Antioxidant Activity and Total Phenolics in Selected Cereal Grains and Their Different Morphological Fractions

H. Zieliński* and H. Kozłowska

Division of Food Science, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima 10, P.O. Box 55, 10-747 Olsztyn, Poland

The purpose of this study was to examine the antioxidant properties of water and 80% methanolic extracts of cereal grains and their different morphological fractions. Wheat (*Triticum aestivum L.*) cv. Almari and cv. Henika, barley (*Hordeum vulgare L.*) cv. Gregor and cv. Mobek, rye (*Secale cereale L.*) cv. Dańkowskie Zlote, oat (*Avena sativa L.*) cv. Slawko and buckwheat (*Fagopyrum esculentum* Moench) cv. Kora were used. PC (L-α-phosphatidylcholine) liposome system and spectrophotometric assay of total antioxidant activity (TAA) were used to evaluate the antioxidative activity of extracts. Among the water extracts, only the one prepared from buckwheat exhibited antioxidant activity at the concentration analyzed. The following hierarchy of antioxidant activity was provided for 80% methanolic extracts originated from whole grain: buckwheat > barley > oat > wheat ≅ rye. The antioxidant activity was observed in extract prepared from separated parts of buckwheat and barley. In respect to hulls, the antioxidant hierarchy was as follows: buckwheat > oat > barley. The correlation coefficient between total phenolic compounds and total antioxidative activity of the extracts was −0.35 for water extracts and 0.96, 0.99, 0.80, and 0.99 for 80% methanolic extracts originated from whole grains, hulls, pericarb with testa fractions and endosperm with embryo fractions, respectively.

Keywords: *Antioxidant activity; total phenolic compounds; lipid peroxidation; liposomes; cereal grain extracts*

INTRODUCTION

The data from both experimental and epidemiological studies show that grains, vegetables and fruits contain a large variety of substances called “plant chemicals” or “phytochemicals” (Pratt, 1992). The term “phytochemical” refers to every naturally occurring chemical substance present in plants, especially those that are biologically active (Caragay, 1992). Major phytochemicals include phenolic acids, flavonoids, and coumarin derivatives as well as many other polyphenols. The antioxidant activities in these phytochemicals range from extremely slight to very great. The natural antioxidants may have one or more of the following functions: free-radical scavengers, reducing agents, potential complexers of prooxidant metals, and quenchers of singlet oxygen (Kinsella et al., 1993; Przybylski et al., 1998; Xing and White, 1997; Lasztity, 1998; Watanabe, 1998). Consequently, phytochemical dietary components may actively contribute to the control of oxidative reactions and provide protection in vivo; however, this is still questionable. Antioxidants play an important role in preventing undesirable changes in flavor and nutritional quality of foods. Cereal grains provide significant quantities of energy, protein, and selected micronutrients to the animal and human diet. The chemical composition and bioavailability of nutrients varies between species and varieties of grains and may be affected by forms of processing as feed and food. Cereal grains are rich in phenolic acids; the total amounts may approach 500 mg/kg of edible cereals (Senter et al., 1983). Other phytochemicals occurring in cereals are phytosterines, saponins, and phytoestrogens. In cereals, flavonoids are present in small quantities. Barley contains measurable amounts of catechins and some di- and trimer procyanidins (McMurrough and Baert, 1994). In contrast, cereals are the major source of dietary lignans in human nutrition. Lignans are potent antioxidants and exert anticancer effects. They decrease the production of reactive oxygen species by tumor cell types and cells of the immune system (Cassidy, 1996). Recent reports have described antioxidants and compounds with radical-scavenging activity present in peabean (Tsuda et al., 1993), peanut (Yen and Duh, 1993), rice (Ramarathanam et al., 1998), buckwheat (Watanabe, 1998), and oat (Xing and White, 1997). The solvent extraction using different solvents has been the major method used to isolate cereal antioxidants or to obtain cereal grain extracts rich in antioxidants.

One objective of this study was to determine total antioxidant capacity of water and 80% methanolic extracts of cereal grains by testing the ability of these extracts to inhibit AAPH-induced lipid peroxidation in vitro using the phosphatidylcholine (PC) liposome system in comparison to synthetic antioxidant such as butylated hydroxytoluene (BHT). The second objective was to determine the contents of total phenolics in water and methanolic extracts of cereal grains and to explore relationship between phenolic content and total antioxidant capacity of investigated extracts and their ability to inhibit AAPH-induced lipid peroxidation.

* To whom correspondence should be addressed [telephone (4889) 5234682; fax (4889) 5240124; e-mail haziel@food.irzbz.pan.olsztyn.pl].

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MATERIALS AND METHODS

Reagents. L-α-Phosphatidylcholine (type III-E, PC; Sigma Co.), diethylenetriaminepentoacetic acid (DTPA; Sigma), tris-(hydroxymethyl)aminomethate (Sigma), 2,6-di-*tert*-butyl-4-methylphenol (BHT; Aldrich Chem. Co.), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH; Wako Pure Chemical Industries, Ltd., Osaka, Japan), (±)-catechin (Sigma Chem. Co.), sodium carbonate (Na_2CO_3; POCh, Gliwice, Poland), Folin−Ciocalteu reagent (POCh, Gliwice, Poland), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; Aldrich Chem. Co.), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS; Aldrich Chem. Co.), Total Antioxidant Status kit (catalog no. NX2332, Randox Laboratories Ltd., U.K.). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland.

Analytical Instruments. The instruments used are listed as follows: (HPLC) detector, UV SPD-10A; pump, LC-10AD; recorder, C−R6A, column C8 Nova Pak (3.9 × 150 mm Waters Millipore), (Shimadzu); LiposoFast-Basic (Avestin, Canada); spectrophotometer Beckman DU 7500.

Cereal Samples. Single cereal grain samples grown in 1997 were obtained from local plant breeding station in the North-East Poland. The samples included two cultivars of wheat (winter cv. Almari and spring cv. Henika), two cultivars of barley (winter cv. Gregor and spring cv. Mobek), one cultivar of rye cv. Dańkowskie Zlote, one cultivar of oat cv. Slawko and one cultivar of buckwheat cv. Kora. Samples from two replications were chosen for analysis. Whole-grain samples were dehulled using a laboratory dehuller and fractions of hull, pericarb and testa, and endosperm with embryo were separated manually by sieving through a set of sieves. All samples were grounded in a laboratory mill type WZ-1 (Factory of machines and mechanisms for the food industry, Żnin, Poland). Ground samples were stored at −30 °C until extraction.

Analysis. All samples were analyzed in duplicate for dry matter, nitrogen, and ash using the methods of the AOAC (1990). Lipids (crude fat) were extracted from 1 g samples by shaking with 10 mL of petroleum ether for 30 min. The solvent was decanted after centrifuging (2000*g*, 10 min), and the extraction was repeated on the residue. The combined supernatants were evaporated in a rotary evaporator at 50 °C under vacuum, and the petroleum ether-extractable lipid was weighted gravimetrically. The hull content of the oat, barley and buckwheat was determined by hulling 5 g samples of seeds by hand and then calculating hull weight from total weight. The pericarb with testa fraction contents were determined according to the Carra's method in respect to the 50 g of free caryopse of rye and wheat grain and according to the Luffa's method in relation to the 50 g of free caryopse of barley grain (Jakubczyk and Habera, 1981). Results are the means of triplicates.

Preparation of Extracts. The rye grain was taken for the determination of the most effective extraction system. Four different solvents and different temperatures were investigated. Extraction was performed using the following sequence of solvents: acetone−water (4:1, v/v), ethanol−water (4:1, v/v), methanol−water (4:1, v/v), and water. The extraction temperature was set at 20, 35, and 50 °C. On the grounds of data provided, water and 80% methanol were chosen for the extraction of the remaining cereal grains and their morphological fractions.

The cold-water extracts were prepared from ground whole grains according to the *Official Methods of Analysis* (AOAC, 1990). Water (100 mL) was gradually added to 10 g of sample at ca. 0 °C and the mixture was continuously shaked. Then, the sample was left to stand for 40 min at 0 °C, shaking occasionally, centrifuged at 4000 rpm with centrifuge type MPW-360 (Factory of precise mechanics, Warsaw, Poland). Finally, the supernatant was filtered rapidly, returning filtrate to filter until clear, and then the filtrate was lyophilized. The lyophilizate was dissolved in Tris-HCl buffer, pH 7.4 (2.5 mg/mL) for measuring the inhibition of AAPH-induced lipid peroxidation in unilamellar liposomes or in phosphate saline buffer 0.01 M, pH 7.0 (10 mg/mL) for the determination of the total antioxidant activity of the lyophilizates. For the determination of the total phenolic compounds, lyophilizates were dissolved in methanol (2.5 mg/mL). To prepare 80% methanolic extracts of cereals and their morphological fractions, ground, freeze-dried samples (5 g) were extracted with 80% aqueous methanol (50 mL) on a shaker water bath for 30 min at 20 °C. Extracts were centrifuged at 4000 rpm for 15 min then filtered and methanol was evaporated under vacuum. The residues were refrigerated and freeze-dried. The lyophilizate was dissolved in methanol (2.5 mg/mL) for measuring the inhibition of AAPH-induced lipid peroxidation in unilamellar liposomes and for the determination of total phenolic compounds. For the determination of the total antioxidant activity of the lyophilizates, exactly 5 mg was dissolved in 1 mL of pure methanol.

Measurement of Total Antioxidant Activity (TAA). Two methods were used in this study. In the first one, the antioxidative activity was determined by a liposome method (Terao et al., 1994). An aliquot (50 μL) of cold-water extracts in Tris-HCl buffer, pH 7.4 (2.5 mg/mL), or methanolic extracts in methanol (2.5 mg/mL) was added to the solution (0.7 mL) of peroxide-free egg-yolk phosphatidylcholine (PC) dissolved in chloroform. After removing solvents in the stream of nitrogen followed by vacuum, the residue was dissolved in Tris-HCl buffer (0.7 mL, 10 mM/L, pH 7.4) with 0.5 mM/L-diethylenetriaminepentaacetic acid (DTPA), vortexed, and exposed to ultrasonic waves for 30 s. The liposomes were standardized by extruding the sample (0.6 mL) 21 times in a Liposo Fast Basic apparatus with a polycarbon membrane of 100 nm pore size. The liposomes (0.5 mL) were suspended in 0.5 mL Tris-HCl buffer and placed in a light-protected shaker water bath at 37 °C. After 5 min, AAPH was added to the sample (final concentration was 1 mM) as an initiator of radicals. The amount of phosphatidylcholine peroxides (PC−OOH), analyzed during 7 h of incubation at 72 min intervals, was determined at 235 nm by the HPLC method using the Shimadzu system, a C8 Nova Pak column (3.9 × 150 mm, Waters Millipore), and a methanol−water mixture (96:4, v/v) as a mobile phase. The amount of peroxides was calculated from a standard curve prepared with standard PC−OOH which was obtained from the oxidized PC. The butylated hydroxytoluene (BHT) at final concentration of 0.52 mM was used as a standard, and methanol was used as the blank.

In the second method (Miller and Rice-Evans, 1996), the relative abilities of antioxidants to scavenge $ABTS^{\bullet+}$ was measured by a spectrophotometric technique in comparison with the antioxidant potency of standard amounts of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The radical cation $ABTS^{\bullet+}$, produced by the ferrylmyoglobin radical generated from metmyoglobin and H_2O_2 in the presence of the peroxidase, is a blue/green chromogen with characteristic absorption at 734 nm. The determination of the TAA was carried out using the Randox kit. Twenty microliters of cold-water extract lyophilizate diluted in phosphate saline buffer (10 mg/mL) or twenty microliters of 80% methanolic extract lyophilizate diluted in pure methanol (5 mg/mL) was added to 1 mL of chromogen solution previously incubated at 37 °C for 6 min. At the start of the reaction and after 3 min, the absorbance was measured and compared with that of 1.55 mM Trolox and the TAA of each extract was calculated. The TAA of each extract was calculated according to the following equations:

$$A_2 - A_1 = A \text{ of blank or sample or 1.55 mM Trolox}$$

$$\text{TAA (mM Trolox)} = [(1.55 \text{ mM Trolox})(A_{\text{blank}} - A_{\text{sample}})]/A_{\text{blank}} - A_{\text{Trolox}}$$

In these equations A_1 is the absorbance at the start of the reaction and A_2 is the absorbance at 3 min.

Table 1. Means of Hull and Pericarb with Testa Fraction (%) in the Whole Grain of Selected Cereals

source	hull content[a] (%)	pericarb with testa content[b] (%)
wheat cv. Almari	no contain	18.9
wheat cv. Henika	no contain	20.0
barley cv. Mobek	7.6	12.3
barley cv. Gregor	9.8	8.3
rye cv. Dañkowskie Zlote	no contain	26.5
oat cv. Slawko	23.8	?
buckwheat cv. Kora	23.6	nd[a]

[a] nd, not determined. As buckwheat hull is reffered to the pericarb, it was difficult to obtain testa of buckwheat grain. Therefore, the testa fraction was included to the endosperm with embryo fraction. [b] Pericarb with testa fraction content was determined by chemical treatment of 50 g of free caryopse according to the Carra and Luffa method.

Determination of Total Phenolic Compounds (TPCs). TPCs were determined according to the method of Shahidi and Naczk (1995). A 0.25 mL aliquot of the extract solution (2.5 mg/mL methanol) was mixed with 0.25 mL of Folin−Ciocalteu reagent (previously diluted with water 1:1 v/v) and 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution and 4 mL of water. Mixture was allowed to stand at room temperature for 25 min and then it was centrifuged at 5000 rpm for 10 min. Supernatant absorbance was measured at 725 nm using a spectrophotometer. The results were expressed as (±) catechin equivalents.

RESULTS AND DISCUSSION

The means of hull and pericarb with testa fraction (%) in whole grain of selected cereals are presented in Table 1. The highest content of hull was noted for buckwheat and oat. Similar content of buckwheat and oat hulls was reported by Przybylski et al. (1998) and by Xing and White (1997), respectively. In the case of barley grain, small differences in hull content were observed. It was noted that these differences were connected with type of cultivar used (7.6 and 9.8% for barley cv. Mobek and Gregor, respectively). Despite these, hull content in barley grain was in the range reported by other authors (Gąsiorowski, 1997). The content of pericarb with testa fraction in wheat, barley, and rye was higher than it has been reported in the literature. Probably it was caused by using drastic chemical methods for their separation and possible moving a some part of aleurone layer into pericarb fraction.

Daniels and Martin (1961), Daniels et al. (1963), and Daniels and Martin (1967) used light petroleum ether to defat the whole oat kernel and diethyl ether to extract the phenolic compounds. Sosulski and co-workers (1982) extracted free phenolic acids and soluble phenolic acid esters from oat and other cereals by using a methanol−acetone−water solvent. Collins and co-workers (1991) used aqueous methanol to extract phenolics from oat groats and hulls. They found that the greatest activities were obtained with methanolic extracts. Previous studies of other authors revealed that the amounts of components extracted by solvents increased as the polarity of the solvent used increased (Duh et al., 1992; Przybylski et al., 1998; Bałasińska and Troszyńska, 1998). To select the best one, the following sequence of solvents were used: acetone−water (4:1, v/v), ethanol−water (4:1, v/v), methanol−water (4:1, v/v), and water. The extraction temperature was set at 20, 35, and 50 °C. On the ground of experiments with rye grain, the 80% methanol was selected for the extraction at 20 °C

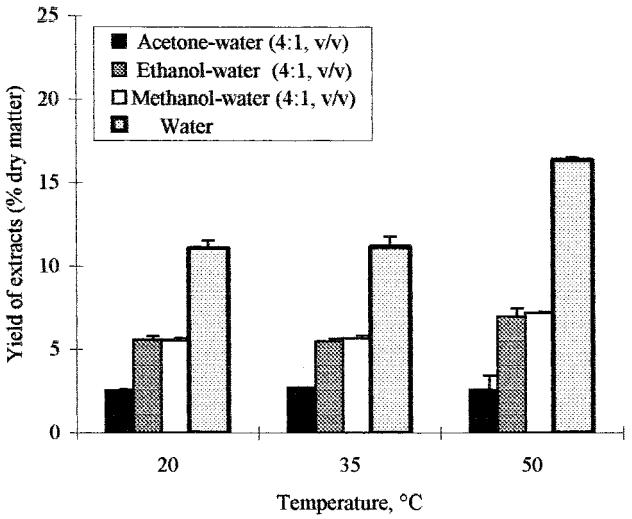

Figure 1. Yields of extracts obtained from whole-grain of rye. Ground, freeze-dried samples (5 g) were extracted with indicated solvents (50 mL) for 30 min at 20, 35, and 50 °C. Extracts were centrifuged at 4000 rpm for 15 min and then filtered, and solvents were evaporated under vacuum. The residues were freeze-dried and weighed. The results are the mean from triplicate determinations.

Table 2. Yield of Water and 80% Methanolic Extracts Obtained from the Whole Grain (% Dry Matter ± SD)[a]

source	water extracts (4 °C)	methanolic extracts (20 °C)
wheat cv. Almari	6.62 ± 0.16	5.21 ± 0.05
wheat cv. Henika	5.75 ± 0.03	5.38 ± 0.14
barley cv. Mobek	5.96 ± 0.46	4.60 ± 0.33
barley cv. Gregor	5.37 ± 0.37	4.17 ± 0.06
rye cv. Dañkowskie Zlote	11.47 ± 0.23	7.44 ± 0.06
oat cv. Slawko	6.08 ± 0.02	3.82 ± 0.08
buckwheat cv. Kora	8.87 ± 0.24	4.23 ± 0.19

[a] Average from triplicate determinations; percentage based on dry matter.

within a period of 30 min (Figure 1). As the water extract contained the highest yield of dry matter, it was also taken for the further studies. In this case, however, the extraction was conducted at 4 °C in order to prevent water-soluble antioxidants and enzymes against degradation of their biological activity.

The yields of water and 80% methanol extracts obtained from whole-grain of investigated cereals are shown in Table 2. The amount of components extracted by water generally was higher as compared to that extracted by 80% methanol. It is worthy to notice that the differences were the most visible in the case of buckwheat and oat grain-cereals having the highest content of hull.

Antioxidant Activities of Water Extract. Only the extract prepared from the whole grain of buckwheat exhibited a slight antioxidant activity at the concentration analyzed (Figure 2) under PC (L-α-phosphatidylcholine) liposome system. However, this activity was limited to the first 2 h of the experiment. The remaining extracts prepared from the whole grain of wheat, barley, rye, and oat exhibited a prooxidant effect (Figure 2). The comparisons were done in respect to the butylated hydroxytoluene (BHT). BHT is synthetic antioxidant with phenolic structure and it has been used in various food systems because of its high stability, low cost, and other practical advantages. However, its application has been reassessed because of possible toxic or carcinogenic compounds formed during its degradation (Shahidi et

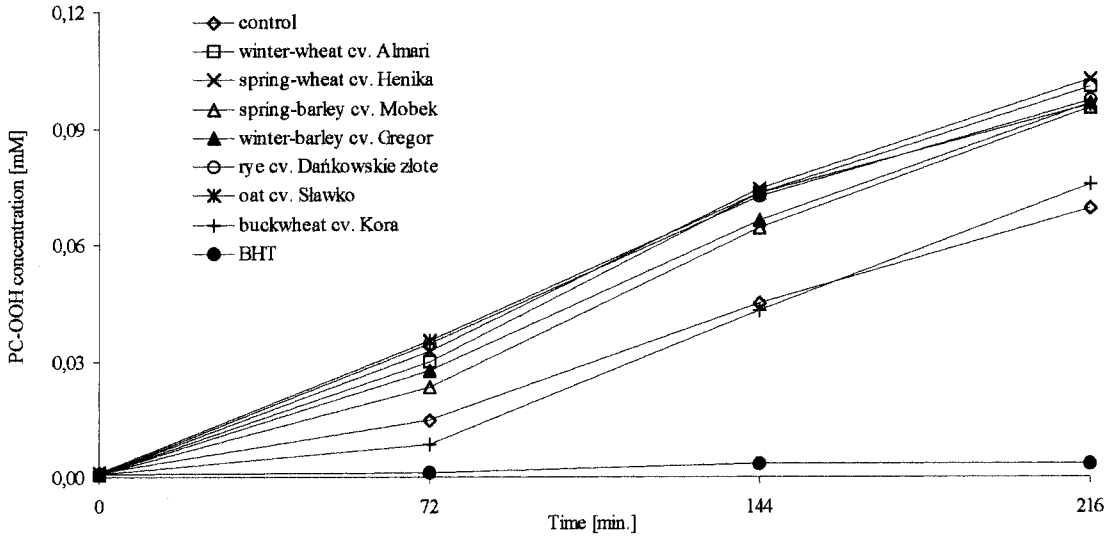

Figure 2. Time-related changes in peroxides content (PC−OOH) during incubation of phosphatidylcholine with water extracts of different cereal whole-grain samples. AAPH (1 mM) was used as an oxidant. The results are the mean from three replications.

Table 3. Contents of Total Phenolic Compounds and Total Antioxidant Activity Values of Liophilyzed Water and 80% Methanol Extracts Obtained from the Whole Grain

source	total phenolic compds [μg of (±)-catechin/ mg of lyophilizate]	total antioxidant activity (μmol Trolox/ mg of lyophilizate)	total antioxidant activity (μmol Trolox/ mg of TPC)
wheat cv. Almari			
water extract	7.30	0.027	3.6
80% methanol extract	9.05	0.054	5.9
wheat cv. Henika			
water extract	8.0	0.023	2.9
80% methanol extract	10.16	0.150	14.7
barley cv. Mobek			
water extract	11.30	0.059	5.2
80% methanol extract	26.90	0.244	9.1
barley cv. Gregor			
water extract	10.20	0.064	6.2
80% methanol extract	24.34	0.222	9.1
rye cv. Dañkowskie Zlote			
water extract	8.20	0.003	0.4
80% methanol extract	8.86	0.056	6.3
oat cv. Slawko			
water extract	1.47	0.032	21.8
80% methanol extract	17.60	0.081	4.6
buckwheat cv. Kora			
water extract	1.85	0.138	74.6
80% methanol extract	117.72	0.587	5.0

al., 1992). The contents of total phenolic compounds and total antioxidant activity (TAA) values of lyophilizates of water extracts obtained from the whole grain are shown in Table 3. The highest amounts of phenolic components extracted by water originated from barley, wheat, and rye, respectively. The oat and buckwheat grain contained about five times smaller total phenolics extracted by water as compared to barley. The estimated values of TAA based on the relative abilities of the extracts to scavenge the $ABTS^{\bullet+}$ in comparison with Trolox are weak and showed a higher antioxidant activity of extract prepared from buckwheat. Twofold decreased activity in this system was noted for extract from barley and about 5-fold smaller activity for extracts from wheat. The water extract prepared from rye did not show any antioxidant properties in this system. There is no linear relation between total antioxidant activity (TAA) of water extracts and their content of phenolics. However, for a same level of TPC, the following hierarchy of antioxidant activities is noted: buckwheat > oat > barley > wheat > rye. Hence, the TAA is very high for the buckwheat water extract, and for rye water extract is very low, when compared to all others (Table 3). It can be explained by the fact that the Folin−Ciocalteu method measures other constituents than phenolics, and its specificity is poor. The Folin−Ciocalteu reagent detects all phenolic groups found in extracts, including those found in the extractable proteins (Shahidi and Naczk, 1995). It is confirmed by the lack of correlation coefficient between total antioxidant activity (TAA) of water extracts and their content of phenolics ($r = -0.35$). This indicates also that factors other than total phenolics may play a role in the antioxidant activity of water extracts. Moreover, all the phenolics do not have the same antioxidant activity, some are powerful, others are weak, they develop antagonistic or synergistic effects with themselves or with the other constituents of extracts (Rice-Evans et al., 1996; Moran et al., 1997; Lien et al., 1999) For example, it may originate from the combined action of phenolic and protein in the sample. Cereal protein has been reported to exert strong antioxidant activities

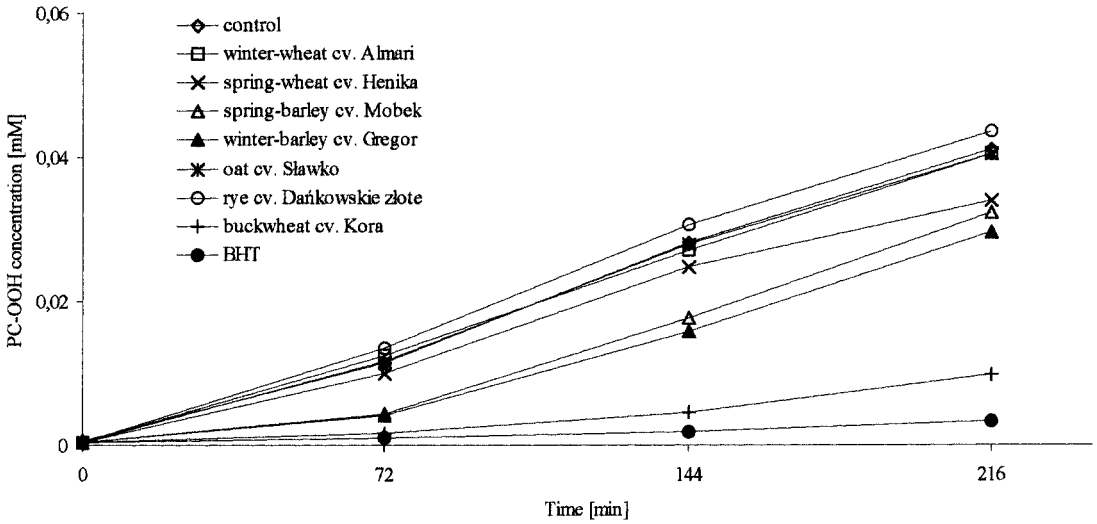

Figure 3. Time-related changes in peroxides content (PC−OOH) during incubation of phosphatidylcholine with the same amount of 80% methanol extracts of different cereal whole-grain samples. AAPH (1 mM) was used as an oxidant. The results are the mean from three replications.

(Iwama et al., 1987). The proteins in buckwheat are one of the best known sources of high biological value in the plant kingdom (Sure, 1955). Buckwheat contains also a high level of crude fiber and tannins (Eggum et al., 1981). The results obtained for buckwheat extract suggest that testing the ability of antioxidants to inhibit AAPH-induced lipid peroxidation in vitro using the phosphatidylcholine (PC) liposome system and the relative abilities of antioxidants to scavenge the $ABTS^{\bullet+}$ radicals can produce a similar picture.

Antioxidant Activity of 80% Methanol Extracts Originated from the Whole Grain. Methanol appears to be the best solvent for extracting compounds such as phenolics, flavonoids, and other polar material in cereals (Przybylski et al., 1998; Xing and White, 1997; Watanabe, 1998; Velioglu et al., 1998). Our studies showed that 80% methanolic extracts prepared from the whole grain of buckwheat, two cultivars of barley, and wheat cv. Henika exhibited antioxidant activity at the concentration analyzed under AAPH-induced lipid peroxidation in unilamellar liposomes system (Figure 3). The highest antioxidant properties were observed in the extract from buckwheat. In contrast, extract from rye, similar to the water extract, exhibited also small prooxidant effect. The rest of extracts did not show any antioxidant properties under the system used.

The contents of total phenolic compounds and total antioxidant activity values of lyophilizates of 80% methanol extracts obtained from the whole grain are shown in Table 3. The highest amounts of phenolic extracted by 80% methanol were originated from buckwheat. The content of phenolics was five and seven times smaller in barley and oat, respectively, as compared to buckwheat. Moreover, the wheat and rye grain contained only 8.6% and 7% of total phenolic, in comparison to the content in buckwheat. The obtained values of total antioxidant activity (TAA) based on the relative abilities of antioxidants originated from the 80% methanolic extracts to scavenge the $ABTS^{\bullet+}$ in comparison with Trolox showed the following hierarchy of antioxidant activities: buckwheat > barley > wheat cv. Henika > oat > rye ≅ wheat cv. Almari. This hierarchy is in agreement with content of total phenolic compounds (correlation coefficient $r = 0.96$), indicating strong links between phenolic compounds and TAA. Moreover, the data obtained from AAPH-induced lipid peroxidation in unilamellar liposomes system showed a similar hierarchy of antioxidant activity, excluding extract from oat. However, for the same level of TPC, the following hierarchy of antioxidant activities is noted: wheat cv. Henika > barley > rye ≅ wheat cv. Almari ≅ buckwheat ≅ oat (Table 3). The different picture of links between TPC and TAA into the two kinds of extracts may be explained by different ability of phenolics to be extracted by 80% methanol (Xing and White, 1997) and their structure−antioxidant activity relationships (Przybylski et al., 1998; Watanabe, 1998; Rice-Evans et al., 1996).

Antioxidant Activity of 80% Methanolic Extracts of Different Cereal Dehulled-Grain. Methanolic extracts (80%) prepared from dehulled grains exhibited approximatively the same behavior than whole grain extracts toward AAPH-induced lipid peroxidation in unilamellar liposomes system. A high antioxidant activity was observed in the extract from buckwheat and similarly from barley, while extract from dehulled oat did not show any antioxidant properies (Figure 4). It was also found that inhibition of AAPH-induced lipid peroxidation in unilamellar liposomes system increased with the amounts of buckwheat groat and hull extracts (data not shown).

The contents of total phenolic compounds and total antioxidant activity values of lyophilizates of 80% methanolic extracts are shown in Table 4. These values were near those obtained for whole grain. The highest content of total phenolic compounds and the highest TAA were found again in dehulled buckwheat (buckwheat groats). However, for the same level of TPC, oat groats had higher TAA than whole grain.

Antioxidant Activity of 80% Methanolic Extracts of Different Grain Hull Samples. Methanolic extracts (80%) prepared from hull samples originated from buckwheat, and oat and barley exhibited antioxidant activity at the concentration analyzed under AAPH-induced lipid peroxidation in unilamellar liposomes system. The antioxidant activity was decreased in the following order: buckwheat hull, oat hull, and barley hull (Figure 5). These findings were quite in agreement with TAA values and total phenolic compounds which decreased in the same order (Table 4). The correlation

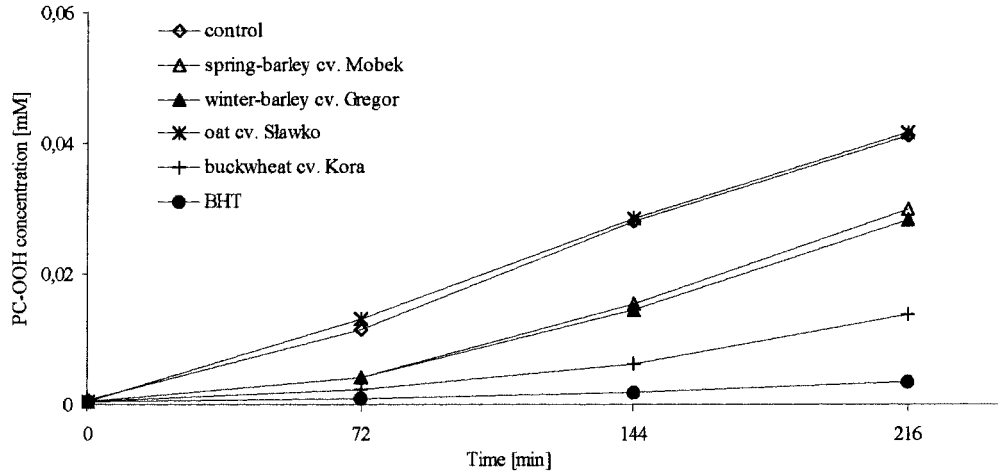

Figure 4. Time-related changes in peroxides content (PC−OOH) during incubation of phosphatidylcholine with 80% methanol extracts of different cereal dehulled-grain samples. AAPH (1 mM) was used as an oxidant. The results are the mean from three replications.

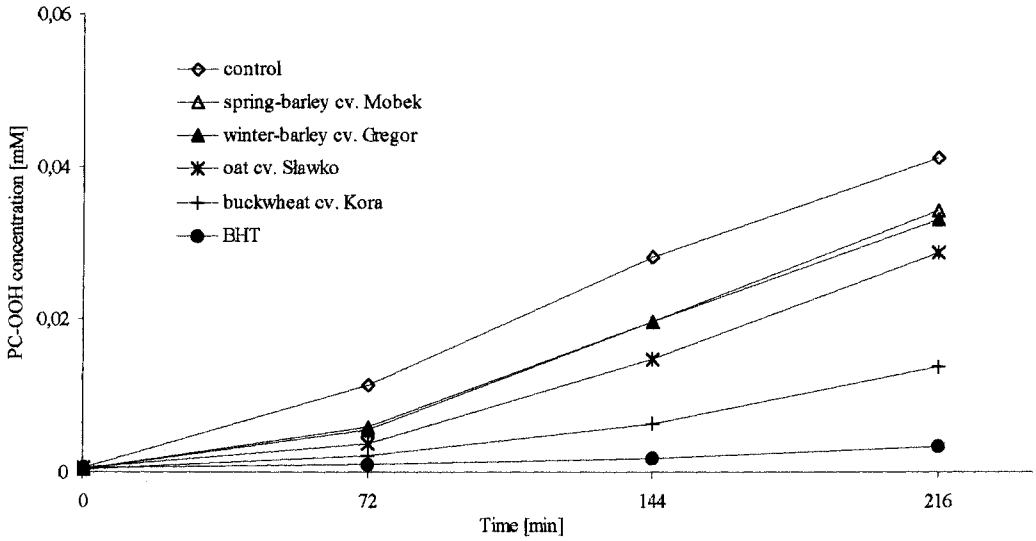

Figure 5. Time-related changes in peroxides content (PC−OOH) during incubation of phosphatidylcholine with 80% methanol extracts of different grain hulls samples. AAPH (1 mM) was used as an oxidant. The results are the mean from three replications.

Table 4. Contents of Total Phenolic Compounds and Total Antioxidant Activity Values in Lyophilizates of 80% Methanol Extracts of Different Cereal Dehulled-Grain and Different Grain Hulls Samples

source	total phenolic compds [μg of (±)-catechin/ mg of lyophilizate]	total antioxidant activity (μmol Trolox/ mg of lyophilizate)	total antioxidant activity (μmol Trolox/ mg of TPC)
barley cv. Mobek			
dehulled grain	28.67	0.218 ± 0.037	7.6
hulls	36.81	0.224 ± 0.002	6.9
barley cv. Gregor			
dehulled grain	27.96	0.246 ± 0.008	8.8
hulls	31.33	0.224 ± 0.002	7.1
oat cv. Slawko			
dehulled grain	16.33	0.77 ± 0.001	47.2
hulls	45.00	0.264 ± 0.001	5.9
buckwheat cv. Kora			
dehulled grain	90.72	0.548 ± 0.018	6.0
hulls	381.86	1.175 ± 0.020	3.1

coefficient between these parameters was $r = 0.99$, indicating strong links between antioxidants properties of hulls and the content of phenolic compounds. In respect to buckwheat hull, our data are in contrast to those presented by Przybylski (1998) who found that methanol extract from buckwheat hulls showed prooxidant activity toward canola oil. However, data provided here are in agreement with those reported by Watanabe et al. (1997), who found that crude ethanolic extracts from buckwheat hull had antioxidant activity which was attributed to phenolic compounds including flavonoids. In our study, we found that inhibition of AAPH-induced lipid peroxidation in unilamellar liposomes system was dependent on the amounts of buckwheat hull extracts. Moreover, extract prepared from buckwheat hull had a higher TAA and TPC values when compared to those

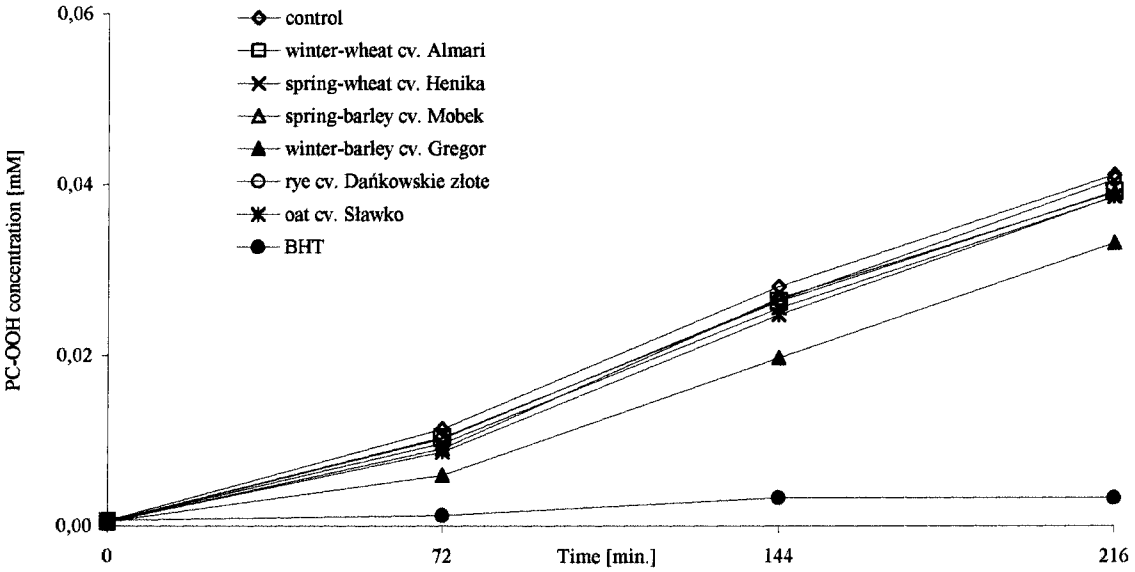

Figure 6. Time-related changes in peroxides content (PC−OOH) during incubation of phosphatidylcholine with 80% methanol extracts of pericarb and testa fractions samples. AAPH (1 mM) was used as an oxidant. The results are the mean from three replications.

for buckwheat groat (Table 4). In contrast, for a same amount of TPC, the hull extracts had a lower TAA than that of the groat extracts. Xing and White (1997) found that crude methanol extract from oat hull had higher TPC content that of the groat and the groat extract had a greater antioxidative effect than did the hull extract at the same concentration. These findings agree with data reported in this study. Moreover, we found that dehulled oat for a same level of TPC, had very high TAA, when compared to the same quantity of phenolics in dehulled buckwheat and barley (Table 4). These differences in TAA may be because of the composition of phenolic acids in groat and hull extracts. Xing and White (1997) reported in respect to oat, that it might be because of the presence of caffeic acid, which was only found in the groat extract, but not the hull extracts. The latest contained additionally *o*-coumaric, sinapic, and salicylic acids in contrast to the groat extract. Moreover, in oat, there is another group of phenolic antioxidants which are cinnamoyl-anthranilic acid derivatives called avenanthramides can enhance the TAA (Dimberg et al., 1993). Oat groat contains at least 25 avenanthramides, while hull extracts contain only 20 of these compounds (Collins, 1989). The antioxidant capacity of aventhramide-2 is about 60% of that of α-tocopherol and seems to be quite heat stable (Lasztity, 1998).

Antioxidant Activity of 80% Methanolic Extracts of Pericarb and Testa Fractions Samples. Methanolic extracts (80%) prepared from pericarb and testa fractions originated from wheat, rye, and oat exhibites neither antioxidant nor prooxidant activities at the concentration analyzed under AAPH-induced lipid peroxidation in unilamellar liposomes system. Only extracts prepared from pericarb and testa fraction originated from winter barley cv. Gregor showed antioxidant activity (Figure 6). This finding was in agreement with TAA value and total phenolic compounds which had the highest values (Table 5). The correlation coefficient between TAA values and total phenolic compounds of investigated samples was $r = 0.80$. It indicates a weaker dependence between TAA and TPC than in relation to the hull samples. However, for the the same level of TPC, hull extracts and pericarb and testa fraction extracts had similar TAA values (Table 4 and Table 5). No data was provided in respect to buckwheat pericarb and testa because it was difficult to obtain testa from buckwheat grain by using laboratory dehuller. Therefore, the testa fraction was included to the endosperm with embryo fraction.

Antioxidant Activity of 80% Methanolic Extracts of Endosperm with Embryo Fractions Samples. It was shown that, using AAPH-induced lipid peroxidation in a unilamellar liposomes system, only 80% methanolic extracts prepared from endosperm with embryo fractions from buckwheat and from winter barley cv. Gregor exhibited antioxidant activity at the concentration analyzed (Figure 7). The contents of total phenolic compounds and TAA values of lyophilizates of 80% methanolic extracts are shown in Table 5. As it was shown in respect to data obtained for the whole grain, dehulled grain and hulls, the highest content of total phenolic compounds and the highest TAA was noted for endosperm with embryo fraction from buckwheat (Table 5). The fraction of endosperm with embryo from two cultivars of barley had about three times lower contents of total phenolics and their TAA values were also decreased about 3-fold. However, fraction from oat had a similar TAA value in comparison to the ones from barley but the level of TPC was about 3-fold less. On the other hand, for a same level of TPC, endosperm and embryo extract from oat and wheat cv. Henika had the highest TAA. The correlation coefficient between TAA values and TPC of investigated samples was $r = 0.99$, indicating strong links between antioxidant properties and content of phenolic compounds.

CONCLUSIONS

The total phenolic content of the cereal grain 80% methanolic extracts and their morphological fractions investigated in this study varied from 5.08 (endosperm with embryo fraction from wheat cv. Henika) to 381.86 (hull from buckwheat grain) expressed as micrograms of TPC per milligram of freeze-dried extract. The relationship between TPC and total antioxidant activity

Table 5. Contents of Total Phenolic Compounds and Total Antioxidant Activity Values in Lyophilizates of 80% Methanol Extracts of Pericarb and Testa Fractions and Endosperm with Embryo Fractions Samples

source	total phenolic compds [μg of (±)-catechin/mg]	total antioxidant activity (μmol Trolox/mg)	total antioxidant activity [μmol Trolox/mg of TPC]
wheat cv. Almari			
pericarb and testa	15.98	0.125 ± 0.013	7.8
endosperm and embryo	6.84	0.068 ± 0.023	9.9
wheat cv. Henika			
pericarb and testa	16.72	0.110 ± 0.011	6.6
endosperm and embryo	5.08	0.079 ± 0.004	15.6
barley cv. Mobek			
pericarb and testa	22.65	0.170 ± 0.017	7.5
endosperm and embryo	17.35	0.165 ± 0.001	9.5
barley cv. Gregor			
pericarb and testa	23.01	0.175 ± 0.001	7.6
endosperm and embryo	20.80	0.192 ± 0.006	9.2
rye cv. Dańkowskie Zlote			
pericarb and testa	17.38	0.103 ± 0.007	5.9
endosperm and embryo	10.29	0.070 ± 0.011	6.8
oat cv. Slawko			
pericarb and testa	23.85	0.139 ± 0.004	5.8
endosperm and embryo	8.80	0.155 ± 0.018	17.6
buckwheat cv. Kora			
pericarb and testa[a]	nd	nd	nd
endosperm and embryo	64.77	0.598 ± 0.053	9.2

[a] nd, not determined. As hull is referred to the pericarb, it was difficult to obtain testa of buckwheat grain. Therefore the testa fraction was included to the endosperm with embryo fraction.

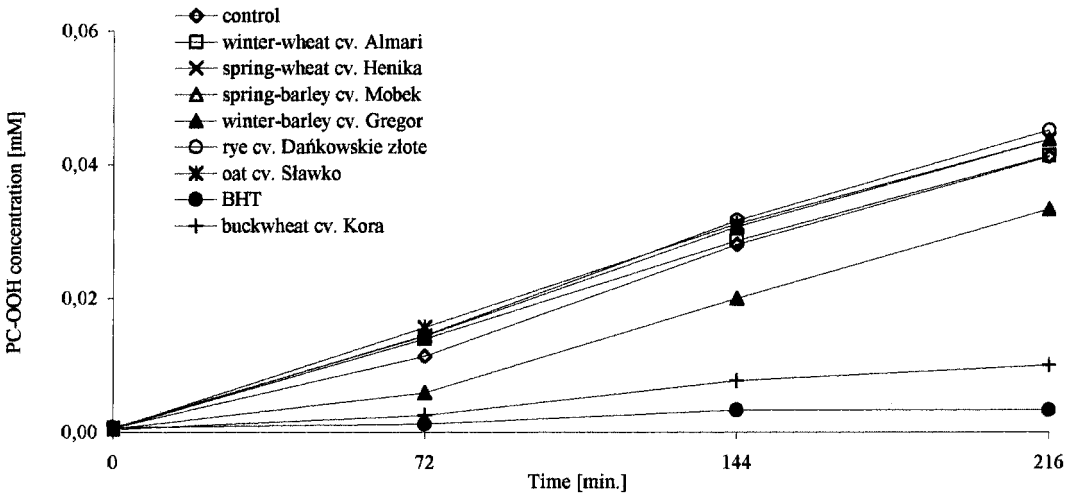

Figure 7. Time-related changes in peroxides content (PC−OOH) during incubation of phosphatidylcholine with 80% methanol extracts of endosperm with embryo fractions samples. AAPH (1 mM) was used as an oxidant. The results are the mean from three replications.

of all 80% methanolic extracts and their morphological fractions is given by the equation $y = 233.66x - 20.336$, with $R^2 = 0.69$. This result indicates that when all investigated materials were included in the statistical analysis, there was a positive relationship between total phenolics and antioxidant activity. However, the relationship would be more significant if the results for oat hull and buckwheat endosperm with embryo fractions are omitted ($R^2 = 0.93$). When the antioxidant activities of all investigated extracts were reported to the same quantity of phenolics and compared, the final conclusion is that dehulled oat and endosperm extracts appeared as the most potent antioxidants; however, from among them only dehulled oat methanol extract exhibited antioxidant activity using AAPH-induced lipid peroxidation in unilamellar liposomes system. This indicates that factors such as phenolics composition, their individual antioxidant activity, and solubility together with other water and lipid-soluble antioxidant can play a major role in the total antioxidant activity of cereal grains. In conclusion, evaluation of antioxidant properties of cereal grain extracts as well as their separated part based on the measurement of the inhibition of AAPH-induced lipid peroxidation in unilamellar liposomes system and the relative abilities of extracts to scavenge the $ABTS^{\bullet+}$ should be expanded, including also such properties of cereal constituents as reducing agents, potential complexers of prooxidant metals, and quenchers of singlet oxygen. For this reason, further nutritive antioxidant present in cereals should be taken into account including phytosterines, saponins, phytoestrogens, carotenoids, tocopherols, and tocotrienols (Andlauer and Furst., 1998), soluble proteins containing sulfhydryls, and glutathione (Zieliński et al., 1999). It seems more necessary to well know the composition of the extracts and to identify the antioxidant compounds in the extracts in order to fully explain the obtained results. These studies are in progress in our laboratory.

LITERATURE CITED

Andlauer, W.; Furst, P. Antioxidative power of phytochemicals with special reference to cereals. *Cereal Foods World* **1998**, *5*, 356−360.

Association of Official Analytical Chemists. *AOAC Official Methods of Analysis*; Washington DC, 1990; Vol. 2, 15th ed., p 777.

Bałasińska, B.; Troszyńska, A. Total antioxidative activity of evening primrose (*Oenothera paradoxa*) cake extract measured in vitro by liposome model and murine L1210 cells. *J. Agric. Food Chem.* **1998**, *46*, 3558−3563.

Caragay, A. B. Cancer-preventive foods and ingredients. *Food Technol.* **1992**, *46* (4), 65−69.

Cassidy, A. Physiological effects of phytoestrogens in relation to cancer and other human health risks. *Proc. Symp. On Physiologically Active Substances in Plant Foods (Non-Nutrive Nutrient)*; Cambridge University Press: London, 1996.

Collins, F. W. Oat phenolics: avenanthramides, novel substituted N-cinnamoylanthranilate allkaloids from oat groats and hulls. *J. Agric. Food. Chem.* **1989**, *37*, 60−65.

Collins, F. W.; McLachlan, D. C.; Blackwell, B. A. Oat phenolics: Avenalumic acids, a new group of bound phenolic acids from oat groats and hulls. *Cereal Chem.* **1991**, *68*, 184−189.

Daniels, D. G.; Martin, H. F. Isolation of a new antioxidant from oats. *Nature* **1961**, *191*, 1302.

Daniels, D. G.; Martin, H. F. Antioxidants in oats: mono-esters of caffeic and ferrulic acids. *J. Sci. Food Agric.* **1967**, *18*, 589−595.

Daniels, D. G.; King, H. G. C.; Martin, H. F. Antioxidants in oats: esters of phenolic acids. *J. Sci. Food Agric.* **1963**, *14*, 385−390.

Dimberg, L. H.; Theander, O.; Lingnert, H. Avenanthramides − a group of phenolic antioxidants in oats. *Cereal Chem.* **1993**, *70* (6), 637−641.

Duh, P. D.; Yeh, D. B.; Yen, G. C. Extraction and Identification of an Antioxidative Component from Peanut Hulls. *J. Am. Oil Chem. Soc.* **1992**, *69*, 814−818.

Eggum, B. O.; Kreft, I.; Javornik, B. Chemical composition and protein quality of buckwheat (*Fagopyrum esculentum* Moench). *Qual. Plant. Plant Foods Hum. Nutr.* **1981**, *30*, 175−179.

Gąsiorowski, H.; *Jęczmień − chemia i technologia*; PWRiL: Poznań, 1997; pp 72−76.

Iwama, K.; Hattori, M.; Ibuki, F. Prominent antioxidant effect of wheat gliadin on linoleate peroxidation in powder model systems at high water activity. *J. Agric. Food Chem.* **1987**, *35*, 628−631.

Jakubczyk, T.; Haber, T. Analiza zbóż i przetworów zbożowych. Warsaw Agriculture University Press: Warsaw, Poland, 1981; 64−65.

Kinsella, J. D.; Frankel, E.; German, B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, *47* (4), 85−93.

Lasztity, R. Oat grain − a wonderful reservoir of natural nutrients and biologically active substances. *Food Rev. Int.* **1998**, *14* (1), 99−119.

Lein, E. J.; Ren, S.; Bui, H. H.; Wang, R. Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radical Biol. Med.* **1999**, *26* (3/4), 285−294.

McMurrough, I.; Baert, T. Identification of proanthocyanidins in beer and their direct measurement with a dual electrode electrochemical detector. *J. Inst. Brew.* **1994**, *100*, 409−413.

Miller, N. J.; Rice-Evans, C. A. Spectrophotometric determination of antioxidant activity. *Redox Rep.* **1996**, *2* (3), 161−171.

Moran, J. F.; Klucas, R. V.; Grayer, R. J.; Abian, J.; Becana, M. Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radical Biol. Med.* **1997**, *22* (5), 861−870.

Naczk, M.; Shahidi, F. The effect of methanol-ammonia treatment on the content of phenolic acids in cande. *Food Chem.* **1989**, *31*, 159−164.

Pratt, D. E. Natural antioxidants from plant material. In *Phenolic Compounds in Food and Their Effects on Health*; Huang, I. M. T., Ho, C. T., Lee, C. Y., Eds.; Am. Chemical Society: New York, 1992.

Przybylski, R.; Lee, Y. C.; Eskin, N. A. M. Antioxidant and radical-scavenging activities of buckwheat seed components. *J. Am. Oil Chem. Soc.* **1998**, *75* (11), 1595−1601.

Ramarathanam, N.; Osawa, T.; Namiki, M.; Kawakishi, S.; Ohshima, K. Chemical studies on novel rice hull antioxidants. 1. Isolation, fractionation, and partial characterization. *J. Agric. Food Chem.* **1988**, *36*, 732−737.

Rice-Evans, C. A.; Miller, N. M.; Paganda, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20* (7), 933−956.

Senter, S. D.; Horvat, R. J.; Forbus, W. R. Comparative GLC-MS analysis of phenolic acids of selected tree nuts. *J. Food Sci.* **1983**, *48*, 798−803.

Shahidi, F.; Naczk, M. Methods of analysis and quantification of phenolic compounds. *Food Phenolic: Sources, Chemistry, Effects and Applications*; Technomic Publishing Company, Inc.: Lancaster, PA, 1995; pp 287−293.

Shahidi, F.; Janitha, P. K.; Wanasandura, P. D. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67−103.

Sosulski, F.; Krygier, K.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereals and potato flours. *J. Agric. Food Chem.* **1982**, *30*, 330−340.

Sure, B. Nutritive value of proteins in buckwheat and their role as supplements to proteins in cereal grains. *J. Agric. Food Chem.* **1995**, *3*, 793−795.

Terao, J.; Piskuła, M.; Yao, Q. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.* **1994**, *308* (1), 278−284.

Tsuda, T.; Osawa, T.; Nakayama, T.; Kawakishi, S.; Ohshima, K. Antioxidant activity of pea bean (*Phaseolus vulgaris*) extract. *J. Am. Oil Chem. Soc.* **1993**, *70*, 909−913.

Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.

Watanabe, M. Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. *J. Agric. Food Chem.* **1998**, *46*, 839−845.

Watanabe, M.; Sato, A.; Osawa, R.; Terao, J. Antioxidative activity of buckwheat seed extracts and its rapid estimate for evaluation of breeding materials. *J. Jpn. Soc. Food Sci. Technol.* **1995**, *42*, 649−655.

Watanabe, M.; Ohshita, Y.; Tsuhida, T. Antioxidant compounds from buckwheat (*Fagopyrum esculantum* Moench) hulls. *J. Agric. Food Chem.* **1997**, *45*, 1039−1044.

Xing, Y.; White, P. J. Identification and function of antioxidants from oat groats and hulls. *J. Am. Oil Chem. Soc.* **1997**, *74* (3), 303−307.

Yen, G. C.; Duh, P. D. Antioxidant properties of methanolic extracts from peanut hulls. *J. Am. Oil Chem. Soc.* **1993**, *70*, 383−386.

Zieliński, H.; Honke, J.; Troszyńska, A.; Kozłowska, H. The reduced/oxidized glutathione status as a potential index of oxidative stress in mature cereal grain. *Cereal Chem.* **1999**, *76* (6), 944−948.

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