JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Comparison of Phenolic Acids Profile and Antioxidant Potential of Six Varieties of Spelt (*Triticum spelta* L.)

Urszula Gawlik-Dziki,*[,†] Michał Świeca,[†] and Dariusz Dziki[‡]

[†]Department of Biochemistry and Food Chemistry, University of Life Sciences, Skromna Str. 8, 20-704 Lublin, Poland

[‡]Department of Thermal Technology, University of Life Sciences, Doświadczalna Str. 44, 20-280, Lublin, Poland

ABSTRACT: Phenolic acids profile and antioxidant activity of six diverse varieties of spelt are reported. Antioxidant activity was assessed using eight methods based on different mechanism of action. Phenolic acids composition of spelt differed significantly between varieties and ranged from 506.6 to 1257.4 μg/g DW. Ferulic and sinapinic acids were the predominant phenolic acids found in spelt. Total ferulic acid content ranged from 144.2 to 691.5 μg/g DW. All analyzed spelt varieties possessed high antioxidant potential. In spite of the fact that bound phenolic acids possessed higher antioxidant activities, analysis of antioxidant potential and their relationship with phenolic acid content showed that free phenolics were more effective. Eight antioxidant methods were integrated to obtain a total antioxidant capacity index that may be used for comparison of total antioxidant capacity of spelt varieties. Total antioxidant potential of spelt cultivars were ordered as follows: Ceralio > Spelt INZ ≈ Ostro > Oberkulmer Rotkorn > Schwabenspelz > Schwabenkorn.

KEYWORDS: *spelt, phytochemicals, antioxidant activity, nutraceutical value, phenolic acids*

INTRODUCTION

Spelt (*Triticum aestivum* ssp. *spelta* L.) is an ancient and distant cousin to modern wheat (*Triticum aestivum*). Spelt was the predominant bread cereal from the fifth century until the beginning of the 20th century. Nowadays, it is an alternative crop in relation to wheat. Spelt is a more environmentally friendly crop compared to wheat because it requires lower nitrogen fertilization levels and is well-adapted to cool and wet conditions. Additionally, spelt wheat is a low-input plant, suitable for growth without the use of pesticides, in harsh ecological conditions and in marginal areas of cultivation.[1] For many years, cultivation of spelt declined, but recent interest in use of spelt for ecologically grown foods has led to resurgence in its cultivation.

Epidemiological studies strongly suggest that diets play a significant role in the prevention of many chronic diseases. Grain consumption has been associated with reduced risk of certain chronic diseases, including cancer, and this has been attributed in part to the unique phytochemicals in grains.[2] Phenolic acids and their associated antioxidant activity are one of the important constituents of wheat, widely studied in recent years. Simple phenolic acids of wheat include mainly ferulic, vanillic, syringic, sinapinic, caffeic, and *p*-coumaric acids, which have been demonstrated to be a source of nutritional antioxidants.[3] Previously, the phenolic content of whole grains had been underestimated, as most research only determined the free phenolic content and not the content of phenolics that were bound to the cell wall material.[2]

Grain phytochemicals exert their health benefits through multifactorial physiological mechanisms including antioxidant activity. A majority of the phenolics in wheat are found predominantly in the outer bran layer. Upon digestion, even though most of the phenolics are ester- or ether-linked to polymers in the plant cell wall, intestinal microbes are able to cleave the bonds and free many of the phenolics, thereby making them nutritionally available.[4] Many of these phenolics exhibit strong antioxidant properties in that they scavenge or neutralize free radicals and thereby reduce or minimize oxidative damage to proteins, DNA, and lipids. This reduction in oxidative damage to cells and cell components may explain their preventive effect against diseases related to oxidative stress.[5]

Current interest in the health benefits provided by grain consumption has led to an increased focus on the phytochemical content of different grains and grain varieties. Recently there has been some renewed interest in ancient grains by health-conscious groups, as well as the health food market, with the aim to utilize the unique nutraceutical values offered by these ancient grains.[2] In spite of the growing interest in spelt products, review of recent literature shows a lack of comprehensive and comparative elaborations concerning phenolic acids profile and antioxidant activities demonstrated by spelt varieties. The objectives of this study were to determine the profiles of total phenolic acids including free and bound forms and the antioxidant activity profiles of six spelt varieties.

MATERIALS AND METHODS

Plant Material. The examined grain of spelt wheat (*Triticum aestivum* ssp. *spelta* L.) varieties (Ceralio, Ostro, Spelt INZ Droogendijk, Oberkulmer Rotkorn, Schwabenspelz and Schwabenkorn) derived from the Experimental Farm (51°19′ N; 23°26′ E) belonging to Agroecology Department of University of Life Sciences in Lublin, Poland. The experiments were set up on mixed rendzina soil formed in the Cretaceous age. Cultivar samples were grown in the

Received: November 28, 2011
Revised: April 1, 2012
Accepted: April 15, 2012
Published: April 15, 2012

ACS Publications © 2012 American Chemical Society

2011 crop year. Grains (three samples per variety) were dehulled, grounded in a labor mill, and sieved (60 mesh).[6]

Chemicals. Ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin–Ciocalteau reagent, linoleic acid, ammonium thiocyanate, nitroblue tetrazolium (NBT), phenazinemetholsulphate (PMS), pyrogallol, NADH, and hemoglobin were purchased in Sigma-Aldrich Co. USA. All others chemicals were of analytical grade.

Extraction of Free and Bound Phenolic Compounds. Wholemeal fractions (1 g) were mixed with 10 mL of 80% chilled ethanol for 20 min with continuous shaking at room temperature. The suspension was centrifuged, and the supernatant (free phenolic extracts) was collected. The pellet was re-extracted twice with 10 mL of 80% chilled ethanol, and all supernatants were combined. The free phenolic extracts were concentrated using a vacuum evaporator and then filled up with methanol to a final volume of 10 mL. The free phenolic compounds were then stored at −40 °C until further use. After extraction of free phenolics, 20 mL of 2 M NaOH was added directly to the pellet and shaken for 90 min at 60 °C. After alkaline hydrolysis, the solution was acidified to pH 2 with 6 M HCl and centrifuged to separate cloudy precipitates. The free fatty acids and other lipid contaminants in the clear solution were removed by extraction with hexane (five times). The liberated phenolic acids were then extracted six times with ethyl acetate. The pooled ethyl acetate extracts were evaporated to dryness, and then bound phenolic compounds were reconstituted in 10 mL of methanol and stored at −40 °C until further use.[3]

HPLC Analysis of Phenolic Acids Profile. Phenolics were analyzed according to the method described previously.[7] Samples were analyzed with a Varian ProStar HPLC system separation module (Varian, Palo Alto, CA) equipped with ProStar 325 UV–vis detector. The column used was a 250 mm × 4.6 mm i.d., 5 μm Varian ChromSep C18 SS, with a guard column ChromSpher 5 C18 SS 10 mm × 2 mm. The column thermostat was set at 40 °C. The mobile phase consisted of 4.5% acetic acid (solvent A) and 50% acetonitrile (solvent B) at a flow rate of 0.8 mL/min. At the end of the gradient, the column was washed with 50% acetonitrile and equilibrated to the initial condition for 10 min. Quantitative determinations were carried out with the external standard calculation, using calibration curves of the standards. The gradient elution was used as follows: 0 min 92% A, 30 min 70% A, 45 min 60%, 80 min 61% A, 82 min 0% A, 85 min 0% A, 86 min 92% A, 90 min 92% A. Detection was carried out at 270 and 370 nm. Comparison of their retention times with those of the standard compounds was conducted to identify phenolic compounds in a sample. Phenolics were expressed in mg/g of dry weight (DW).

Determination of DPPH Radicals Scavenging Activity (DPPH). Forty μL of methanolic extracts was mixed with 1.80 mL of 6 μM solution of DPPH$^{\bullet}$ in methanol. Absorbance at 715 nm was measured immediately and after 10 min of incubation.[8] The affinity of test material to quench DPPH free radical was evaluated according to equation:

$$\text{scavenging\%} = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \times 100$$

where: $A_{C(0)}$, absorbance of control at 0 min; $A_{A(t)}$, absorbance of sample after 10 min.

Reducing Power Assay (RED). Sample (2.5 mL) was mixed with 2.5 mL of 200 mM phosphate buffer (pH 6.6, prepared by dilution of $NaH_2PO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}7H_2O$ stock solutions mixed with proper proportions) and 2.5 mL of 1% (w/v) aqueous solution of potassium ferricyanide $K_3[Fe(CN_6)]$. The mixture was incubated at 50 °C for 20 min. A portion (0.5 mL) of 10% (w/v) trichloroacetic acid was added to the mixture, which was then centrifuged at 25*g* for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of 0.1% (w/v) $FeCl_3$, and the absorbance was measured at 700 nm.[9] Data were expressed as a quercetin equivalent (QE) μg/mg of DW.

Chelating Power Assay (CHEL). The extract samples (0.5 mL) was added to a 0.1 mL of 2 mM $FeCl_2$ solution and 0.2 mL 5 mM ferrozine, and the mixture was shaken vigorously and left standing at room temperature for 10 min.[10] Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was given below formula:

$$\%\text{inhibition} = [1 - (A_A/A_c)] \times 100$$

where: A_c, absorbance of control; A_A, absorbance of sample

Inhibition of Linoleic Acid Peroxidation (LPO). The antioxidant activity was determined as a degree of inhibition on the hemoglobin-catalyzed peroxidation of linoleic acid.[11] Ten μL of sample were mixed with 0.37 mL of 50 mM phosphate buffer (pH 7) containing 0.05% (v/v) Tween 20 and 4 mM linoleic acid and then equilibrated at 37 °C for 3 min. The peroxidation of linoleic acid was initiated by adding 0.02 mL 0.035% (w/v) hemoglobin (in water) followed by incubation in a shaking bath at 37 °C for 10 min and stopped by adding 5 mL 0.6% HCL (v/v; in ethanol). The hydroperoxide formed was assayed according to a ferric thiocyanate method with mixing in order of 20 mM $FeCl_2$ (0.1 mL) and 30% (w/v) ammonium thiocyanate (0.1 mL). The absorbance at 480 nm (A_s) was measured for 5 min. The absorbance of blank (A_0) was obtained without addition hemoglobin to the above reaction mixture; the absorbance of control (A_{100}) was obtained with no sample addition to the above mixture. The antioxidative activity of the sample was calculated as:

$$AA[\%] = [1 - (A_s - A_0)/(A_{100} - A_0)] \times 100$$

where: A_0, absorbance of blank; A_s, absorbance of sample; A_{100}, absorbance of control

Superoxide Anion Scavenging Activity (SASA). One mL of nitroblue tetrazolium (NBT) solution (156 μg NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468 μg in 100 mM phosphate buffer, pH 7.4), and 0.1 mg of sample solution were mixed. The reaction started by adding 100 μL of phenazinemetholsulphate (PMS) solution (60 μg NBT in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm against blank samples were measured. Decreased of absorbance indicated an increased superoxide anion scavenging activity.[12]

The affinity of test material to quench superoxide anion was evaluated according to equation:

$$\text{SASA\%} = [(A_{(0)} - A_A)/A_{(0)}] \times 100$$

where: $A_{(0)}$, absorbance of control; A_A, absorbance of sample

Assay for Superoxide Dismutase-Like Activity (SOD-like). Superoxide dismutase SOD-like activity was assayed by the method described previously.[13] The reaction mixture was prepared by mixing 0.2 mL of the sample solution, 2.6 mL of the Tris–HCl buffer (50 mM TRIZMA+ 10 mM EDTA, pH 8.5), and 0.2 mL of 7.2 mM pyrogallol and kept at 25 °C for 10 min. The oxidized pyrogallol was measured at 420 nm after stopping the reaction by adding 0.1 mL of 1.0 M HCl. The SOD-like activity was calculated using the following equation:

$$\text{SOD-like activity}[\%] = [1 - (A_s/A_c)] \times 100$$

where: A_s, absorbation of tested sample; A_c, absorbation of control sample

Inhibition of Lipoxygenase (LOXI). Lipoxygenase activity was determined spectrophotometrically at temperature 25 °C by measuring the increase of absorbance at 234 nm over a 2 min period.[14] Briefly, the reaction mixture contained 2.45 mL 1/15 mol/L phosphate buffer, 0.02 mL of lipoxygenase solution (167 U/mL), and 0.05 mL of inhibitor (spelt extract) solution. After preincubation of the mixture at 30 °C for 10 min, the reaction was initiated by adding 0.08 mL 2.5 mmol/L linoleic acid. One unit of LOX activity was defined as an increase in absorbance of 0.001/min at 234 nm.

OH$^{\bullet}$ Scavenging Assay (OH). OH$^{\bullet}$ scavenging assay was performed according to procedure previously described.[15] Hydroxyl radicals were generated by Fenton reaction in the system of $FeSO_4$ and H_2O_2. The reaction mixture was consisted of 0.5 mL of $FeSO_4$ (8 mM), 0.8 mL of H_2O_2 (6 mM), 0.5 mL distilled water, 1.0 mL of extract, and 0.2 mL sodium salicylate (20 mM). The total mixture

Table 1. Phenolic Acids Profile of Spelt Varieties Extracts[a]

	spelt cultivar											
	Ostro		Schwabenspelz		Spelt INZ		Schwabenkorn		Ceralio		Oberkulmer Rotkorn	
	concentration [μg/g DW, ± SD]											
phenolic acid	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound
G[b]	0 ± 0.00^{a}	78.5 ± 6.34^{B}	16.3 ± 0.98^{d}	0 ± 0.00^{A}	8.8 ± 0.34^{c}	0 ± 0.00^{A}	5.4 ± 0.33^{b}	0 ± 0.00^{A}	46.4 ± 2.78^{e}	0 ± 0.00^{A}	0 ± 0.00^{a}	0 ± 0.00^{A}
PK[b]	47.0 ± 3.78^{d}	16.8 ± 0.76^{F}	9.8 ± 0.78^{c}	13.9 ± 0.87^{E}	86.5 ± 5.22^{a}	0.4 ± 0.07^{A}	78.2 ± 3.99^{a}	7.1 ± 0.22^{C}	0.8 ± 0.56^{b}	11.04 ± 1.01^{D}	60.21 ± 4.59^{e}	5.1 ± 0.21^{B}
PHB[b]	2.0 ± 0.32^{c}	12.4 ± 0.54^{A}	0.8 ± 0.29^{a}	14.0 ± 0.57^{AB}	0.3 ± 0.09^{a}	12.5 ± 0.54^{A}	13.4 ± 1.87^{d}	16.2 ± 1.56^{C}	6.9 ± 0.45^{b}	5.0 ± 0.42^{D}	7.3 ± 0.44^{b}	14.5 ± 1.76B^{C}
C[b]	0.2 ± 0.09^{a}	2.1 ± 0.22^{B}	0.5 ± 0.05^{bc}	6.2 ± 0.45^{D}	0.4 ± 0.02^{ab}	3.4 ± 0.31^{A}	1.0 ± 0.24^{c}	3.8 ± 0.23^{A}	2.7 ± 0.61^{e}	2.8 ± 0.13^{C}	1.7 ± 0.11^{d}	6.1 ± 0.33^{E}
V[b]	0.1 ± 0.07^{a}	1.5 ± 0.09^{C}	0.2 ± 0.06^{ab}	3.5 ± 0.21^{A}	1.2 ± 0.08^{de}	3.6 ± 0.17^{A}	0.3 ± 0.05^{c}	2.7 ± 0.07^{E}	1.4 ± 0.08^{e}	2.0 ± 0.02^{D}	1.0 ± 0.10^{d}	3.1 ± 0.12^{B}
SYR[b]	13.0 ± 0.67^{b}	0.5 ± 0.20^{B}	3.0 ± 0.18^{c}	2.7 ± 0.32^{A}	1.3 ± 0.07^{a}	3.0 ± 0.12^{A}	1.0 ± 0.18^{a}	2.10 ± 0.11^{C}	12.9 ± 2.21^{b}	0.8 ± 0.24^{B}	50.1 ± 3.78^{d}	2.8 ± 0.17^{A}
PC[b]	0 ± 0.00^{b}	2.4 ± 0.54^{C}	0.6 ± 0.12^{ac}	13.6 ± 0.55^{A}	0 ± 0.00^{b}	13.0 ± 0.34^{A}	0.3 ± 0.03^{a}	9.8 ± 0.77^{D}	0.4 ± 0.06^{a}	0.8 ± 0.07^{B}	0.8 ± 0.20^{c}	14.1 ± 0.78^{A}
SYN[b]	2.1 ± 0.08^{c}	95.3 ± 3.87^{B}	5.9 ± 0.45^{d}	445.4 ± 16.89^{D}	3.0 ± 0.11^{b}	287.6 ± 9.44^{C}	0.6 ± 0.07^{a}	262.5 ± 13.76^{C}	0.8 ± 0.10^{a}	87.4 ± 5.87^{AB}	2.9 ± 0.23^{b}	76.5 ± 3.56^{A}
FER[b]	1.2 ± 0.04^{a}	215.2 ± 10.12^{A}	23.9 ± 1.21^{c}	667.9 ± 23.11^{E}	14.5 ± 0.76^{b}	614.5 ± 11.23^{D}	1.1 ± 0.32^{a}	549.0 ± 30.00^{C}	41.5 ± 2.43^{d}	219.5 ± 17.00^{A}	11.7 ± 1.77^{b}	132.5 ± 9.22^{B}
SAL[b]	0 ± 0.00^{a}	0.8 ± 0.02^{B}	9.2 ± 0.65^{c}	0 ± 0.00^{A}	5.8 ± 0.45^{b}	3.0 ± 0.32^{D}	7.3 ± 1.00^{b}	1.6 ± 0.22^{C}	0 ± 0.00^{a}	0 ± 0.00^{A}	12.7 ± 0.87^{d}	0 ± 0.00^{A}
OC[b]	0 ± 0.00^{a}	0.3 ± 0.03^{D}	0 ± 0.00^{a}	0 ± 0.00^{B}	0.04 ± 0.02^{b}	0.5 ± 0.03^{A}	0 ± 0.00^{a}	0.5 ± 0.02^{A}	0 ± 0.00^{a}	0.1 ± 0.01^{C}	0.1 ± 0.02^{b}	1.7 ± 0.11^{E}
CH[b]	6.4 ± 0.35^{bc}	8.4 ± 0.39^{C}	5.5 ± 0.52^{ab}	28.6 ± 1.78^{B}	7.5 ± 0.59^{c}	26.2 ± 2.89^{B}	4.4 ± 0.64^{a}	17.1 ± 0.56^{D}	15.0 ± 0.77^{d}	3.5 ± 0.45^{A}	13.1 ± 0.23^{c}	4.2 ± 0.22^{A}
∑	72.6	434.0	75.6	1181.8	129.3	967.5	112.9	872.4	128.8	332.9	161.7	260.5
∑TPA	506.6		1257.4		1096.77		985.25		461.7		422.2	
F/B ratio	0.17		0.06		0.13		0.13		0.39		0.62	

[a]Means in the lines followed by different small letters (concerning free phenolic acids) are significantly different at $p < 0.05$. Means in the lines followed by different capital letters (concerning bound phenolic acids) are significantly different at $p < 0.05$. [b]G, gallic acid; PK, protocatechuic acid; PHB, *p*-hydroxybenzoic acid; C, caffeic acid; V, vanillic acid; SYR, syringic acid; PC, *p*-coumaric acid; SYN, sinapinic acid; FER, ferulic acid; SAL, salicylic acid; OC, *o*-coumaric acid; CH, chlorogenic acid.

dx.doi.org/10.1021/jf3011239 | J. Agric. Food Chem. 2012, 60, 4603−4612

(3.0 mL) was incubated at 37 °C for 1 h, and then the absorbance of the mixture was recorded at 562 nm. The scavenging activity was calculated using the following equation:

$$\text{scavenging activity}[\%] = [1 - (A_1 - A_2)/A_0] \times 100$$

where: A_0, absorbance of the control (without extract); A_1, absorbance of the extract addition, and A_2, absorbance without sodium salicylate.

Total Antioxidant Capacity Index (AI). Sample concentration (mg DW/mL) providing 50% inhibition (EC_{50}) was calculated from the graph plotting inhibition percentage. Lower EC_{50} means a higher potency. Additionally, phenolic acid concentration providing 50% activity (PAC_{50}) was determined in a dose-dependent manner (data not shown) of spelt antioxidant action.

Eight antioxidant methods were integrated to obtain the total antioxidant capacity index (AI) that may be used for comparison of total antioxidant capacity of spelt varieties. The AI of each spelt was calculated as the sum of relative activities (RA) for each antioxidant chemical methods. RA was calculated as follows:

$$RA = A_m/A_{max}$$

where: A_m, measured activity (EC_{50}); A_{max}, maximal activity determined for the method

Statistical Analysis. All experimental results were mean ± SD of three (three extracts and three measurements for each extract) parallel experiments (n = 9). Statistical tests were evaluated by using the Statistica 6.0 software (StatSoft, Inc., Tulsa, OK, USA). The obtained data were subjected to statistical analysis and the consequent evaluations were analyzed for variance analysis. The statistical differences were estimated through Tukey's test. All the statistical tests were carried out at a significance level of α = 0.05.

RESULTS

Phenolic Acids Profile. As presented in Table 1, phenolic acids composition of spelt differs significantly between varieties. It could be seen that dominant free phenolic acids were protocatechuic, ferulic, and chlorogenic. Protocatechuic acid was the dominant free phenolic acid in Ostro, Spelt INZ, Schwabenkorn, and Oberkulmer Rotkorn grains (47.5, 86.5, 78.2, and 60.2 μg/g, respectively). The free phenolic fractions of the Ostro, Ceralio, and Oberkulmer Rotkorn grains contained larger amounts of syringic acid than the bound phenolic extracts. Gallic acid mainly occurred in free phenolic fraction. Only in Ostro grains, bound phenolics extract contained this compound. In all samples, a relatively low level of caffeic acid was determined, however the bound phenolics extract contained larger amounts of this compound as compared to the free phenolics extracts. It is noteworthy that phenolic acids were strongly bound with matrix and were effectively released during hydrolysis. For example, *p*-coumaric, *p*-hydroxybenzoic, ferulic, sinapinic, and chlorogenic acids occurred mainly in the bound phenolics. Profile of phenolic acids released after hydrolysis showed that ferulic and sinapinic acids were dominant components of bound phenolic acids fraction. For Schwabenspelz, Spelt INZ, and Schwabenkorn, these phenolics constituted 80%, 78%, and 88% of bound phenolic acids, respectively. It is worth mentioning that in Ceralio, no salicylic acid was detected, whereas Schwabenspeltz grain did not contain *o*-coumaric acid. An example of HPLC analysis of phenolic acids is presented in Figure 1. Total phenolic acid level and contribution of individual phenolic acid in the phenolics extract of spelt grain differ significantly between cultivars (Table 1). The highest total phenolic acids (TPA) content was determined for Schwabenspelz (1257.4 μg/g), whereas the lowest was for Ceralio (461.7 μg/g). The highest amount of free phenolic acids were found for Oberkulmer Rotkorn (161.8 μg/g), whereas the lowest was in Ostro and Schwabenspelz grains (72.6 and 75.6 μg/g, respectively). Contrary to this, the highest amounts of bound phenolic acids were determined in Schwabenspelz (1181.8 μg/g) and Spelt INZ (967.5 μg/g). In our study, the free/bound phenolic acid ratio was determined. Taking into account this parameter, spelt varieties were ordered as follows: Oberkulmer Rotkorn > Ceralio > Ostro > Spelt INZ ≈ Schwabencorn > Schwabenspelz (Table 1).

Antioxidant Activity. Considering the chemical diversity of the antioxidant compounds present in food and the interactions occurring among those different molecules, the evaluation of total antioxidant capacity seems to be a more useful marker as opposed to the evaluation of a single compound. In this experiment, six methods (LPO, antiradical, chelating, and reducing power estimation) were applied to analyze the antioxidant activity of spelt grains. Additionally, SOD-like activity and LOX inhibition activity were determined. In the light of data presented in Table 2, all tested spelt cultivars possessed high nutraceutical potential based on various mechanisms of antioxidant action. It was observed that all analyzed spelt samples possessed the ability to prevent lipids against oxidation. Taking into account the free phenolics fraction, the highest activity was observed for Ceralio extract (EC_{50} = 18.9 mg/mL) whereas the lowest was for Schwabenkorn extract (EC_{50} = 125.8 mg/mL). It was noted that $OH^{\bullet}$, O^{2-}, and DPPH radicals were effectively scavenged by free phenolics extracts from Spelt INZ (EC_{50} = 20.6, 30.2, and 154.3 mg/mL, respectively). High ability to quench O^{2-} radicals and inhibit LOX was also determined for Ostro free phenolics fraction. Free phenolics from Ceralio and Oberkulmer Rotkorn possessed a high ability to chelate metal ions. Irrespective of the spelt variety and methods used, higher antioxidant activity was demonstrated by bound phenolics fraction. It is worth noting that bound phenolics from all studied varieties, except Schwabenkorn, effectively prevent lipids from oxidation. Significantly higher SOD-like activity and ability to neutralize $OH^{\bullet}$ radicals were determined for bound phenolics fraction from Schwabenkorn, Ceralio, Oberkulmer Rotkorn, and Spelt INZ. It should be noted that extracts of Spelt INZ and Oberkulmer Rotkorn inhibited LOX activity by a lesser degree than the other studied cultivars. As it can be observed from the Table 2 that the highest DPPH scavenging activity was determined for Schwabenspelz (EC_{50} = 74.9 mg/mL) whereas the lowest was for Schwabenkorn (EC_{50} = 182.7 mg/mL). On the other hand, when antioxidant activity was expressed as PAC_{50}, it is clearly visible that in all spelt varieties, except Oberkulmer Rotkorn, free phenolic acids comprised the most active fraction (Table 3).

For better estimation of total antioxidant potential, the antioxidant index (AI) was determined. Data presented in Table 4 show that the highest relative activity (RA) values were obtained in both free and bound phenolics extracts from Ceralio, whereas the lowest were in Schwabenkorn extracts. Total antioxidant potential of spelt cultivars were ordered as follows: Ceralio > Spelt INZ ≈ Ostro > Oberkulmer Rotkorn > Schwabenspelz > Schwabenkorn.

DISCUSSION

Phenolic Acids Profile. The health benefit (lower risk of cardiovascular disease, ischemic stroke, type II diabetes, metabolic syndrome, and gastrointestinal cancers) of whole grain consumption may be due to their unique phytochemical

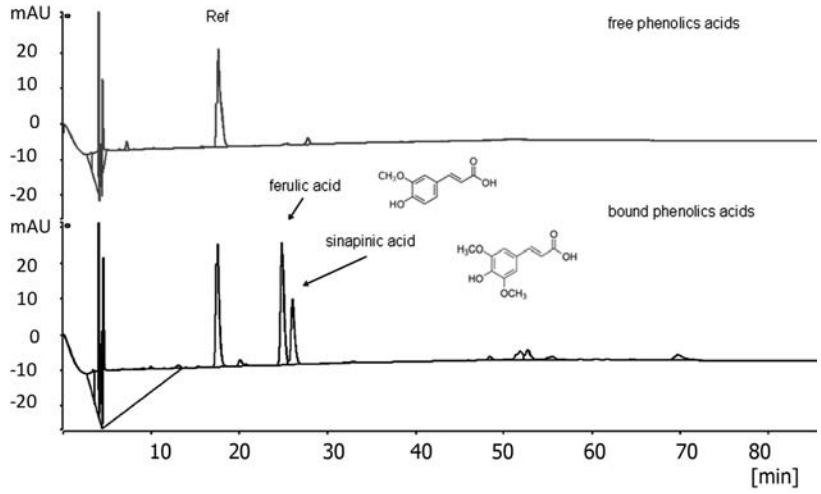

Figure 1. HPLC profile of free and bound phenolic acids of spelt (Schwabenspelz). The arrows indicate the main constituents of phenolic acids fraction: ferulic and sinapinic acids.

Table 2. Antioxidant Activity of Spelt Cultivars

		spelt cultivar					
activity	extract	Ostro EC_{50} [mg DW/mL]	Schwabenspelz EC_{50} [mg DW/mL]	Spelt INZ EC_{50} [mg DW/mL]	Schwabenkorn EC_{50} [mg DW/mL]	Ceralio EC_{50} [mg DW/mL]	Oberkulmer Rotkorn EC_{50} [mg DW/mL]
LPO	free	39.4 ± 1.32^{c}	20.3 ± 0.89^{a}	26.4 ± 1.99^{b}	125.9 ± 9.08^{d}	19.0 ± 0.38^{a}	20.7 ± 1.40^{a}
	bound	24.2 ± 1.71^{c}	13.2 ± 0.88^{a}	14.4 ± 0.54^{a}	113.8 ± 8.11^{d}	8.7 ± 0.88^{b}	13.2 ± 1.20^{a}
CHEL	free	73.3 ± 9.11^{b}	101.1 ± 7.22^{c}	74.0 ± 6.43^{b}	127.9 ± 2.79^{d}	48.6 ± 1.90^{a}	55.5 ± 7.10^{a}
	bound	40.1 ± 3.19^{b}	91.4 ± 7.12^{c}	33.7 ± 2.32^{a}	83.1 ± 4.16^{c}	29.8 ± 4.33^{a}	35.5 ± 2.01^{ab}
OH	free	26.2 ± 2.23^{b}	29.4 ± 3.11^{b}	20.7 ± 0.99^{a}	20.6 ± 1.29^{a}	27.5 ± 1.56^{b}	20.6 ± 2.08^{a}
	bound	23.7 ± 1.45^{d}	18.0 ± 0.67^{c}	11.3 ± 0.34^{a}	11.8 ± 0.11^{a}	12.5 ± 0.78^{ab}	13.1 ± 0.61^{b}
SASA	free	21.1 ± 1.30^{b}	33.3 ± 1.00^{a}	30.2 ± 2.06^{a}	40.9 ± 1.56^{c}	58.8 ± 2.09^{d}	30.5 ± 2.54^{a}
	bound	11.5 ± 1.78^{a}	27.6 ± 2.34^{d}	14.2 ± 2.11^{ab}	22.4 ± 1.41^{c}	34.0 ± 0.77^{e}	17.3 ± 0.98^{b}
SOD-LIKE	free	27.3 ± 0.45^{c}	20.7 ± 1.87^{b}	18.5 ± 0.11^{a}	18.4 ± 0.21^{a}	18.5 ± 0.55^{a}	18.4 ± 1.04^{a}
	bound	15.7 ± 0.71^{c}	15.8 ± 0.66^{c}	8.8 ± 1.23^{b}	11.3 ± 0.39^{a}	11.3 ± 0.22^{a}	11.8 ± 2.01^{ab}
LOXI	free	64.2 ± 3.34^{a}	113.2 ± 9.11^{b}	641.6 ± 23.10^{d}	110.0 ± 3.07^{b}	72.6 ± 5.11^{a}	350.0 ± 11.23^{c}
	bound	41.7 ± 1.11^{a}	62.7 ± 3.87^{b}	350.6 ± 23.11^{d}	72.4 ± 8.00^{b}	44.5 ± 3.45^{a}	166.7 ± 8.65^{c}
DPPH	free	228.3 ± 11.23^{a}	158.7 ± 9.43^{b}	154.3 ± 5.99^{b}	277.8 ± 19.07^{a}	248.7 ± 10.30^{a}	245.1 ± 8.88^{a}
	bound	139.2 ± 8.22^{a}	74.9 ± 2.78^{b}	95.3 ± 6.00^{c}	182.7 ± 12.05^{d}	150.8 ± 9.11^{a}	140.0 ± 5.44^{a}
RED^{a}	free	26.5 ± 1.56^{ab}	26.5 ± 2.00^{ab}	23.9 ± 1.87^{a}	23.1 ± 1.23^{a}	29.1 ± 1.23^{b}	25.6 ± 2.11^{ab}
	bound	56.4 ± 3.33^{c}	45.9 ± 0.99^{a}	44.0 ± 1.10^{a}	40.7 ± 2.29^{ab}	35.1 ± 1.96^{b}	46.9 ± 2.78^{a}

^{a}Expressed as quercetin equivalent [μgQ/mL]. ^{b}Means followed by different letters in the lines are significantly different at $p < 0.05$. ^{c}LPO, inhibition of linoleic acid peroxidation; CHEL, chelating power; OH, OH$^{\bullet}$ radicals scavenging assay; SASA, superoxide anion scavenging activity; SOD-LIKE, superoxide dismutase-like activity; LOXI, ability to inhibition of lipoxygenase activity; DPPH, DPPH radicals scavenging activity; RED, reducing power.

composition. The common phenolic acids found in whole grains were ferulic acid, vanillic acid, caffeic acid, syringic acid, and *p*-coumaric acid.[4] Ferulic and other phenolic acids protect wheat kernels by providing both physical and chemical barriers through cross-linking carbohydrates, antioxidant activities to combat destructive radicals, and astringency that deters consumption by insects and animals.[16] Differences in phenolic acid composition among cultivars have been observed.[17] Ferulic acid, a hydroxycinnamic acid derivative, is the predominant phenolic acid found in whole grains.[18] Okarter et al.[2] reported that the total ferulic acid content of 11 wheat varieties ranged from 286.5 to 587.8 μg/g DW, with the bound ferulic acid accounting for more than 97% of the total ferulic acid content. The total ferulic acid content of eight Maryland-grown soft wheat samples ranged from 455.5 to 620.8 μg/g DW, with the bound ferulic acid accounting for more than 89% of the total ferulic acid content.[17] Li et al.[19] reported that the total ferulic acid contents of spring wheat and winter wheat were 787.3 and 773.6 μg/g DW, respectively. In our study, significantly higher ferulic acid contents were observed for Schwabenspelz, Spelt

dx.doi.org/10.1021/jf3011239 | J. Agric. Food Chem. 2012, 60, 4603−4612

Table 3. Antioxidant Activity of Spelt Cultivars Expressed As PAC_{50}[a]

		spelt cultivar					
	phenolics fraction	Ostro PAC_{50} [μg phenolic acids/mL]	Schwabenspelz PAC_{50} [μg phenolic acids/mL]	Spelt INZ PAC_{50} [μg phenolic acids/mL]	Schwabenkorn PAC_{50} [μg phenolic acids/mL]	Ceralio PAC_{50} [μg phenolic acids/mL]	Oberkulmer Rotkorn PAC_{50} [μg phenolic acids/mL]
LPO[b]	free	2.9 ± 0.10^{d}	1.5 ± 0.07^{b}	3.4 ± 0.26^{a}	14.20 ± 1.02^{e}	2.4 ± 0.05^{c}	3.3 ± 0.23^{a}
	bound	10.5 ± 0.74^{b}	15.6 ± 1.04^{cd}	14.0 ± 0.52^{bc}	99.2 ± 7.08^{e}	2.9 ± 0.29^{a}	3.4 ± 0.31^{a}
CHEL[b]	free	5.3 ± 0.66^{b}	7.6 ± 0.55^{a}	9.6 ± 0.83^{a}	14.4 ± 0.31^{c}	6.3 ± 0.24^{b}	9.0 ± 1.15^{a}
	bound	17.4 ± 1.38^{b}	108.0 ± 8.41^{e}	32.6 ± 2.24^{c}	72.5 ± 3.63^{d}	9.9 ± 1.44^{a}	9.3 ± 0.52^{a}
OH[b]	free	1.9 ± 0.16^{b}	2.2 ± 0.24^{abc}	2.7 ± 0.13^{a}	2.3 ± 0.15^{ac}	3.5 ± 0.20^{d}	3.3 ± 0.34^{d}
	bound	10.3 ± 0.63^{a}	21.3 ± 0.79^{c}	10.9 ± 0.33^{a}	10.3 ± 0.10^{a}	4.2 ± 0.26^{b}	3.4 ± 0.16^{b}
SASA[b]	free	1.5 ± 0.09^{c}	2.5 ± 0.08^{d}	3.9 ± 0.27^{a}	4.6 ± 0.18^{ab}	7.6 ± 0.27^{e}	4.9 ± 0.41^{b}
	bound	5.09 ± 0.77^{a}	32.7 ± 2.77^{d}	13.7 ± 2.04^{b}	19.5 ± 1.23^{c}	11.3 ± 0.26^{b}	4.5 ± 0.26^{a}
SOD-LIKE[b]	free	2.0 ± 0.03^{a}	1.6 ± 0.14^{c}	2.4 ± 0.01^{b}	2.1 ± 0.02^{a}	2.4 ± 0.07^{b}	3.0 ± 0.17^{d}
	bound	6.8 ± 0.31^{b}	18.7 ± 0.78^{d}	8.5 ± 1.19^{bc}	9.9 ± 0.34^{c}	3.8 ± 0.07^{a}	3.1 ± 0.52^{a}
LOXI[b]	free	4.7 ± 0.24^{b}	8.6 ± 0.69^{a}	83.0 ± 2.99^{d}	12.4 ± 0.35^{a}	9.4 ± 0.66^{a}	56.6 ± 1.82^{c}
	bound	18.1 ± 0.48^{a}	74.1 ± 4.57^{b}	339.2 ± 22.36^{d}	63.1 ± 6.98^{b}	14.8 ± 1.015^{a}	43.4 ± 2.25^{c}
DPPH[b]	free	16.6 ± 0.81^{c}	12.0 ± 0.71^{b}	20.0 ± 0.77^{d}	31.4 ± 2.22^{a}	32.0 ± 1.33^{a}	39.6 ± 1.44^{e}
	bound	60.4 ± 3.57^{a}	88.5 ± 3.29^{b}	92.2 ± 5.80^{b}	159.4 ± 10.51^{d}	50.2 ± 3.03^{a}	36.5 ± 1.42^{c}
RED[b]	free	365.2 ± 21.50^{c}	350.4 ± 26.45^{c}	185.1 ± 1.81^{ab}	204.5 ± 10.90^{a}	225.7 ± 9.55^{a}	158.6 ± 13.05^{b}
	bound	130.1 ± 7.67^{c}	38.8 ± 0.84^{a}	45.5 ± 1.06^{a}	46.7 ± 3.26^{a}	105.3 ± 5.89^{b}	180.0 ± 10.67^{d}

[a] PAC_{50} – define the phenolic acids concentration providing 50% of antioxidant activity. Means followed by different letters in the lines are significantly different at $p < 0.05$. [b] LPO, inhibition of linoleic acid peroxidation; CHEL, chelating power; OH, $OH^{\bullet}$ scavenging assay; SASA, superoxide anion scavenging activity; SOD-LIKE, superoxide dismutase-like activity; LOXI, ability to inhibition of lipoxygenase activity; DPPH, DPPH radicals scavenging activity; RED, reducing power.

dx.doi.org/10.1021/jf3011239 | J. Agric. Food Chem. 2012, 60, 4603−4612

Table 4. Relative Activities and Total Antioxidant Potential of Spelt

antioxidant activity	phenolics fraction	spelt cultivar					
		Ostro	Schwabenspelz	Spelt INZ	Schwabenkorn	Ceralio	Oberkulmer
		Rotkorn Relative Activity (RA)					
LPO[a]	free	0.22	0.43	0.33	0.07	0.46	0.42
	bound	0.36	0.66	0.60	0.08	1.00	0.65
CHEL[a]	free	0.41	0.29	0.40	0.23	0.61	0.54
	bound	0.74	0.33	0.88	0.36	1.00	0.84
OH[a]	free	0.43	0.38	0.55	0.55	0.41	0.55
	bound	0.48	0.63	1.00	0.96	0.90	0.86
SASA[a]	free	0.54	0.34	0.38	0.28	0.20	0.38
	bound	1.00	0.42	0.81	0.51	0.34	0.66
SOD-LIKE[a]	free	0.32	0.42	0.47	0.48	0.47	0.48
	bound	0.56	0.55	1.00	0.77	0.77	0.74
LOXI[a]	free	0.65	0.37	0.06	0.38	0.57	0.12
	bound	1.00	0.67	0.12	0.58	0.94	0.25
DPPH[a]	free	0.33	0.47	0.49	0.27	0.30	0.31
	bound	0.54	1.00	0.79	0.41	0.50	0.53
RED	free	0.47	0.47	0.42	0.41	0.51	0.45
	bound	1.00	0.81	0.78	0.72	0.62	0.83
		Antioxidant Index (AI)					
free		3.37	3.18	3.11	2.66	3.54	3.24
bound		5.67	5.06	5.98	4.38	6.07	5.38
total		9.04	8.24	9.08	7.05	9.61	8.61
free/bound ratio		0.59	0.63	0.52	0.61	0.58	0.60

[a]LPO, inhibition of linoleic acid peroxidation; CHEL, chelating power; OH, OH• scavenging assay; SASA, superoxide anion scavenging activity; SOD-LIKE, superoxide dismutase-like activity; LOXI, ability to nhibition of lipoxygenase activity; DPPH, DPPH radicals scavenging activity; RED, reducing power.

INZ, and Schwabenkorn, whereas Oberkulmer Rotkorn, Ostro, and Ceralio extracts contained significantly lower amount of ferulic acid (144.2, 216.2, and 261.1 μg/g DW, respectively). Values obtained in our study were also generally in agreement with data provided by Mpofu et al.[20] Significantly higher values in comparison to our studies were obtained by Siebenhandl et al.[18] and Mattila et.al.[21] In our study, bound ferulic acid contributed 84−99% to the total ferulic acid content. These findings are similar to those reported by Okarter et al.[2] Data concerning durum wheat showed that its bran contained protocatechuic, *p*-hydroxybenzoic, caffeic,vanillic, chlorogenic, syringic, *p*-coumaric, and ferulic acids at concentrations of 226, 124, 116, 637, 84, 130, 580, and 764 μg/g, respectively.[22] Zhou et al.[23] reported similar phenolic acids in durum wheat but at lower levels. These results were confirmed by our study. Grains of analyzed spelt cultivars contained above-mentioned phenolic acids, but their amounts differ significantly. Sinapinic acid contents reported in the present study are higher than those previously reported.[3] Schwabenspelz, Spelt INZ, and Schwabencorn varieties were especially rich in this phenolic acid. Okarter et al.[2] stated that *p*-coumaric acid was the second most abundant phenolic acid found in whole wheat. In our study, the highest content of this compound was observed in Schwabenspelz, Spelt INZ, and Oberkulmer Rotkorn kernels (14.4, 12.9, and 14.9 μg/g, respectively). These data are in agreement with those provided previously.[17] In whole wheat hydroxybenzoic acid derivatives (protocatechuic, *p*-hydroxybenzoic, salicylic, vanillic, and syringic acids) have also been reported.[4] These reports are in agreement with our results. The dominant hydroxybenzoic acid derivatives in spelt extracts were protocatechuic and syringic acids. In the study of Moore et al.,[17] only two of the eight wheat varieties had any syringic acid in the free fraction. Contrary to this, in our studies, syringic acid was determined in free phenolics fraction of all spelt varieties. Additionally, in Ostro, Ceralio, Schwabenspelz, and Oberkulmer Rotkorn, amounts of free acids were significantly larger than the bound ones (Table 1).

Antioxidant Activity. The antioxidant activity is of a fundamental importance to human life. Many of the biological functions, including antimutagenicity, anticarcinogenicity, and antiaging, among others, may originate from this property.[24] In vitro methods used to measure antioxidant activity (i.e., ABTS, DPPH, ORAC, or FRAP) do not provide information about the bioavailability or metabolism of these compounds in biological system. However, these methods are useful to screen and compare antioxidant activity levels among a wide variety of samples. The different contribution of individual phenolic acid in the phenolic extracts resulted in the different antioxidant capacity of the extracts.[2] Comparison of the antioxidant capacity of the spelt varieties used in these studies is difficult because the different antioxidant capacity assay has not been standardized.

Reduction of DPPH radicals reveals that examined extracts possessed radical inhibitors or scavengers acting as primary antioxidants. They might react with free radicals, particularly with the peroxy radicals, which are the major propagators of the auto-oxidation chain of fat, thereby terminating the chain reaction.[4] Hung et al.[3] stated that percentage DPPH$^{\bullet}$ scavenging of the free and bound phenolics extracted from whole waxy wheat were 15.5% and 27.9%, respectively. These results were higher than those provided by our study. DPPH radical scavenging activity of spelt bound phenolics were comparable to the activity of white wheat flour. Also, Sedej et al.[25] proved that IC_{50} value of wheat ethanolic extract was 31.2 mg/mL. In our study, the most active fraction of bound phenolics from Schwabenspelz and Spelt INZ average about 74.9 and 95.3 mg/mL, respectively. Reactive oxygen species (ROS) and free radicals such as superoxide anion (O^{2-}) and hydroxyl radical (OH^{-}) are constantly formed in the human body by normal metabolic action and have been implicated in the pathogenesis of certain human diseases. Their action is opposed by a balanced system of antioxidant defenses including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death. All analyzed spelt varieties showed high abilities to scavenge OH$^{\bullet}$ and O^{2-} radicals. Most importantly, activity of free and bound phenolics fractions differ less than in other methods (Tables 2−3). The highest activities against OH$^{\bullet}$ were demonstrated by Spelt INZ, Schwabenkorn, and Oberkulmer Rotkorn grains, whereas O^{2-} radicals were most effectively scavenged by Ostro grain phytochemicals. In the light of previous data,[26] this fact can testified that antiradical-active compound could be highly bioavailable and can prevent upper gastrointestinal tract against ROS damages.

In addition, in the present study, SOD-like activity of spelt extracts were determined. Free phenolics of all samples demonstrated high activity with EC_{50} values ranging from 27.3 to 18.4 mg/mL. These data are in agreement with those obtained during SASA and OH$^{\bullet}$ scavenging assays and indicate that free phenolics of spelt grain demonstrated higher activity against ROS than other antioxidant actions such as lipid prevention, chelating, and reducing power, strongly connected with bound phenolics.

The normal cellular physiology is reliant on the intactness of the plasma membrane. Oxidative damage of membrane lipids by peroxidation may modulate the signal transduction pathways that may consequently affect various downstream processes. Oxidative damage to membrane may also disrupt the ionic channels, membrane transport proteins, and inactivate membrane-associated enzymes, and the lipid bilayer itself may become more permeable due to oxidative damage. A number of chronic diseases are indeed characterized by an intense increase in LPO products.[27] In recent literature, there is a lack of data concerning the inhibition of lipid peroxidation by spelt varieties. Data showed in the studies clearly indicated that spelt grains contained phytochemicals able to prevent lipid against oxidation. In all the varieties, activity (expressed as EC_{50}) of bound phenolic fraction was higher than activity demonstrated by free phenolics. The higher ability to inhibit lipid peroxidation was observed for Ceralio grains. Additionally, all spelt varieties demonstrated the ability to inhibit lipoxygenase activity. It is very interesting that both extracts (free and bound phenolics) for Ostro and Ceralio grains demonstrate high LOXI activity. These results may indicate their potential anti-inflammatory properties.

Because ferrous and cupric ions are the most effective prooxidant in food systems, and ferrous ions are commonly found in food systems, high chelating activity of investigated extracts would be beneficial in retarding metal-catalyzed oxidation.[28] In the present study, higher activity was observed for bound phenolics fractions and ranged from 35.0 to 56.4 μg QE/mL. Reducing power of the free phenolics fraction did not exceed 30 μg QE/mL. In recent study, there is a lack of data concerning the reducing power of spelt varieties. The presence of some more potent chelating component(s) in wheat flour extract might be responsible for its higher chelating capacity. Significant Fe^{2+} chelating activities and inhibitory effects were detected in wheat grain extracts.[22] Chelating power of free and bound phenolics spelt extracts differ significantly. Higher activity was observed in bound phenolic fractions. These results were lower than those obtained by Sedej et al.[25] In recent literature, there is a lack of data concerning chelating abilities of spelt grains.

Bioactivity of Free and Bound Phenolic Acids. Bound phytochemicals can not be digested by human enzymes, could survive stomach and small intestine digestion, and therefore may possibly reach the colon. The colonic microflora may release the bound phytochemicals through fermentation and thus provide site-specific health benefits in colon or other tissues after absorption.[2] For this reason, bound phenolics are often defined as main antioxidants of cereals. On the other hand, Mateo Anson et al.[26] stated that the bioaccessibility of ferulic acid from unprocessed aleurone, bran, and flour was low, which confirms the in vivo low bioavailability from cereal bran consumption. Free ferulic acid was highly bioaccessible, while only a very small part of bound fraction was apparently released during gastrointestinal digestion. The total bioaccessibility remained under 1%. Nevertheless, free ferulic acid in the intestine was extensively absorbed, thus absorption itself is probably not the limiting factor for this compound. In the light of these facts, more attention must be given to free phenolic acids content. Probably the health-promoting properties of grains also depend on free phenolics due their high bioavailability. For this reason, in this study, the free/bound phenolic acid ratio (F/B) was determined. The highest value of this parameter was obtained for Oberkulmer Rotkorn and Ceralio (0.6 and 0.4, respectively), whereas the lowest was for Schwabenspelz (0.06) grains extract (Table 1). High value of this parameter may indicate bioavailability and bioactivity of phenolic nutraceuticals. Hung et al.[3] proved that antioxidant capacity of the bound phenolic extracts also mostly contributed to total antioxidant of the whole grain flour. The total antioxidant capacity of all graded milling fractions were mainly contributed by the bound phenolic extracts (72.5−83.2%). On the other hand, the antioxidant capacity of the bound phenolics of the white flour was only slightly higher than that of the free phenolic extracts. These data are in agreement with those concerning lipid prevention, reducing potential, chelating power, and DPPH-scavenging presented in this study but in opposite to those concerning ROS neutralization. Previous studies[3,29] have proved that the bound phenolic compounds were the primary antioxidants of wheat. Citied investigators stated that these compounds, not extractable by aqueous methanol or ethanol but released upon alkaline, acid, or enzyme hydrolysis, show significant health benefits as compared to the free phenolics in wheat grains. As opposed to this, Mateo Anson et al.[26] proved that binding of ferulic acids to arabinoxylans and other indigestible polysaccharides restricts

its release in the small intestine. Thus, bioavailability of bound phenolics could be questionable. In the light of this data, high anti-ROS activity of free phenolic compounds from spelt can indicate their pro-health capacity. Results obtained in this work seem to confirm the assumption that antioxidant potential of spelt depends on free phenolic acids (Table 4). Because multiple reaction mechanisms and different phase locations are usually involved in measuring the antioxidant capacity of a complex food system, there is no simple universal method by which "total antioxidant capacity" can be measured accurately and quantitatively. The consensus is that multiple methods, based upon different reaction mechanisms, should be used.[30] For this reason, in our study, total antioxidant index was proposed.

As it can be seen in Table 4, the highest AI value was obtained for Ceralio. The similar, comparable AI values were estimated for Ostro and Spelt INZ varieties, whereas the lowest TAI value was determined for Schwabenkorn variety. The lowest free/bound phenolics activity ratio was estimated for Spelt INZ. In other samples, free/bound ratios average about 0.60. These results showed that in Spelt INZ grain, contrary to others varieties, total antioxidant activity was strongly depended on bound phenolics.

Obtained data showed that free and bound phenolic profiles of spelt varieties were comparable to those reported in the literature for wheat. In the light of results obtained, it can be concluded that hydroxybenzoic acid derivatives dominated in the free phenolics fraction, whereas hydroxycinnamic acid derivatives were predominant in the bound phenolics fraction. It was noted that Oberkulmer Rotkorn, Ostro, and Ceralio contained the lowest total phenolic acid content in comparison to other analyzed varieties but their antioxidant potential was the highest. All analyzed spelt varieties possessed high antioxidant potential (Tables 2–4). In spite of the fact that bound phenolic acids possessed higher antioxidant activities (expressed as EC_{50}), analysis of antioxidant potential and their relationship with phenolic acid content showed that free phenolics were more effective (antioxidant activities expressed as PAC_{50}). The phytochemicals found in spelt grains may be responsible for the health benefits associated with whole grain consumption. Therefore, high antioxidant potential of potentially easily bioaccessible and bioavailable phenolics (free phenolic acids), sensory properties comparable to common wheat products, and environmental and ecological aspects could be the additional positive argument for the consumers to buy spelt products. However, future research, especially concerning bioaccessibility and bioavailability of active compounds, is needed to provide high-quality evidence for health benefits of spelt nutraceutical compounds.

AUTHOR INFORMATION

Corresponding Author

*Phone:+ 48-81- 4623327. Fax: +48-81-462332. E-mail: urszula.gawlik@up.lublin.pl.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Agroecology Department of University of Life Sciences in Lublin for plant material.

REFERENCES

(1) Bonafaccia, G.; Galli, V.; Francisci, R.; Mair, V.; Skrabanja, V.; Kreft, I. Characteristics of spelt wheat products and nutritional value of spelt wheat-based bread. *Food Chem.* **2000**, *68*, 437–441.

(2) Okarter, N.; Liu, C.-S.; Sorrels, M.; Liu, R. H. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. *Food Chem.* **2010**, *119*, 249–257.

(3) Hung, P. V.; Hatcher, D. W.; Barker, W. Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food Chem.* **2011**, *126*, 1896–1901.

(4) Dykes, L.; Rooney, L. W. Phenolics compounds in cereal grains and their health benefits. *Cereal Foods World* **2007**, *51*, 105–111.

(5) Verma, B.; Hucl, P.; Chibbar, R. N. Phenolic content and antioxidant properties of bran in 51 wheat cultivars. *Cereal Chem.* **2008**, *58*, 544–548.

(6) Dziki, D.; Laskowski, J. Study to analyze the influence of sprouting of the wheat grain on the grinding process. *J. Food Eng.* **2010**, *96*, 562–567.

(7) Gawlik-Dziki, U.; Świeca, M. Effect of various pH conditions simulated in vivo on the activity of lipophilic antioxidants isolated from selected spices. *Polish J. Food Nutr. Sci.* **2007**, *57*, 19–22.

(8) Brand-Williams, W.; Cuvelier, E.; Berset, C. M. Use of free radical method to evaluate antioxidant activity. *LWT—Food Sci. Technol.* **1995**, *28*, 25–30.

(9) Oyaizu, M. Studies on products of browning reaction—antioxidative activities of products of browning reaction prepared from glucosamine. *Japan. J. Nutr.* **1986**, *44*, 307–315.

(10) Guo, J.-T.; Lee, H.-L.; Chiang, S.-H.; Lin, H.-I.; Chang, C.-Y. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. *J. Food. Drug Anal.* **2001**, *9*, 96–101.

(11) Kuo, J.-M.; Yeh, D.-B.; Pan, B. Rapid photometric assay evaluating antioxidative activity in edible part material. *J. Agric. Food Chem.* **1997**, *47*, 3206–3209.

(12) Liu, F.; Ooi, V. E. C.; Chang, S. T. Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci.* **1997**, *60*, 763–71.

(13) Marklund, G.; Marklund, S. Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **1974**, *47*, 469–474.

(14) Axelroad, B.; Cheesborough, T. M.; Laakso, S. Lipoxygenases in soybeans. *Methods Enzymol.* **1981**, *71*, 441–451.

(15) Su, X.-Y.; Wang, Z.-Y.; Liu, J.-R. In vitro antioxidant activity of *Pinus korainensis* seed extract containing phenolic compounds. *Food Chem.* **2009**, *117*, 681–686.

(16) Arnason, J. T.; Gale, J.; Conilh de Beyssac, B.; Sen, A.; Miller, S. S.; Philogene, B. J. R.; Lambert, J. D. H.; Fulcher, R. G.; Serratos, A.; Mihm, J. Role of phenolics in resistance of maize grain to stored grain insects, *Prostphanus truncatus* (Horn) and *Sitophilus Zeamais* (Motsch). *J. Stored. Prod. Res.* **1992**, *28*, 119–126.

(17) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, *53*, 6649–6657.

(18) Siebenhandl, S.; Grausgruber, H.; Pellegrini, N.; Del Rio, D.; Fogliano, V.; Pernice, R.; Berghofer, E. Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley. *J. Agric. Food Chem.* **2007**, *55*, 8541–8547.

(19) Li, L.; Shewry, P. R.; Ward, J. L. Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9732–9739.

(20) Mpofu, A.; Sapirstein, H. D.; Beta, T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *J. Agric. Food Chem.* **2006**, *54*, 1265–1270.

(21) Mattila, P.; Pihlava, J. M.; Hellstrom, J. Contents of phenolic acids, alkyl and alkenylresorcinols, and avenanthramides in commercial grain products. *J. Agric. Food Chem.* **2005**, *53*, 8290–8295.

(22) Yu, L.; Haley, S.; Perret, J.; Harris, M. Antioxidant properties of hard winter wheat extracts. *Food Chem.* **2002**, *78*, 457−462.
(23) Zhou, K.; Yu, L. Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT—Food Sci. Technol.* **2004**, *37*, 717−721.
(24) Boivin, D.; Lamy, S.; Lord-Dufour, S.; Jackson, J.; Beaulieu, E.; Côté, M.; Moghrabi, A.; Barrette, S.; Gingras, D.; Béliveau, R. Antiproliferative and antioxidant activities of common vegetables: a comparative study. *Food Chem.* **2009**, *112*, 374−380.
(25) Sedej, I. J.; Sakac, M. B.; Misan, A. C.; Mandic, A. I. Antioxidant activity of wheat and buckwheat flours. *Prog. Nat. Sci.* **2010**, *188*, 59−68.
(26) Mateo Anson, N.; van den Berg, R.; Havenaar, R.; Bast, A.; Haenen, G. R. M. M. Bioavailability of ferulic acid is determined by its bioaccessibility. *J. Cereal Sci.* **2009**, *49*, 296−300.
(27) Santanam, N.; Ramachandran, S.; Parthasarathy, S. Oxygen radicals, antioxidants, and lipid peroxidation. *Semin. Reprod. Endocr.* **1998**, *16*, 275−280.
(28) Kehrer, J. P. The Haber−Weiss reaction and mechanisms of toxicity. *Toxicology* **2000**, *149*, 43−50.
(29) Liyana-Pathirana, C. M.; Shahidi, F. Importance of insoluble-bound phenolics to antioxidant properties of wheat. *J. Agric. Food Chem.* **2006**, *54*, 1256−1264.
(30) Sun, T.; Tanumihardjo, S. A. An integrated approach to evaluate food antioxidant capacity. *J. Food Sci.* **2007**, *72*, 159−165.