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## Phenolic acids in kernels of different coloured-grain wheat genotypes

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**Abstract:** The content of free, conjugated and bound phenolic acids in 12 wheat (*Triticum aestivum* L.) genotypes of 4 different grain colours (standard red, yellow endosperm, purple pericarp and blue aleurone) from 2-year field trial was analysed in the present study. Significant increase (8%) in the total phenolic acid content was observed in the dryer year 2017. Five phenolic acids (ferulic, sinapic, *p*-coumaric, vanillic and 4-hydroxybenzoic) and *cis*-isomers of ferulic and sinapic acid were determined by HPLC-DAD (high-performance liquid chromatography with a diode-array detector) in grain samples. The total phenolic acid content of coloured wheat groups varied: blue aleurone > purple pericarp > yellow endosperm > red colour (798 > 702 > 693 > 599 µg/g). The fraction of bound phenolic acids was the major contributor to the total phenolic acid content (91.7%) with ferulic acid predominating (85.2%). Conjugated phenolic acids accounted for 7.9% of the total with sinapic and ferulic acid predominating (47.6% and 19.9%). The composition of individual phenolic acids was similar within these two fractions. The remaining 0.4% was represented by the fraction of free phenolic acids in which the phenolic acid profile varied among the individual coloured groups. Ferulic acid prevailed in red and yellow wheats, vanillic in blue and *p*-coumaric in purple wheats.

**Keywords:** cereal; phenolics; antioxidant; anthocyanins, dietary source

Cereal-based foods are an important source not only of essential nutrients (saccharides, proteins) but also of many other nutritionally valuable substances such as phenolic acids (PAs), carotenoids and anthocyanins (Lachman et al. 2017).

The amount of PAs in different types of cereals is highly variable. The highest content was found in maize > barley > wheat > oat. PAs are predominantly located in the aleurone layer of cereals, where they account for 4 309 µg/g on average (av.), while in the endosperm they are significantly scarcer (122 µg/g on av.). PAs in the wheat kernel are present in three different forms: soluble free, soluble conjugated and insoluble bound PAs. Free PAs (FPAs) usually have

a very small share (0.4–1%) in the total phenolic acid content (TPA) of wheat grains (Martini et al. 2015). Conjugated PAs (CPAs) are esterified to sugars or other low molecular weight components e.g. maleic, quinic or tartaric acid. This fraction represents approximately 13–22% of TPA. Bound PAs (BPAs) are covalently linked to polysaccharides, lignin or other structural components of plant cell walls (Li et al. 2008) and were recognized as the most abundant PAs in cereal kernels, accounting for over 70% of TPA (Fernandez-Orozco et al. 2010).

Throughout their development, plants naturally synthesize PAs in response to various stress factors such as: UV light exposure, insect bite, attack of virus

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or bacteria (Acosta-Estrada et al. 2014, Martini et al. 2015). In the human body, they act as strong antioxidants and their appropriate consumption is linked to prevention of many chronic diseases such as diabetes, cardiovascular diseases or cancer (Verma et al. 2008).

The aim of this study is therefore: (1) to determine the TPA and the distribution of PAs among the individual fractions (FPAs, CPAs, BPAs) in selected genotypes and groups of genotypes according to grain colour, and (2) to determine profiles of individual PAs in groups of wheat with different kernel colour: purple pericarp (Pp), blue aleurone (Ba), yellow endosperm (Ye) and standard red.

## MATERIAL AND METHODS

**Plant material.** In total, 12 wheat genotypes (2 red, 2 Ye, 4 Pp, 4 Ba) were harvested in 2016 and 2017 from the precise field trials at the Agricultural Research Institute (Agrotest Fyto, Ltd.) in Kroměříž, Czech Republic (Table 1). Experimental field parameters: GPS location 49.2851172N; 17.3646269E; 235 m a.s.l.; luvisc chernozem/loamic soils; long-term annual av. temperature of 9.2 °C and av. precipitations of 576 mm; mean temperature in the vegetation period 2015/16 was 9.8 °C and 8.9 °C in 2016/17; sum of precipitation was 536 mm in 2015/16 and 426 mm in 2016/17. Cultivars were grown on small experimental plots (10 m<sup>2</sup>) using conventional growing technology.

The forecrop was winter rape; fertilisation 3 times with LAV 27 (NH<sub>4</sub>NO<sub>3</sub> and finely ground limestone) 100 kg/ha (27 kg total N/ha); morphoregulator Spatial Plus with active ingredients (AI) chlormequat chloride and ethephon, 1.5 L/ha once; herbicides Cougar Forte (AI diflufenican and flufenacet) 0.54 L/ha; Glean XP (AI chlorsulfuron) 6 g/ha; Nurelle D (AI chlorpyrifos and cypermethrin) 0.6 L/ha once and fungicides Topsin (AI thiophanate-methyl) 0.7 L/ha and Impulse (AI spiroxamine) 0.6 L/ha twice. After harvest, samples were stored in paper bags in the dark at 20 °C for 2 months before being analysed.

**Chemicals.** *Trans*-ferulic acid (FeA, 98%), *trans*-sinapic acid (SiA, ≥ 98%), *trans*-*p*-coumaric acid (*p*-CoA, ≥ 98%), vanillic acid (VaA, ≥ 97%), 4-hydroxybenzoic acid (4-HBA, 99%) and acetic acid (ACS reagent, ≥ 99.7%) were obtained from Sigma Aldrich, Germany. Acetonitrile (HPLC grade), methanol (GR grade), ethyl acetate (GR grade) and NaOH (GR grade) were purchased from Lachner s.r.o., Czech Republic. HPLC grade water was prepared using the Simplicity UV (Merck Millipore, Germany).

**Sample preparation.** Wheat grain samples were ground in an IKA analytical mill (Janke & Kunkel Co., Germany). For extraction and chromatographic separation, a method published by Martini et al. (2015) was used, with some modifications.

**Isolation and analysis of FPAs.** 1 g of a finely ground sample was placed into a plastic falcon tube

Table 1. Description of wheat (*Triticum aestivum* L.) genotypes

Cultivar	Growth type <sup>b</sup>	Country of origin <sup>c</sup>	Cultivar status <sup>d</sup>	Grain colour	Pedigree	Gene symbol
Bohemia	W	CZ	RC	Red	(540i-92 × 6192a-92) × (540i-92 × Kontrast)	R genes (R1–R3)
Annie	W	CZ	RC	Red	Meritto × CH11.12772 × Eurofit	R genes (R1–R3)
Citrus	W	DE	RC	Ye	(Sunnan × Monopol) × Stamm GI 912	Psy1; Psy2
Bona Vita	W	SK	RC	Ye	(SO-690 × Arida) × Arida	Psy1; Psy2
AF Jumiko	W	CZ	RC	Pp	ANK-28A × Meritto	Pp1; Pp2
PS Karkulka	W	SK	RC	Pp	ANK-28A × PS 11	Pp1; Pp2
Konini	S	NZ	RC	Pp	(Fortuna × Arawa) × (Kopara × Purple Hilgendorf)	Pp genes
Purple	S	CD	RG	Pp	unknown	Pp1; Pp3 (Pp3a)
V1 131–15	W	CZ	BL	Ba + Ye	(RU-440 × V1-702) × (Citrus × Bona Dea)	Ba2; + ?
Skorpion	W	CZ	RC	Ba	line B5 × Versailles	Ba2
UC 66049	S	US	GR	Ba	HS 152-2 × Sonora 64	Ba1
TBS <sup>a</sup>	S	AT	RG	Ba	from the heritage of Erich von Tschermak	Ba2

<sup>a</sup>Tschermaks Blaukörniger Sommerweizen; <sup>b</sup>W – winter, S – spring; <sup>c</sup>CZ – Czech Republic, DE – Germany, SK – Slovakia, NZ – New Zealand, CD – Canada, US – United States of America, AT – Austria; <sup>d</sup>RC – released cultivar, RG – research germplasm, BL – breeding line, GR – genetic resource

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with 10 mL of methanol/water (80:20; v/v). The sample was subsequently vortexed for 30 s, sonicated for 10 min in an ultrasonic bath and finally centrifuged at 3 214 rcf for 10 min. Supernatant was transferred into 25 mL volumetric flask and the sediment was re-extracted with 10 mL of the extraction solution. Both supernatants were combined and the volume was adjusted to 25 mL. The obtained extract was used for FPAs and CPAs analysis. For FPAs analysis the extract was directly filtered through a syringe filter (PVDF, 0.45 µm) into an amber HPLC vial.

**Isolation and analysis of CPAs.** 10 mL of the methanol/water (80:20; v/v) extract from the previous step was mixed with 10 mL of NaOH (2 mol/L) and the obtained mixture was shaken for 1 h at 20 °C in the dark. Afterwards, 5 mL of HCl (4 mol/L) was added to adjust the pH value to 1–2. 2 mL of hydrolysate was transferred into glass vial and 2 mL of ethyl acetate was added. The mixture was shaken for 15 min and then centrifuged for 2 min at 2 057 rcf for separation of an aqueous and organic phase. Organic phase was transferred to another glass vial and the aqueous residuum was re-extracted with 2 mL of ethyl acetate. Combined organic phases were evaporated to dryness under nitrogen (40 °C), reconstituted with 1 mL of 80% methanol and filtered through a syringe filter (PVDF; 0.45 µm) into an amber HPLC vial.

**Isolation and analysis of BPAs.** The solid residuum after FPAs and CPAs extraction was dried to a constant weight. 14 mL of NaOH (2 mol/L) was added into a plastic falcon tube with 0.25 g of dry material. The samples were vortexed for 30 s and sonicated for 10 min. The mixture was shaken for 1 h at 20 °C. Afterwards, 7 mL of HCl (4 mol/L) was added to adjust the pH value to 1–2, followed by centrifugation (3 214 rcf; 4 °C; 15 min). 2 mL of supernatant was extracted twice using 2 mL of ethyl acetate. Further steps were identical with the CPA sample preparation.

**HPLC-DAD analysis.** The analyses were performed by an Ultimate 3000 HPLC system (Thermo Fisher Scientific, USA) coupled with a diode array detector. The analytes were separated using the gradient elution on an Omnispher C18 column (250 × 4.6 mm; particle size 5 µm; Agilent, Inc., USA). The operating conditions were: flow rate 0.8 mL/min; column temperature 25 °C; autosampler temperature 10 °C; injection volume 10 µL; detection wavelengths  $\lambda_1 = 280$  nm and  $\lambda_2 = 325$  nm. The mobile phase A: water + 0.1% acetic acid, mobile phase B: acetonitrile + 0.1%

acetic acid. The multi-step gradient (in terms of eluent B): at time 0–5 min, 10%; at 7 min, 20%; at 25 min, 30%; at 28–30 min, 75%; at 32–36 min, 10%.

**Identification and quantification.** The analytes were identified based on the retention time and UV-Vis absorption spectra. The peak area and external calibration (8 different levels spanning 0.02–20 µg/mL per analyte) were used for *trans*-forms of PAs (*t*-PAs) quantitation. Detection limits (LOD) of FeA, *p*-CoA, SiA, 4-HBA, VaA (0.025; 0.02; 0.02; 0.05; 0.05 µg/mL, respectively) were calculated using the formula  $3.3 \times (\sigma/S)$ , where *S* is the mean of the slopes and  $\sigma$  is the standard deviation of the intercepts obtained from five (three-point) calibration curves around the limit of detection (Q2B CH, 1996).

Standards for quantification of *cis*-forms of PAs (*c*-PAs) were prepared from the corresponding *t*-PAs solutions. 4 mL of methanolic stock solution (20 µg/mL) of each *t*-PA was placed into glass vial and exposed to UV radiation ( $\lambda = 365$  nm). Approximately 56% of *t*-PAs was isomerised to *c*-PAs after 30 min of exposure to UV light. The exact concentration of each *c*-PA standard solution was calculated based on a decrease in the chromatographic peak areas of corresponding *t*-PAs. The alternative six-point calibration curves for *cis*-ferulic (*c*-FeA) and *cis*-sinapic acid (*c*-SiA) were constructed from the obtained solution of *c*-PAs in the range 0.05–10 µg/mL.

All analyses were performed in triplicates, and the concentrations of individual PAs and TPA were expressed in µg/g DW (dry weight) of sample.

**Statistical analysis.** Data were processed employing Chromeleon (Thermo Fisher Scientific, Inc., USA) and Excel (Microsoft, USA). Statistical evaluation was performed in the Statistica software using a simple sorting method at a significance level of  $\alpha = 0.05$ . The Tukey's test was used for detailed evaluation.

## RESULTS AND DISCUSSION

Cereal grains were analysed for their PA contents and profiles. 5 PAs were detected across the investigated wheat samples (Figure 1). The PAs identified were: FeA, SiA, *p*-CoA, VaA and 4-HBA, and also *c*-FeA and *c*-SiA.

**TPA.** TPA was established as the sum of the individual fractions (FPAs, CPAs and BPAs) and ranged from 567 to 868 µg/g. The av. TPA for all analysed genotypes was 715 µg/g (Table 2). The wheats with blue aleurone showed exceptionally high TPA: cv. Skorpcion had the highest TPA (868 µg/g), along

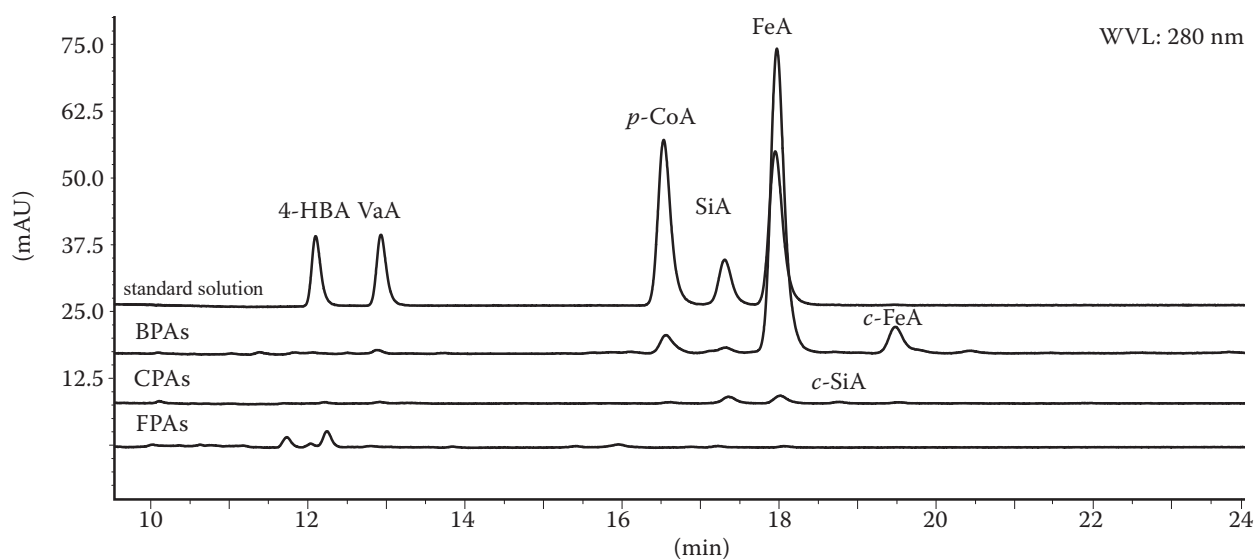


Figure 1. Chromatogram of phenolic acids in blue aleurone (Ba) wheat breeding line V1 131–15 (RU-440 × V1-702) × (Citrus × Bona Dea), 2016, (HPLC-DAD;  $\lambda = 280$  nm); BPAs – bound phenolic acids; CPAs – conjugated phenolic acids; FPAs – free phenolic acids; 4-HBA – 4-hydroxybenzoic acid; VaA – vanillic acid; *p*-CoA – *trans-p*-coumaric acid; SiA – *trans*-sinapic acid; FeA – *trans*-ferulic acid; *c*-FeA – *cis*-ferulic; *c*-SiA – *cis*-sinapic acid

with cv. TBS and V1 131–15 (852; 837  $\mu\text{g/g}$ , respectively); the only exception was cv. UC 66049 with the third lowest TPA (637  $\mu\text{g/g}$ ). On the contrary, the lowest TPA was found in cultivars with standard red colour: Annie and Bohemia (567; 632  $\mu\text{g/g}$ , respectively).

The TPA found in red wheats is nearly 10% higher compared to the results of Zuchowski et al. (2011) who measured 547  $\mu\text{g/g}$ . Other authors (Li et al. 2008, Guo and Beta 2013) reported higher values (654; 676  $\mu\text{g/g}$ ). Red wheats analysed by Fernandez-Orozco

Table 2. Free phenolic acids (FPAs), conjugated phenolic acids (CPAs), bound phenolic acids (BPAs) and total phenolic acid content (TPA) (av. contents  $\pm$  standard deviation and the contribution of individual phenolic acid (PA) fractions to TPA)

Cultivar	FPAs	CPAs	BPAs	TPA	FPAs	CPAs	BPAs
	( $\mu\text{g/g}$ DW)				(% of total)		
Bohemia	0.95 $\pm$ 0.03 <sup>h</sup>	71.1 $\pm$ 0.42 <sup>b</sup>	560 $\pm$ 36.0 <sup>ef</sup>	632 $\pm$ 36.3 <sup>e</sup>	0.2	11.3	88.6
Annie	0.63 $\pm$ 0.09 <sup>h</sup>	43.7 $\pm$ 1.20 <sup>fg</sup>	522 $\pm$ 16.2 <sup>f</sup>	567 $\pm$ 14.9 <sup>f</sup>	0.1	7.7	92.2
Citrus	2.51 $\pm$ 0.16 <sup>ef</sup>	59.8 $\pm$ 1.89 <sup>d</sup>	673 $\pm$ 52.8 <sup>b</sup>	735 $\pm$ 52.4 <sup>b</sup>	0.3	8.1	91.5
Bona Vita	2.26 $\pm$ 0.19 <sup>fg</sup>	64.5 $\pm$ 3.61 <sup>c</sup>	584 $\pm$ 30.7 <sup>de</sup>	650 $\pm$ 27.3 <sup>de</sup>	0.3	9.9	89.7
AF Jumiko	3.05 $\pm$ 0.20 <sup>de</sup>	55.6 $\pm$ 1.98 <sup>e</sup>	663 $\pm$ 6.28 <sup>b</sup>	722 $\pm$ 7.70 <sup>bc</sup>	0.4	7.7	91.9
PS Karkulka	1.79 $\pm$ 0.25 <sup>g</sup>	42.7 $\pm$ 2.40 <sup>gh</sup>	627 $\pm$ 17.4 <sup>bcd</sup>	671 $\pm$ 19.5 <sup>cde</sup>	0.3	6.4	93.4
Konini	1.11 $\pm$ 0.04 <sup>h</sup>	53.4 $\pm$ 4.75 <sup>e</sup>	642 $\pm$ 33.1 <sup>bc</sup>	697 $\pm$ 28.3 <sup>bcd</sup>	0.2	7.7	92.2
Purple	0.63 $\pm$ 0.12 <sup>h</sup>	56.8 $\pm$ 1.56 <sup>de</sup>	660 $\pm$ 9.16 <sup>b</sup>	717 $\pm$ 10.5 <sup>bc</sup>	0.1	7.9	92.0
V1 131–15	3.33 $\pm$ 0.11 <sup>cd</sup>	67.9 $\pm$ 1.85 <sup>bc</sup>	766 $\pm$ 47.7 <sup>a</sup>	837 $\pm$ 46.1 <sup>a</sup>	0.4	8.1	91.5
Skorpion	5.60 $\pm$ 0.69 <sup>b</sup>	44.7 $\pm$ 0.19 <sup>f</sup>	817 $\pm$ 27.5 <sup>a</sup>	868 $\pm$ 27.1 <sup>a</sup>	0.6	5.2	94.2
UC 66049	3.72 $\pm$ 0.16 <sup>c</sup>	38.3 $\pm$ 1.09 <sup>h</sup>	595 $\pm$ 54.6 <sup>cde</sup>	637 $\pm$ 55.4 <sup>e</sup>	0.6	6.0	93.4
TBS	6.59 $\pm$ 1.05 <sup>a</sup>	78.7 $\pm$ 1.49 <sup>a</sup>	766 $\pm$ 26.1 <sup>a</sup>	852 $\pm$ 27.3 <sup>a</sup>	0.8	9.2	90.0
Mean	2.68 $\pm$ 1.91	56.4 $\pm$ 12.6	656 $\pm$ 89.3	715 $\pm$ 94.7	0.4	7.9	91.7

TBS – Tschermaks Blaukörniger Sommerweizen; DW – dry weight; Different letters above the columns indicate significant difference between the analysed wheat varieties at  $P \leq 0.05$

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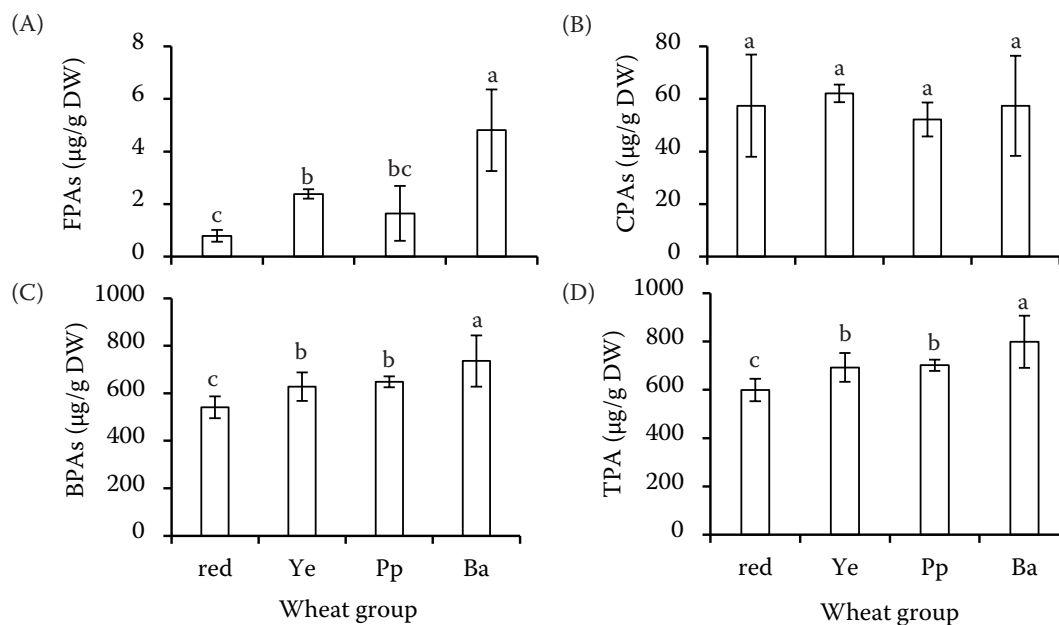


Figure 2. Comparison of total free phenolic acids (FPAs), conjugated phenolic acids (CPAs), bound phenolic acids (BPAs) and total phenolic acid (TPA) among coloured-grain wheat groups; red – standard red; Ye – yellow endosperm; Pp – purple pericarp; Ba – blue aleurone; DW – dry weight. <sup>a,b,c</sup>Different letters above the columns indicate significant difference between the four coloured-grain wheat groups at  $P \leq 0.05$

et al. (2010) attained significantly higher values (20–50%), as their TPA ranged from 728 to 900 µg/g.

Among the groups of wheats according to the grain colour, the highest TPA was determined in wheats with Ba (798 µg/g). Lower TPA was measured in genotypes with Pp and Ye (701; 692 µg/g). Standard red wheats showed the lowest TPA of all investigated genotypes (599 µg/g) (Figure 2D). Contrary to our results, Li et al. (2005) found higher values of PAs in Pp compared to Ba wheats (929 vs. 706 equivalent of FeA).

Coloured-grain wheats are able to synthesize and store anthocyanins in the outer layers of grain (Lachman et al. 2017). Their biosynthesis derives from the phenylpropanoid pathway, the common means of synthesis of a wide range of natural phytochemicals like PAs, flavonoids and others (Robbins 2003). Therefore, it is assumed that high concentration of anthocyanins and PAs in coloured wheat grains could be the result of increased activity of the phenylpropanoid biosynthetic pathway.

Studies by other authors confirm an increased PA content in coloured wheat cultivars. Ma et al. (2016) reported 20% higher TPA in Pp wheats (1 008 µg/g) compared to the conventional wheats analysed in their study. Besides, it is 30% more in comparison with the results of Pp wheats measured in the present study. On the other hand, Guo and Beta's (2013)

study provides slightly lower values of the TPA in Pp wheats (689 µg/g).

Other results that indicate a higher TPA in coloured wheats are not directly comparable to our values because they come from analyses of individual parts of the grain: bran (Li et al. 2005), pericarp, aleurone and endosperm (Ndolo et al. 2013), or the values of PAs are expressed as a FeA equivalent (Li et al. 2005). Ndolo et al. (2013) determined the TPA in the Pp wheats pericarp to be 20% higher in comparison to unpigmented wheat kernels (3 815 vs. 3 194 µg/g). In compliance with the results of Li et al. (2005), coloured wheat groups may be classified according to the TPA in bran in descending order as follows: Pp > Ba > black > white wheats (3 084 > 2 842 > 2 824 > 1 984 µg/g, respectively). As the consumers' demand for wholegrain bakery products has grown significantly in recent years, it is important to identify the content of PAs not only in bran but in the entire grain, which is why our study analysed wholegrain flour.

A significant impact of the crop year (weather conditions) on the TPA was found. The TPA was 8% higher in the drier year 2017, which is in accordance with the results of Zrcková et al. (2019) who reported a 14% increase of the TPA due to the drought.

**Total PA profile.** The individual groups of coloured-grain wheats differed significantly in their

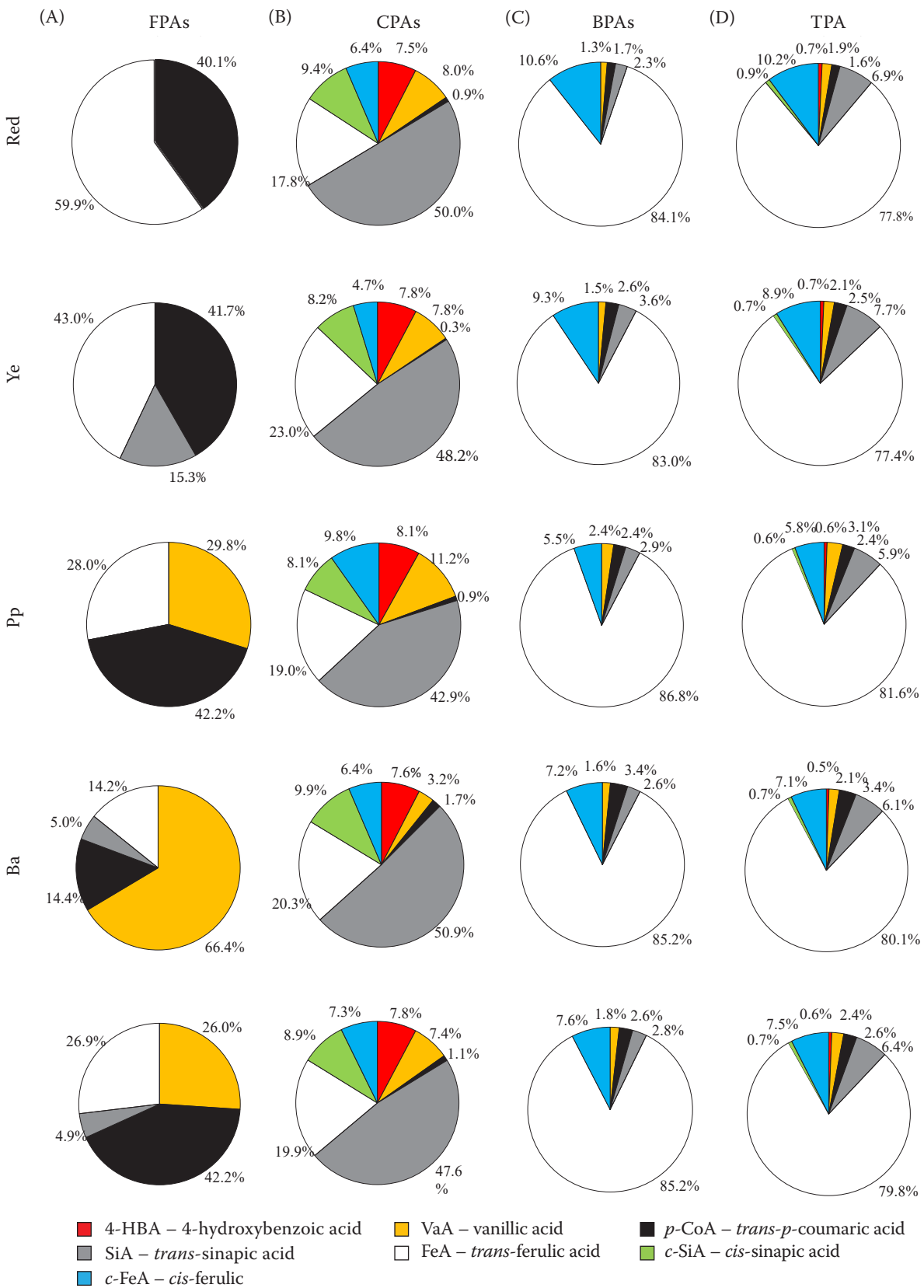


Figure 3. Phenolic acid profiles of individual coloured-grain wheat groups (the last line pie charts express av. free phenolic acids (FPAs), conjugated phenolic acids (CPAs), bound phenolic acids (BPAs) and total phenolic acid (TPA) of all analysed wheat samples). red – standard red; Ye – yellow endosperm; Pp – purple pericarp; Ba – blue aleurone

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TPA, but the share of individual PAs in TPA was very similar across all groups (Figure 3D). FeA was predominant among all PAs (79.8%), followed by *c*-FeA and SiA (7.5%; 6.4%). The results of various studies on PAs in wheat grains are consistent about the dominant presence of FeA (Zuchowski et al. 2011, Brandolini et al. 2013, Ndolo and Beta 2014). Other identified PAs, *p*-CoA, VaA, *c*-SiA and 4-HBA, formed only a minimal percentage (2.6, 2.4, 0.7, 0.6%) of TPA. A similar profile but without the distinction of *c*-FeA/FeA and *c*-SiA/SiA was also found by Zuchowski et al. (2011). The dominant FeA (87.1%) was accompanied by SiA, *p*-CoA, VaA and 4-HBA (5.7, 2.9, 2.4, 1.9%).

**Individual PA fractions.** Three different fractions of PAs (FPAs, CPAs, BPAs) had a share on the TPA in wheat grains. BPAs represented the majority of the TPA (91.7%); the share of CPAs was considerably lower (7.9%) and FPAs constituted a fairly small proportion of the TPA (0.4%). The contents of the individual PA fractions measured in the analysed wheat genotypes, with their percentage of the TPA are shown in Table 2. Our results (BPAs, CPAs, FPAs) correspond to the values reported by Martini et al. (2015) (86.1, 13.5, 0.4%, respectively). Slightly higher share of CPAs is reported by Li et al. (2008) (77, 22, 1%, respectively).

The largest amounts of FPAs were found in Ba wheats (4.81 µg/g), less in Ye and Pp wheats (2.39, 1.65 µg/g, respectively). Red wheat grains contained the lowest amount of FPAs (0.79 µg/g) (Figure 2A).

No statistically significant differences were found in the CPA content among the coloured-grain wheat groups: Ye, Ba, red, Pp (62.1, 57.4, 57.4, 52.1 µg/g, respectively; Figure 2B).

The individual groups of coloured-grain wheats differed significantly in BPA content. Listed in descending order of the measured values: Ba > Pp > Ye > red (736 > 648 > 628 > 541 µg/g, respectively; Figure 2C).

**FPA profile.** Four different PAs were identified in the FPA fraction. Listed in descending order according to their average value: *p*-CoA, FeA, VaA and SiA (42.2, 26.9, 26.0, 4.9%, respectively; Figure 3A). Free FeA prevailed in red (59.9%) and Ye wheats (43.0%). *p*-CoA (42.2%) formed the major proportion in the Pp grain, and VaA was predominant in Ba wheats (66.4%). However, the FPA spectrum across the individual genotypes showed considerable variability. Martini et al. (2015) reported that the most frequently occurring FPAs in durum wheat were VaA and FeA

(34.1, 34.0%, respectively), accompanied by SiA, 4-HBA and *p*-CoA (15.9, 9.2, 6.8%, respectively). Li et al. (2008) found a similar share of FeA (31.7%) in non-coloured wheat.

**CPA profile.** The CPA profile is depicted in Figure 3B. Almost half (47.6%) of the total CPA content consisted of SiA. Highly represented was also FeA (19.9%), followed by *c*-SiA, 4-HBA, VaA and *c*-FeA, which were present in very similar quantities (8.9, 7.8, 7.4, 7.3%, respectively). Only a tiny share of the CPAs was represented by *p*-CoA (1.1%). Martini et al. (2015) published similar results for durum wheat, SiA, FeA and VaA represented 64.2, 20.9 and 7.6%, respectively, followed by 4-HBA, syringic and *p*-CoA (3.5, 2.1, 1.6%, respectively). Findings by Fernandez-Orozco et al. (2010) also confirmed the dominant position of SiA and FeA averaging 47.6% and 20.7% of the total CPA content, respectively. The data measured by Li et al. (2008) differed significantly both in terms of the species and in terms of the representation of the identified PAs. They reported that the most prominent CPA was 2,4-dihydroxybenzoic acid (33.7%), followed by SiA (23.7%) and FeA (18.1%). Substantially less represented were VaA, syringaldehyde and 4-HBA (8.8, 6.9, 3.7%, respectively).

**BPA profile.** The BPA profile of all studied coloured-grain wheat groups was very similar (Figure 3C). FeA, representing 85.2% on av., prevailed in this fraction. Zhang et al. (2012) found a slightly lower share of FeA (70.7%). On the contrary, higher percentage of FeA (89.7%) was reported by Martini et al. (2015) and 94.9% by Brandolini et al. (2013) who further disclosed *p*-CoA (3.3%) and other acids (4-HBA, VaA, syringic), all representing less than 1% of the total BPA content. According to our results, FeA in the BPA fraction was accompanied by *c*-FeA (7.6%). Other acids were present in substantially lower amounts: SiA, *p*-CoA, VaA (2.8, 2.6, 1.8%, respectively). Martini et al. (2015) found a relatively similar representation of these three acids (7.2, 2.2, 0.7%). Zhang et al. (2012) identified also caffeic (11.3%) and chlorogenic acid (3.8%) in the BPA fraction.

In conclusion, less traditional, pigmented cereal grains including purple- and blue-grained wheat cultivars are now being investigated primarily for anthocyanins and are presumed to provide health benefits. It is apparent from our results that Ba wheats contain relatively large amounts of phenolic acids as compared to other groups of coloured-grain wheats. The newly-bred cultivar V1 131–15 is worth special attention because its aleurone layer contains a lot of anthocyanins and phenolic acids,

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and an unusually high concentration of carotenoids (as previously published by Paznocht et al. 2018) can be found in the endosperm. Wheats with blue aleurone and purple pericarp are, according to our results, a potentially significant dietary source of these nutritionally valuable substances and could be a suitable alternative to traditionally used cultivars in future.

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