Total phenolic contents and antioxidant capacities of cereal and pseudocereal genotypes

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Summary

Total phenolic contents and antioxidant capacities of 20 oat genotypes, 13 wheat genotypes, 6 barley genotypes and 2 buckwheat genotypes were studied. Buckwheat genotypes showed the highest contents of phenolic compounds and the highest antioxidant capacity. Contents of phenolic compounds, expressed as gallic acid equivalents per 1 kilogram of cereal dry weight, ranged from 2.456 $g.kg^{-1}$ to 2.624 $g.kg^{-1}$ in buckwheat genotypes, from 1.429 $g.kg^{-1}$ to 1.806 $g.kg^{-1}$ in barley genotypes, from 0.758 $g.kg^{-1}$ to 1.244 $g.kg^{-1}$ in oat genotypes and from 0.459 $g.kg^{-1}$ to 1.07 $g.kg^{-1}$ in wheat genotypes. The order of antioxidant capacities was as follows: buckwheat > barley > oat > wheat (2,2-diphenyl-1-picrylhydrazyl, DPPH test); buckwheat > barley > wheat > oat (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate], ABTS test); buckwheat > barley > oat ≈ wheat (electron paramagnetic resonance, EPR/spin-trapping test). The particular genotypes of cereals differed in parameters tested ($P < 0.05$). A good correlation was found between antioxidant capacities and contents of phenolic compounds in wheat genotypes ($R = 0.872$) and in oat genotypes ($R = 0.794$), though in barley genotypes there was a weak correlation between the analysed parameters ($R = 0.574$).

Keywords

wheat; oat; barley; buckwheat; genotypes; antioxidant capacity; phenolic compounds

Cereals belong to the most important food groups supplying significant amounts of energy, macronutrients, micronutrients and non-nutrients to the human diet [1]. Epidemiological studies conclude that there exists a relationship between high intakes of whole grains and reduced incidence of chronic diseases such as cardiovascular disease, diabetes and cancer [2]. Cereal grains contain a wide variety of biologically active compounds, including dietary fibre, microelements, sterols, phenolic compounds, peptides, vitamins, and the effects of these have been associated with antioxidant properties [3, 4].

Phenolic compounds are secondary metabolites with different biosynthetic pathways. A large and heterogeneous group of polyphenols including phenylpropanoids, condensed tannins, lignin, flavonoids and hydroxycinnamic acids, are derived from the same precursor, L-phenylalanine. The second major group represented by hydrolysable tannins has gallic acid as a precursor [5]. The major portion of phenolic compounds is located in outer parts of grains, where they are involved in defense against ultraviolet radiation, pathogen invasion and in modification of mechanical properties. Moreover, phenolics, predominantly ferulic and coumaric acid, play an important role in limiting polysaccharide degradation by exogenous enzymes, where they act as a cross-link between polysaccharides, and between polysaccharides and lignin [6]. Phenolic compounds have strong antioxidant properties in vitro, associated with their ability to scavenge free radicals and chelate transition metal ions [4]. Their efficiency depends on many structural factors, such as number and position of hydroxyl groups bonded to the aromatic ring, character of substituents and the position of substituents in relation to the hydroxyl groups [7].

Some crops are a unique source of several compounds, which are not present in other cereals

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and pseudocereals, such as avenanthramides and avenalumic acids in oat grains [8, 9] or rutin in buckwheat grains. Rutin exerts a variety of pharmacologically demonstrated activities including antiinflammatory, antimutagenic, antitumoral, anticarcinogenic, antihemorrhagic, antioxidative, hypotensive, estrogen receptor binding activities, supports smooth muscle relaxation, and it is known to reduce fragility of blood vessels [10-16].

Cereals of primary economic importance include wheat, barley, oat, rye, corn, rice, sorghum and millet [1]. According to previous studies, individual crop genotypes differ in their antioxidant capacities and phenolic contents [17, 18]. Apart from genetic factors, the maturity and environmental factors such as abiotic stress, nutrient availability, weather, water supply and other growing conditions may play important roles in the biosynthesis of secondary metabolites, including phenolics [19, 20]. Therefore, understanding the properties of crop genotypes may lead to the production of food products containing enhanced levels of natural antioxidants and consequently may improve agricultural economy, quality and safety of food products and human health [18].

In this study, contents of phenolic compounds were determined in cereal and pseudocereal genotypes using Folin-Ciocalteu reagent and three different methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) and electron paramagnetic resonance (EPR)/spin-trapping assays, were applied to characterize their antioxidant capacities.

MATERIAL AND METHODS

Samples

Grain samples of 20 oat varieties (belonging to two different oat species, *Avena sativa* L. - covered yellow oats, and *Avena sativa nuda* L. - naked oats), 6 barley varieties (*Hordeum vulgare* L.), 13 wheat varieties (belonging to species - *Triticum aestivum* L. - spring and winter wheat, *Triticum durum* L. - durum spring wheat and *Triticum spelta* L. - spelt), 2 buckwheat varieties (*Fagopyrum esculentum*) were obtained from the gene bank of the Research Institute of Plant Production (Piešťany, Slovakia). Variety samples were grown in localities Vígľaš Pstruša, Borovce and Piešťany (Slovakia) in the crop year 2003.

Preparation of cereal and pseudocereal extracts

The samples were milled and ground to pass through a 0.5 mm screen. Moisture content was determined and all data were expressed on a dry weight basis. The fine flour was extracted with 65% ethanol (1:20, w/v) at 80 °C for three hours [21]. Extracts were filtered and used for DPPH test and total phenolic content determination. For ABTS and EPR/spin-trapping tests, filtrates were evaporated to dryness under vacuum at 40 °C and the residues were dissolved in dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany).

DPPH test

Antioxidant capacity determination using the free DPPH radical (DPPH$^{\bullet}$, 95%, Aldrich, Steinheim, Germany) was carried out according to Yen and Chen [22]. Amounts of 3 ml ethanol and 1 ml DPPH solution (120 mg DPPH in 100 ml of 96% ethanol) were added to 1 ml of appropriately diluted cereal extracts. In the reference sample, 1 ml of ethanol was used instead of the extract, and all spectra were measured against ethanol. The difference in the absorbance after 10 min at 517 nm relative to the reference spectrum was used to calculate total antioxidant capacity as the amount of scavenged DPPH radicals per 1 kg of cereal dry weight and in Trolox (Sigma-Aldrich, Steinheim, Germany) equivalents per 1 kg of cereal dry weight, respectively.

ABTS test

Antioxidant capacities were measured using modified versions of the methods of Re et al. [23] and Arts et al. [24]. To prepare ABTS cation radical solution (ABTS$^{+\bullet}$), a potassium persulphate ($K_2S_2O_8$; p. a. purity, Merck) aqueous solution (3.3 mg $K_2S_2O_8$ in 5 ml distilled water) was added to 17.2 mg ABTS (purum, > 99%, Fluka Chemie, Buchs, Switzerland) and the resulting solution was stored for 14 h in the dark. A volume of 1 ml of the final dark-green radical solution was then diluted with 60 ml distilled water and used in ABTS tests. Here, a volume of 50 μl of the cereal extract in DMSO was added to 2 ml of ABTS$^{+\bullet}$ solution in 1 cm UV-cuvette and vigorously mixed. UV-VIS spectra were recorded at intervals of 1 min for 10 min using UV-VIS 1700 spectrophotometer (Shimadzu, Kyoto, Japan). UV-VIS spectrum of initial ABTS$^{+\bullet}$ solution measured against distilled water was taken as a reference spectrum. The difference in the absorbances (ΔA) after 10 min at 730 nm relative to the reference spectrum was used to calculate the Trolox equivalents.

EPR/spin-trapping test

Thermal decomposition of $K_2S_2O_8$ in DMSO at 60 °C was used as a source of reactive radicals. EPR/spin-trapping method [25] employing the 5,5-dimethylpyrroline-N-oxide (DMPO, Sigma-

Aldrich) spin trap, was used for determination of the radical scavenging ability in cereal samples. 200 μl of DMSO extract (or pure DMSO in reference measurements) was mixed with 25 μl of 0.2 M DMPO in DMSO and 25 μl of 0.01 M $K_2S_2O_8$ (aq.) and filled into an EPR flat cell. A time course of EPR spectra of the DMPO spin adducts was recorded using EPR X-band EMX spectrometer from Bruker (Rheinstetten, Germany) at intervals of 1 min for 20 min at 60 °C (each spectrum was an accumulation of 3 scans). The EPR intensity (double integral) after 20 min, recorded for the sample solutions, was compared to the reference. The difference between the integral EPR intensities of the reference and the cereal samples after 20 min characterized the amount of radicals scavenged by the scavengers present in the corresponding sample. The value for the virtual conditions when all radicals were scavenged was set to 100% of radical scavenging capacity (RSC). The RSC values were calculated as a percentage of scavenged radicals relative to the reference sample (DMSO). Trolox solutions in DMSO were used to obtain the calibration curve in order to express the RSC in Trolox equivalents similar to DPPH and ABTS tests.

Total phenolic compounds

Total contents of phenolic compounds were measured in the ethanol extract with a standard Folin-Ciocalteu reagent [26]. The reaction mixture contained 100 μl of cereal extracts and 500 μl of the Folin-Ciocalteu reagent (Merck) and 1.5 ml of 20% sodium carbonate. The sample was then mixed on a vortex mixer and diluted with distilled water to the final volume of 10 ml. After 2 h of reaction, the absorbance at 765 nm was measured and used to determine the phenolic contents using the calibration curve of gallic acid (Sigma-Aldrich). The results were expressed in gallic acid equivalent per 1 kg of cereal dry weight.

Statistical analysis

Data were reported as means ± standard deviation. Results were subjected to correlation analysis, descriptive statistics and analysis of differences among mean values by Student test. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Antioxidant capacities and the contents of phenolic compounds of four crops and their genotypes were determined. The results obtained for 41 analysed cereal samples are shown in Tab. 1. Antioxidant capacities determined by DPPH test are expressed as the amount of scavenged DPPH radicals and the contents of phenolic compounds as gallic acid equivalents per 1 kg of cereal dry weight.

Antioxidant capacities of oat genotypes ranged from 1.930 g.kg^{-1} to 3.092 g.kg^{-1} and the contents of phenolic compounds from 0.758 g.kg^{-1} to 1.244 g.kg^{-1}. Xing and White [27] and Emmons and Peterson [28] obtained total phenolic values of only 0.2-0.4 g.kg^{-1} (as gallic acid equivalents) from oat grains. Higher concentration (1.138 g.kg^{-1}, as gallic acid equivalents) was reported by Holasova et al. [29]. Differences between the data in literature and our data may be due to genotype variability, growing location, sample preparation and extraction procedure, respectively. Naked oat genotypes showed by 9.4% lower antioxidant capacities and by 14.7% lower contents of phenolic compounds compared to covered genotypes on average. Mean contents of phenolic compounds were 0.882 g.kg^{-1} in naked oat genotypes and 1.035 g.kg^{-1} in covered genotypes. It was reported in previously published papers that phenolic acids are localized particularly in seed hulls and that the oat endosperm has better antioxidant properties in comparison with the whole grain [27, 28, 30], thereby it is possible to elucidate our results.

In wheat genotypes, similar levels were determined for both antioxidant capacities (1.096–3.133 g.kg^{-1}) and phenolic contents (0.459–1.07 g.kg^{-1}) as in oat samples. Spelt *Triticum spelta*, one of the oldest crops that has become ever more interesting nowadays, was also tested. Spelt was found to contain 0.522 g.kg^{-1} of phenolic compounds and to have a weaker radical scavenging capacity of 1.293 g.kg^{-1}, as determined by the DPPH free-radical assay. Considerable differences between genotypes of wheat and durum wheat were observed. The mean phenolic content (0.803 g.kg^{-1}) in durum wheat was near to the value determined for wheat (0.833 g.kg^{-1}); however, the antioxidant capacity was lower by about 23.1%. This indicated that not only the quantitative but also qualitative occurrence of phenolic compounds and other synergistically-acting compounds is responsible for the antioxidant properties.

In this manner, the results obtained for the barley genotypes could be explained. Their antioxidant capacities (mean value 12.68 g.kg^{-1}) were markedly higher (approximately 6 times) although the contents of phenolic compounds (mean value 1.620 g.kg^{-1}) were only approximately 2 times greater than in genotypes of oat and wheat. Barley

Tab. 1. Antioxidant capacities determined by DPPH test [g.kg^{-1}] and the contents of phenolic compounds [g.kg^{-1}] in cereals.

Crop				DPPH test [g.kg^{-1}]	Phenolic compounds [g.kg^{-1}]
Genotypes of oat	covered (*Avena sativa*)	SV 5		3.092 ± 0.008	1.103 ± 0.012
		Euro		2.974 ± 0.023	1.225 ± 0.022
		Zvolen		2.921 ± 0.015	1.204 ± 0.004
		Expander		2.764 ± 0.017	1.040 ± 0.010
		Roxtron		2.748 ± 0.147	1.029 ± 0.006
		Dalimil		2.708 ± 0.063	1.244 ± 0.020
		Ardo		2.663 ± 0.041	1.102 ± 0.008
		Adam		2.523 ± 0.048	0.865 ± 0.012
		Cyril		2.442 ± 0.022	0.963 ± 0.012
		Neklan		2.270 ± 0.067	0.998 ± 0.014
		Flämingsstern		2.253 ± 0.036	0.996 ± 0.018
		Azur		2.028 ± 0.021	0.851 ± 0.016
		Auron		1.930 ± 0.032	0.836 ± 0.020
	naked (*Avena nuda*)	Detvan		2.904 ± 0.051	1.055 ± 0.024
		Ábel		2.390 ± 0.084	0.874 ± 0.024
		PS 106		2.365 ± 0.083	0.935 ± 0.024
		PS 9 0		2.296 ± 0.091	0.792 ± 0.006
		Izák		2.122 ± 0.076	0.984 ± 0.024
		Jakub		2.155 ± 0.024	0.758 ± 0.002
		PS 100		2.013 ± 0.045	0.780 ± 0.024
Genotypes of wheat	*Triticum aestivum*	spring	Leguan	2.590 ± 0.019	1.023 ± 0.018
			Aranka	1.814 ± 0.076	0.832 ± 0.009
		winter	Kris	3.133 ± 0.023	1.070 ± 0.007
			Astella	2.213 ± 0.047	0.903 ± 0.004
			Niagara	2.110 ± 0.047	0.786 ± 0.011
			Banquet	1.744 ± 0.011	0.755 ± 0.014
			Ekspromt	1.096 ± 0.024	0.459 ± 0.017
	Triticum durum	Yavaros Tall		2.203 ± 0.054	0.887 ± 0.007
		Altar 84		1.733 ± 0.017	0.937 ± 0.009
		Aconchi Tall		1.502 ± 0.044	0.795 ± 0.010
		Olinto		1.424 ± 0.011	0.724 ± 0.014
		Kucuk		1.204 ± 0.044	0.670 ± 0.007
	Spelt (*Triticum spelta*)			1.293 ± 0.035	0.522 ± 0.008
Genotypes of barley	Virac			15.451 ± 0.196	1.806 ± 0.020
	Luxor			14.268 ± 0.619	1.735 ± 0.031
	Regina			13.163 ± 0.298	1.429 ± 0.015
	Barolo			11.699 ± 0.421	1.564 ± 0.015
	Okal			11.027 ± 0.498	1.691 ± 0.032
	Oriflame			10.471 ± 0.229	1.492 ± 0.025
Genotype of buckwheat	Špačinská			17.237 ± 0.326	2.624 ± 0.012
	Pyra			15.120 ± 0.214	2.456 ± 0.032

Results of the DPPH test are expressed as the amount of scavenged DPPH radicals and phenolic compounds as gallic acid equivalents. Values are presented as mean of 4 parallels ± standard deviation. Relative standard deviation (RSD) for DPPH test: 0.25–5.34%; RSD for the contents of phenolic compounds: 0.41–3.6%.

contains a flavonoid catechin, which has a significant antioxidant effect and its contribution to antioxidant capacity is higher than the contribution of most phenolic acids. Eventually, this result may be explained by a synergistic action of individual compounds.

Buckwheat genotypes showed the highest antioxidant capacities (mean value 16.178 g.kg^{-1}) and the highest contents of phenolic compounds (mean value 2.54 g.kg^{-1}). Buckwheat is a rich source of flavonoids, mainly rutin, compared to other cereals and pseudocereals.

Tab. 2. Statistical data for DPPH radical scavenging capacities [g.kg^{-1}] of oat, wheat and barley genotypes.

Crop	Mean	Standard deviation	Variation coefficients	Median	Minimum	Maximum
Oat	2.478	0.351	14.172	2.413	1.930	3.092
naked	2.321	0.291	12.553	2.296	2.013	2.904
covered	2.563	0.361	14.104	2.663	1.930	3.092
Wheat	1.851	0.588	31.747	1.744	1.096	3.133
spring wheat	2.202	0.549	24.922	2.202	1.814	2.590
winter wheat	2.059	0.743	36.067	2.110	1.096	3.133
spring and winter wheat	2.100	0.650	30.962	2.110	1.096	3.133
durum wheat	1.613	0.380	23.568	1.502	1.204	2.203
Barley	12.680	1.950	15.379	12.431	10.471	15.451

DPPH radical scavenging capacity is expressed as the amount of scavenged DPPH radicals.

Tab. 3. Statistical data of the contents of phenolic compounds [g.kg^{-1}] in oat, wheat and barley genotypes.

Crop	Mean	Standard deviation	Variation coefficients	Median	Minimum	Maximum
Oat	0.981	0.147	14.991	0.989	0.758	1.244
naked	0.882	0.113	12.858	0.873	0.758	1.054
covered	1.035	0.138	13.328	1.029	0.835	1.244
Wheat	0.797	0.178	22.282	0.795	0.459	1.070
spring wheat	0.928	0.135	14.522	0.928	0.832	1.023
winter wheat	0.795	0.225	28.269	0.786	0.459	1.070
spring and winter wheat	0.833	0.202	24.284	0.832	0.459	1.070
durum wheat	0.803	0.111	13.786	0.795	0.670	0.937
Barley	1.620	0.147	9.104	1.628	1.429	1.806

Content of phenolic compounds is expressed as gallic acid equivalents.

Tab. 2 and Tab. 3 show the results of descriptive statistics of the obtained data on antioxidant capacities by DPPH test and total phenolic contents data in crops. Variation coefficients, which characterize the variability of data sets studied, are higher in value, particularly for wheat genotypes. The lowest variation coefficient was obtained for phenolic contents of barley varieties, suggesting that these possess higher degree of stability of phenolic compounds biosynthesis in comparison to wheat and oat varieties. The genetic make up of different genotypes, which leads to different levels of phenolic synthesis, is probably responsible for differences observed [19].

Particular genotypes of cereals differed in the tested parameters ($P < 0.05$). A good correlation was found between antioxidant capacity and contents of phenolic compounds in wheat genotypes ($R = 0.872$) and in oat genotypes ($R = 0.794$), though in barley genotypes, there was a weak correlation between the analysed parameters ($R = 0.574$).

Cereal genotypes with the highest and the lowest activities in terms of antioxidant capacities obtained by DPPH test, by ABTS and EPR/spin-trapping analyses were also analysed (Fig. 1). The determined antioxidant capacities are expressed as Trolox equivalents in order to allow quantitative comparison between different methods used.

The particular cereals tested differed in the ability to scavenge the stable $ABTS^{+\bullet}$ radical as

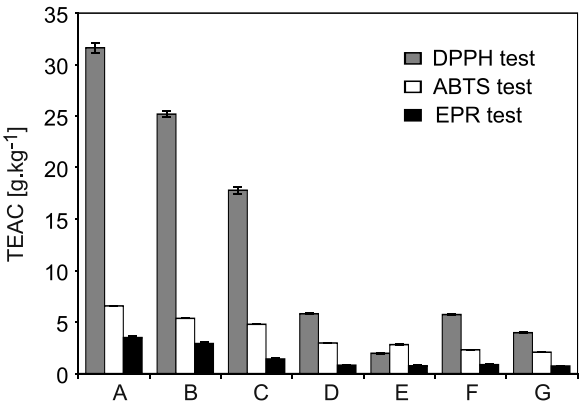

Fig. 1. Antioxidant capacities of individual crop varieties determined by DPPH, ABTS and EPR/spin-trapping tests.
TEAC - Trolox equivalent antioxidant capacity. A - Buckwheat Špačinska, B - Barley Virac, C - Barley Oriflame, D - Wheat Kris, E - Wheat Expromt, F - Oat SV5, G - Oat Auron.

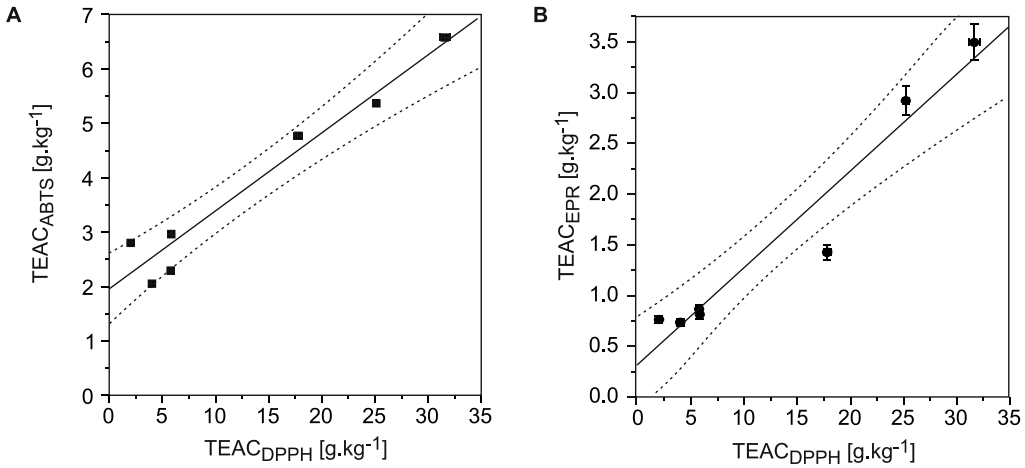

Fig. 2. Correlations between antioxidant capacities of crop varieties determined by different tests at a 95% confidence level.
Confidence bands are marked by dotted lines. TEAC - Trolox equivalent antioxidant capacity. A - TEAC determined by DPPH and ABTS tests, B - TEAC determined by DPPH and EPR/spin-trapping tests. Results are expressed with error bars corresponding to standard deviations.

well as to terminate the reactive oxygen- and carbon-centred radicals generated by the thermal decomposition of $K_2S_2O_8$. Similar to DPPH analysis, the highest radical-scavenging effect was found for buckwheat grains followed by barley grains. The order of antioxidant capacities was as follows: buckwheat > barley > oat > wheat (DPPH test); buckwheat > barley > wheat > oat (ABTS test); buckwheat > barley > oat ≈ wheat (EPR/spin-trapping test). Contents of phenolic compounds decreased in the order: buckwheat > barley > oat ≈ wheat.

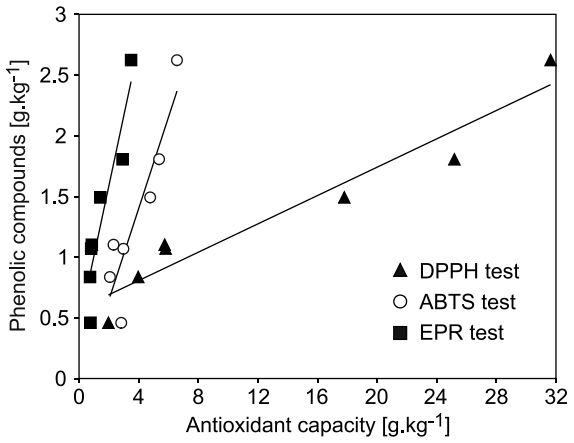

Fig. 3. Correlation between the contents of phenolic compounds and the antioxidant capacities of crop varieties determined by DPPH, ABTS and EPR/spin-trapping tests.
Antioxidant capacities are expressed as Trolox equivalents and phenolic contents as gallic acid equivalents.

Good correlations were found among the antioxidant capacities determined by DPPH, ABTS and EPR/spin-trapping tests (Fig. 2). The correlation coefficient between DPPH and ABTS procedures, and between DPPH and EPR/spin-trapping procedures were 0.974 and 0.969, respectively. The correlation between ABTS and EPR/spin-trapping procedures was also high (R = 0.938). Results of correlation analyses suggest that these methods have similar predictive capacity for cereal antioxidant activities.

The applied in vitro methods are not specific for individual antioxidant compounds but they provide a total view of the antioxidant capacities of the samples. Total phenolic contents determined in the samples and their antioxidant capacities measured by DPPH, ABTS and EPR/spin-trapping tests correlated significantly as well (R = 0.958, 0.907 and 0.934, respectively) as shown in Fig. 3, indicating that phenolic compounds distinctively contribute to the total antioxidant capacities of cereals and pseudocereals.

CONCLUSION

This study deals with determination of the contents of phenolic compounds in oat, wheat, barley and buckwheat genotypes and their contribution to the total antioxidant capacities. The highest contents of phenolics was observed in buckwheat varieties (2.54 g.kg^{-1}, on average) followed by barley varieties (1.620 g.kg^{-1}, on average). Furthermore,

the best antioxidant capacities were observed for buckwheat and barley grains. Our results confirmed that the particular genotypes of cereals differed in tested parameters. From the individual crops, barley genotypes Virac, Luxor, wheat genotypes Kris (winter wheat), Leguan (spring wheat), Altar 84, Yavaros Tall (durum wheat), covered oat Dalimil, Euro, Zvolen and naked oat Detvan could be the most suitable source of health-beneficial phenolic compounds. Selection of genotypes is essential from agricultural and food industry viewpoint and our data may offer valuable additional information for classification of various cereal varieties.

Good correlations were found among the antioxidant capacities of cereal samples measured by DPPH, ABTS and EPR/spin-trapping methods. In addition, the antioxidant properties highly correlated with total phenolic contents. Based on these findings, it was possible to deduce a significant contribution of phenolics to the antioxidant effects of cereals and pseudocereals.

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