH 2), and the samples were centrifuged (2000×g, 5 min). The supernatant was washed three times with ethyl acetate (7 mL) and centrifuged between each step (10000×g, 5 min, 10 °C). The combined extracts were dried in a rotary evaporator (200 rpm, 40 °C) (Laborota 4000 Heidolph) coupled to a chiller. All dried extracts were resuspended in 1.5 mL of 2% methanol, 5% acetonitrile and 93% ultrapure water and then filtered (13 mm, 0.22 μm), transferred to vials, and stored at –20 °C until analysis.

2.5. Folin–Ciocalteu reducing capacity

Total PC in the obtained extracts were estimated by measuring their capacity to reduce Folin–Ciocalteu reagent, it was estimated in triplicate in free and bound extracts of wheat flours based on the original method (Singleton et al., 1999) adapted to microplates. Absorbance was determined at 750 nm on a microplate reader (FlexStation III, Molecular Devices). The standard curve was carried out with gallic acid (5–130 mg/L) in the reaction mixture. Results were expressed as mg gallic acid equivalents (GAE) per 100 g of sample (dry basis).

2.6. Determination of the phenolic compounds profile by UPLC-MS^E

For each analysis, a mixed solution of standard compounds (10 ppm) or extracts (2 μL) was injected in triplicate into the system UPLC Acquity (Waters, Milford, MA) coupled to the Xevo G2-S Q-TOF (Waters, Manchester, UK), which was equipped with an electrospray ionization source according to Santos et al. (2019). The separation was carried out using a UPLC HSS T3 C18 column (100 × 2.1 mm, 1.8 μm particle diameter) (Waters) at 30 °C, with a flow of 0.6 mL/min of ultra-pure water and 5 mM ammonium formate (mobile phase A) and acetonitrile (mobile

phase B), both containing 0.3% formic acid. The gradient method was applied as follows: 0 min - 97% A; 6.78 min - 50% A; 7.36 min - 15% A; 8.51 min - 15% A; and 9.09 min - 97% A. The capillary and cone voltage were set at 2.0 kV and 30 V, respectively. The desolvation gas (N₂) was set at 800 L/h and 500 °C, and the cone gas was set at 50 L/h and the source at 120 °C. Data was acquired through the centroid mode using a multiplexed MS/MS acquisition with alternating low and high-energy acquisition (MS^E), from *m/z* 50 to 1000, operating in negative ion mode ESI (-). MS/MS experiments were performed with a collision energy ramp (30–55 eV) and ultrapure argon (Ar) as collision gas. All acquisitions were performed using leucine encephalin (Leu-Enk) for calibration and MassLynx 4.1 (Waters) software for the data.

2.7. UPLC-MS^E data processing

The putative identifications followed the levels of identification according to Sumner et al. (2007). The software Progenesis QI (Waters) was used under the following conditions to analyze the data set: all runs, automatic limits, centroid data, and resolution of 30000, and negative ion mode. For the identification, neutral mass isotope distribution, retention time, and MS/MS fragments from standards were used by applying MetaScope based on the comparison with polyphenols database from PubChem, Kegg and the online database Phenol Explorer as per Santos et al. (2019). The non-targeted identifications followed these parameters, in descending order of importance: comparison between the experimental and theoretical *m/z*; isotopic similarity (>80); exact mass error (<10 ppm); score >30; highest fragmentation score; and all parameters generated by the software used. In addition, other factors such

as comparison sample characteristics, retention time, literature data and chemical characteristics of the molecule were used as criteria for tentative identification of multiple or unknown compounds.

2.8. Statistical analysis

All analyses were performed in technical triplicate, and the results are reported as mean values ± standard deviation (SD). The XLSTAT software (Addinsoft, France) was used to perform the statistical analysis among samples (Tukey's test, *p* < 0.05, and one-way ANOVA) and the heatmaps. The omics data was exported to the EZInfo software (Waters, USA) for multivariate analysis to Principal Components Analysis (PCA) and S-plot. The graphic representation of the results was created using the GraphPad Prism (5.0) software.

3. Results and discussion

3.1. Physico-chemical characterization of wheat flours

Ash content, usually, indicates the contamination of flour with bran particles during milling and thus provides an estimation of the degree of separation of bran and germ from endosperm during milling. The presence of bran darkens the color of the products (Katyál et al., 2016). The ash content averaged 0.77% and ranged from 0.54 to 1.24% across the 14 flours, in accordance with Brazilian legislative guideline for refined wheat flour (maximum value of ash: 1.4%) (BRASIL, 2005).

Although studies do not relate the ash content to the technological quality, but external factors such as soil and, weather conditions.

Table 1

Contents of ash, starch, protein, colorimetric parameters, and phenolic contents in the different wheat flours.

Genotype	Ash (% db)	Protein (% db)	Starch (% db)	Colorimetric analysis					Folin-Ciocalteu (mg GAE/100 g db)				
				L*	a*	b*	C*	h _{ab}	Free (F)	Ratio (F/T)	Bound (B)	Ratio (B/T)	Total (T)
<i>Alpaca</i>	0.54 ± 0.06 ^e	9.30 ± 1.33 ^f	62.99 ± 0.71 ^e	91.17 ± 0.08 ^a	0.31 ± 0.01 ^j	7.64 ± 0.02 ^h	7.65 ± 0.02 ^{bcd}	1.53 ± 0.04 ^a	28.43 ± 0.44 ^g	0.60	18.92 ± 0.47 ⁱ	0.40	47.35 ± 0.91 ^h
<i>Ametista</i>	0.79 ± 0.11 ^{bcd}	14.89 ± 0.02 ^a	69.24 ± 1.80 ^{bcd}	85.48 ± 0.02 ⁱ	1.02 ± 0.00 ^d	9.99 ± 0.02 ^e	10.04 ± 0.02 ^f	1.47 ± 0.01 ⁱ	30.93 ± 0.30 ^{ef}	0.51	29.77 ± 0.30 ^{de}	0.49	60.70 ± 0.60 ^e
<i>Campeiro</i>	0.71 ± 0.04 ^{cde}	10.93 ± 0.01 ^{def}	70.45 ± 0.73 ^{abcde}	89.95 ± 0.09 ^d	0.55 ± 0.01 ^h	7.78 ± 0.08 ^h	7.80 ± 0.08 ^d	1.50 ± 0.02 ^c	31.83 ± 0.72 ^e	0.44	40.47 ± 1.47 ^a	0.56	72.29 ± 2.19 ^b
<i>Iguaçu</i>	0.80 ± 0.05 ^{bcd}	8.97 ± 0.72 ^f	65.09 ± 0.94 ^{cde}	86.93 ± 0.01 ^g	0.88 ± 0.01 ^e	11.28 ± 0.03 ^a	11.31 ± 0.03 ^c	1.49 ± 0.04 ^e	29.60 ± 0.15 ^{fg}	0.44	37.21 ± 0.73 ^b	0.56	66.81 ± 0.88 ^{cd}
<i>Jadeite</i>	0.78 ± 0.03 ^{bcd}	13.65 ± 0.51 ^{ab}	72.36 ± 1.92 ^{ab}	85.80 ± 0.03 ^h	1.05 ± 0.00 ^c	10.19 ± 0.02 ^d	10.24 ± 0.02 ^{cd}	1.47 ± 0.01 ⁱ	30.91 ± 0.44 ^{ef}	0.53	27.03 ± 1.65 ^{fg}	0.47	57.94 ± 2.09 ^f
<i>Marfim</i>	0.66 ± 0.02 ^{de}	15.60 ± 0.19 ^{ab}	67.18 ± 1.98 ^{bcd}	90.24 ± 0.05 ^c	0.53 ± 0.00 ^h	7.30 ± 0.05 ⁱ	7.32 ± 0.05 ^g	1.50 ± 0.03 ^d	25.31 ± 0.14 ^h	0.52	22.99 ± 0.77 ^h	0.48	48.31 ± 0.91 ^h
<i>Noble</i>	0.58 ± 0.04 ^e	14.00 ± 0.21 ^{ab}	67.85 ± 0.90 ^{bcd}	89.92 ± 0.17 ^d	0.59 ± 0.02 ^g	8.09 ± 0.16 ^g	8.12 ± 0.16 ^{ab}	1.50 ± 0.07 ^d	52.36 ± 0.06 ^a	0.68	24.68 ± 0.31 ^{gh}	0.32	77.04 ± 0.91 ^a
<i>ORS1401</i>	0.59 ± 0.09 ^e	13.50 ± 0.00 ^{abc}	72.57 ± 1.85 ^{abc}	87.32 ± 0.02 ^f	0.76 ± 0.00 ^f	10.59 ± 0.04 ^c	10.61 ± 0.04 ^{bcd}	1.50 ± 0.01 ^{cd}	44.74 ± 0.74 ^b	0.65	23.82 ± 0.00 ^h	0.35	68.56 ± 0.74 ^g
<i>ORS1402</i>	0.85 ± 0.03 ^{bc}	13.65 ± 0.21 ^{cde}	70.61 ± 2.96 ^{abc}	87.60 ± 0.02 ^e	0.89 ± 0.01 ^e	9.42 ± 0.02 ^f	9.46 ± 0.02 ^{de}	1.48 ± 0.03 ^g	30.04 ± 0.33 ^f	0.51	28.64 ± 0.90 ^{ef}	0.49	58.68 ± 1.23 ^b
<i>ORSVintecino</i>	0.95 ± 0.02 ^b	13.62 ± 0.50 ^{ab}	71.61 ± 3.25 ^{abc}	89.94 ± 0.05 ^d	0.60 ± 0.00 ^g	7.42 ± 0.01 ⁱ	7.45 ± 0.01 ^h	1.49 ± 0.00 ^f	24.39 ± 0.42 ^b	0.52	22.75 ± 0.44 ^h	0.48	47.14 ± 0.86 ^h
<i>ORSVintese</i>	0.56 ± 0.02 ^e	13.52 ± 0.38 ^{abc}	76.94 ± 7.28 ^a	90.71 ± 0.07 ^b	0.41 ± 0.01 ⁱ	7.46 ± 0.05 ⁱ	7.47 ± 0.05 ⁱ	1.52 ± 0.03 ^b	31.94 ± 0.76 ^e	0.59	22.15 ± 0.39 ^{cd}	0.41	54.09 ± 1.15 ^{de}
<i>Sintonia</i>	1.24 ± 0.10 ^{bcd}	10.90 ± 0.39 ^{def}	67.73 ± 1.04 ^{bcd}	87.63 ± 0.05 ^e	0.87 ± 0.00 ^e	11.08 ± 0.03 ^b	11.11 ± 0.03 ^{cd}	1.49 ± 0.01 ^e	32.27 ± 0.39 ^e	0.58	23.76 ± 0.98 ^h	0.42	56.04 ± 1.37 ^g
<i>Sossego</i>	0.81 ± 0.02 ^b	10.57 ± 0.69 ^{ef}	64.24 ± 0.40 ^{de}	85.25 ± 0.06 ^j	1.08 ± 0.00 ^b	11.17 ± 0.07 ^{ab}	11.22 ± 0.07 ^a	1.47 ± 0.04 ^h	36.55 ± 0.00 ^c	0.54	31.32 ± 0.73 ^d	0.46	67.87 ± 0.73 ^c
<i>Topázio</i>	0.95 ± 0.02 ^a	12.79 ± 0.34 ^{bcd}	70.71 ± 0.76 ^{abcd}	85.14 ± 0.02 ^j	1.12 ± 0.01 ^a	10.07 ± 0.03 ^{de}	10.13 ± 0.03 ^{abc}	1.46 ± 0.02 ^j	34.67 ± 0.08 ^d	0.50	34.24 ± 0.84 ^c	0.50	68.91 ± 0.92 ^c
Low*	0.69 ± 0.03 ^B	11.84 ± 0.22 ^B	70.38 ± 1.60 ^A	90.44 ± 0.07 ^A	0.47 ± 0.00 ^C	7.58 ± 0.03 ^B	9.57 ± 0.06 ^B	1.51 ± 0.00 ^A	29.15 ± 0.21 ^C	0.50	28.93 ± 0.30 ^B	0.50	58.08 ± 0.50 ^C
Medium*	0.77 ± 0.02 ^A	12.19 ± 0.10 ^B	68.72 ± 1.50 ^A	87.61 ± 0.02 ^B	0.83 ± 0.00 ^B	9.91 ± 0.03 ^A	10.92 ± 0.08 ^A	1.49 ± 0.00 ^B	33.78 ± 0.09 ^B	0.56	26.16 ± 0.43 ^C	0.44	59.89 ± 0.38 ^B
Superior*	0.84 ± 0.04 ^A	12.85 ± 0.17 ^A	69.12 ± 0.36 ^A	86.65 ± 0.04 ^C	0.93 ± 0.00 ^A	9.92 ± 0.05 ^A	11.04 ± 0.05 ^A	1.48 ± 0.00 ^C	35.69 ± 0.14 ^A	0.54	30.82 ± 0.54 ^A	0.46	66.51 ± 0.59 ^A

C*: croma, h_{ab}: hue angle. a-j subscripted letters indicated a significant difference (*p* < 0.05) between the genotypes in the same columns and A-C indicates a significant difference (*p* < 0.05) between the technological qualities. Low represents Alpaca, Campeiro, ORS25 and ORS27 varieties; Medium represents Marfim, ORS1401, ORS1402, Sintonia and Sossego varieties; and Superior represents Ametista, Jadeite, Iguaçu, Noble and Topázio varieties. Data are means ± SD (n = 3).

Concerning the technological quality effect, this study showed a significant difference in the ash content ($p < 0.05$) between the medium/superior flours and the low flour. The ash content ranged from 0.54 to 0.95% in low, 0.59–0.95% in medium, and 0.58–1.24% in superior wheat flours (Table 1). The ash content of low wheat flours (average 0.69%) was similar to the findings of the previous literature (0.66 ± 0.03) for refined wheat flour (Dhiraj & Prabhasankar, 2013). Conversely, medium and superior flours had an average ash content that was 12% and 20% higher than expected, respectively. Refined wheat flours with high ash content are associated with non-endosperm (e.g., bran) contamination during refining. However, all flour varieties were submitted to the same extraction rate (white wheat flour: 50%). In addition, contamination would also have impacted the protein and starch analyses. Nevertheless, they did not show a good correlation, and the starch content did not change between the three technological qualities. Therefore, this difference can be explained by the following two hypotheses: a) particular characteristics of the genotype, wheat class, and cultivar determine mineral/ash content variability (Czaja et al., 2020); and b) there is a correlation between flour technological quality and ash content, corroborating the findings of Yousaf et al. (2019).

In general, L^* , a^* , and b^* coordinates of flours ranged from 85 to 91, 0.3 to 1.1, and 7.3 to 11.3, respectively (Table 1). The same reported trends for ash content were obtained for colorimetric results. A significant impact of technological quality on colorimetric parameters ($p < 0.05$) was also found. According to parameters, the superior flour is darker (L^*) and redder (a^*) compared to the others, while the b^* axis is similar to medium flour, indicating that both have the same yellow color. In contrast, low flours are lighter, less red, and less yellow. As expected, a positive correlation between ash content with a^* and b^* ($r = 0.90$, $p = 3.5 \cdot 10^{-6}$ and $r = 0.84$, $p = 0.03$) was observed. At the same time, the brightness (L^*) and hue angle were negatively correlated with ash content ($r = 0.90$, $p = 2.37 \cdot 10^{-4}$ and $r = 0.92$, $p = 2.47 \cdot 10^{-4}$, respectively). Previous studies have also shown a similar correlation between ash and CIELAB parameters (Katyal et al., 2016).

The increase of ash content in flour is attractive from a nutritional point of view; however, it negatively impacts the flour's technological characteristics (Carson & Edwards, 2009; Hemery et al., 2011). As observed in the colorimetric analyses, the high-ash flour was characterized by a darker color, resulting in the possible rejection of the final product (Bucselia et al., 2016). Furthermore, the ash content was also associated with greater activity of proteolytic and amylolytic enzymes; in other words, dietary fiber and non-gluten proteins disintegrated, weakening the protein matrix during dough formation (Bucselia et al., 2016; Carson & Edwards, 2009). According to commercial classification (BRASIL, 2010), the superior quality is equivalent to improved wheat mixed with (low quality) basic flours for bakery products (e.g., bread, biscuits, and cakes). With this blend, the high ash content and colorimetric parameters of superior quality flours would not impact the final product.

3.2. Total phenolic content in different cultivars of wheat flour

Although the Folin-Ciocalteu method may present interferences due to the reaction of other reducing molecules present in the extracts (Górnas et al., 2016; Huang et al., 2005), in this work, the total phenolic content was estimated by measuring the reducing capacity of the obtained extracts, enriched in phenolic compounds, considering free and bound phenolics. Table 1 shows the TPC of the different wheat flours. The TPC averaged 62 mg GAE/100 g and ranged between 47 and 77 mg GAE/100 g for the 14 wheat flours. As expected, these values were lower than those previously found for the same cultivars in mature wheat whole grains (Santos et al., 2019). However, the TPC of the present study was greater than the values found for some wheat grains (53.1 mg GAE/100 g) and different winter wheat flours (11.3–37.1 mg GAE/100 g) (Alvarez-Jubete et al., 2010; Yu et al., 2004). The technological

quality effect was again evidenced; the TPC of medium wheat flours was significantly higher (3%) than low flours, and superior wheat flours were significantly higher than low and medium flours (15% and 11%, respectively), corroborating the results found for ash content and colorimetric parameters. More generally in our data, TPC of flours showed a positive but not significant correlation with ash content ($r = 0.85$, $p = 0.43$), and with redness (a^*) ($r = 0.81$, $p = 0.03$), while the brightness (L^*) was inversely correlated to TPC ($r = 0.82$, $p = 2.92 \cdot 10^{-7}$).

An analysis of each extract (Table 1) showed that free extract generally had 16% more phenolic content than bound extract ($p < 0.05$). Although, the majority of previous studies have reported that PC are mostly found in bound forms (60–75%) in wheat grains (*Triticum* spp.), Liyana-Pathirana and Shahidi (2006) have shown that the presence of bran influences. These authors reported that the highest percentage of bound PC was found in bran (84%), while 59% was found in whole grain and only 49% in wheat white flour, corroborating our data. The superior sample had 22% and 6% more free PC than low and medium flours, respectively. Similarly, free PC were higher in superior flours, and medium showed the lowest value (7% and 18% less than low and superior samples, respectively).

The mean ratio of free-to-bound (F/B) PC in low flours was 1.0–1.3 in medium and 1.2 in superior flours, showing different profiles between the technological qualities. Although the bakery effect was not evaluated in our study, the literature has shown that this processing is able to change the F/B ratio. Bread, cookie and muffin production releases bound PC, increasing the free PC content and boosting its bioavailability (Abdel-Aal & Rabalski, 2013). However, it is important to note that this effect appeared dependent on the baking recipe, heating conditions and PC class.

3.3. Identification of phenolic compounds by UPLC-MS^E

3.3.1. Comparison of different Brazilian genotypes

This work tentatively identified a total of 43 PC including isomers. Some compounds were fully confirmed by reference standards, such as: caffeic acid, ferulic acid, *p*-coumaric and sinapic acid, present in both extracts (free and bound). Overall, five classes of PC were found in this study: flavonoids (32%), phenolic acids (30%), other polyphenols (26%), stilbenes (7%) and lignans (5%).

Table 2 presents the complete table with all information about the putative compounds, including the experimental exact mass [M-H], the retention time, the isotopic similarity, the mass error of precursors, the fragmentation score and the fragments ions (MS/MS) generated. The MS/MS data that resulted from the specific fragmentation of precursor ions from the breaking of structural bonds in the collision cell is an important parameter for confirmation the identified PC in a non-targeted approach (Ncube et al., 2014).

Fifteen PC were identified in all 14 wheat flours, showing that despite genetic variability and the influence of external factors on phenotype, wheat contains a core content of PC. Some of these identified compounds, such as *p*-coumaric acid, caffeic acid and ferulic acid, stand out since they are present in the main synthetic pathways of other PC (phenylpropanoid biosynthesis map available at <http://www.genome.jp/dbget-bin/www.bget?map00940>). In our previous study, the phenolic profile was followed along with the development of wheat grains in seven genotypes of wheat (Campeiro, ORS Vintecinco, ORS1401, ORS1402, Marfim, Jadeite 11, Ametista); furthermore, a total of 100 PC were identified even in mature grains (used for flour production), including isomeric forms (Santos et al., 2019). In the current work, it was possible to compare the phenolic profile of the same wheat mature grains with the respective flours in these seven genotypes, that showed the presence of 26 PC. It means that a high number of PC remains present even after the grain milling process.

A high number of PC in refined wheat flours, including a high amount of ferulic acid and its isoforms, is of great importance and further reinforces the positive aspects attributed to wheat consumption,

Table 2
Putative identification of phenolic compounds in Brazilian refined wheat flour by UPLC-MS^E.

Compound	Molecular formula	<i>m/z</i> [M-H]	RT (min)	Score (%)	FS (%)	Fragments	Mass Error (ppm)	IS (%)	
PHENOLIC ACIDS									
1	3,4-dihydroxy-5-methoxybenzoic acid	C ₈ H ₈ O ₅	183.0281	0.94	36.6	ND	–	–6.88	90.65
2	Gallic acid ethyl ester	C ₉ H ₁₀ O ₅	197.0442	1.31	38.4	13.2	101.0244 (61.76%); 125.0244 (36.51%); 143.0349 (1.47%)	–6.94	86.54
3	<i>Caffeic acid</i> *	C ₉ H ₈ O ₄	179.0332	2.67	56.4	96.3	135.0435 (100%)	–9.08	95.48
4	Diferulic acid I	C ₂₀ H ₁₈ O ₈	385.0913	3.59	49.5	59.9	341.1030 (100%)	–3.60	91.87
5	Diferulic acid II	C ₂₀ H ₁₈ O ₈	385.0913	3.75	36.9	ND	–	–3.50	89.10
6	Diferulic acid III*	C ₂₀ H ₁₈ O ₈	385.0912	3.83	37.7	ND	–	–4.40	93.58
7	Diferulic acid IV*	C ₂₀ H ₁₈ O ₈	385.0913	4.47	37.6	ND	–	–4.12	92.84
8	Diferulic acid V	C ₂₀ H ₁₈ O ₈	385.0911	4.79	36.6	ND	–	–4.69	88.24
9	Ellagic acid	C ₁₄ H ₆ O ₈	300.9971	3.50	51.8	67.7	299.9981 (31.31%)	–6.12	98.16
10	<i>Trans-ferulic acid</i> *	C ₁₀ H ₁₀ O ₄	193.0491	3.53	53.4	77.6	134.0373 (100%); 178.0257 (11.52%); 133.0295 (6.47%); 149.0609 (3.14%);	–8.15	98.70
11	Ferulic acid*	C ₁₀ H ₁₀ O ₄	193.0488	3.69	55	87.4	134.0373 (100%); 178.0255 (4.29%); 149.0608 (1.64%)	–9.33	97.62
12	<i>p-Coumaric acid I</i> *	C₉H₈O₃	163.0384	3.29	53.2	82.7	119.0490 (100%)	–9.95	94.00
13	<i>Sinapic acid</i>	C ₁₁ H ₁₂ O ₅	223.0594	3.53	35.7	ND	–	–8.12	87.65
FLAVONOIDS									
14	3,7-Dimethylquercetin	C ₁₇ H ₁₄ O ₇	329.0650	5.71	36	ND	–	–5.22	86.23
15	Apigenin 7- <i>O</i> -apiosyl-glucoside I*	C ₂₆ H ₂₈ O ₁₄	563.1400	2.87	56.8	92.1	353.0667 (100%); 383.0772 (66.69%)	–1.17	93.48
16	Apigenin 7- <i>O</i> -apiosyl-glucoside II*	C ₂₆ H ₂₈ O ₁₄	563.1399	3.01	56.1	87.2	353.0653 (19.03%); 383.0760 (14.10%)	–1.29	94.64
17	Auriculoside I	C ₂₂ H ₂₆ O ₁₀	449.1426	1.15	36.2	0.11	71.0138 (9.76%)	–6.03	88.01
18	Auriculoside II	C ₂₂ H ₂₆ O ₁₀	449.1492	6.31	35.5	ND	–	8.60	86.83
19	Carlinoside	C ₂₆ H ₂₈ O ₁₅	579.1354	2.74	37	ND	–	–1.29	86.80
20	Daidzein	C ₁₅ H ₁₀ O ₄	253.0495	4.73	35.8	ND	–	–4.41	84.19
21	Eupatorin I	C ₁₈ H ₁₆ O ₇	343.0808	4.76	35.7	ND	–	–4.54	83.63
22	Eupatorin II	C ₁₈ H ₁₆ O ₇	343.0817	5.31	35.7	ND	–	–1.81	80.88
23	Koparin	C ₁₆ H ₁₂ O ₆	299.0551	5.76	35.8	ND	–	–3.39	82.87
24	Okanin	C ₁₅ H ₁₂ O ₆	287.0561	2.03	40	16.6	101.0244 (19.31%); 113.2044 (9.29%); 125.0231 (3.51%); 99.0437 (5.11%); 261.0404 (1.99%)	–0.19	83.77
25	Psoralidin	C ₂₀ H ₁₆ O ₅	335.0900	1.01	35.8	ND	–	–7.36	87.21
26	Tectoridin	C ₂₂ H ₂₂ O ₁₁	461.1059	3.21	35.5	ND	–	–6.62	85.17
27	Tetramethylscutellarein	C ₁₉ H ₁₈ O ₆	341.1017	4.90	35.9	ND	–	–3.86	83.77
OTHER POLYPHENOLS									
28	1- <i>O</i> -Sinapoyl-beta-D-glucose	C ₁₇ H ₂₂ O ₁₀	385.1125	3.15	56.3	95.8	113.0244 (100%)	–3.91	90.39
29	1'-Acetoxychavicol acetate	C ₁₃ H ₁₄ O ₄	233.0805	5.97	35.8	ND	–	–6.13	85.98
30	Acetyl Eugenol	C ₁₂ H ₁₄ O ₃	205.0855	5.61	37.9	ND	–	–72.9	97.70
31	Catechol	C ₆ H ₆ O ₂	109.0287	0.77	37	ND	–	–7.14	93.26
32	Elemicin	C ₁₂ H ₁₆ O ₃	207.1011	5.55	35.7	ND	–	–7.67	87.10
33	Esculetin I	C ₉ H ₆ O ₄	177.0177	2.62	42	25.9	133.0295 (19.45%)	–9.22	94.01
34	Esculetin II	C ₉ H ₆ O ₄	177.0176	3.89	35.9	ND	–	–9.66	89.91
35	Leptodactylone	C ₁₁ H ₁₀ O ₅	221.0435	4.32	36.7	ND	–	–9.05	93.46
36	<i>Sinapoyl alcohol</i> *	C ₁₁ H ₁₄ O ₄	209.0802	5.82	36.1	ND	–	–8.09	89.46
37	Syringaldehyde	C ₉ H ₁₀ O ₄	181.0489	4.25	35.8	ND	–	–9.71	89.87
38	Vanillactic acid	C ₁₀ H ₁₂ O ₅	211.0595	1.91	53.3	82.7	136.0166 (100%); 151.0400 (98.56%)	–8.14	92.66
LIGNANS									
39	4'-Demethyldeoxypodophyllotoxin	C ₂₁ H ₂₀ O ₇	383.1130	0.92	36.5	ND	–	–1.54	84.15
40	Flaxseed	C ₂₆ H ₃₈ O ₁₂	541.2290	2.19	38.7	ND	–	0.07	93.41
STILBENES									
41	Astringin	C ₂₀ H ₂₂ O ₉	405.1152	0.95	38.6	13.2	180.0639 (20.27%)	–9.69	90.20
42	Pterostilbene	C ₁₆ H ₁₆ O ₃	255.1044	6.17	36.1	ND	–	6.76	88.00
43	Resveratrol 3- <i>O</i> -glucoside	C ₂₀ H ₂₂ O ₈	389.1220	3.48	35.8	ND	–	–5.72	85.51

m/z: mass/charge; RT: retention time; FS: fragmentation score; IS: isotope similarity. Bold: reference standards; italic: compounds identified in both extracts; *Phenolic compounds found in all samples studied.

even if it is refined flour (Wieser et al., 2020). Indeed, these PC present in flours are likely to resist the baking process (hydration, fermentation, heat treatment) and can be found in the final products. The impact of the baking process on PC will differ depending on whether they are bound or free. High temperatures applied in the baking process can decrease the concentration of bound PC, probably due to broken bonds, but do not eliminate the PC. On the contrary, heat treatments can positively affect some matrices, increasing free PC and as a result, PC bioavailability (Lu et al., 2017). Lu et al. (2014) showed similar results, reporting that PC (initially higher in wholemeal flour than refined flour) remained present in the final product after the fermentation and baking process. The genotypes Ametista, Iguacu, ORS1402, Sossego and Topazio showed the highest number of total PC (Fig. 1A).

Corroborating previous studies (Dinelli et al., 2009; Wang et al., 2013), the number of free PC (28) identified in this study was lower than the number of bound PC (33). This lower diversity of free PC was true for some classes of secondary metabolites identified in this study: phenolic acid, other polyphenols, lignans and stilbenes. Moreover, some compounds such as stilbenes, some lignans and flavonoids and some dimers of ferulic acid were identified only in bound extracts, reaffirming the importance of performing the hydrolysis step for the identification of compounds.

As expected, the isomers of ferulic acid were the most abundant PC in free and bound extracts. The ferulic acid is known to be the most abundant phenolic acid in cereals, especially in wheat, and is known for its potential health benefits (Luthria et al., 2015). The isomers of ferulic

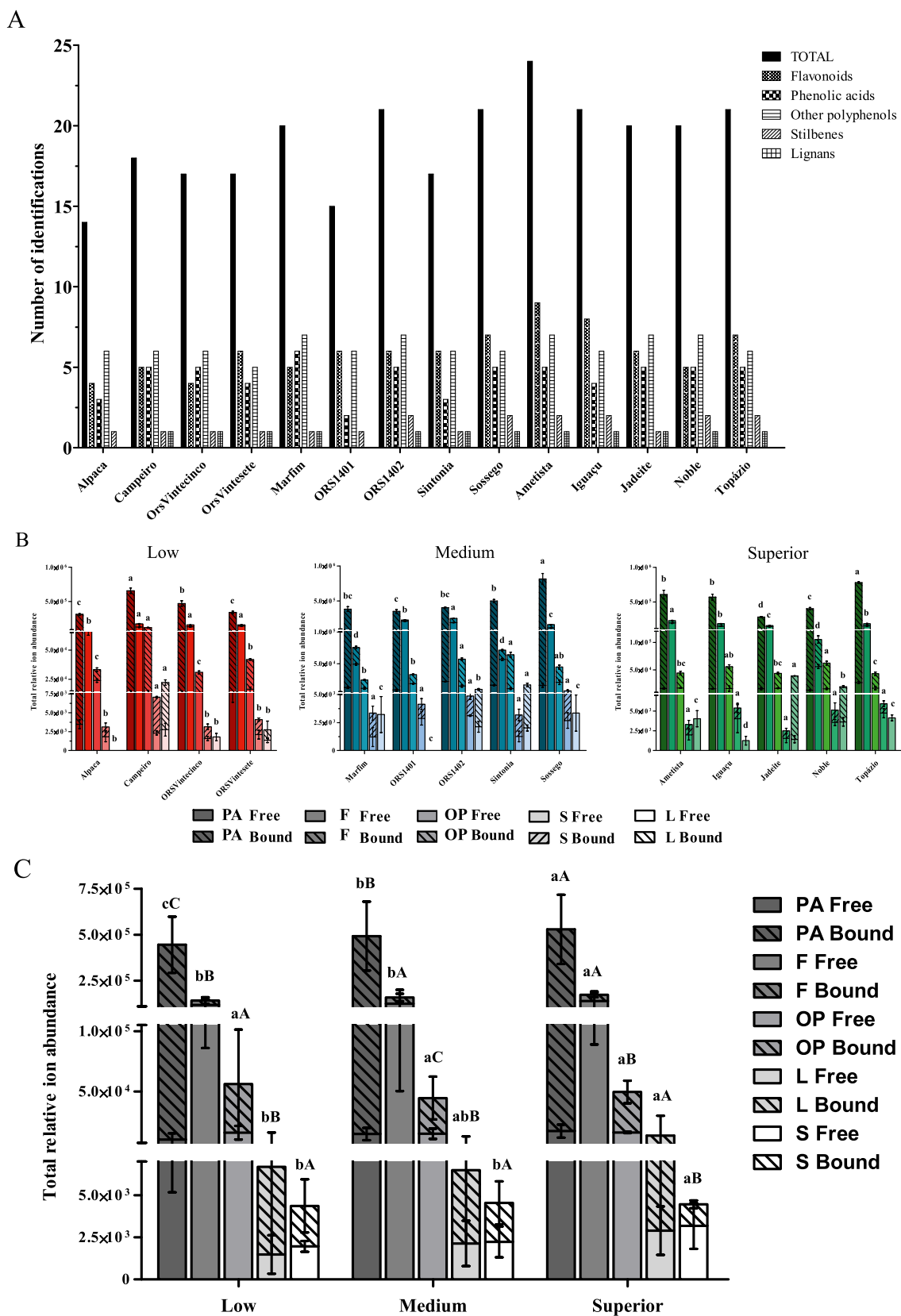


Fig. 1. A. Number of identifications of phenolic classes for each wheat flour. B. Total relative ion abundance of phenolic classes for each wheat flour grouped by technological class (Low, Medium and Superior). C. Average of total relative ion abundance of phenolic classes for wheat flours by technological quality. OP: Other polyphenols; PA: phenolic acid; F: flavonoids; S: stilbenes and L: lignans. Means \pm SD ($n = 3$). Different letters mean a significant difference ($p < 0.05$) between free (lowercase) and bound (uppercase) extract samples, respectively, and within the same group (Fig. B) and comparing different technological classes (Fig. C).

acid were responsible to 25–50% of ion abundance of phenolic compounds depending on genotype (Fig. 2). The next most abundant compounds in free extracts included apigenin 7-O-apiosyl-glucoside (flavonoid), *p*-coumaric (phenolic acid) and diferulic acid (phenolic acid).

In bound extracts, the most abundant compounds belong to the phenolic acids class and the most abundant ferulic acid isomers were followed by ellagic acid, *p*-coumaric and one isomer of diferulic acid. The presence of the carboxylic acid grouping allows these compounds to perform ester-like reactions. Besides this, five isoforms of diferulic acid were detected; among these, four isoforms were detected exclusively in bound extracts. Only the genotype Alpaca did these isoforms. The other genotypes presented all dimers in bound extract and at least one of the dimers in free extracts. These compounds have already been found in immature wheat grains (Santos et al., 2019) and present potential antioxidant activity able to inhibit the lipid peroxidation (García-Conesa et al., 1999). The genotypes Campeiro, Sossego and Topázio showed the highest total relative abundance of PC (Fig. 2). In this study the main classes were the flavonoids and phenolic acids corroborating the literature (Fig. 1B) (Wang et al., 2013; Zhang et al., 2012).

The average of total ion abundance for each compound by genotype is displayed in the supplementary table 2. The difference between the two extracts can be evidenced by applying multivariate data, such as the principal components analysis (PCA), which allows for elucidating differences between the complex samples. In Fig. 3, it is possible to observe the difference in the phenolic profiles found in the free extract and the bound extract.

Looking at each genotype's total relative ion abundance (Fig. 2), the abundance of free PC remained more constant than for bound PC. Previous works have suggested that bound PC are genotypically predetermined, while free PC are more likely influenced by external factors (Silvestro et al., 2016). However, our results have shown that genotypes with the same technological quality demonstrate variations that may be linked with phenotype conditions or individual genotype factors with highly variable bound PC. Thus, it is possible to conclude that external factors influence the relative abundance of PC in wheat flours but have

limited influence on the global profile of PC which is mainly determined by the genotype.

3.3.2. Correlation between the differences in the phenolic profile and the technological quality of refined wheat flour

Concerning the technological quality effect (low vs medium vs superior) and the number of putative PC, superior wheat flours showed higher PC numbers. The number of identifications of phenolic acids and flavonoids were preponderant, as well as the total relative ion abundance. A significant impact of technological quality was observed in total relative abundance of PC ($p < 0.05$) (Fig. 2), where the wheat flour classified as superior presented the most abundance of PC followed by medium and low qualities, corroborating the Folin-Ciocalteu results. Contrary to this result, Indian varieties classified as poor showed higher levels of PC and higher expression of enzymes related to their synthesis pathways when compared to “good” wheat flour (Sharma et al., 2016).

Apart from proteomics studies of metabolic non-prolamin proteins (Victorio et al., 2018) and gluten proteins (Victorio et al., 2021) recently applied to Brazilian wheat flours, there are no studies that have investigated the phenolic profile. To the best of our knowledge, this is the first study to correlate the phenolic profile with the technological quality of Brazilian wheat flours. A multivariate analysis was applied to determine the possible differences between the technological qualities studied (low vs. medium vs. superior).

The covariance $p[1]$ and correlation $p[1]$ loadings from a two-class orthogonal partial least squares discriminant analysis (OPLS-DA) model are displayed in an S-plot format (Fig. 4). Considering the total relative abundance and all compounds putatively identified in the free and bound extract, the upper right quadrant of the S-plot shows the elevation of PC, in a specific technological quality, while the lower-left quadrant presents a comparison of the elevation of PC according to technological quality: low versus medium (Fig. 4A), medium versus superior (Fig. 4B) and low versus superior (Fig. 4C). The further away from the x-axis the PC is, the greater the contribution to the variation between the technological qualities, while the further away from the y-axis, the greater the reliability of the analytical result – thus, the significance.

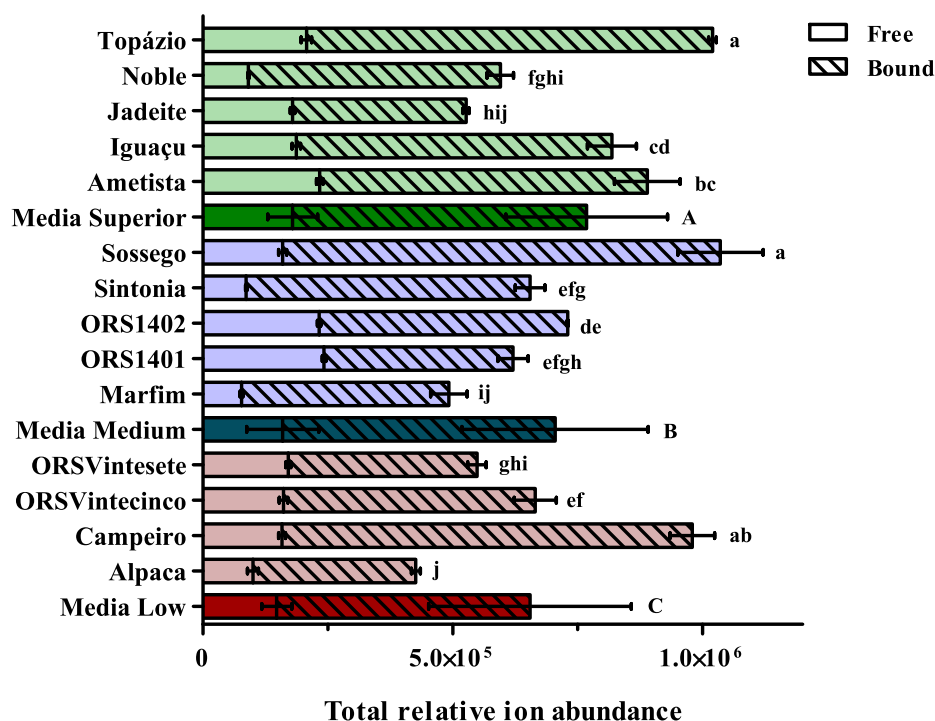


Fig. 2. Total relative ion abundance of phenolic compounds in each genotype. Means ± SD (n = 3). Different lowercase letters mean a significant difference between samples. Uppercase letters mean a significant difference between technological qualities means ($p < 0.05$).

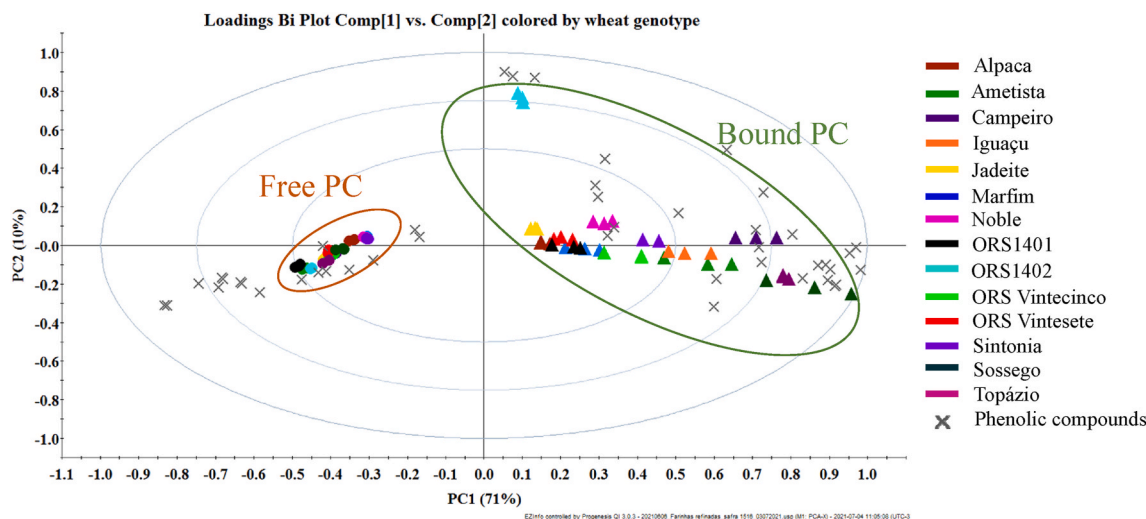


Fig. 3. Principal Components Analysis of all putative identified phenolic compounds in wheat flours of different genotypes. Dot: free phenolic compounds; Triangle: bound phenolic compounds; x means identified phenolic compounds.

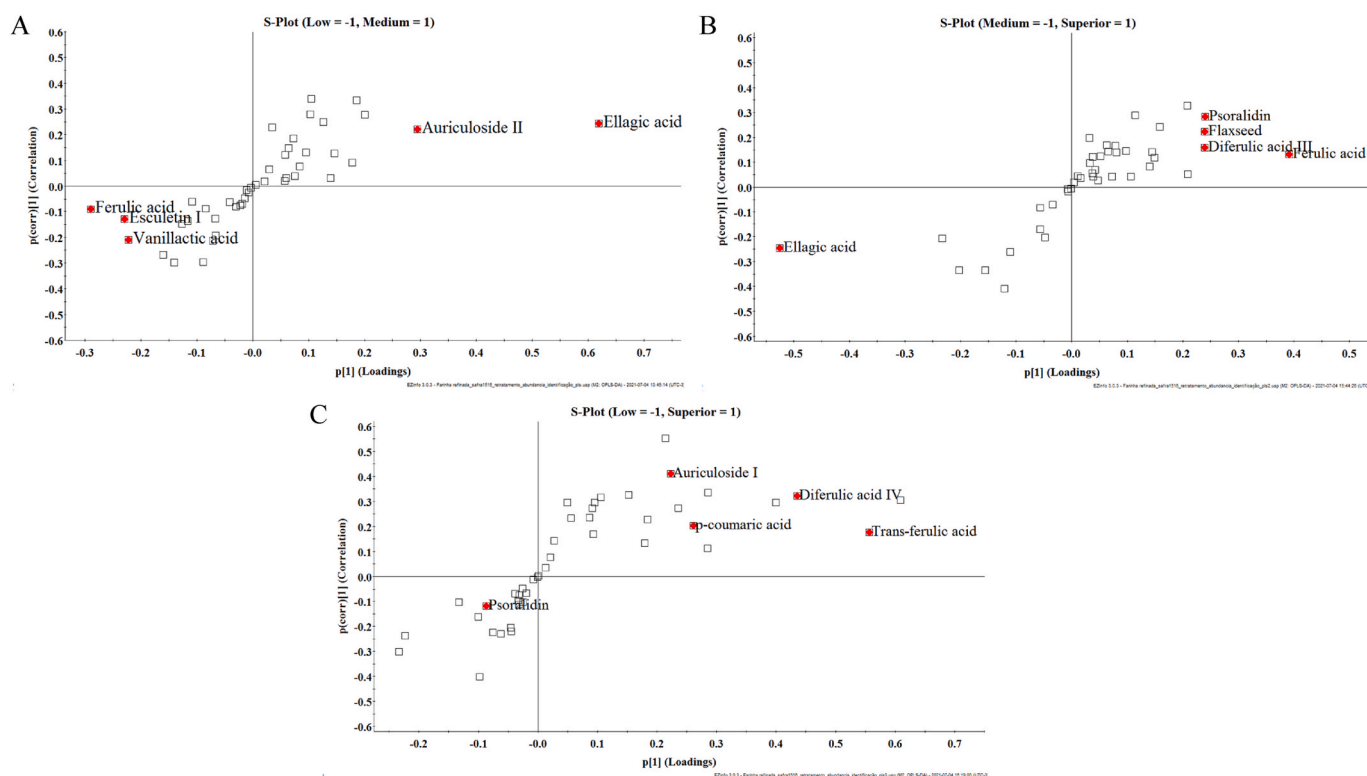


Fig. 4. S-plots comparing the wheat flours by pairs between technological quality. A) Low vs Medium; B) Medium vs Superior and C) Low vs Superior. Marked in red: the five discriminants phenolic compounds - VIP (Variance Important Projection). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Each S-plot evaluated the discriminant PC by variable importance in projection (VIP). In Fig. 4A, the abundance of a glycosylated flavonoids isomer (auriculoside isomer II) and ellagic acid differs from medium to low. Ferulic acid, esculetin I, and vanillic acid were the responsible PC in comparisons of low to medium. Fig. 4B shows the most discriminant PC when comparing medium to superior in the upper right quadrant (superior). While ellagic acid was the most discriminant in medium wheat flours, four PC are highlighted in superior: ferulic acid, diferulic III (phenolic acids), psoralidin (flavonoid) and the lignan flaxseed. Fig. 4C shows the difference between low and superior; the most

discriminant PC selected by VIP are present in superior wheat flours: *trans*-ferulic acid, *p*-coumaric acid, diferulic acid IV and auriculoside I, from phenolic acids and flavonoids classes.

The putative PC were illustrated in two heatmaps for a visual representation of abundance to compare the differences between technological qualities for each extract (Fig. 5). The clusters were built from the quantitative correlations between compounds (on the left) and between wheat flours (at the top). The color gradient represents the variation in the abundance of these compounds, ranging from blue to yellow, where the darkest blue color represents the least abundant compounds and the

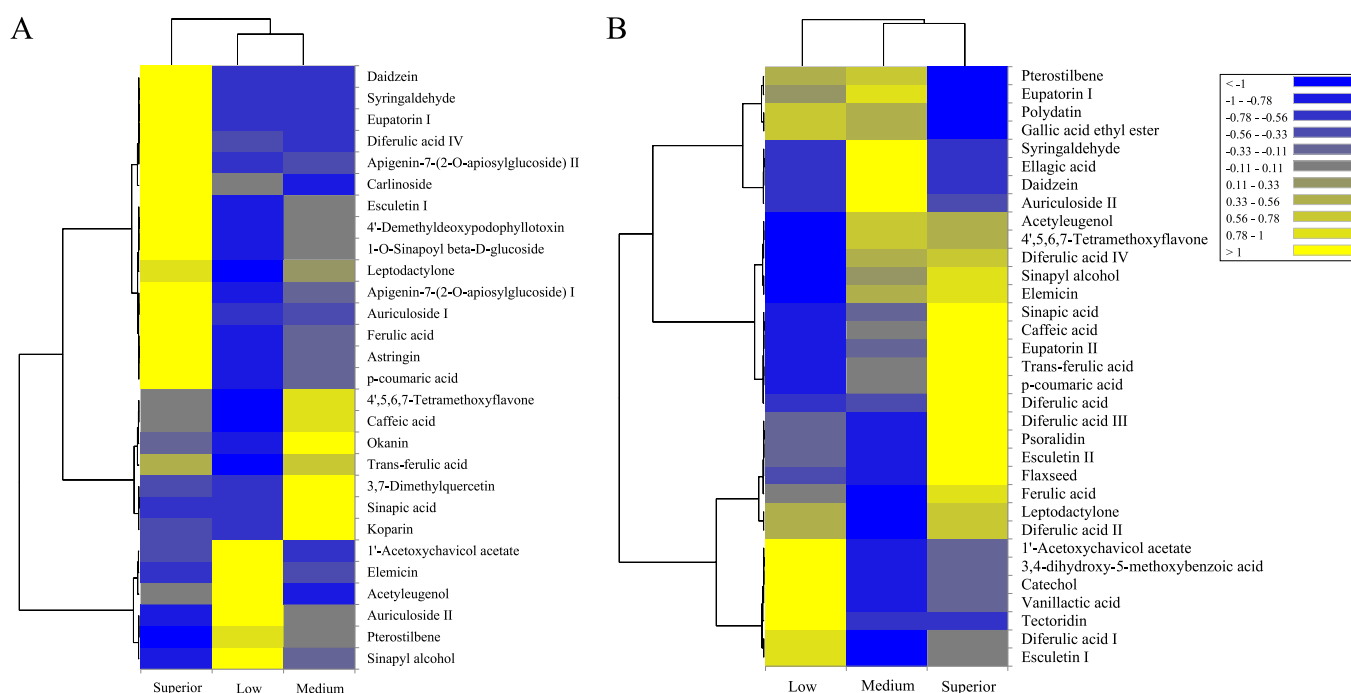


Fig. 5. Heatmap of the putative phenolic compounds identified by UPLC-MS^E in different technological qualities of wheat flours: A: free extracts and B: bound extracts. Color legend generated from XLStat calculations based on the total ion abundances of each compound, which the extreme scores (-1 and >1) represent a scale meaning the maximum and minimum presence of a given compound in each variety. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

darkest yellow the most abundant.

The free PC are displayed in Fig. 5A. The differential compounds lead to the formation of two important clusters separating superior from the medium and low qualities. The low-quality flours showed a higher abundance of non-polar compounds, which mostly belonged to the class of other polyphenols. While in the superior flours, the presence of glycosylated and esterified PC is noteworthy. The clustering separation was different for the bound PC (Fig. 5B) due to the phenolic profile. Low-quality flours were separated from the others due to the highest abundance of other polyphenols and stilbenes classes. The superior grouped with medium wheat flours and showed a higher abundance of dimers of ferulic acid and phenolic acids (*p*-coumaric, isomers of ferulic acid, and caffeic acid).

4. Conclusion

This research is the first study to correlate the phenolic profile with the technological quality of wheat flours. This study showed a characterization of PC and provides the most recent database of the phytochemical composition of common Brazilian wheat flours from different genotypes and technological qualities. The phenolic profile present in refined wheat flour is of significant nutritional importance for human health, considering the large consumption of this flour in the form of bread, cakes, pasta and cookies and the persistence of PC during the baking process. A metabolomics-based characterization could help build a database of wheat flour composition in secondary metabolites, useful for functional bakery products formulation/innovation or even for an improved understanding of the interactions between PC and cereal storage proteins. Despite a similar profile of phenolic compounds, the studied genotypes showed a difference in abundance. The superior technological quality presented highest levels of PC than medium and low. The multivariate analysis allowed to highlight discriminating compounds among the technological qualities. Such comprehensive approaches could help select a genotype and its expression as the wheat phenotype for a given application according to its bioactive compounds'

profile and gluten composition.

CRedit authorship contribution statement

Millena Cristina Barros Santos: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Luciana Ribeiro da Silva Lima:** Formal analysis, Investigation, Writing – original draft. **Carolina Thomaz dos Santos D’Almeida:** Formal analysis, Investigation, Writing – original draft. **Verônica Cristina Mayrinck Victorio:** Investigation, Writing – original draft. **Luiz Claudio Cameron:** Resources, Supervision. **Claire Bourlieu-Lacanal:** Conceptualization, Writing – review & editing, Supervision, Project administration. **Mariana Simões Larraz Ferreira:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2022.112519>.

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