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Relation between polyphenol profile and antioxidant capacity of different Argentinean wheat varieties. A Boosted Regression Trees study

Natalia S. Podio, María V. Baroni, Daniel A. Wunderlin *

ICYTAC, Instituto de Ciencia y Tecnología de Alimentos Córdoba, CONICET and Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Haya de la Torre esq. Medina Allende, 5000 Córdoba, Argentina

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ABSTRACT

We report the polyphenol profile and antioxidant capacity (AC) of 12 Argentinean wheat varieties from different regions. The polyphenol profile was studied by HPLC–MS. The AC was measured by TEAC and FRAP. Twenty-five polyphenols were identified. ACA 315 and KLEIN GUERRERO varieties showed the highest content of polyphenols, whereas BIOINTA 3004, KLEIN CAPRICORNIO and LE 2330 showed the lowest one. ACA 315 presented the highest AC, while BIOINTA 3004 and KLEIN CAPRICORNIO showed the lowest one. Boosted Regression Trees (BRT) analyses helped finding significant correlations between AC and polyphenol profile, being hydroxybenzoic acid diglucoside, tryptophan, chrysoeriol-6,8-di-C-pentoside and isomers 4, 5, 9 and 12 of diferulic acids key compounds to explain the observed AC. To our knowledge, this is the first report on the interaction between the environment and wheat genotypes evaluated by BRT, showing how the whole polyphenol profile can explain the AC in wheat.

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1. Introduction

Wheat, the major cereal in the human diet, is widely consumed worldwide. According to the FAO (Food and Agriculture Organization, 2015), the 2015 world wheat production was 735 million tonnes, 10.5 of which occurred in Argentina, with a cultivated area of 4.2 million hectares.

Different wheat varieties are commercially used for the production of a variety of foods, such as bread, cakes, biscuits, and cookies, satisfying the caloric needs of the population. In addition, epidemiological studies suggest that regular consumption of whole wheat may reduce the incidence of cardiovascular disease, type 2 diabetes, and colon cancer (Verma, Hucl, & Chibbar, 2009).

Some studies, attempting to identify the compounds responsible for this beneficial effect, suggested that it can be attributed to the synergistic action of several compounds within the grain, especially those present in the bran and wheat germ, like dietary fibre and some phytochemicals, like vitamins, minerals, flavonoids, phenolic acids, etc. (Sang et al., 2006). Dietary fibre improves intestinal health, while the antioxidant and *anti*-inflammatory properties of most phytochemicals may help prevent cancer and cardiovascular disease (Fardet, 2010).

Among the phytochemicals found in the wheat grain, phenolic acids and flavonoids are the most important ones. Most of these compounds, primarily hydroxycinnamic acid derivatives, are insoluble or are attached to the cell wall macromolecules, like arabinoxylans and lignin through ester or ether linkages, interlacing (intra- and / or inter-) molecularly to form a net (Liyana-Pathirana & Shahidi, 2006). On the other hand, a small proportion of phenolic acids and flavonoids are present as free or soluble compounds, within cellular vacuoles, or as glycosides (Stalikas, 2007). The distribution of these polyphenolic compounds within the wheat grain is not uniform, the largest proportion is found in the outer layers (aleurone, testa and pericarp) that integrate the wheat bran (Hemery et al., 2010).

The type and content of polyphenols in wheat also depend on other factors, such as variety, climatic conditions and cultural practices, among others (Hernández, Afonso, Rodríguez, & Díaz, 2011). So, the beneficial effect on human health caused by these compounds may result in additive, synergistic or antagonistic associations between these compounds, considering their interaction with the environment.

Numerous studies have examined the profile of polyphenolic compounds in different wheat varieties from Spain (Hernández et al., 2011), United States (Adom, Sorrells, & Liu, 2005; Moore et al., 2005), Canada (Guo & Beta, 2013; Verma et al., 2009), Turkey (Serpen, Gökmen, Karagöz, & Köksel, 2008), China (Li, Shan, Sun,

* Corresponding author.
E-mail address: dwunder@fcq.und.edu.ar (D.A. Wunderlin).

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Corke, & Beta, 2005; Zhang, Wang, Yao, Yan, & He, 2012), Denmark (Liu, Qiu, & Beta, 2010), Germany (Dobberstein & Bunzel, 2010), Italy (Heimler et al., 2010), among others (Fernandez-Orozco, Li, Harflett, Shewry, & Ward, 2010). Additionally, the antioxidant capacity of wheat has been studied (Adom et al., 2005; Guo & Beta, 2013; Heimler et al., 2010; Li et al., 2005; Liu et al., 2010; Moore et al., 2005; Serpen et al., 2008; Verma et al., 2009). Moreover, there are studies that show the influence of the environment on the polyphenol profile of wheat (Fernandez-Orozco et al., 2010; Heimler et al., 2010; Stracke, Eitel, Watzl, Mäder, & Rüfer, 2009; Zhang et al., 2012). However, there are no reports on the polyphenol profile and the antioxidant capacity of Argentinean wheat varieties, or the interaction with the environment in which they are grown.

It is worth to mention that some authors found significant correlations between the trolox equivalent antioxidant capacity (TEAC) assay and the amount of ferulic acid in different wheat extracts (Mateo Anson, van den Berg, Havenaar, Bast, & Haenen, 2008; Siebenhandl et al., 2007). However, these studies did not account for the influence of the whole polyphenol profile on the antioxidant capacity using multivariate statistical techniques. Verma et al. (2009) studied the contribution of different polyphenols found in wheat extracts to the antioxidant capacity (2,2-Diphenyl-1-Picrylhydrazyl radical -DPPH and TEAC assays), but they calculated this contribution to the antioxidant capacity using single polyphenols, which does not reproduce the conditions of an extract, having a mixture of compounds in different proportions, which can modify the antioxidant capacity because of additive, synergic or antagonic effects.

Among the multivariate statistical techniques used to study the influence of a given polyphenol profile on the antioxidant capacity, the Boosted Regression Trees (BRT) model can be mentioned. BRT is a relatively new assembly method, which shuffles the insight and the technique of both traditional statistics and machine-learning, seeking to predict a regressor variable from two or more predictors. Using a boosting technique, this method generates a single (“best”) model from the combination of a number of simple models (classification-regression trees) to optimise the predictive performance of the final model (BRT) (Elith, Leathwick, & Hastie, 2008). BRT is robust for treating collinear variables, anomalous data, lack of data, including both categorical and continuous variables. Additionally, BRT has the ability to model nonlinear responses, identifying and modelling interactions between variables (Leathwick, Elith, Chadderton, Rowe, & Hastie, 2008).

Although the BRT method is applied to various fields, including ecology (Hale, Marshall, Jeppe, & Pettigrove, 2014), epidemiology (Cheong, Leitão, & Lakes, 2014), agriculture (Müller, Leitão, & Sikor, 2013) and highway safety (Saha, Alluri, & Gan, 2015), it has been applied only once in food science (Podio et al., 2015).

Thus, the main goal of this study was to evaluate the antioxidant capacity of different varieties of Argentinean wheat, using BRT to correlate antioxidant capacity with the corresponding phenolic profile. Thus, we look to identify those varieties with higher AC, promoting future studies on the genetic basis that determines the phenolic composition, improving the nutritional quality of wheat.

2. Materials and methods

2.1. Chemicals and materials

Ultra-pure water (<5 $\mu g\ L^{-1}$ TOC) was obtained from a purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany). Folin-Ciocalteu reagent, ABTS (2,2′-azino-bis-(3-thylbenzothiazolne-6-sulfonic acid) diammonium salt), TTPZ (2,4,6-tripyridyl-S-triazine) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were obtained from Sigma Aldrich (Switzerland). Methanol (HPLC grade) and formic acid (puriss. p. a. for mass spectroscopy) were provided by J. T. Baker (Edo. de Mexico, Mexico) and Fluka (Steinheim, Germany), respectively. Commercial standards of *trans*-ferulic acid and caffeic acid were obtained from Extrasynthese (Genay, France), *p*-coumaric acid was provided by Fluka (Dorset, U.K.), catechin, apigenin and tryptophan were purchased from Sigma-Aldrich (Steinheim, Germany). Filters (0.45 μm, HVLP04700) were obtained from Millipore (São Paulo, Brazil). All other reagents were of analytical grade.

2.2. Wheat samples and sampling

Twelve commercial wheat (*Triticum aestivum*) varieties were used in this study: **ACA 303**, **ACA 315**, **ACA 320** and **ACA 903 B** from ASOCIACIÓN de COOPERATIVAS ARGENTINAS (ACA), **BUCK 75 ANIVERSARIO** from BUCK SEMILLAS S. A., **Cronox** from Asociados Don Mario S. A., **BIOINTA 3004** from BIOCERES (Instituto Nacional de Tecnología Agropecuaria-INTA), **BAGUETTE PREMIUM 11** from Nidera S. A., **LE 2330** from SURSEM S. A., **KLEIN CAPRICORNIO**, **KLEIN GUERRERO** and **KLEIN YARARÁ** from Criadero Klein S. A.

Sampling was conducted by the INTA staff. The seeds collection was carried out during two seeding years (2009 and 2010) in 4 areas bounded by INTA: SUB I (Reconquista – 29°08′ S and 59°38′ W), SUB IIN (Marcos Juarez – 32°42′ S and 62°06′ W, and Pergamino – 33°54′ S and 60°34′ W), SUB IV (Balcarce – 37°51′ S and 58°15′ W and Barrow – 38°18′ S and 60°14′ W) and SUB VN (Jesús María – 30°59′ S and 64°05′ W and Manfredi – 31°51′ S and 63°44′ W) (Fig. S1 in Supplementary material).

One hundred forty-six samples were collected: 16 of ACA 303, 17 of ACA 315, 15 of ACA 320, 13 of ACA 903 B, 11 of BAGUETTE PREMIUM 11, 14 of BIOINTA 3004, 15 of BUCK 75 ANIVERSARIO, 8 of CRONOX, 7 of KLEIN CAPRICORNIO, 9 of KLEIN GUERRERO, 8 of KLEIN YARARÁ and 13 of LE 2330.

Samples were transported to the laboratory in dark pre-cleaned plastic bags, fractionated in pre-cleaned plastic pots and stored at −20 °C until analysis.

2.3. Extraction of polyphenols

Before extraction, samples were dried at 35 °C during 3 days. Then, they were ground in a coffee grinder to produce whole grain flour, followed by drying at 35 °C during 60 h.

2.3.1. Extraction of free polyphenols

Five grams of dried whole grain flour were extracted with 20 mL of a cold mixture of acetone/water (4:1) under stirring for 1 h at room temperature in darkness. Then, the supernatant was removed and filtered through a cellulose filter. This procedure was repeated twice. Finally, supernatants were pooled, evaporated at 35 °C in a Rotavapor (BÜCHI) to dryness, and reconstituted with 5 mL of HPLC grade methanol. This free phenolic fraction (FF) was stored at −80 °C until analysis.

2.3.2. Extraction of bounded polyphenols

The solid residue obtained after the extraction of free polyphenols was dried at 35 °C for 2 h. Then, it was treated with 20 mL of 2 M NaOH under stirring for 15 h at room temperature in darkness. After basic hydrolysis, the sample was acidified (pH < 2) with 4 mL of concentrated HCl and stirred for 90 s. Subsequently, the sample was centrifuged in an ultra-centrifuge (Beckman Coulter Avanti J-25 model) at 21,289*g* for 20 min at 4 °C. Bound polyphenols were extracted from the supernatant with 15 mL of a cold mixture of ethyl ether/ethyl acetate (1:1) for 1 min with agitation, and

centrifuged at 5322*g* for 15 min at room temperature (Eppendorf model 5804 centrifuge). Extraction with ethyl acetate/ethyl ether was repeated twice, and the supernatant was evaporated to dryness, and further reconstituted with 5 mL of HPLC grade methanol. The bounded phenolic fraction (BF) was stored at −80 °C until analysis.

2.4. Determination of polyphenol compounds

2.4.1. Total polyphenol content

Total polyphenol (TP) content of wheat extracts (FF and BF) was measured by the Folin-Ciocalteu method, in accordance with the technique reported by Zhou and Yu (2004) with fewer changes. Briefly, 10 μL of wheat extract were mixed with 1.68 μL of ultrapure water and 100 μL of methanol. Then, 100 μL of the Folin-Ciocalteu reagent were added and stirred (vortex). After exactly 1 min, 300 μL of aqueous sodium carbonate (20 g 100 mL^{-1}) were added, stirred (vortex) and allowed to stand 120 min at room temperature in the dark. The absorbance was then read at 750 nm. TP was calculated by linear regression using gallic acid as standard. Results are expressed in mg of gallic acid equivalents (GAE) per 100 g of dry whole grain. All samples were analysed in triplicate.

2.4.2. Polyphenol profile

Polyphenols were analysed in wheat extracts by HPLC–MS/MS according to Podio et al. (2015).

Polyphenols present in samples were characterised according to their retention times, exact mass, UV/Vis spectra, MS and MS/MS spectra, which were compared to authentic standards when available. When authentic standards were not available, a tentative identification was performed using UV–VIS, exact MS and MS/MS, considering reports from tentative compounds in the literature. The quantification of polyphenols was based on external calibration curves from available phenolic standards, using the mass peak areas obtained from the extracted ion chromatograms, at concentrations between 1 and 100 mg L^{-1}. When the corresponding standards were not available, the quantification was performed using an external standard with a similar structure to the tentative compound. Sample and standard solutions were filtered (0.45 μm) and injected in the HPLC–MS/MS system. All samples were analysed in duplicate and the results were expressed in mg of standard equivalent per 100 g of dry whole grain.

2.5. Determination of antioxidant capacity

In vitro antioxidant activity was measured using the trolox equivalent antioxidant capacity (TEAC) assay and ferric reducing ability of plasma (FRAP) assay.

2.5.1. TEAC assay

The TEAC assay was performed using the methodology described by Re et al. (1999) with fewer modifications. Briefly, the ABTS radical was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate (final concentration in 10 mL of water), keeping the mixture in the dark at room temperature for 16 h before use. The aqueous $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of 0.80 ± 0.02 at 734 nm. Five μL of wheat extract were added to 3 mL of the TEAC solution, adding 95 μL of methanol, incubated for 30 min in the dark, and measured at 734 nm. The calibration plot used was linear between 0 and 0.02 mM trolox. Results are expressed in mmol trolox equivalents (TE) per 100 g of dry whole grain. All samples were analysed in triplicate.

2.5.2. FRAP assay

The FRAP assay was performed according to Benzie and Strain (1996) with fewer modifications. Briefly, the fresh working solution was prepared by mixing 25 mL acetate buffer pH 3.6 (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 16 mL $C_2H_4O_2$), 2.5 mL of a 10 mM TTPZ solution in 40 mM HCl, and 2.5 mL of a 20 mM $FeCl_3{\cdot}6H_2O$ solution. Five μL were added to 3 mL of the FRAP solution, additional 95 μL of methanol, incubated for 30 min in the dark, and measured at 593 nm. The calibration plot afforded a linear dynamic range between 0 and 0.02 mM trolox. Results are expressed in mmol TE per 100 g of dry whole grain. All samples were analysed in triplicate.

2.6. Statistical analysis

Results were analysed using the statistical package Statistica 8.0 from StatSoTF Inc. (2007), the Infostat software package (Di Rienzo et al., 2008) and RStudio version 0.98.953 – © 2009–2013 of RStudio, Inc.

2.6.1. Analysis of variance

ANOVA was performed using mixed models (Di Rienzo, Macchiavelli, & Casanoves, 2010); in the case of significance ($P < 0.05$), a DGC (Di Rienzo, Guzmán, & Casanoves, 2002) comparison test was performed to reveal paired differences between means.

2.6.2. Principal Components Analysis (PCA)

PCA was performed to explore the differences between wheat varieties. PCA was based on the correlation matrix, with no rotation factor and average values (each missing value was replaced by the average value of that variable).

2.6.3. Canonical Correlation Analysis (CCA)

CCA was used to study the correlation between antioxidant capacity (TEAC and FRAP) and polyphenol profile in wheat samples.

2.6.4. Boosted Regression Trees Analysis (BRT)

BRT models were performed according to Podio et al. (2015). Model overfitting was avoided by cross validation (CV) (Elith et al., 2008). Three parameters were adjusted to maximise model performance: the "bag fraction", the "learning rate" and the "tree complexity". Model performance was evaluated using the CV correlation (the correlation between predicted and raw data withheld from the model). The importance of predictor variables in BRT models was evaluated using the previously described function, which calculates the contribution to the model fit attributable to each predictor, averaged across all trees (Elith et al., 2008).

3. Results and discussion

3.1. Determination of polyphenols

3.1.1. Total polyphenol (TP) content

TP content of both free and bounded fractions (FF and BF, respectively) in the wheat varieties studied are presented in Fig. 1A. TP content of both summed fractions (FF + BF) is also shown, which is identified as the total fraction (TF).

The TP values obtained in this study show the same order of magnitude than data reported in the literature (Heimler et al., 2010; Liu et al., 2010; Liyana-Pathirana & Shahidi, 2006; Verma et al., 2009). In the FF, the TP content ranged from 48 to 90 mg GAE/100 g. On the other hand, the TP content in the BF ranged from 55 to 84 mg GAE/100 g. With regard to the TF, the TP content

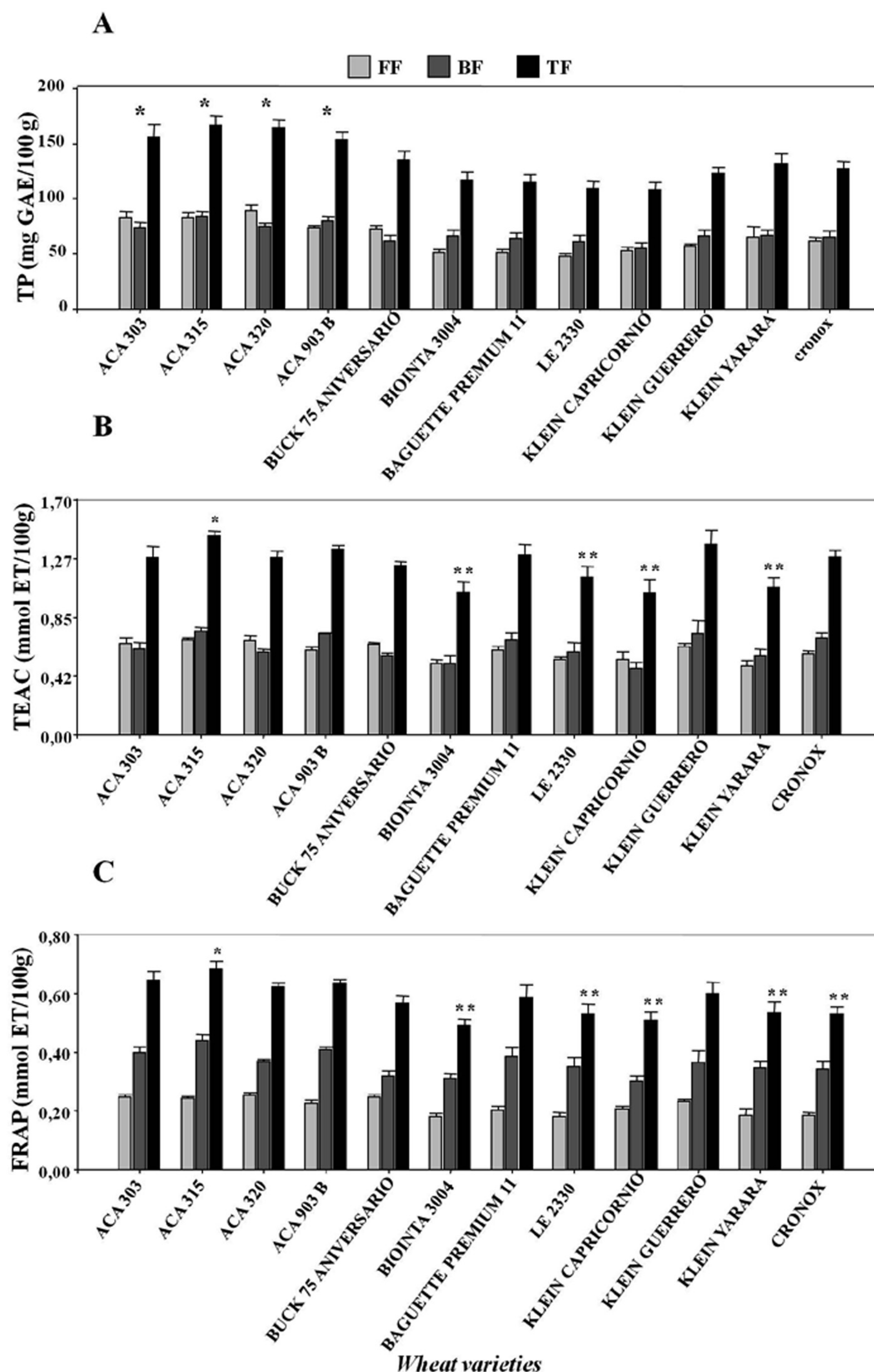

Fig. 1. Total polyphenol content (TP) and *in vitro* antioxidant capacity (AC) by TEAC and FRAP assays (A, B and C, respectively) in free (FF), bounded (BF), and free + bounded (TF) fractions, respectively of each wheat varieties. * means significantly higher ($P < 0.05$) with respect to the rest, while ** means significantly lower ($P < 0.05$) than the rest.

ranged from 108 to 170 mg GAE/100 g. ACA 303, ACA 315, ACA 320 and ACA 903 B varieties showed significantly higher TP values ($P < 0.05$) in the 3 fractions (FF, BF and TF).

3.1.2. Polyphenol profile by HPLC–MS/MS

3.1.2.1. Identification of polyphenols. With respect to individual polyphenolic constituents, 25 compounds were identified in wheat extracts: 3 derivatives from hydroxybenzoic acid, 3 flavones, 18 derivatives from hydroxycinnamic acid and 1 amino acid. Eleven out of these 25 compounds were identified in the FF, while 14 out of 25 were identified in the BF, mostly corresponding to ferulic acid derivatives. Table S1 of Supplementary material shows the parameters used for their identification.

Hydroxybenzoic acid derivatives (2-hydroxy-3-O-β-d-glucopyranosylbenzoic acid-**HGPBA**, hydroxybenzoic acid diglucoside-**HBADG** and hydroxybenzoic acid glucoside-**HBAG**, Table S1), tryptophan (**Try**), flavones (chrysoeriol-6,8-di-C-pentoside-**ChDP**, 8-C-glucosyl-6-C-arabinosyl-apigenin-**8G6AA** and 6-C-glucosyl-8-C-arabinosyl-apigenin-**6G8AA**) and *p*-coumaroyl-feruloyl putrescine (***p*****CoFP**) were only found in the FF. In addition, three compounds were tentatively identified in this fraction as dimers of ferulic acid (**DFA 3**, **DFA 4** and **DFA 6**) considering their UV,

Table 1
Quantification of polyphenols identified in the FF of wheat extracts. Results are expressed in mg/100 g of dry sample.

Varieties	HGPBA	HBADG	HBAG	Try	ChDP	8G6AA	6G8AA	DFA 3	DFA 4	DFA 6	*t*FA	*p*CoFP
ACA 303	<LOQ	12 ± 4 b	24 ± 7 b	4 ± 3 b	0.0077 ± 0.0018 c	0.45 ± 0.20 b	0.24 ± 0.10 b	0.31 ± 0.17 b	1.1 ± 0.3 b	2.2 ± 0.5 b	0.26 ± 0.05 a	<LOQ
ACA 315	<LOQ	16 ± 3 a	27 ± 5 b	6 ± 4 a	0.009 ± 0.004 c	0.25 ± 0.06 c	0.17 ± 0.04 c	0.52 ± 0.20 a	1.4 ± 0.4 a	3.2 ± 1.0 a	0.4 ± 0.7 a	<LOD
ACA 320	<LOQ	13 ± 6 b	22 ± 8 b	4 ± 3 b	0.0113 ± 0.0021 b	0.61 ± 0.26 a	0.33 ± 0.05 a	0.58 ± 0.23 a	1.4 ± 0.6 a	2.5 ± 0.8 b	0.26 ± 0.06 a	<LOQ
ACA 903 B	0.10 ± 0.06	12 ± 6 b	27 ± 10 b	2.9 ± 2.0 b	0.0061 ± 0.0016 d	0.40 ± 0.14 b	0.27 ± 0.08 b	<LOQ	0.8 ± 0.4 b	1.7 ± 0.7 b	0.20 ± 0.04 b	0.22 ± 0.17
BAGUETTE PREMIUM 11	0.12 ± 0.04	13.5 ± 2.5 b	27 ± 3 b	3 ± 3 b	0.0056 ± 0.0025 d	0.27 ± 0.13 c	0.19 ± 0.07 c	0.33 ± 0.18 b	0.9 ± 0.4 b	2.5 ± 1.3 b	0.30 ± 0.07 a	0.4 ± 0.6
BIOINTA 3004	0.14 ± 0.15	11 ± 4 b	27 ± 9 b	8 ± 4 a	0.032 ± 0.011 a	0.7 ± 0.6 a	0.4 ± 0.3 a	0.23 ± 0.17 c	0.6 ± 0.3 b	1.8 ± 0.6 b	0.28 ± 0.12 a	0.17 ± 0.26
BUCK 75 ANIVERSARIO	<LOQ	15.3 ± 2.6 a	32.0 ± 2.4 a	3 ± 3 b	0.015 ± 0.004 b	0.93 ± 0.15 a	0.41 ± 0.08 a	0.20 ± 0.13 c	0.98 ± 0.15 b	1.93 ± 0.24 b	0.30 ± 0.03 a	0.3 ± 0.3
cronox	<LOQ	10 ± 7 b	20 ± 15 b	6 ± 4 a	0.026 ± 0.016 a	0.8 ± 0.8 a	0.5 ± 0.4 a	0.20 ± 0.22 c	0.9 ± 0.6 b	2.0 ± 1.5 b	0.23 ± 0.15 a	<LOD
KLEIN CAPRICORNIO	<LOQ	10.0 ± 2.4 b	27 ± 5 b	1.5 ± 1.4 b	0.0115 ± 0.0021 b	0.82 ± 0.12 a	0.38 ± 0.08 a	0.32 ± 0.05 b	0.7 ± 0.4 b	1.4 ± 0.6 b	0.229 ± 0.025 a	0.32 ± 0.19
KLEIN GUERRERO	<LOQ	14.7 ± 1.7 a	28 ± 7 b	4.9 ± 1.7 b	0.022 ± 0.003 a	0.58 ± 0.23 a	0.38 ± 0.12 a	0.40 ± 0.14 b	0.9 ± 0.3 b	2.3 ± 1.4 b	0.24 ± 0.03 a	0.32 ± 0.14
KLEIN YARARA	<LOQ	9 ± 5 b	23 ± 11 b	3 ± 3 b	0.024 ± 0.006 a	0.7 ± 0.5 a	0.48 ± 0.20 a	<LOQ	0.5 ± 0.4 b	1.4 ± 0.5 b	0.23 ± 0.06 a	<LOQ
LE 2330	0.10 ± 0.05	10.9 ± 1.7 b	28 ± 8 b	3.7 ± 2.0 b	0.0130 ± 0.0022 b	0.66 ± 0.20 a	0.37 ± 0.08 a	0.20 ± 0.05 c	0.83 ± 0.24 b	1.4 ± 0.3 b	0.24 ± 0.03 a	0.16 ± 0.17

Abbreviations: HGPBA, 2-hydroxy-3-*O*-*β*-D-glucopyranosylbenzoic acid; HBADG, hydroxybenzoic acid diglucoside; HBAG, hydroxybenzoic acid glucoside; Try, tryptophan; ChDP, chrysoeriol-6,8-di-C-pentoside; 8G6AA, 8-C-glucosyl-6-C-arabinosyl-apigenin; 6G8AA, 6-C-glucosyl-8-C-arabinosyl-apigenin; DFA 3, 4 and 6, diferulic acid isomer 3, 4 and 6, respectively; *t*FA, *trans*-ferulic acid; *p*CoFP, *p*-coumaroyl-feruloylputrescine; <LOD, below limit of detection; <LOQ, below limit of quantification. Different letters (a > b > c > d) in the same column indicate significant differences ($P < 0.05$) among wheat varieties. Compounds **HGPBA**, **HBADG** and **HBAG** were quantified using gallic acid as reference compound; compound **Try** was quantified using tryptophan; **ChDP**, **8G6AA** and **6G8AA** using apigenin; **DFA 3**, **DFA 4**, **DFA 6**, ***t*FA** and ***p*CoFP** using *trans*-ferulic acid. MDL = 0.05 mg/100g to compound ***p*CoFP**. MQL = 0.08 mg/100g to compound **HGPBA**; 0.2 mg/100g to **DFA 3** and 0.14 mg/100g to ***p*CoFP**.

Table 2
Quantification of polyphenols identified in the BF of wheat extracts. Results are expressed in mg/100 g of dry sample.

Varieties	DFA 1	DFA 2	DFA 5	DFA 7	*p*CoA	FAD	*t*FA	*c*FA	DFA 8	DFA 9	DFA 10	TFA	DFA 11	DFA 12
ACA 303	1.4 ± 0.6	2.8 ± 0.9 b	3.6 ± 1.8 b	4.5 ± 1.3 b	0.47 ± 0.17 b	2.2 ± 1.0 a	19 ± 5 b	3.8 ± 1.6 b	5 ± 3 b	10 ± 5 b	4.6 ± 2.1 b	<LOQ	5.3 ± 2.2 b	2.2 ± 0.9 b
ACA 315	2.0 ± 0.7	5.2 ± 2.5 a	5.3 ± 2.0 a	8 ± 4 a	0.71 ± 0.23 a	2.0 ± 0.6 a	21.5 ± 2.5 b	5.1 ± 1.7 a	8 ± 4 a	18 ± 8 a	9 ± 4 a	3.1 ± 1.7 a	10 ± 5 a	4.2 ± 1.7 a
ACA 320	1.3 ± 0.7	2.7 ± 1.5 b	3.6 ± 1.6 b	4.7 ± 1.8 b	0.38 ± 0.07 b	2.5 ± 0.9 a	21 ± 4 b	3.8 ± 1.6 b	5 ± 3 b	12 ± 4 b	4 ± 3 b	2.0 ± 1.2 b	5 ± 3 b	2.3 ± 0.7 b
ACA 903 B	1.6 ± 0.6	2.9 ± 0.6 b	4.4 ± 1.1 a	6.1 ± 1.3 a	0.44 ± 0.13 b	2.2 ± 0.9 a	24.6 ± 1.9 a	5.6 ± 1.9 a	6.1 ± 2.0 b	12.8 ± 1.5 a	5.8 ± 1.5 a	2.5 ± 0.7 b	7.2 ± 2.4 a	3.2 ± 0.9 b
BAGUETTE PREMIUM 11	1.6 ± 0.8	3 ± 3 b	4 ± 3 a	6 ± 5 a	0.9 ± 0.6 a	1.4 ± 0.4 b	16.6 ± 2.2 c	4.8 ± 1.8 a	6 ± 5 b	13 ± 9 a	6 ± 6 a	2.1 ± 1.9 b	8 ± 6 a	2.8 ± 1.4 b
BIOINTA 3004	<LOQ	2.6 ± 1.6 b	<LOQ	3.9 ± 2.4 b	0.6 ± 0.3 b	1.7 ± 0.9 a	14 ± 6 c	3.1 ± 1.6 b	4.0 ± 2.0 b	9 ± 5 b	3.8 ± 2.1 b	<LOQ	5 ± 3 b	2.2 ± 1.2 b
BUCK 75 ANIVERSARIO	1.6 ± 0.8	2.8 ± 1.3 b	3.6 ± 1.5 b	4.4 ± 1.2 b	0.55 ± 0.20 b	2.3 ± 0.9 a	21 ± 3 b	5.1 ± 1.4 a	6 ± 3 b	11 ± 2 b	4.6 ± 2.0 b	<LOQ	5.5 ± 1.8 b	2.1 ± 0.8 b
cronox	<LOQ	2.6 ± 1.0 b	<LOQ	4.1 ± 1.2 b	0.64 ± 0.24 a	2.9 ± 0.8 a	21.2 ± 2.2 b	6.2 ± 0.8 a	5.0 ± 1.4 b	12.8 ± 1.1 a	3.8 ± 0.7 b	<LOQ	5.7 ± 2.0 b	2.2 ± 0.9 b
KLEIN CAPRICORNIO	<LOQ	<LOQ	<LOQ	<LOQ	0.41 ± 0.13 b	2.7 ± 0.9 a	14 ± 6 c	2.7 ± 1.1 b	4 ± 5 b	8 ± 4 b	<LOQ	<LOQ	4.3 ± 2.6 b	1.6 ± 0.9 c
KLEIN GUERRERO	1.7 ± 1.1	5 ± 5 a	6 ± 5 a	8 ± 8 a	0.7 ± 0.4 a	2.2 ± 1.0 a	19 ± 4 b	4.5 ± 2.5 a	10 ± 9 a	18 ± 13 a	10 ± 10 a	4 ± 3 a	11 ± 10 a	4 ± 3 a
KLEIN YARARA	1.6 ± 1.0	3.2 ± 2.0 b	<LOQ	5 ± 3 b	0.54 ± 0.19 b	2.7 ± 1.1 a	22 ± 3 b	5.6 ± 1.6 a	6 ± 4 b	14 ± 5 a	5 ± 4 b	2.3 ± 1.9 b	8 ± 4 a	2.6 ± 1.0 b
LE 2330	<LOQ	<LOQ	<LOQ	<LOQ	0.50 ± 0.11 b	2.6 ± 1.1 a	16 ± 6 c	4.1 ± 1.1 b	3.9 ± 2.1 b	9 ± 3 b	<LOQ	<LOQ	4.2 ± 1.4 b	1.3 ± 0.6 c

Abbreviations: DFA, diferulic acid; pCoA, *p*-coumaric acid; FAD, ferulic acid derivative; *t*FA, *trans*-ferulic acid; *c*FA, *cis*-ferulic acid; TFA, triferulic acid; <LOD, below limit of detection; <LOQ, below limit of quantification. Different letters (a > b > c) in the same column indicate significant differences ($P < 0.05$) among wheat varieties. All compounds were quantified using *trans*-ferulic acid as reference compound, except compound ***p*CoA** that was quantified with *p*-coumaric acid. MDL = 1.2 mg/100 g to compound **DFA 1**; 2.6 mg/100 g to **DFA 2**; 3.4 mg/100 g to **DFA 5** and **DFA 7**; 3.5 mg/100 g to **DFA 10** and 2.0 mg/100 g to compound **TFA**.

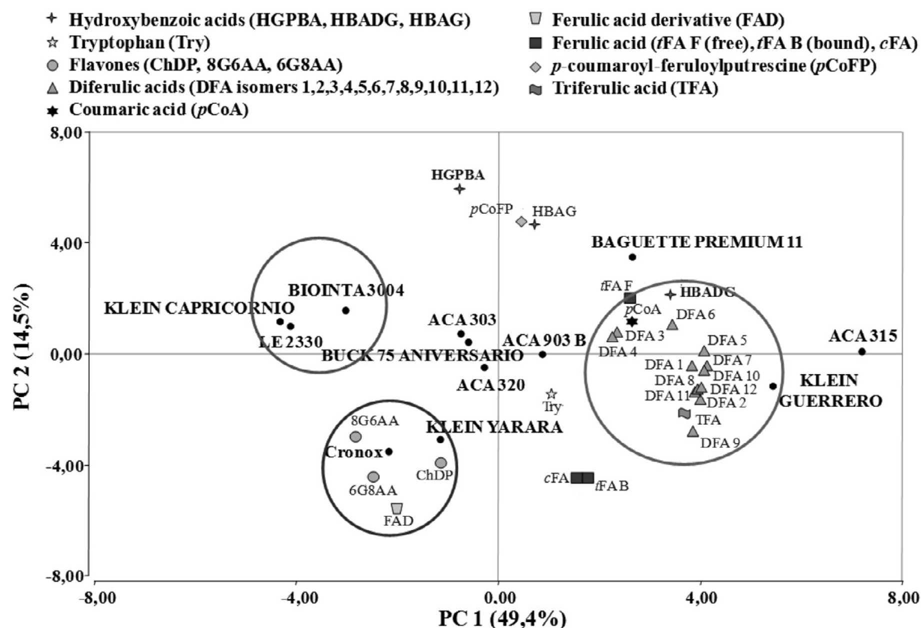

Fig. 2. Biplot obtained from PCA illustrating the relationship between phenolic profile and wheat varieties.

MS and MS/MS spectra that were concordant with this type of compounds, despite the errors in the determination of their exact mass due to the low intensity of the compound. Tryptophan (**Try**) was included in this study even though it is not a polyphenolic compound, because it has antioxidant capacity when measured by the TEAC method (Bauer, Harbaum-Piayda, & Schwarz, 2012). Moreover, **Try** also reacts with the Folin Ciocalteu reagent, contributing to the total polyphenol values (Verma et al., 2009). With respect to the BF, 3 hydroxycinnamic acid derivatives were identified: *p*-coumaric acid (***p*CoA**); *trans*-ferulic acid (***t*FA**), and *cis*-ferulic acid (***c*FA**). Additionally, 8 dimers (**DFA isomers 1**, **2**, **5**, **7**, **8**, **9**, **10**, **11** and **12**) and 1 trimer (**TFA**) of ferulic acid were also tentatively identified in this fraction. Finally, **FAD** was also tentatively identified as a ferulic acid derivative.

To our knowledge, **HBADG** as well as the presence of **DFAs** in the FF and **FAD** in BF of wheat extracts are reported for the first time in this work.

3.1.2.2. Quantification of polyphenols and differences between varieties. Quantification of the polyphenolic compounds in the FF (Table 1) showed that the most abundant compound was **HBAG**. The BUCK 75 ANIVERSARIO variety showed significantly higher values ($P < 0.05$) for this compound, while the other varieties did not show significant differences between them. The rest of the quantified polyphenols (except **HGPBA** and ***p*CoFP**) showed significant differences between varieties.

Regarding the quantification of the polyphenolic compounds in the BF (Table 2); the main compound found was *trans*-ferulic acid (***t*FA**), followed by **DFA 9**. Both compounds showed significant differences ($P < 0.05$) between wheat varieties. The ACA 903 variety showed the highest values of ***t*FA**. ACA 903 in addition to ACA 315, BAGUETTE PREMIUM 11, CRONOX, KLEIN GUERRERO and KLEIN YARARÁ showed the highest values ($P < 0.05$) of **DFA 9**. The rest of the BF quantified polyphenols (except **DFA 1**) showed significant differences between wheat varieties.

Thus, we were interested in evaluating whether the polyphenol profile could help to differentiate between wheat varieties. Therefore, we applied principal component analysis (PCA) using 25 compounds identified in our work. The PCA model was obtained using four principal components (PCs), which explained 84% of the variability found in the analysed data. Fig. 2 shows a biplot obtained from the first 2 principal components (PC 1 and PC2). PC 1 explained 49.4% of the variability found among the different wheat varieties studied, while CP 2 explained 14.5%. Diferulic acids from the BF were the largest contributors to the CP1 (e1 eigenvalue in Table S2 of supplementary material), allowing to differentiate ACA 315 and KLEIN CAPRICORNIO varieties from LE 2330 and BIOINTA 3004 varieties. Furthermore, **HGPBA**, followed by **FAD**, ***p*CoFP**, **HBAG**, **6G8AA**, ***t*FA** (from the BF), ***c*FA** and **ChDP** explained the variability found in the PC 2, which allowed to separate the BAGUETTE PREMIUM 11 variety from the CRONOX (Table S2 in Supplementary material).

This analysis shows that the polyphenol profile depends on the wheat variety studied, being the ferulic acid derivatives (DFAs, ***c*FA**, ***t*FA**, and **FAD**), hydroxybenzoic acids (**HGPBA** and **HBAG**) and flavones (**ChDP** and **6G8AA**) the most important compounds to differentiate between studied varieties.

3.1.2.3. Effects of the environment on the polyphenol profile. In addition to the genotype, environmental and growing conditions can also affect the content and composition of polyphenolic compounds in wheat samples (Heimler et al., 2010; Stracke et al., 2009; Zhang et al., 2012). Knowing the effect of the environment on the polyphenol profile can provide additional evidence on the complexity of such effects. Furthermore, understanding these effects can provide crucial information to select the appropriate genotype-location combination affording of the better polyphenol profile. Because of this, we performed an analysis of variance considering the wheat variety (V), cultivation area (C) and the interaction between both (V × C) (Table S3 in supplementary material). Furthermore, changes in the polyphenol profile resulting from temporal differences (from year to year) were also analysed (variance), considering the variety (V), seeding year (Y) and the interaction between both (V × Y) (Table S3).

Results show that nine (**HBADG**, **Try**, **ChDP**, **8G6AA**, **6G8AA**, **DFA 3**, **DFA 4**, **DFA 6** and ***p*CoFP**) out of 12 compounds found in the FF were strongly influenced by the wheat variety, showing significant changes in the quantitative phenolic profile. On the other hand, hydroxybenzoic acid derivatives (**HGPBA**, **HBADG** and **HBAG**) and DFAs (**isomers 3**, **4** and **6**) were significantly influenced by the

cultivation area (SUB I, SUB IIN, SUB IV and SUB VN). Only **DFA 6** was affected by the interaction between the variety and the cultivation area (V × C). On the other hand, the phenolic profile found in the BF showed a significant influence of the genotype, but also from the cultivation area showed a significant influence on several phenolics, except for ***t*FA** and ***c*FA**. Additionally, ***p*CoA**, ***t*FA**, **TFA**, and the isomers **1**, **7**, **8**, **10**, **11** and **12** of **DFAs** were influenced by the interaction between the variety and the cultivation area (V x C). Most of these compounds (except ***p*CoA** and **DFA 10**) were also important for the differentiation of wheat samples by variety (Table S2 and Fig. 2).These results demonstrate that the phenolic compounds most affected by the cultivation area are those present in the BF, being the SUB IV zone the most favourable for growing wheat with high contents of polyphenolic compounds (Table S4 in supplementary material).

After analysing differences in the phenolic profile in the BF due to wheat variety (Table 2), and studying variations between cultivation areas (Table S4), it seems evident that the varieties with better antioxidant properties are ACA 315 and KLEIN GUERRERO from the SUB IV zone, showing the highest values for polyphenolic compounds, particularly of ADFs, in this zone.

On the other hand, the seeding year showed a significant influence on **Try**, **8G6AA**, **DFA 3** and ***p*CoFP** from the FF, and on **FAD** from the BF (Table S3). However, there was not an important interaction between the variety and the seeding year (V x Y), which was only significant for flavones (**ChDP**, **8G6AA** and **6G8AA**) in the FF, and for **DFA 1** in the BF.

These results demonstrate that the polyphenol content in the wheat grain is influenced by both the genotype and the environment (crop zone and seeding year), affecting mainly those phenolics that are part of the cell wall, such as DFAs.

So, finding a genotype with higher content of polyphenolic compounds, not only involves knowing the genetic characteristics of a wheat crop, but also providing a suitable environment that promotes the formation of these compounds (namely, an appropriate phenotype).

3.2. Evaluation of the antioxidant capacity (AC)

AC was evaluated by two methods: radical scavenging (TEAC) and reducing power (FRAP).

Fig. 1 B shows TEAC results for both FF and BF, corresponding to 12 wheat varieties analysed. Fig. 1 C shows FRAP results. Total amounts (TF = FF + BF) for phenolics (TP) and AC (TEAC and FRAP) are also shown in the Fig. 1.

Values obtained by TEAC in the FF showed a range between 0.49 and 0.69 mmol Trolox equivalent (TE) 100 g^{-1} (dry weight; DW). Furthermore, the BF showed a variation between 0.48 and 0.75 mmol ET 100 g^{-1} DW. TF showed AC values between 1.02 and 1.44 mmol ET 100 g^{-1}.

The ACA 315 variety showed the highest TEAC values in the FF, BF and TF; however, it was only significantly different ($P < 0.05$) from the rest in the TF. On the other hand, BIOINTA 3004, LE 2330, KLEIN YARARÁ and KLEIN CAPRICORNIO presented the lowest TEAC values in the 3 fractions analysed, differing significantly from the rest in FF and TF.

When AC was evaluated by FRAP, it ranged fron 0.18 to 0.25 mmol TE 100 g^{-1}, while the BF ranged from 0.30 to 0.44 mmol TE 100 g^{-1}. Furthermore, TF ranged from 0.49 to 0.68 mmol TE 100 g^{-1}.

So far, among the 12 varieties studied, ACA 315 showed the highest AC by FRAP in both BF and TF, while BIOINTA 3004 and KLEIN CAPRICORNIO presented the lower FRAP values.

Analysing both FRAP and TEAC, the ACA 315 variety was significantly different from the rest, presenting the highest FRAP and TEAC values in the TF. On the other hand, the BIOINTA 3004 and KLEIN CAPRICORNIO varieties showed the lowest AC values by both methods. These results are in agreement with those discussed in the Section 3.1, where the ACA 315 variety differentiated from BIOINTA 3004, KLEIN CAPRICORNIO and LE 2330 in the total polyphenol content and in the polyphenol profile, mainly in ferulic acid derivatives. For this reason, we propose that AC is closely related to the polyphenol profile. So far, the next step is evaluating the association between AC and particular compounds within the polyphenol profile, looking for a more detailed explanation on which compounds are mainly responsible for the observed AC.

3.3. Relationship between antioxidant capacity and polyphenol profile

The simple analysis of the content of single phenolic compounds, or even the analysis of a given family, is not adequate to indicate which wheat variety has outstanding AC. Such simple analysis is also not appropriate to evaluate how a particular compound contributes to AC. To this point, we have to consider that AC is the result of synergic, antagonic and additive interactions between different polyphenols, including their interaction with other compounds present in the food matrix. Hence, we need to evaluate the effect of the entire polyphenol profile on AC to explain the antioxidant behavior of the different wheat genotypes (Lingüa, Fabani, Wunderlin, & Baroni, 2016; Podio et al., 2015). Thus, we applied a Boosted Regression Trees (BRT) analysis looking for evidences on the contribution of individual compounds to AC.

Table S5 (in supplementary material) shows the BRT adjusted parameters (bag fraction, learning rate and tree complexity), performance (CV correlation and number of trees), and the relative influence of single polyphenols on each AC model (TEAC and FRAP), considering both FF and BF. BRT models showed a good performance correlating AC (CV correlation), measured by both TEAC and FRAP, with polyphenols present in both FF and BF (Table S5).

Eight out of 12 quantified compounds in the FF can explain almost 89% of the variability found by TEAC (**HBADG**, **HBAG**, **Try**, **ChDP**, **8G6AA**, **DFA 4**, **TFA** and ***t*FA**). On the other hand, 7 polyphenols (**HBADG**, **HBAG**, **Try**, **ChDP**, **8G6AA**, **DFA 4**, and ***t*FA**) plus **DFA 3** can explain 84% of the variability found by FRAP.

HBAG was the most significant compound, contributing 22.5% to the TEAC BRT model in the FF. On the other hand, **DFA 4** was the most significant variable for the FRAP BRT model (with a contribution of almost 18%). Additionally, other predictor variables (**HBADG**, **Try**, **ChDP**, **8G6AA** and ***t*FA**) were also important in both AC models (TEAC and FRAP). Although TEAC and FRAP explain different mechanisms by which polyphenols perform their antioxidant capacity, compounds previously mentioned appear to be the most relevant to explain the total AC found in the FF of wheat extracts.

On the other hand, 7 out of 14 compounds quantified in the BF can explain 83% of the AC variability by TEAC, while 6 compounds can explain 83% of the AC variability found by FRAP. **DFA 12** was the most significant predictor for the TEAC BRT model (26%), whereas **DFA 10** showed the highest contribution to the FRAP BRT model (22%). It is worth mentioning that **isomers 5**, **9** and **12** of **DFA** were present in both models.

Differences found in AC between different wheat varieties can be explained by analysing the partial dependence plots of polyphenols on TEAC (Fig. 3) and FRAP (Fig. 4) BRT models, considering both FF (Figs. 3A and 4A) and BF (Figs. 3B and 4B).

BRT plots demonstrate a complex pattern of variation between polyphenols and AC in the four models performed (TEAC: FF and BF; FRAP: FF and BF). For instance, AC by TEAC in the FF is high when the contents of **HBADG**, **HBAG** and **DFA 4** are greater than 14, 18 and 1.1 mg 100 g^{-1}, respectively (Fig. 3A, dotted lines). The concentration of **Try** producing high AC is between 6 and 8 mg 100 g^{-1}. Conversely, compounds **ChDP**, **8G6AA**, **DFA 6** and ***t*FA** require concentrations lower than 0.014, 0.25, 0.9 and

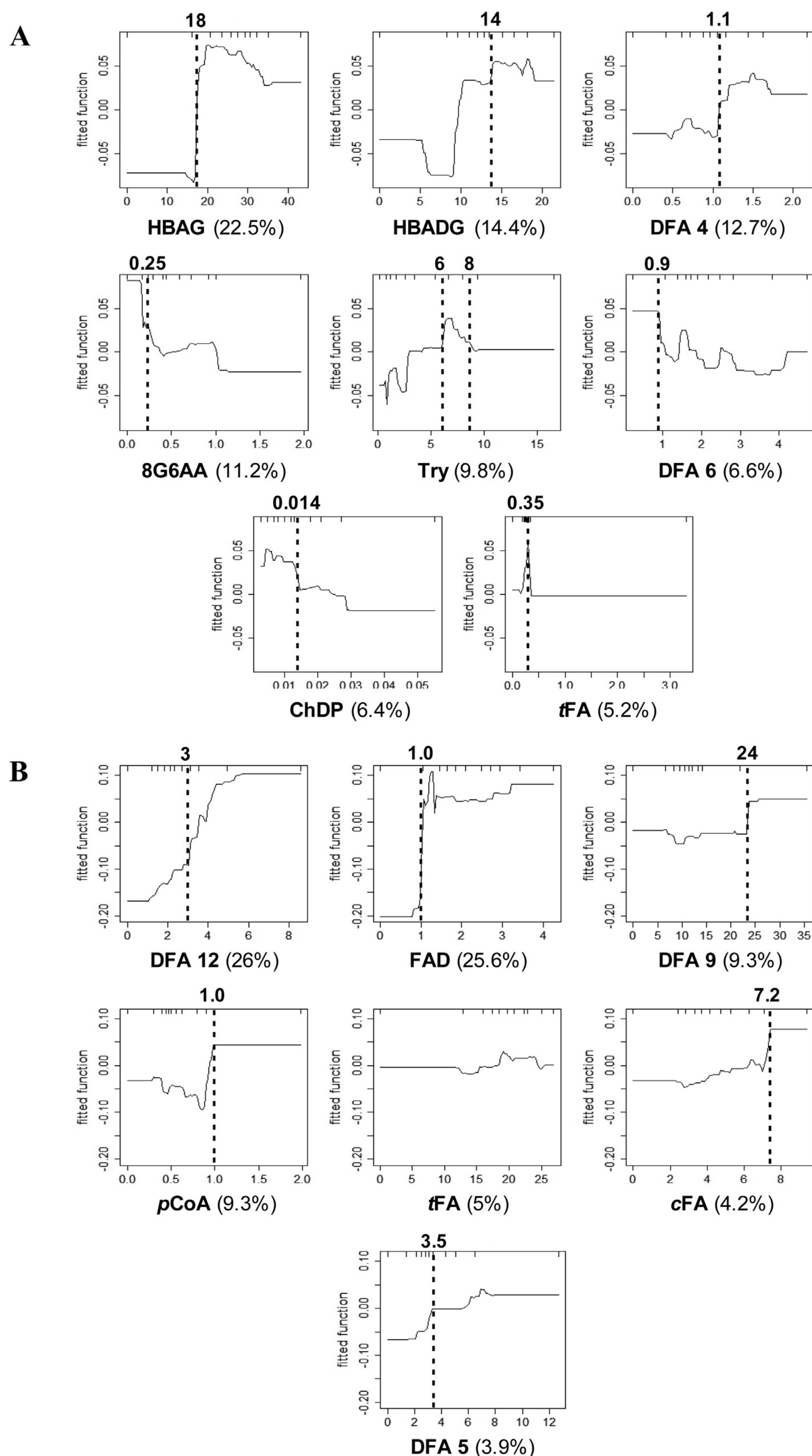

Fig. 3. Functions fitted for the BRT model, showing the influence of polyphenols (mg 100 g^{-1}), and their contribution (between square brackets) to fit the TEAC BRT models in FF (A) and BF (B). The dotted lines indicate changes on the fit function of Antioxidant capacity.

0.35 mg 100 g^{-1}, respectively to significantly contribute to AC measured by TEAC (Fig. 3 A). The same analysis can be performed for the TEAC antioxidant capacity in the BF (Fig. 3 B, dotted lines) as well as for the FRAP antioxidant capacity in both FF (Fig. 4 A, dotted lines) and BF (Fig. 4 B, dotted lines). These analyses demonstrate that these optimal conditions are satisfied mostly by the ACA

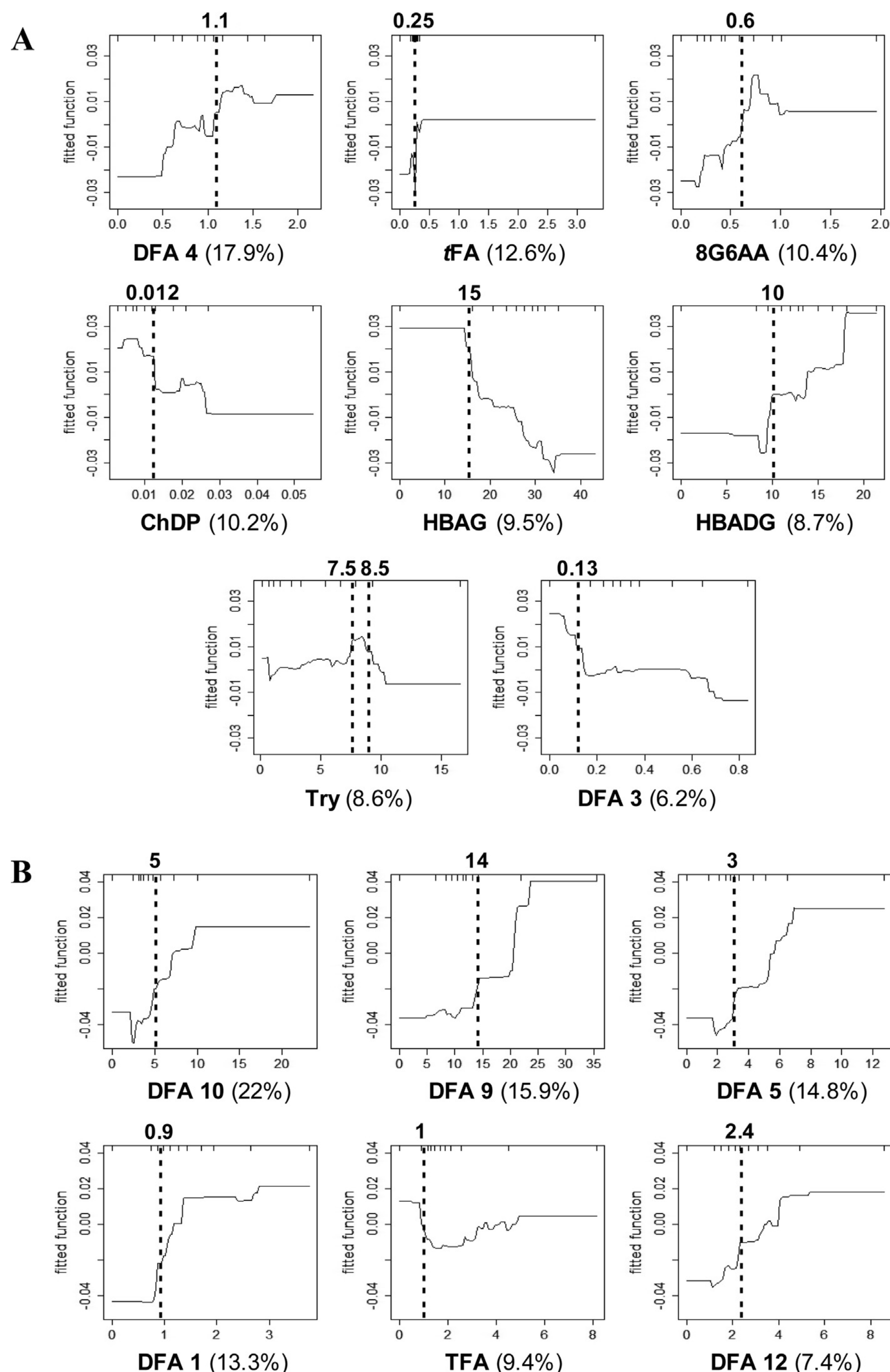

Fig. 4. Functions fitted for the BRT model, showing the influence of polyphenols (mg 100 g^{-1}) and their contribution (between square brackets) to fit the FRAP BRT models in FF (A) and BF (B). The dotted lines indicate changes on the fit function of Antioxidant capacity.

315 variety in both TEAC and FRAP assays, and in both FF and BF extracts (Tables 1 and 2).

This would explain the higher AC found in the TF of ACA 315 with respect to the rest, measured by both TEAC and FRAP (Fig. 1B and Fig. 1C).

Additionally, **HBADG**, **Try**, **ChDP** and **DFA 4** from the FF, and **DFA 5**, **DFA 9** and **DFA 12** from the BF showed similar dependence on the AC in both TEAC (Fig. 3) and FRAP (Fig. 4) BRT models, regardless of the antioxidant mechanism that these methods evaluated. This makes them particularly important, because these compounds could exert their antioxidant action through two ways: quenching free radicals and/or reducing oxidant compounds.

It is worth to remark that isomers **5**, **9** and **12** of **DFA** were also important to discriminate among different wheat varieties (Table S2). Besides, they provide additional evidence that the antioxidant capacity is strongly influenced by the type and content of polyphenolic compounds (polyphenol profile).

4. Conclusions

To our knowledge, this is the first report linking the polyphenol profile and antioxidant properties of Argentinean wheat (*Triticum aestivum*) varieties.

Different wheat varieties showed a characteristic polyphenol profile. We found 25 polyphenolic compounds, four of which are reported for the first time in wheat extracts (**HBADG** and **DFAs** in FF; **FAD** in BF).

DFAs, *cis* and *trans*-ferulic acids, ferulic acid derivative, 2-hydroxy-3-*O*-*β*-D-glucopyranosylbenzoic acid, hydroxybenzoic acid glucoside, chrysoeriol-6,8-di-C-pentoside and 6-C-glucosyl-8-C-arabinosyl-apigenin were key compounds to differentiate between wheat varieties. In addition to wheat variety (genotype), the environment (cultivation area and seeding year) showed significant influence on the polyphenol profile, being with DFAs strongly affected by environmental conditions (phenotype). These findings provide crucial information to outline which genotype-location combinations could be used to favour the production of wheat with remarkable antioxidant properties.

On the other hand, the study of antioxidant properties showed that the ACA 315 variety presented the highest values of antioxidant capacity. Hydroxybenzoic acid diglucoside, tryptophan, chrysoeriol-6,8-di-C-pentoside and isomers 4, 5, 9 and 12 of diferulic acids seem to be key compounds to explain the higher AC found in ACA315, measured by both TEAC and FRAP assays. This could indicate that these compounds exert their antioxidant capacity through two ways: quenching free radicals and/or reducing oxidant compounds. However, further research is needed to fully assess the real effects of these polyphenols, and other secondary metabolites present in foods derived from wheat, on the antioxidant capacity.

Finally, the use of multivariate statistical techniques, particularly Boosted Regression Trees analysis, enable to interpret the relationship between AC and the polyphenol profile, presenting a promissory method for future research in food chemistry and nutrition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.123.

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