UDC 575:633.11
DOI:10.2298/GENSR1301087Z
Original scientific paper

SOLUBLE FREE PHENOLIC COMPOUND CONTENTS AND ANTIOXIDANT CAPACITY OF BREAD AND DURUM WHEAT GENOTYPES

Slađana ŽILIĆ[1], Vesna HADŽI-TAŠKOVIĆ ŠUKALOVIĆ[2], Dejan DODIG[1], Vuk MAKSIMOVIĆ[2], Vesna KANDIĆ[1]

[1]Maize Research Institute, "Zemun Polje", Belgrade-Zemun, Serbia
[2]Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia

Zilic S., V. Hadzi –Taskovic Šukalovic, D. Dodig, V.Maksimovic and V. Kandic (2013): *Free soluble phenolic compound contents and antioxidant capacity of bread and durum wheat genotypes.* Genetika, Vol 45, No. 1, 87-100.

The objective of this study was to determine phenolic compounds and the total antioxidant capacity in the grain of ten bread (*T. aestivum* L.) and ten durum (*T. durum* Desf.) wheat genotypes. Soluble free forms of total phenolics, flavonoids, PVPP (polyvinylpolypyrrolidone) bound phenolics, proanthocyanidins and phenolic acids were investigated. In addition, the correlation coefficients between total antioxidant capacities and the concentration of different soluble free phenolic compounds, as well as between soluble free total phenolics and phenolic acids, flavonoids and PVPP bound phenolics were determined. Significant differences in the content of aceton/water extractable total phenolics, PVPP bound phenolics and phenolic acids between and within two wheat species were found. On the average, durum wheat samples had about 1.19-fold higher total phenolic compounds and about 1.5-fold higher PVPP bound phenolics than bread wheat samples. Three phenolic acids, ferulic, caffeic and chlorogenic, were detected in wholemeal bread wheat. Caffeic acid was not found in durum wheat samples whilst ferulic acid was the most abundant. Proanthocyanidins in bread and durum wheat genotypes were not detected. The antioxidant capacity measured as the DPPH radical scavenging activity was similar in wholemeal of bread and durum wheat, however, significant differences were observed among genotypes within species.

Key words: bread and durum wheat, phenolic compounds, antioxidant capacity.

Corresponding author: Slađana Žilić, Maize Research Institute, "Zemun Polje", Department of Technology, Slobodana Bajića 1, 11000 Belgrade-Zemun, Serbia; tel.:+381113756704; fax: +381113754994; E-mail: szilic@mrizp.rs

INTRODUCTION

Cereals, in general, play an important role in human nutrition. The importance of wheat for the production of flour and semolina, which form the basic ingredients of bread and other bakery products and pasta, has been well recognised (BELDEROK, 2000). However, little attention has been given to the nutritional and health beneficial properties of grains and its improvement through breeding programmes.

Health-beneficial properties of wheat have been ascribed to the levels of natural antioxidants, including phenolic acids, flavonoids other polyphenols and lipid soluble antioxidants. Wheat grains are a source of phenolics with the potential health benefits, but the nutritional properties will only be fully exploited if whole-wheat products are available. Early research found antioxidants in wheat concentrated mostly in the aleurone layer of bran with some in the pericarp, nucellar envelope and germ (FULCHER and DUKE, 2002; ŽILIĆ *et al.,* 2012a). These tissues could contribute greatly to increasing the nutritional quality of human food if included in flours or used as food ingredients. Phenolic acids and flavonoids represent the most common form of phenolic compounds found in whole wheat grains, and they are among the major and most complex groups of phytochemicals in cereal grain, with a number of types that exist as soluble free compounds, soluble conjugates that are esterified to sugars and other low molecular mass components, and insoluble bound forms (PIIRONEN *et al.,* 2009; ŽILIĆ et al., 2012b). The latter are the major form in wheat and are involved in cross-linking polymers, particularly arabinoxylans in the grain cell walls (SAULNIER *et al.,* 2007). The ferulic acid is dominant phenolic acid of wheat grain and a major contributor to the antioxidant capacity. The aleurone layer and the pericarp of wheat grain contain 98% of the total ferulic acid (ZHOU *et al.,* 2004a). Wheat grains also contain *p*-coumaric, vanillic, caffeic, chlorogenic, syringic, and *p*-hydroxybenzoic acids (MPOFU *et al.* 2006). Flavonoids as the predominant class of phenols have a strong antioxidant capacity. For flavonoids, efficiency as free-radical scavengers seems to depend mainly on the number of hydroxyl groups and their position on the molecule. Other known properties of the flavonoids include inhibition of hydrolytic and oxidative enzymes (phospholipase A2, cyclooxygenase, lipoxygenase), anti-carcinogenic and anti-inflammatory action (ZHISHEN *et al.,* 1999).

More data are needed regarding phenolic compounds in wheat genotypes, as this could lead to new opportunities for breeding and eventual commercial production of value-added varieties rich in health-beneficial components for making nutraceuticals and other functional foods. For this reason the objective of the present study was to investigate the content of different phenolics of 20 diverse bread and durum wheat genotypes including soluble free total phenolics, flavonoids, PVPP bound phenolics, proanthocyanidins and phenolic acids. Also, the correlation analysis between total antioxidant capacities and concentration of different phenolic compounds was done.

MATERIALS AND METHODS

Plant Material

Experimental materials consisted of 10 bread (*Triticum aestivum* L.) and 10 durum (*Triticum durum* Desf.) wheat genotypes. From a total of 20 wheat genotypes, 15 were developed at the Maize Research Institute, Zemun Polje (MRIZP). The remaining five are cultivars originated from Serbia, Slovakia, Italy, Austria and France. Name, pedigree, origin and growth type of the 20 genotypes are given in Table 1. They were chosen on the basis of their

differences in agronomic traits such as yield and its components. Grains were collected at the full maturity stage from plants grown under the equal conditions in a field-trial at the location of MRIZP during the growing season 2009-2010. The experiment was set up according to the randomised complete block (RCB) design with two replications. Standard cropping practices were applied to provide adequate nutrition and to keep the disease-free plots.

Table 1. Name, pedigree, growth type and origin of bread and durum genotypes; country code from the UN website

Genotypes	Parents (Origin)	Country	Growth type
Bread wheat			
ZP 87/I	L-99 (SRB) x Pobeda (SRB)	SRB	winter
ZP 87/II	L-99 (SRB) x Pobeda (SRB)	SRB	winter
ZP DK-07/P17	Jasenica (SRB) x Studenica (SRB)	SRB	winter
ZP Zlatna	Jasenica (SRB) x Rodna (SRB)	SRB	winter
ZP AU 12	Proteinka (MKD) x Orovčanka (MKD)	MKD	winter
ZP 224	L-4 (SRB) x Dulus/Metso (CIMMYT)	SRB	facultative
ZP Zemunska rosa	Skopljanka (MKD) x Proteinka (SRB)	SRB	winter
Pobeda	Sremica (SRB) x Balkan (SRB)	SRB	winter
Ludwig		AUT	winter
Apache		FRA	winter
Durum wheat			
ZP 34/I	SOD 55 (SVK) x Korifla (ICARDA)	SRB	facultative
ZP 34/IL	SOD 55 (SVK) x Korifla (ICARDA)	SRB	facultative
ZP 10/I	Windur (DEU) x Rodur (ROU)	SRB	winter
ZP 120/I	Windur (DEU) x Kavadarka (ROU)	SRB	winter
ZP DSP/01	Windur (DEU) x SOD 64 (SVK)	SRB	winter
ZP 7820	ZP 34/I (SRB) x Altar 84 (CIMMYT)	SRB	facultative
ZP 7858	Mina (MKD) x Mexicali 75 (CIMMYT)	SRB	facultative
ZP 7879	Zitka (SRB) x Mexicali 75 (CIMMYT)	SRB	facultative
SOD 55		SVK	winter
Varano		ITA	facultative

ICARDA = International Centere for Agricultural Research in the Dry Areas (SYR)

CIMMYT = International Maize and Wheat Improvement Centre (MEX)

Analytical Procedures

Extraction of soluble free phenolic compounds

The wholemeal (particle size < 500 μm) obtained by grinding wheat grains on a Perten 120 lab mill (Perten, Sweden) was used for the analyses.

For the detection of the DPPH$^{\bullet}$ scavenging activity, total phenolics, PVPP bound phenolics and proanthocyanidins, wheat extracts were prepared by continuous shaking of 0.3 g of wholemeal in 10 ml of 70% (by volume) acetone for 30 min at room temperature. After centrifugation (20 min at 15000 *g*) supernatant was used for experiments. For the detection of flavonoids, 1 g of wholemeal was extracted in 10 ml of 40% (by volume) ethanol for 30 min at room temperature. The supernatant after centrifugation for 20 min at 15,000 rpm was used in

experiments. Free phenolic acids were extracted twice with 80% methanol at a 1:8 ratio (by mass per volume) for 1 h at room temperature, according to KIM *et al.* (2006). The supernatants obtained after centrifugation (15 min at 15000 *g*), were combined and concentrated to 3.0 ml for the HPLC analysis.

DPPH radical scavenging activity

The DPPH$^{\bullet}$ scavenging activity was determined according to the ABE *et al.* (1998) assay. Briefly, an aliquot of extract (0.3 ml) was mixed with the DPPH reagent (0.5 mM in ethanol, 0.25 ml) and the acetate buffer (100 mM, pH 5.5, 0.5 ml). After standing for 30 min in the dark, the absorbance was measured at 517 nm against a blank containing absolute ethanol instead of a sample aliquot. The results were expressed as an IC_{50} value that represents the amount of wholemeal (in mg of dry matter) providing 50% inhibition of DPPH$^{\bullet}$.

Determination of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu procedure (SINGLETON *et al.,* 1999). Aliquots (0.2 ml) of aqueous acetone extracts were transferred into test tubes and their volumes made up to 0.5 mL with distilled water. After addition of the Folin-Ciocalteu reagent (0.25 ml) and 20% aqueous sodium carbonate solution (1.25 ml), tubes were vortexed. After 40 min the absorbance of the resulting blue colored mixtures was recorded at 725 nm against a blank containing only an extraction solvent (0.2 ml). The total phenolic content of each sample was determined by means of a calibration curve prepared using catechin and expressed as mg catechin equivalents (CE) per g of dry matter (d.m.).

Determination of PVPP bound phenolics

The PVPP bound phenolics were determined according to MAKKAR *et al.* (1993). Two milliliters of aqueous acetonic extracts were mixed with 200 mg of insoluble, crosslinked PVPP. After 15 min at 4°C, tubes were vortexed and centrifuged for 10 min at 15000 *g*. Aliquots of the supernatant (0.2 ml) were transferred into test tubes and non-adsorbed phenolics determined by the same procedure used for total phenolics (SINGLETON *et al.,* 1999). The content of PVPP bound phenolics was calculated as the difference between total and non-adsorbed phenolics and expressed in mg CE per g of d.m.

Determination of proanthocyanidins

Proanthocyanidins were determined by a butanol-HCl assay (HAGERMAN *et al.* 2000). Briefly, 0.5 ml of the extract was mixed with 3.0 ml of butanol-HCl reagent (95:5 by volume) and 0.1 ml ferric reagent (2% ferric ammonium sulfate in 2.0 M HCl). Test tubes were vortexed and placed in a boiling water-bath for 60 min. After cooling, the absorbance was recorded at 550 nm against a blank containing the solvent (0.5 ml) instead of an extract. The content of proanthocyanidins was calculated as a leucocyanidin equivalent (LE) according to the formula: ($A_{550nm} \times 78.26 \times$ dilution factor) / (% dry matter) given by PORTER *et al.* (1986).

Determination of flavonoid content

Flavonoid content was determined using a colorimetric method described previously (JIA *et al.* 1999). Briefly, 0.5 ml of the ethanol extract was diluted with 1 mL of distilled water. Then, 0.075 mL of a 5% $NaNO_2$ solution was added to the mixture. After 6 min, 0.15 ml of a

10% $AlCl_3 \times 6H_2O$ solution was added, and the mixture was allowed to stand for another 5 min. Half of a millilitre of 1 M NaOH was added, and the volume was made up to 2.5 ml with distilled water. The solution was well mixed, and the absorbance was measured immediately against the blank (containing the extraction solvent instead of a sample) at 510 nm. The results are expressed as mg CE per g of d.m.

Measurement of individual phenolic acids

Quantification of phenolic acids was done by HPLC. The phytochemical methanol extracts were injected in a Waters HPLC system consisting of 1525 binary pumps, thermostat and 717+ autosampler connected to a Waters 2996 diode array detector (Waters, Milford, USA). The separation of phenolics was performed on a 125 × 4 mm Symmetry C-18 RP column with the 5-µm particle size (Waters) with an appropriate guard column. Two mobile phases, A 0.1% phosphoric acid and B acetonitrile (J. T. Baker, Deventer, the Netherlands), were used at a flow of 1 mL/min with the following gradient profile: 20 min from 10–22% B, 20 min with a linear rise to 40% B, 5 min reverse to 10% B, and additional 5 min equilibration time. Amount of detected compounds were estimated from calibration curves obtained by injecting mixtures of pure phenolic compounds (Sigma Co. St. Louis, MO) as standards. Identified peaks were confirmed and quantified by data acquisition and spectral evaluation using Waters Empower 2 chromatographic software (Waters).

Statistical Analysis

All chemical analyses were performed in two replicates per plot and the results were statistically analysed. Results are presented as means ± standard deviation (SD). Significant differences between genotype means were determined by the Fisher´s least significant differences (LSD) test, after the analysis of variance (ANOVA) for trials set up according to the RCB design. T-test was performed to test the significance of differences between the species means. Differences with $P<0.05$ were considered significant. The coefficient of variation (CV) was determined for each trait. Correlations between parameters were examined using the Pearson's coefficient of correlation.

RESULTS AND DISCUSSION

It is known that the antioxidant properties of wheat grain are significantly influenced by the genotype and environmental conditions (ONYENEHO and HETTIARACHCHY, 1992; ADOM *et al.,* 2003), and that phenolic compounds may significantly contribute to the overall antioxidant capacity of wheat grains (KIM *et al.,* 2006). In this study, for most of the traits, considerable variations within bread and durum wheat genotypes were found. Because grains were collected at the full maturity stage from plants grown under the equal conditions in a field-trial at the same location during the growing season 2009-2010, the influence of environmental factors could be ignored.

The content of aceton/water extractable phenolics was significantly higher in durum than bread wheat (Table 2). The average value was 1.20 CE mg g^{-1} (ranged from 0.99 to 1.60) and 1.43 CE mg g^{-1} (ranged from 1.27 to 1.65) in bread and durum wheat, respectively. However, the mean content did not vary much among durum wheat genotypes (9.27%), but relatively a high variation was found among bread wheat genotypes (14.80%). Values and ranges for the soluble free total phenolic content in both cultivated wheat species were similar to those previously reported by ŽILIĆ *et al.* (2010) for Serbian growing conditions (1.02 to 1.17 and 1.21

to 1.59 CE mg g^{-1} for bread and durum wheat, respectively). The genotype differences reported for the total phenolic content of wheat grain likely indicated that genotype significantly affected the biosynthesis and accumulation of one or more of wheat phenolic compounds, such as flavonoids, PVPP bound phenolics and free phenolic acids.

Table 2. The Content of total free phenolics, flavonoids, PVPP bound phenolics and proanthocyanidins in the soluble free fraction of bread and durum wheat grain

Genotype	Total phenolics[1]	Flavonoids[1]	PVPP bound phenolics [1]	Proanthocyanidins[2]
Bread wheat				
ZP 87/I	1.26±0.012^{c}	0.035±0.0005^{bc}	0.237±0.028^{a}	n.d.
ZP 87/II	1.23±0.002^{d}	0.030±0.0006^{cd}	0.214±0.016^{ab}	n.d.
ZP DK-07/P17	1.07±0.010^{g}	0.028±0.0013^{d}	0.163±0.010^{bc}	n.d.
Apache	1.02±0.019^{i}	0.030±0.0006^{cd}	0.114±0.017^{c}	n.d.
Zemunska rosa	1.60±0.030^{a}	0.034±0.0001^{bc}	0.025±0.021^{d}	n.d.
Pobeda	0.99±0.019^{j}	0.028±0.0007^{d}	0.192±0.011^{ab}	n.d.
Ludwig	1.14±0.002^{f}	0.036±0.0006^{b}	0.204±0.041^{ab}	n.d.
ZP AU 12	1.21±0.016^{e}	0.035±0.0001^{bc}	0.108±0.028^{c}	n.d.
ZP 224	1.03±0.001^{h}	0.036±0.0024^{b}	0.172±0.040^{abc}	n.d.
Zlatna	1.34±0.026^{b}	0.042±0.0011^{a}	0.155±0.042^{bc}	n.d.
F test	***	n.s.	*	
CV/%	14.80	13.44	40.38	
Durum wheat				
ZP 34/I	1.54±0.028^{abc}	0.030±0.0019^{bc}	0.303±0.0001^{ab}	n.d.
ZP 34/IL	1.49±0.023^{abcd}	0.032±0.0001^{abc}	0.300±0.023^{ab}	n.d.
ZP 10/I	1.52±0.077^{abcd}	0.031±0.0001^{bc}	0.137±0.035^{d}	n.d.
ZP 120/I	1.37±0.014^{bcd}	0.030±0.0013^{bc}	0.234±0.021^{bc}	n.d.
ZP DSP/01	1.61±0.019^{ab}	0.028±0.0001^{bc}	0.226±0.007^{c}	n.d.
SOD 55	1.27±0.027^{d}	0.027±0.0013^{c}	0.249±0.051^{bc}	n.d.
Varano	1.65±0.009^{a}	0.027±0.0006^{c}	0.127±0.014^{d}	n.d.
ZP 7858	1.38±0.016^{bcd}	0.037±0.0017^{a}	0.338±0.035^{a}	n.d.
ZP 7879	1.32±0.042^{cd}	0.033±0.0011^{ab}	0.228±0.008^{c}	n.d.
ZP 7820	1.27±0.023^{d}	0.030±0.0006^{bc}	0.277±0.019^{abc}	n.d.
F test	***	n.s.	*	
CV/%	9.27	10.10	29.11	
Mean (bread wheat)	1.20^{b}	0.033^{a}	0.158^{b}	-
Mean (durum wheat)	1.43^{a}	0.030^{a}	0.241^{a}	-

[1]CE mg g^{-1} d.m., [2]LE mg g^{-1} d.m., Mean of genotypes and species followed by the same letter within the same column are not significantly different (P<0.05), * = significant at P<0.05, *** = significant at P<0.001, CV = coefficient of variation, n.d.-not detected.

Flavonoids are an important class of phytochemicals in wheat, contributing to the health beneficial properties. Although significant differences in free flavonoid contents between bread and durum species were not found, variations among the genotypes existed (Table 2). The content of flavonoids in the bread wheat genotypes ranged from 0.028 to 0.042 CE mg g^{-1}. Similar results were reported by ADOM and LIU (2002) for wheat genotypes grown in Minnesota (0.026 CE mg g^{-1}). According to LIU *et al.* (2010) the flavonoid content in the white, red and yellow wheat grown in Denmark was lower (0.096, 0.107 and 0.134 CE mg g^{-1}, respectively), but still higher than in presented genotypes. These results indicate that dark-colored wheat comprises more free flavonoids than light-colored wheat. Obtained ranges for flavonoids in ZP durum (0.027 to 0.033 CE mg g^{-1}) wheat genotypes were rather lower than those reported by DINELLI *et al.* (2009) for bread wheat (0.084 to 0.281 CE mg g^{-1}). The same authors reported the contribution of free to the total flavonoid contents from 27 to 49%, and that the content of bond flavonoids did not statistically differ among genotypes.

The content of aceton/water extractable PVPP bound phenolics in durum and bread wheat samples is presented in Table 2. The PVPP bound phenolics content of bread wheat samples ranged from 0.025 CE mg g^{-1} (ZP Zemunska rosa) to 0.237 CE mg g^{-1}. (ZP 87/I) with an average value of 0.158 CE mg g^{-1}. Among the tested durum wheat samples, the highest PVPP bound phenolics content of 0.338 CE mg g^{-1} was detected in ZP 7858, whereas the lowest level of 0.127 CE mg g^{-1} was detected in Varano. The average value of durum wheat samples for the PVPP bound phenolics content was 0.241 CE mg g^{-1}, which was about 1.5-fold higher than that of bread wheat. The difference was statistically significant between two species ($P<0.05$). Further research is needed to elucidate the nature of PVPP bound phenolics.

The presence of proanthocyanidins in grain of our bread and durum wheat genotypes could not be established (Table 2), which may suggest that they are not common phenolic constituents in wheat. This observation was in agreement with ones previously reported (LIU *et al.* 2010; ŽILIĆ *et al*., 2011).

Significant differences between bread and durum genotypes for individual free phenolic acid contents were determined by the HPLC method ($P<0.05$). In both species, two phenolic acids, ferulic and chlorogenic acid, were detected in wholemeal wheat samples. Caffeic acid was detected only in bread wheat (Table 3). Free phenolic acids make the smallest (typically <0.5 to 1%) contribution to the total phenolic acid content in cereals (ADOM and LIU, 2002). In the present study, ferulic acid was the major free phenolic acid in bread and durum wheat, representing about 62 and 72% of the total detected free phenolic acids, respectively, which is in accordance with previous studies (MOORE *et al.,* 2005; MPOFU *et al.,* 2006). A much higher variation existed in ferulic acid contents within bread (CV = 55.67%) than within durum wheat genotypes (CV = 16.04%). Obtained ranges for free ferulic acid in ZP bread (0.027 to 0.175 μg g^{-1}) and durum (0.085 to 0.151 μg g^{-1}) wheat genotypes were rather lower to those reported by LI *et al.* (2008) for bread (1.2 to 6.2 μg g^{-1}) and durum wheat (2.1 to 3.8 μg g^{-1}) and LIYANA-PATHIRANA and SHAHIDI (2007) for bread wheat (on the average 0.54 μg g^{-1}) and durum wheat (on the average 0.43 μg g^{-1}). Nevertheless, our results have similarities to those reported by ADOM *et al.* (2003) for the free ferulic concentration of wheat varieties grown in the USA (0.19 to 1.42 μg g^{-1}). ADOM and LIU (2002) reported that the ratio of free, soluble conjugated, and bound ferulic acid in wheat was 0.1:1:100. Free and soluble conjugated ferulic acids made very small contributions (<0.6% and <7.0%, respectively), while bound ferulic acid was the prevalent form of ferulic acid present in the grains (>93%). A free caffeic acid range (0.024 to 0.051 μg g^{-}

[1]) detected in 10 bread wheat varieties is comparable to that of 0.0 to 3.3 μg g^{-1} reported by LI *et al.* (2008) for 130 winter varieties of bread wheat. Like in our study, these authors did not detect free caffeic acid in 10 durum varieties. A considerable variation for the chlorogenic acid content was found within both, bread (CV = 88.88%) and durum (CV = 46.53%) wheat genotypes. Durum wheat samples had 2.09-fold higher content of chlorogenic acid than bread wheat genotypes, on the average.

Table 3. Free phenolic acid contents of bread and durum wheat grain (μg g^{-1} d.m.)

Genotype	Ferulic acid	Chlorogenic acid	Caffeic acid	Total detected free phenolic acids
Bread wheat				
ZP 87/I	0.027±0.001^{h}	n.d.^{f}	0.026±0.001^{bc}	0.053±0.002^{h}
ZP 87/II	0.030±0.003^{h}	n.d.^{f}	0.051±0.002^{a}	0.081±0.005^{g}
ZP DK-07/P17	0.038±0.002^{g}	n.d.^{f}	0.049±0.002^{a}	0.087±0.004^{f}
Apache	0.088±0.002^{e}	0.019±0.001^{d}	0.027±0.001^{bc}	0.134±0.003^{e}
Zemunska rosa	0.099±0.003^{d}	0.039±0.002^{b}	0.031±0.002^{b}	0.169±0.002^{c}
Pobeda	0.063±0.002^{f}	0.040±0.003^{b}	0.024±0.001^{c}	0.127±0.003^{e}
Ludwig	0.160±0.004^{b}	0.027±0.002^{c}	0.032±0.004^{b}	0.219±0.009^{b}
ZP AU 12	0.102±0.003^{d}	0.011±0.001^{e}	0.032±0.002^{b}	0.145±0.002^{d}
ZP 224	0.175±0.005^{a}	0.024±0.002^{c}	0.027±0.001^{bc}	0.226±0.008^{a}
Zlatna	0.121±0.002^{c}	0.068±0.002^{a}	0.027±0.002^{bc}	0.216±0.006^{b}
F test	***	***	***	***
CV/%	55.76	88.88	29.22	40.82
Durum wheat				
ZP 34/I	0.149±0.004^{a}	0.060±0.003^{c}	n.d.	0.209±0.006^{c}
ZP 34/IL	0.131±0.003^{bc}	0.040±0.001^{d}	n.d.	0.171±0.003^{e}
ZP 10/I	0.114±0.002^{e}	0.037±0.002^{de}	n.d.	0.151±0.001^{g}
ZP 120/I	0.099±0.002^{f}	0.022±0.001^{g}	n.d.	0.121±0.002^{h}
ZP DSP/01	0.126±0.003^{cd}	0.067±0.003^{b}	n.d.	0.193±0.001^{d}
SOD 55	0.133±0.003^{b}	0.029±0.002^{f}	n.d.	0.162±0.003^{f}
Varano	0.085±0.002^{g}	0.032±0.002^{ef}	n.d.	0.117±0.001^{h}
ZP 7858	0.133±0.003^{b}	0.085±0.003^{a}	n.d.	0.218±0.004^{b}
ZP 7879	0.126±0.003^{d}	0.028±0.002^{f}	n.d.	0.154±0.005^{g}
ZP 7820	0.151±0.005^{a}	0.080±0.002^{a}	n.d.	0.231±0.007^{a}
F test	***	***	-	***
CV/%	16.04	46.53	-	22.03
Mean (bread wheat)	0.090^{b}	0.023^{b}	0.033^{a}	0.145^{b}
Mean (durum wheat)	0.125^{a}	0.048^{a}	-	0.173^{a}

Mean of genotypes and species followed by the same letter within the same column are not significantly different (P<0.05), *** = significant at P<0.001, CV = coefficient of variation, n.d.-not detected.

The antioxidant capacity of bread and durum wheat wholemeal was measured as the DPPH$^{\bullet}$ scavenging activity. The results are presented as IC_{50} values in Figure 1 and 2.

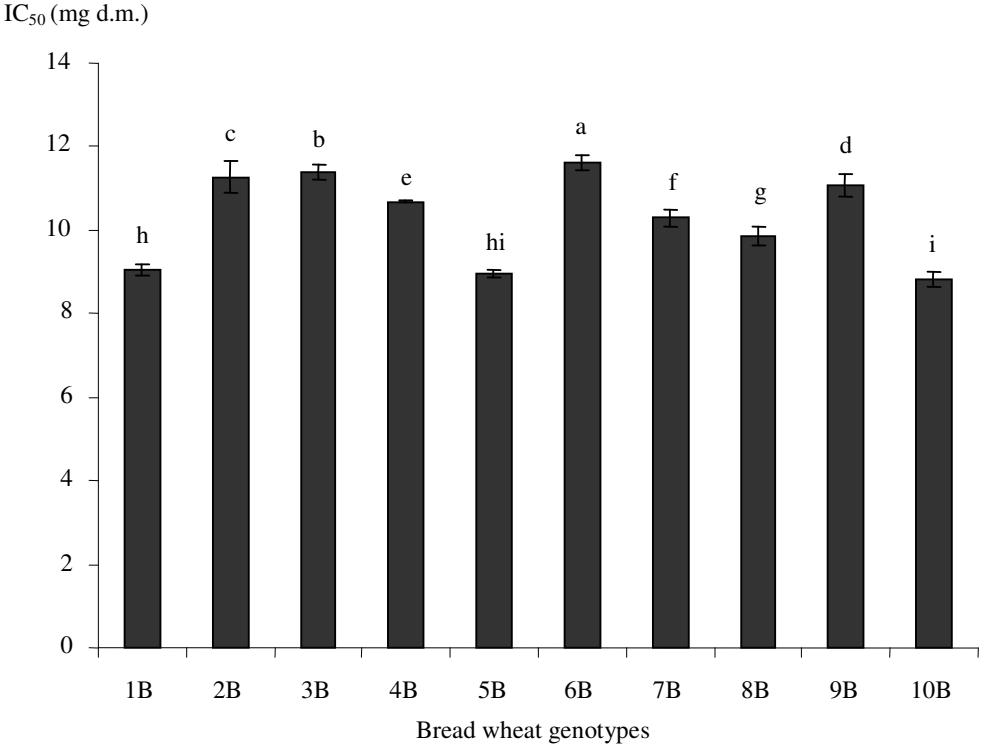

Fig. 1. DPPH$^{\bullet}$ scavenging activity of bread wheat genotypes. 1B-ZP 87/I, 2B-ZP 87/II, 3B-ZP DK-07/P17, 4B-Apache, 5B-Zemunska rosa, 6B-Pobeda, 7B-Ludwig, 8B-ZP AU 12, 9B-ZP 224, 10B-ZP Zlatna. Bars with different letters are statistically significantly different (P<0.05).

A lower IC_{50} value is associated with a stronger DPPH$^{\bullet}$ scavenging activity. There was a similar radical scavenging activity (P>0.05) between wholemeal of bread and durum wheat, although significant differences (P<0.05) were observed among genotypes within each species. The IC_{50} values ranged from 8.82 (ZP Zlatna) to 11.60 mg. (Pobeda) and 8.88 (ZP DSP/01) to 11.62 mg (ZP 7858) in bread and durum genotypes, respectively. The mean total antioxidant activity obtained by the (DPPH$^{\bullet}$) assay was rather two times higher than that of reported for red wheat genotypes grown in Switzerland (ZHOU *et al.,* 2004b), or that of eight winter soft wheat genotypes grown in Maryland (MOORE *et al.,* 2005). Although the DPPH test has been used for evaluating the antioxidant activity of wheat wholemeal and bran extracts in many studies (ZHOU *et al.,* 2004b; LI *et al.,* 2005) a comparison between published results is difficult because of differences in the extraction protocols and units of measure. According to literature data, the total phenolic content strongly correlates with the total antioxidant activity (ZHOU *et al.*, 2004b;

VERMA *et al.,* 2008), which is confirmed in our study. The total antioxidant capacity had a positive correlation with the free total phenolics in bread wheat and durum wheat (r^2=0.76 and 0.80, P<0.01) (Table 4). However, considering that most of the phenolic compounds in cereals are bound to the insoluble polysaccharide, the antioxidant capacity of wheat is mostly dependent on this phenolic form. The content of total phenolics found in the alkali hydrolyzates of the bran of same bread and durum genotypes like in this study were found as 9208 and 9798 mg GAE kg^{-1}, respectively, with high antioxidant capacity (ŽILIĆ *et al.,* 2012a). Besides differences in the scavenging activity of individual phenolic, additive and/or synergistic effects of the mixture of antioxidants could highly influence the antioxidant capacity (EBERHARDT *et al.,* 2000).

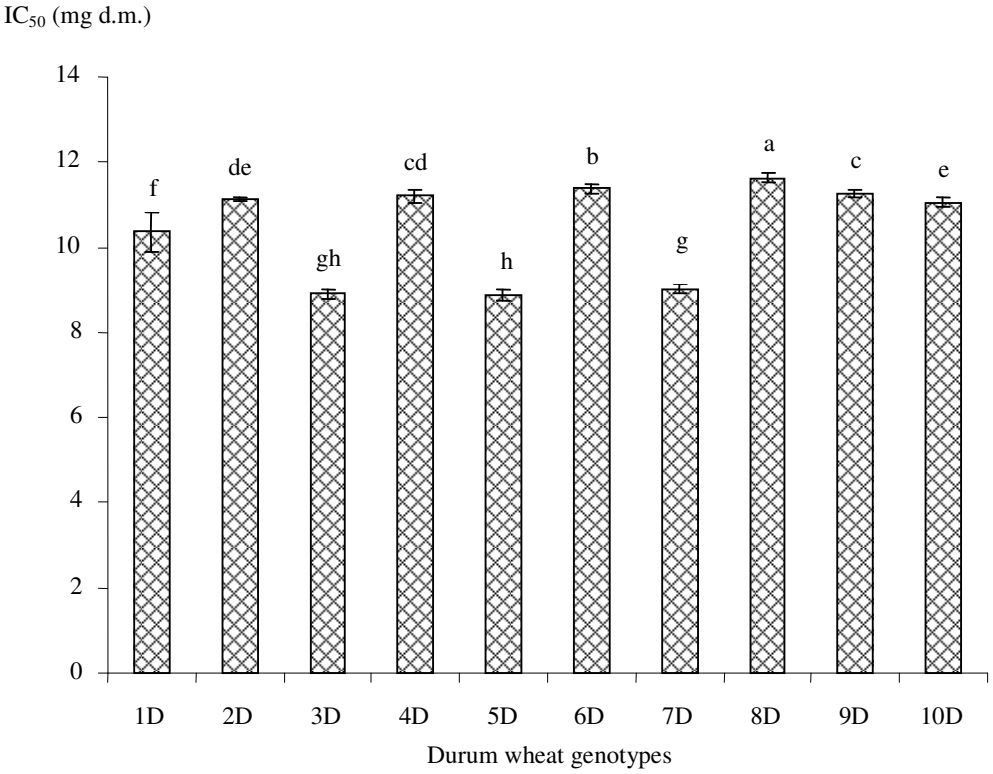

Fig. 2. DPPH$^{\bullet}$ scavenging activity of durum wheat genotypes. 1D-ZP 34/I, 2D-ZP 34/IL, 3D-ZP 10/I, 4D-ZP 120/I, 5D-ZP DSP/01, 6D-SOD 55, 7D-Varano, 8D-ZP 7858, 9D-ZP 7897, 10D-ZP 7820. Bars with different letters are statistically significantly different (P<0.05).

Table 4. Linear correlations between the content of soluble free phenolic compounds and DPPH• scavenging activity of bread and durum wheat genotypes

Variables	Total phenolics		DPPH• scavenging activity	
	Bread wheat	Durum wheat	Bread wheat	Durum wheat
Total phenolics			0.76*	0.80*
PVPP bound phenolics	-0.52	-0.43	-0.34	-0.74*
Flavonoids	0.39	-0.25	0.73*	-0.48
Ferulic acid	-0.08	-0.43	0.13	-0.42
Chlorogenic acid	0.25	-0.02	0.38	-0.05
Caffeic acid	0.17	-	-0.36	-

*significant at $P < 0.05$; **significant at $P < 0.01$

CONCLUSIONS

Significant differences were detected in the content of acetone/water extractable total phenolics, PVPP bound phenolics and all detected phenolic acids between and within two wheat species. Further study is needed to explore how these parameters change across environments, as genotype by environment interactions may have high implication on the anti-oxidative capacity in plants. Although relatively small number of genotypes was studied, high variability for most phenolic compounds and the total antioxidant capacity was obtained in both species. The finding suggests that there are opportunities for developing new varieties with high phenolics and enhanced phytochemical content

ACKNOWLEDGEMENT

This study was supported by the Ministry of Science and Developmental of the Republic Serbia (Grants no. 31069). .

Received July 24h, 2012
Accepted November 07th, 2012

REFERENCES

ABE, N., MURATA, T., A. HIROTA (1998): Novel DPPH radical scavengers, bisorbicillinol and demethyltrichodimerol, from a fungus. Biosci. Biotechnol. Biochem. *62,* 661-666.

ADOM, K.K., R.H. LIU (2002): Antioxidant activity of grains. J. Agric. Food Chem. *50,* 6182-6187.

ADOM, K.K., SORRELLS, M.E., R.H. LIU (2003): Phytochemical profiles and antioxidant activity of wheat varieties. J. Agric. Food Chem. *51,* 7825-7834.

BELDEROK, B. (2000): Developments in bread-making processes Plant Food Hum. Nutr. *55,* 1–14.

DINELLI, G., CARRETERO, A.S., DI SILVESTRO, R., MAROTTI, I., FU, S., BENEDETTELLI, S., GHISELLI, L., A.F. GUTIÉRREZ (2009): Determination of phenolic compounds in modern and old varieties of durum wheat using liquid chromatography coupled with time-of-flight mass spectrometry. J. Chromat. A *1216,* 7229–7240.

EBERHARDT, M.V., LEE, C.Y., R.H. LIU (2000): Antioxidant activity of fresh apples. Nature *405,* 903-904.

FULCHER, R.G., T.K. DUKE (2002): Whole-grain structure and organization: Implications for nutritionists and processors. In: Whole-Grain Foods in Health and Disease. Marquart, L., Slavin, L. and Fulcher, R.G. eds., American Association of Cereal Chemists, St. Paul, MN. pp 9-45.

HAGERMAN, A., HARVEY-MUELLER, I., H.P.S. MAKKER (2000): Quantification of tannins in the foliage-a laboratory manual. ed. FAO/IAEA, Vienna, Austria pp. 4-7.

JIA, Z., MENGCHENG, T., J. WU (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. *64,* 555-559.

KIM, K.-H., TSAO, R., YANG, R., S.W. CUI (2006): Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem. *95,* 466–473.

LI, L., SHEWRY, P.R., J.L. WARD (2008): Phenolic acids in wheat varieties in the healthgrain diversity screen J. Agric. Food Chem. *56,* 9732–9739.

LI, W., SHAN, F., SUN, S., CORKE, H., T. BETA (2005): Free radical scavenging properties and phenolic content of Chinese black-grained wheat. J. Agric. Food Chem. *53,* 8533-8536.

LIU, Q., QIU, Y., T. BETA (2010): Comparison of antioxidant activities of different colored wheat grains and analysis of phenolic compounds. J. Agric. Food Chem. *58,* 9235–9241.

LIYANA-PATHIRANA, C. M., F. SHAHIDI (2007): The antioxidant potential of milling fractions from breadwheat and durum. J. Cereal Sci. *45,* 238–247.

MAKKAR, H. P. S., BLUEMMEL, M., BOROWY, N. K., K. BECKER (1993): Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61:161–165.

MOORE, J., HAO, Z., ZHOU, K., LUTHER, M., COSTA J., L. YU (2005): Carotenoid, tocopherol, phenolic acid and antioxidant properties of Maryland-grown soft wheat. J. Agric. Food Chem. *53,* 6649-6657.

MPOFU, A., SAPIRSTEIN, H.D., T. BETA (2006): Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J. Agric. Food Chem. *54,* 1265-1270.

ONYENEHO, S.N., N.S. HETTIARACHCHY (1992): Antioxidant activity of durum wheat bran. J. Agric. Food Chem. *40,* 1496–1500.

PIIRONEN, V., LAMPI, A.M., EKHOLM, P., SALMENKALLIO-MARTTILA, M., K.-H. LIUKKONEN (2009): Micronutrients and phytochemcials in wheat grain. In: Wheat: Chemistry and Technology. Khan K. and Shewry, P.R. eds., American Association of Cereal Chemists, St. Paul, MN. pp 179–222.

PORTER, L.J., HRSTICH, L. N., B.G. CHAN (1986): The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry *25,* 223–230.

SAULNIER, L., SADO, P.-E., BRANLARD, G., CHARMET, G., F.J. GUILLON (2007): *Wheat arabinoxylans: exploiting variation in amount and composition to develop enhanced varieties***.** Cereal Sci. *46,* 261-281.

SINGLETON, V.L., ORTHOFER R., R.M. LAMUELA-RAVENTOS (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. *299,* 152–178.

VERMA, B., HUCL, P., R.N. CHIBBAR (2008): Phenolic content and antioxidant properties of bran in 51 wheat cultivar. Cereal Chem. *85,* 544–549.

ZHISHEN, J., MENGCHENG, T., W. JIANMING (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. *64,* 555-559.

ZHOU, K., LAUX, J. J., L. YU (2004b) : Comparison of Swiss red wheat grain, and fractions for their antioxidant properties. J. Agric. Food Chem. *52,* 1118-1123.

ZHOU, K., SU, L., L. YU (2004a): Phytochemicals and antioxidant properties in wheat bran. J. Agric. Food Chem. *52,* 6108-6114.

ŽILIĆ, S., DODIG, D., HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, V., MAKSIMOVIĆ, M., SARATLIĆ, G., B. ŠKRBIĆ (2010): Bread and durum wheat compared for antioxidants contents, and lipoxygenase and peroxidase activities. Int. J. Food Sci. Tech. *45,* 1360–1367.

ŽILIĆ, S., HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, V., DODIG, D., MAKSIMOVIĆ, V., MAKSIMOVIĆ, M., Z. BASIĆ (2011): Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. J. Cereal Sci. *54,* 417-424.

ŽILIĆ, S., SERPEN, A., AKILLIOĞLU, G., GÖKMEN, V., J. VANČETOVIĆ (2012b): Phenolic compounds, carotenoids, anthocyanins and antioxidant capacity of colored maize (*Zea mays* L.) kernels. J. Agric. Food Chem. *60,* 1224-1231.

ŽILIĆ, S., SERPEN, A., AKILLIOĞLU, G., JANKOVIĆ, M., V. GÖKMEN (2012a): Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. J. Cereal Sci. *56,* 652-658.

SADRŽAJ RASTVORLJIVIH SLOBODNIH FENOLNIH KOMPONENATA I ANTIOKSIDATIVNI KAPACITETA ZRNA GENOTIPOVA HLEBNE I DURUM PŠENICE

Slađana ŽILIĆ, Vesna HADŽI-TAŠKOVIĆ ŠUKALOVIĆ[2], Dejan DODIG[1], Vuk MAKSIMOVIĆ[2], Vesna KANDIĆ[1]

[1]Institut za kukuruz, "Zemun Polje", Beograd, Sebija
[2]Institut za multidisciplinarna istraživanja, Univerzitet u Beogradu, Beograd, Srbija

Izvod

Cilj ovih istraživanja bio je da se odredi sadržaj fenolnih komponenata i ukupni antioksidativni kapacitet zrna 10 genotipova hlebne (*T. aestivum* L.) i 10 genotipova durum (*T. durum* Desf.) pšenice. Rastvorljive slobodne forme ukupnih fenola, flavonoida, PVPP (polyvinylpolypyrrolidone) vezanih fenola, proantocijanidina i fenolnih kiselina bile su analizirane. Pored toga, korelacioni koeficijent između ukupnog antioksidativnog kapaciteta i koncentracije različitih slobodnih fenolnih komponenata, kao i između rastvorljivih slobodnih ukupnih fenola i fenolnih kiselina, flavonoida i PVPP vezanih fenola bio je određen. Značajne razlike nađene su u sadržaju aceton/voda ekstraktibilnih ukupnih fenola, PVPP vezanih fenola i fenolnih kiselina kako između dve vrste pšenice (durum i hlebne), tako i unutar vrste, odnosno između ispitivanih genotipova iste vrste. U proseku, durum pšenica imala je oko 1.2 puta više ukupnih fenola i oko 1.5 puta više PVPP vezanih fenola nego hlebna pšenica. Tri fenolne kiseline, ferulinska, kafeinska i hlorogena, bile su detektovane u etanolskom ekstraktu celog zrna hlebne pšenice. Kafeinska kiselina nije nađena u genotipovima durum pšenice, dok je ferulinska kiselina bila najzastupljenija u genotipovima obe vrste pšenice. Prisustvo proantocijanidina nije utvrđeno u genotipovima hlebne i durum pšenice. Antioksidativni kapacitet meren kao DPPH radikal vezujuća aktivnost bio je sličan između hlebne i durum vrste pšenice, ali su značajne razlike bile utvrđene između genotipova unutar vrste.

Primljeno 24. VII. 2012.
Odobreno 07. XI. 2012.