Radical Scavenging Activity of Canola Hull Phenolics

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ABSTRACT: Possible use of canola hulls as a source of natural anti-oxidants was explored. Cyclone canola hulls were extracted with methanol (30 to 80%, vol/vol) and acetone (30 to 80%, vol/vol). The free radical-scavenging activity of phenolic extracts so prepared was evaluated using the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical ion ($ABTS^{\circ-}$), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, and chemiluminescence assays. The total content of phenolics in prepared extracts from canola hulls ranged from 15 to 136 mg sinapic acid equivalents per gram of extract. Higher levels of condensed tannins were detected in the acetone extracts than in the corresponding methanolic counterparts. Seventy and 80% (vol/vol) acetone extracts displayed markedly stronger antioxidant activity than any of the other extracts investigated. Statistically significant linear correlations were found between TEAC (Trolox equivalent antioxidant capacity) values (expressed in mM of Trolox equivalents per gram of extract) and total phenolics, TEAC and total condensed tannins (i.e., determined using the modified vanillin and proanthocyanidin assays), as well as TEAC and protein precipitation activity of phenolic extracts (i.e., measured using the dye-labeled assay). The antioxidant activities of extracts as determined by the $ABTS^{\circ-}$ radical ion assay correlated highly with those of the chemiluminescence and DPPH radical assays.

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KEY WORDS: ABTS, antioxidant, canola hulls, chemiluminescence, DPPH, phenolics, radical scavenging activity, solvent systems.

Advances in dehulling technology of canola/rapeseed (1) may bring about the introduction of dehulling to the canola/rapeseed industry in the near future. Canola and rapeseed contain 14 to 18% hulls. The hulls may contain up to 20% oil, 19.1% crude proteins (N × 6.25), 4.4% minerals, and 48% dietary fiber. Other constituents include simple sugars and oligosaccharides, polyphenolics, phytates, and residual polar lipids (2). The phenolic acids and their derivatives as well as soluble and insoluble condensed tannins are the predominant phenolic compounds found in canola and rapeseed (1,2). Canola and rapeseed hulls have been reported to contain from 89 to 1847 mg soluble condensed tannins/100 g of hulls (2). Therefore, the use of hulls, after dehulling, as a potential source of natural antioxidants may offer a commercial means for their utilization.

Alcoholic extracts of rapeseed meal exhibited strong antioxidant activity in a β-carotene–linoleate model system (3). Wanasundara and Shahidi (4) reported that the antioxidant activity of ethanolic extracts of canola meal in canola oil was equivalent to that of TBHQ and stronger than that of BHA, BHT, and BHA/BHT/monoacylglycerol citrate. Meanwhile, 1-*O*-β-D-glucopyranosyl sinapate was found to be the most active component of these extracts (5). Recently, Amarowicz *et al.* (6) reported that crude tannin extracts isolated from high-tannin canola hulls exerted a significantly ($P \leq 0.025$) greater scavenging activity on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical than those from low-tannin rapeseed hulls. Crude tannin extracts of canola hulls contained 10 to 40 times more condensed tannins than those of rapeseed hulls. Therefore, synergism of phenolics among one another and/or other components present in the extract may be responsible for a stronger free radical-scavenging activity of crude tannin extracts isolated from high-tannin canola hulls. Amarowicz *et al.* (7) isolated five major phenolic fractions from non-tannin canola hull phenolics using Sephadex LH-20 column chromatography and 95% (vol/vol) ethanol as the mobile phase. Of these, only fractions I, III, and V exhibited marked scavenging effects against the DPPH radical.

The objective of this study was to investigate the radical-scavenging activity of phenolics extracted from Cyclone canola hulls as affected by different solvent systems.

MATERIALS AND METHODS

Materials. Cyclone canola hulls were prepared according to the procedure described by Sosulski and Zadernowski (8). Hulls were extracted with hexanes for 12 h using a Soxhlet apparatus and then dried by air at room temperature.

Preparation of phenolic extracts. Cyclone canola hulls were blended with 0, 30, 50, 70, and 80% (vol/vol) aqueous acetone or methanol at solvent-to-hulls ratio of 10:1 (vol/mass) at room temperature using a Waring blender (Waring Products Division; Dynamics Corporation of America, Hartford, CT) for 2 min at maximum speed. The slurry was then filtered through Whatman No. 540 filter paper, and the residue was re-extracted once more. The final temperature of the blended slurries did not increase by more than 2°C. The filtered extracts were combined, evaporated to near-dryness under vacuum at 40°C, and then lyophilized.

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Chemical analysis. The total content of phenolic compounds in the extracts was estimated using the Folin–Denis reagent (2) and expressed as sinapic acid equivalents per gram of extract. The content of condensed tannins in the prepared extracts was determined using the modified vanillin assay (2) and expressed as milligrams condensed tannins per gram of extract and by the proanthocyanidin assay (2), in which the results were expressed in absorbance units per gram of extract.

Dye-labeled protein assay. The effect of phenolic extract concentrations on the formation of insoluble phenolic–protein complexes was determined by the dye-labeled BSA assay as described by Naczk *et al.* (9). A series of methanolic solutions of extracts (0.1 to 2.0 mg/mL) were prepared. The protein precipitating potential of phenolic extracts was reported as the slope of a line reflecting the percent concentration of the dye-labeled protein precipitated as phenolic–protein complex vs. the amount of extract added (9).

DPPH radical assay. The scavenging effect of phenolic extracts on DPPH radical was monitored as described by Chen and Ho (10). Briefly, 0.1 mL of methanolic solution containing one of the following—20 to 200 mg of 70 or 80% acetone phenolic extract; 20 to 600 mg of 50% acetone or 70 or 80% methanol phenolic extract, or 20 to 1400 mg of the remaining phenolic extracts—was mixed with 2 mL of distilled water and then added to a methanolic solution of the DPPH radical (1 mM, 0.25 mL). The mixture was vortexed for 1 min, left to stand at room temperature for 30 min, and then the absorbance of this solution was read at 517 nm. The free radical-scavenging activity of the tested extract, $C_{50\%}$, was expressed in milligrams of extract required for a 50% reduction of the DPPH radical present in the reaction mixture and was calculated from the dose–inhibition curve.

2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical ion assay. The scavenging effect of phenolic extracts from canola hulls on ABTS radical ion was monitored as described by Kim *et al.* (11). The concentration of ABTS radical ion solution was adjusted to an absorbance of 0.50 at 734 nm. The ABTS radical ion-scavenging activity of canola hull extracts was expressed in millimoles Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) values per gram of extract. The TEAC value was expressed as the slope of a line reflecting the amount of Trolox (expressed in millimolar equivalents per assay) as a function of the amount of phenolic extract added to the reaction mixture. The ABTS radical ion solutions were freshly prepared each day.

Chemiluminescence assays. The inhibition of photo-induced chemiluminescence (PCL) of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in both water and methanol by constituents of canola hull phenolic extracts was monitored using a Photochem® instrument coupled to a computer running PCLsoft® control and analysis software (Analytik Jena USA, The Woodlands, TX). Chemiluminescence was brought about by the oxidation of luminol and was delayed in the presence of antioxidants. The antioxidant capacity of water-soluble (ACW) and lipid (methanol)-soluble (ACL) constituents of extracts was determined as described by Popov and Lewin (12,13). The control sample was prepared without any extract addition. Standardized kits and protocols, supplied by Analytik Jena USA, were used to prepare working reagent solutions for these assays. Presence of water-soluble constituents delayed chemiluminescence and a lag-time was observed before a PCL signal was measured. The lag-time was used as a parameter for the quantification of radical-scavenging activity of tested extracts and was calculated using the PCLsoft® control and analysis software. The ACW was expressed as ascorbic acid equivalent antioxidant activity per gram of extract and calculated as follows: ACW= [AA]/TS; where AA is the millimolar concentration of ascorbic acid showing the same lag-time as that of the tested extract and found from the calibration curve, and TS is the amount of tested extract used, expressed in grams. On the other hand, the presence of lipid (methanol)-soluble constituents did not delay the PCL signal but markedly reduced its intensity. Hence, this inhibition, expressed as the integral of PCL intensities, was used as a parameter for the quantification of the radical-scavenging activity of tested extracts. The kinetic light emission curve was monitored for 180 s, and the area under the curve was calculated using the PCLsoft® control and analysis software. The ACL was expressed as TEAC per gram of extract and calculated as follows: ACL = TX/TS; where TX is the millimolar concentration of Trolox displaying a similar inhibitory effect to that of tested sample and found from the calibration curve, and TS is the amount of tested extract used, in grams.

Data treatment. The results presented in graphs and tables are mean values of at least three experiments. Statistical analysis of data was carried out using SigmaStat v.3.0 (SSPS Science Inc., Chicago, IL). Each extract, for the purpose of statistical analysis, was referred to as a treatment. The statistical analysis of all treatments was performed using the ANOVA and linear regression tests. In addition, the *t*-test was used among the treatments when a statistically significant difference ($P \leq 0.05$) was found using the ANOVA test. Treatments followed by the same subscript letter in tables are not significantly different ($P > 0.05$; *t*-test).

RESULTS AND DISCUSSION

The total content of phenolics in crude phenolic extracts was determined by the Folin–Denis assay and expressed as sinapic acid equivalent per gram of extract. The Folin–Denis reagent is sensitive to all classes of phenolics, and sinapic acid is the major phenolic compound detected in canola and rapeseed (1). The total phenolics content in canola hull extracts ranged from 15.1 to 136.2 mg/g and was much higher than that reported by Matthäus (14) for extracts obtained from residues of eight oilseeds. The content of condensed tannins was estimated by the vanillin and proanthocyanidin assays, which are commonly used for tannin determination. The contents of condensed tannins reported in Table 1 are in the range of previously published values (1,9). In addition, the data shown in Table 1 indicate that 70 and 80% acetone–water systems were the most efficient solvents for the extraction of phenolics (i.e.,

both total phenolics and condensed tannins) from canola hulls.

A number of methods have been proposed for the estimation of protein-precipitating potential of plant-based phenolic extracts. Of these assays, the dye-labeled BSA assay (9) was chosen for the estimation of the protein precipitation potential of crude phenolic extracts isolated from canola hulls. The dye-labeled BSA assay measures the amount of protein precipitated by phenolics. The protein precipitation potential of phenolic extracts from canola hulls was expressed as slopes of lines (i.e., as titration curves) depicting the amount of phenolic–protein complex precipitated as a function of the amount of extract added to the reaction mixture. According to Naczk *et al.* (9), the slope is a more meaningful measure of protein-precipitating potential of plant phenolic extracts than the measurement carried out at one nonstandardized phenolic/protein ratio (16). The numerical values for the slopes from the titration curves are given in Table 1. Only 70 and 80% (vol/vol) acetone extracts of canola hull phenolics exhibited strong affinities for the dye-labeled protein. According to Porter and Woodruffe (16), the ability of phenolics to precipitate proteins is influenced by the degree of their polymerization. The chemical structures of canola hull phenolics are unknown, and more detailed analyses of these compounds are still needed.

Crude extracts from plant material are a complex mixture of phenolics with different molecular structures. It has been well established that the antioxidant potential of phenolics is greatly influenced by their molecular structures (17). The chemiluminescence, DPPH radical, and ABTS radical ion assays were selected for determination of the radical-scavenging potentials of crude extracts of canola hulls.

The DPPH radical assay has been widely used for the estimation of antioxidant activity of plant phenolics (6,10,18). The ratio of the decrease in absorbance of the DPPH radical solution at 517 nm to the absorbance of the DPPH radical solution in the absence of phenolics at 517 nm was proposed as a measure of the radical-scavenging activity of the antioxidant used (10). This procedure was modified by Brand-Williams *et al.* (18) to take into account the different kinetic behavior of antioxidants. Unfortunately, this modified protocol is not suitable for evaluation of scavenging activity of crude phenolic extracts, since knowledge of molecular structures of compounds is essential. Therefore, in this study, the amount of crude extract required for a 50% depletion of the DPPH radical present in the reaction mixture, i.e., $C_{50\%}$, was used as a measure for the radical-scavenging activity. The $C_{50\%}$ values for tested extracts ranged from 107 to 1250 mg/assay (Table 2). Strong radical-scavenging activity is associated with low $C_{50\%}$ values. The 70 and 80% (vol/vol) crude acetone extracts exhibited greater radical-scavenging activities than any of the other phenolic extracts examined. In this case, less than 140 mg/assay of extract was required to scavenge 50% of the DPPH radicals. Similarly, Matthäus (14) reported that less than 200 mg/assay of a 70% acetone or 70% methanol extract from rapeseed residues was needed to reduce the level of DPPH radicals by 50%.

The ABTS radical ion assay, now commonly used for the estimation of antioxidant activities of plant phenolics, is based on the inhibition of the ABTS radical anion ($ABTS^{\circ-}$) (11) or the ABTS radical cation ($ABTS^{+}$) (19). The assay used in this study is based on the inhibition of $ABTS^{\circ-}$. The TEAC values listed in Table 2 are the slopes of lines depicting the relationship between the antioxidant activity of an extract, expressed as Trolox equivalents (TE)/assay, and the amount (in grams) of extract added to the reaction mixture. The value of the slope is a meaningful measure of the TEAC value because it is based on statistical analysis (linear regression) of experimental data involving the measurement of antioxidant activity of the extract at a minimum of four different antioxidant concentrations. The TEAC values of crude phenolic extracts from canola hulls ranged from 0.180 for the 30% (vol/vol) methanol extract to 0.790 for the 70% (vol/vol) acetone extract (Table 2). The TEAC values of acetone crude phenolic extracts were about two to three times higher than those found for methanolic extracts. The difference in the TEAC values reported here may be due to a higher level of both total phenolics and condensed tannins in acetone extracts than those in the corresponding methanol extracts (Table 1). In a

TABLE 1
Total Phenolic and Total Condensed Tannin Contents of Crude Extracts

Solvent system (%; vol/vol)		Total phenolics (mg sinapic acid equiv/g)	Condensed tannins (CT)		
			VAN[a]	PROA[b]	DLP[c]
Acetone	80	103.8 ± 2.7	96.1 ± 5.3	221.0 ± 8.6[a]	9.66 ± 0.92[a]
	70	94.3 ± 3.0	127.0 ± 2.5	224.4 ± 8.6[a]	9.54 ± 0.06[a]
	50	41.5 ± 0.3	40.1 ± 1.4	95.3 ± 2.0	3.44 ± 0.65[b]
	30	21.2 ± 0.2	10.8 ± 0.1	32.9 ± 2.0	ND
Methanol	80	40.4 ± 0.2	6.0 ± 0.2	45.6 ± 1.6	4.32 ± 0.36
	70	36.5 ± 0.6	6.9 ± 0.2	41.4 ± 0.9	3.04 ± 0.41[b]
	50	23.9 ± 0.2	4.4 ± 0.2	25.0 ± 0.6	2.40 ± 0.04
	30	15.1 ± 0.1	3.5 ± 0.6	16.8 ± 1.0	0.70 ± 0.05

[a]VAN, total CT as determined by the vanillin assay; units are in mg tannins/g extract.
[b]PROA, total CT as determined by the proanthocyanidin assay; units are in absorbance units/g extract.
[c]DLP, protein precipitating potential as determined by the dye-labeled assay; values are expressed as the slope of the line reflecting the percent concentration of the dye-labeled protein precipitated (PP) as a phenolic–protein complex vs. the amount of extract added, in milligrams. Units are percent PP per mg of extract. Values in each column sharing the same roman superscript letter are not significantly different ($P > 0.05$; *t*-test).

TABLE 2
Radical-Scavenging Activity of Crude Phenolic Extracts of Canola Hulls as Determined by the DPPH, ABTS, and Chemiluminescence Assays

Solvent system (%; vol/vol)		DPPH $C_{50\%}$[a]	ABTS TEAC[b,c]	Chemiluminescence ACW[b,d]	Chemiluminescence ACL[b,e]
Acetone	80	107	0.73 ± 0.02	0.94 ± 0.02	1.73 ± 0.13
	70	140	0.79 ± 0.03	0.66 ± 0.04	1.47 ± 0.05
	50	374	0.39 ± 0.03	0.53 ± 0.01	0.61 ± 0.03
	30	703	0.25 ± 0.01	0.29 ± 0.03	0.43 ± 0.04
Methanol	80	360	0.30 ± 0.01[a]	0.85 ± 0.05	0.73 ± 0.03[a]
	70	505	0.29 ± 0.02[a]	0.60 ± 0.04	0.70 ± 0.05[a]
	50	794	0.24 ± 0.01	0.38 ± 0.03	0.48 ± 0.02
	30	1250	0.18 ± 0.01	0.15 ± 0.01	0.27 ± 0.01

[a]Results are the mean values of two experiments; units are in µg extract/assay.
[b]Results are the mean values of 4–6 experiments ± SD.
[c]Values are expressed as the slope of the line reflecting the amount of Trolox equivalents (mM) per assay vs. the amount of phenolic extract added (g); units are in mM Trolox equivalents/g extract.
[d]ACW, antioxidant activity of water-soluble constituents of extract; units are in mM ascorbic acid/g extract.
[e]ACL, antioxidant activity of lipid (methanol)-soluble constituents of extracts; units are in mM Trolox/g extract. Values in each column sharing the same roman superscript letter are not significantly different ($P > 0.05$; *t*-test). DPPH, 1,1-diphenyl-2-picrylhydrazyl; $C_{50\%}$, µg of extract required for 50% reduction of the DPPH radical present in the reaction mixture; ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate); TEAC, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity.

separate experiment, the 70% (vol/vol) acetone extract of canola hulls was fractionated on a Sephadex LH-20 column into nontannin and tannin fractions (7). The antioxidant activity displayed by the tannin fraction was four and eight times greater than that of the corresponding crude phenolic extract and nontannin fraction, respectively. Also, Beninger and Hosfield (20) reported that the condensed tannin fraction of a methanolic extract from *Phaseolus vulgaris* L. plays an important role in the antioxidant activity of the extract.

The Photochem® is the first system that can quantify the antioxidant capacity of water- and lipid (methanol)-soluble substances. It combines the very fast photochemical excitation of radical generation with highly sensitive luminometric detection. Because of the high sensitivity of the assay, only nanomolar concentrations of antioxidant substances are required. The photosensitizer substance (i.e., luminol) is optically excited to produce the superoxide anion radical ($O_2^{-\bullet}$), which generates luminescence. The antioxidant, added to the reaction mixture, scavenges a part of the $O_2^{-\bullet}$ and the remaining luminescence is measured. The antioxidant capacity of phenolic extracts is expressed as ascorbic acid equivalents (i.e., ACW) or TE (i.e., ACL). Table 2 summarizes the antioxidant capacity of the canola hull extracts as evaluated by the chemiluminescence assays. The water-soluble (ACW) and methanol (lipid)-soluble (ACL) constituents of acetone extracts exhibited markedly stronger antioxidant activity than their methanolic counterparts. The acetone extracts contained more condensed tannins than the corresponding methanolic extracts (Table 1). This suggests that condensed tannins may be responsible for this increase in antioxidant activity. The contribution of condensed tannins to the overall antioxidant activity of the extract is more evident for 70 and 80% (vol/vol) acetone extracts, as these extracts contained 3 to 60 times more tannins than the other preparations.

The antioxidant activities of crude phenolic extracts of canola hulls, as determined by the ABTS radical ion assay, correlated strongly with their total content of phenolics (Fig. 1A). Similar correlations were also found between the total phenolic content of the extracts and their antioxidant activities as determined by the DPPH radical (power function; $r^2 = 0.984$) and chemiluminescence (only for ACL; linear function; $r^2 = 0.987$) assays. Furthermore, several authors (21,22) have reported the existence of a strong correlation between total phenols and their antioxidant activity.

Statistically significant linear correlations existed between the TEAC values of crude phenolic extracts and the total condensed tannins content in the extracts as measured by the vanillin (Fig. 1B) and proanthocyanidin (Fig. 1C) assays as well as the protein-precipitating potential of canola hull phenolics (Fig. 1D). These correlations indicate that condensed tannins (i.e., proanthocyanidins) contribute significantly to the antioxidant activities displayed by the crude phenolic extracts of canola hulls. Luximon-Ramma *et al.* (22) also found a strong correlation between the condensed tannins content in extracts of *Cassia fistula* and their antioxidant activity as measured by the ABTS radical ion and FRAP (i.e., ferric-reducing antioxidant power) assays. Moreover, Bors *et al.* (23) observed that tannins displayed stronger antioxidant activity than other phenolics. Oki *et al.* (24) demonstrated that polymeric procyanidins are the major radical-scavenging component of red-hulled rice. Furthermore, Yilmaz and Toledo (25) reported that oligomeric and polymeric procyanidins accounted for most of the antioxidant activity displayed by grapeseed phenolics. Rösch *et al.* (26) found that antioxidant activity of the oligomeric fraction of condensed tannins constituted about 75% of the total antioxidant activity displayed by sea buckthorn pomace.

Both the DPPH radical and ACL values were highly correlated with the TEAC values (Fig. 2). These correlations indicate

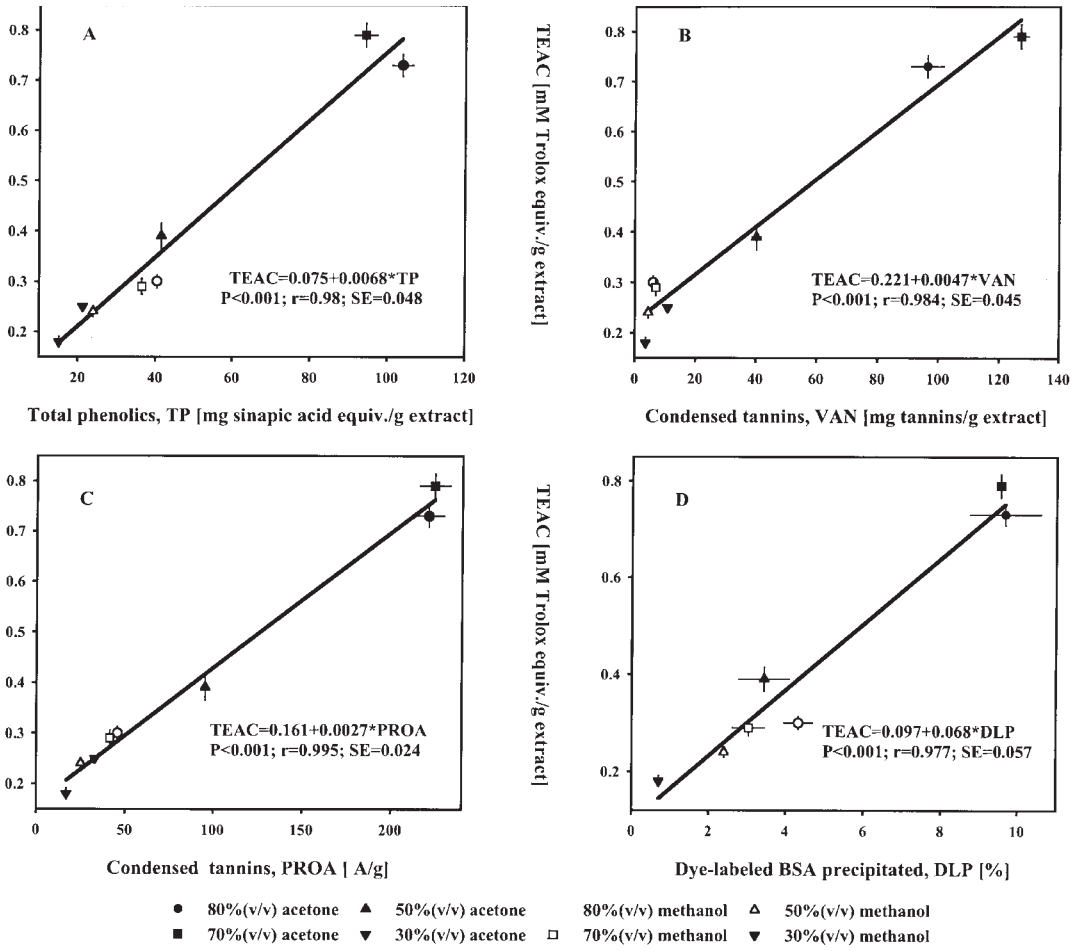

FIG. 1. Effects of total phenolics (A), and proanthocyanidins as determined by the vanillin (VAN) (B), proanthocyanidin (PROA) (C), and dye-labeled protein (DLP) (D) assays. SE, standard error of estimate; TEAC, Trolox(6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity.

that the antioxidant potentials of crude extracts of phenolics from canola hulls, as measured by chemiluminescence DPPH radical and ABTS radical ion assays, are comparable. Statistically significant correlations have also been reported between ABTS radical ion, DPPH radical, and oxygen radical absorbance capacity values for sorghum and sorghum products (21), and between the antioxidant potential of mango seed kernel phenolics as measured by the FRAP and ABTS radical ion assays (27).

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REFERENCES

1. Naczk, M., R. Amarowicz, A. Sullivan, and F. Shahidi, Current Research Developments on Polyphenolics of Rapeseed/Canola: A Review, *Food Chem. 62*:489–502 (1998).
2. Naczk, M., T. Nichols, D. Pink, and F. Sosulski, Condensed Tannins in Canola Hulls, *J. Agric. Food Chem. 42*:2196–2200 (1994).
3. Nowak, H., K. Kujawa, R. Zadernowski, K.B. Roczniak, and H. Kozlowska, Antioxidative and Bactericidal Properties of Phenolic Compounds in Rapeseed, *Fett Wiss. Technol. 94*:149–152 (1994).
4. Wanasundara, U.N., and F. Shahidi, Canola Extract as an Alternative Natural Antioxidant for Canola Oil, *J. Am. Oil Chem. Soc. 71*:817–822 (1994).
5. Wanasundara, U., R. Amarowicz, and F. Shahidi, Isolation and Identification of an Antioxidative Component in Canola Meal, *J. Agric. Food Chem. 42*:1285–1290 (1994).
6. Amarowicz, R., M. Naczk, and F. Shahidi, Antioxidant Activity of Crude Tannins of Canola and Rapeseed Hulls, *J. Am. Oil Chem. Soc. 77*:957–961 (2000).
7. Amarowicz, R., M. Naczk, and F. Shahidi, Antioxidant Activity of Various Fractions of Non-tannin Phenolics of Canola Hulls, *J. Agric. Food Chem. 48*:2755–2759 (2000).
8. Sosulski, F., and R. Zadernowski, Fractionation of Rapeseed Meal into Flour and Hull Components, *J. Am. Oil Chem. Soc. 58*:96–98 (1981).
9. Naczk, M., R. Amarowicz, R. Zadernowski, and F. Shahidi, Protein-Precipitating Capacity of Crude Condensed Tannins of Canola and Rapeseed Hulls, *Ibid. 78*:1173–1178 (2001).
10. Chen, C.-W., and C.-T. Ho, Antioxidant Properties of Polyphenols

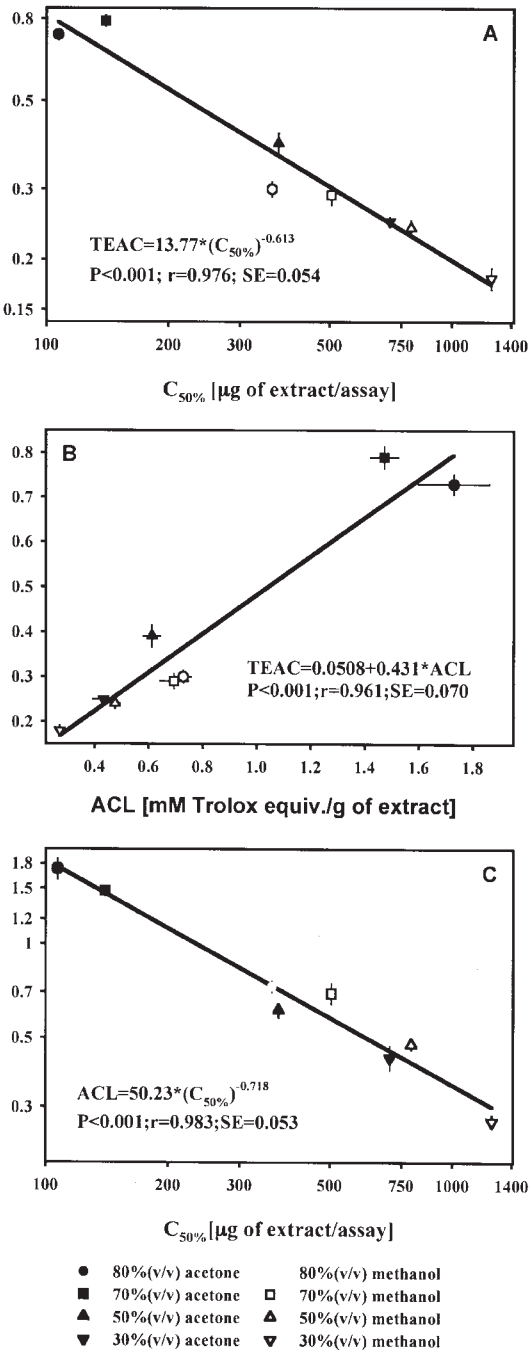

FIG. 2. Correlation between antioxidant activities of canola hull extracts as determined by (A) 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical ion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assays; (B) ABTS radical ion and chemiluminescence (lipid-soluble antioxidant) assays; and (C) DPPH radical and (lipid-soluble antioxidant) assays. For other abbreviations see Figure 1.

Extracted from Green and Black Teas, *J. Food Lipids 2*:35–46 (1995).
11. Kim, D.-O., K.W. Lee, H.J. Lee, and C.Y. Lee, Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Phenolic Phytochemicals, *J. Agric. Food Chem. 50*:3713–3717 (2002).
12. Popov, I.N., and G. Lewin, Photochemiluminescent Detection of Antiradical Activity: II. Testing of Nonenzymic Water-Soluble Antioxidants, *Free Radic. Biol. Med. 17*:267–271 (1994).
13. Popov, I.N., and G. Lewin, Photochemiluminescent Detection of Antiradical Activity; IV: Testing of Lipid-Soluble Antioxidants, *J. Biochem. Biophys. Methods 31*:1–8 (1996).
14. Matthäus, B., Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds, *J. Agric. Food Chem. 50*:3444–3452 (2002).
15. Hagerman, A.E., and L.G. Butler, Protein Precipitation Method for the Quantitative Determination of Tannins, *Ibid. 26*:809–812 (1978).
16. Porter, L.J., and J. Woodruffe, Haemanalysis: The Relative Astringency of Proanthocyanidin Polymers, *Phytochemistry 23*:1255–1256 (1984).
17. Rice-Evans, C.A., N.J. Miller, and G. Paganga, Structure–Antioxidant Activity Relationships of Flavonoids and Phenolic Acids, *Free Radic. Biol. Med. 20*:933–956 (1996).
18. Brand-Williams, W., M.E. Cuvelier, and C. Berset, Use of a Free Radical Method to Evaluate Antioxidant Activity, *Lebensm.-Wiss. Technol. 28*:25–30 (1995).
19. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C.A. Rice-Evans, Antioxidant Activity Applying Improved ABTS Radical Cation Decolorization Assay, *Free Radic. Biol. Med. 26*:1231–1237 (1999).
20. Beninger, C.W., and G.L. Hosfield, Antioxidant Activity of Extracts, Condensed Tannin Fractions, and Pure Flavonoids from *Phaseolus vulgaris* L. Seed Coat Color Genotypes, *J. Agric. Food Chem. 51*:7879–7883 (2003).
21. Awika, J.M., L.W. Rooney, X. Wu, R.L. Prior, and L. Cisneros-Zevallos, Screening Methods to Measure Antioxidant Activity of Sorghum (*Sorghum bicolor*) and Sorghum Products, *J. Agric. Food Chem. 51*:6657–6662 (2003).
22. Luximon-Ramma, A., T. Bahorun, M.A. Soobrattee, and O.I. Aruoma, Antioxidant Activities of Phenolic, Proanthocyanidin, and Flavonoid Components in Extracts of *Cassia fistula, Ibid. 50*:5042–5047 (2002).
23. Bors, W., C. Michel, and K. Stettmaier, Electron Paramagnetic Resonance Studies of Radical Species of Proanthocyanidins and Gallate Esters, *Arch. Biochem. Biophys. 374*:347–355 (2000).
24. Oki, T., M. Masuda, M. Kobayashi, Y. Nishiba, S. Furuta, I. Suda, and T. Sato, Polymeric Procyanidins as Radical-Scavenging Components in Red-Hulled Rice, *J. Agric. Food Chem. 50*:7524–7529 (2002).
25. Yilmaz, Y., and R.T. Toledo, Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin, Epicatechin, and Gallic Acid, *Ibid. 52*:255–260 (2004).
26. Rösch, D., C. Mügge, V. Fogliano, and L.W. Kroh, Antioxidant Oligomeric Proanthocyanidins from Sea Buckthorn (*Hippophäe rhamnoides*) Pomace, *Ibid. 52*:6712–6718 (2004).
27. Soong, Y.-Y., and P.J. Barlow, Antioxidant Activity and Phenolic Content of Selected Fruit Seeds, *Food Chem. 88*:411–417 (2004).

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