Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds†

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Methods for determining primary antioxidant activity were evaluated. A β-carotene bleaching method and a free radical method using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) were modified to rapidly test samples for potential antioxidant activity. Malonaldehyde production in a linoleic acid emulsion system assayed by an HPLC method was also used to determine antioxidant and prooxidant activities initiated by a metal catalyst (Cu^{2+}). All methods were used to assess activity of selected phenolic compounds including several anthocyanidins/anthocyanins and selected berry extracts. Most phenolic compounds had prooxidant activity at low concentrations, unlike synthetic antioxidants (BHA and BHT). Compounds with similar structures exhibited comparable trends in antioxidant activity. Antioxidant activity usually increased with an increase in the number of hydroxyl groups and a decrease in glycosylation. The antioxidant activity of many phenolic compounds and extracts was comparable to those of synthetic antioxidants using the β-carotene bleaching and HPLC methods.

Keywords: *Antioxidant activity; prooxidant activity; phenolics; flavonoids; anthocyanins; berry extracts*

INTRODUCTION

Lipid oxidation occurs when oxygen reacts with lipids in a series of free radical chain reactions that lead to complex chemical changes. Oxidation of lipids in foods causes quality losses. In vivo, lipid oxidation may play a role in coronary heart disease, atherosclerosis, cancer, and the aging process (Jadhav et al., 1996).

Antioxidants are compounds that can delay or inhibit lipid oxidation. When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Jadhav et al., 1996). Recently, interest has been growing in finding naturally occurring antioxidants for use in foods to replace synthetic antioxidants and for possible in vivo use. As one potential source, plant phenolics have primary (chain-breaking) antioxidant activity (Shahidi and Wanasundara, 1992). To evaluate compounds for antioxidant activity, a reliable in vitro method is needed.

Most antioxidant activity assays consist of accelerating oxidation in a lipid system, usually by heat, and then monitoring oxygen consumption, substrate loss, or product formation. Because many factors affect oxidation, including temperature, oxygen pressure, metal catalysts, fat composition, and form of fat, results can vary depending on the oxidation conditions used (Frankel, 1993). Assays to measure substrates or products can also give varying results depending on their specificity. Osawa and Shibamoto (1992) developed a high-performance liquid chromatography (HPLC) method to measure malonaldehyde formed in lipid emulsion systems oxidized by $FeCl_2/H_2O_2$. Malonaldehyde was derivatized by reaction with urea under acidic conditions to form 2-hydroxypyrimidine, which could be measured by HPLC. Tsuda et al. (1994) used this method to measure malonaldehyde formed in various lipid systems. Because the HPLC method is specific for malonaldehyde, combining this method with a model lipid oxidation system could be a good assay for antioxidant activity.

Methods relying on oxidation can be time-consuming to perform depending on conditions used, whereas β-carotene bleaching and reacting compounds with free radicals are quick and simple methods of measuring potential antioxidant activity. Marco (1968) described the use of β-carotene bleaching for ranking compounds for antioxidant activity. In this method, antioxidant activity is measured by the ability of a compound to minimize the loss of β-carotene during the coupled oxidation of linoleic acid and β-carotene in an emulsified aqueous system. The reaction is usually initiated using heat (50 °C). Although the method is simple and sensitive, it was criticized by Frankel (1993) for its nonspecificity, being subject to interference from oxidizing and reducing agents in crude extracts and linoleic acid not being representative of typical food lipids.

Two free radicals that have been used for assessing antioxidant activity are 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH•), also known as 1,1-diphenyl-2-picrylhydrazyl or α,α-diphenyl-β-picrylhydrazyl. Reduction of DPPH• by an antioxidant ($DPPH^{\bullet} + A \rightarrow DPPH{-}H + A^{\bullet}$) or by a radical species ($DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH{-}R$) results in a loss of absorbance at 515 nm. Brand-Williams et al. (1995), using DPPH•, developed a spectrophotometric method that gave results similar to an oxidation method, but comparisons were not quantitative because reaction with DPPH• depended on a compound's structural conformation.

Compounds with antioxidant activity may exhibit prooxidant behavior under certain conditions. Prooxidant activity can accelerate damage to molecules such as DNA, carbohydrates, or proteins (Aruoma et al.,

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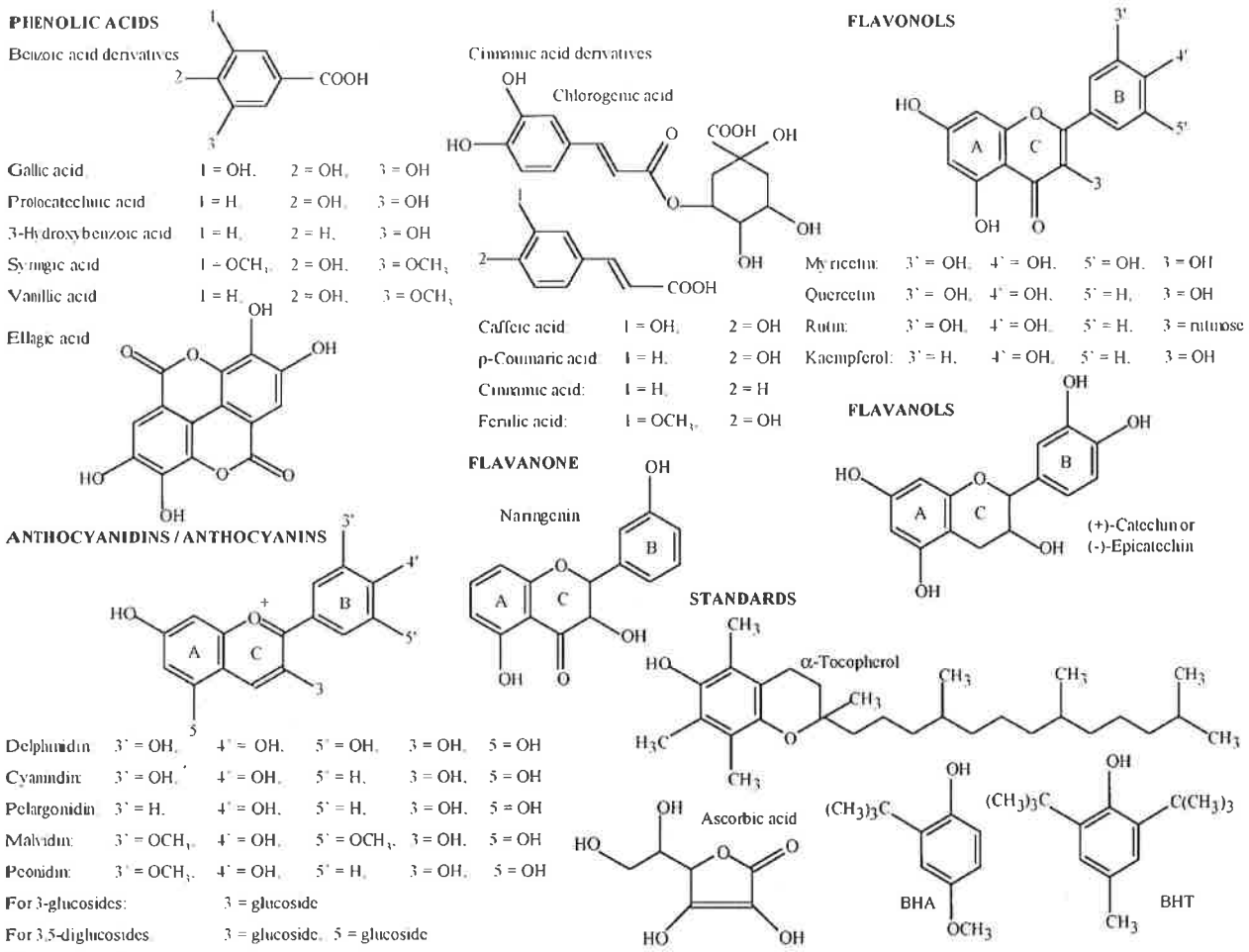

Figure 1. Structures of compounds tested.

1997). Potential antioxidants should therefore be tested for prooxidant activity as well. The deoxyribose, iron–bleomycin–DNA, and copper–1,10 phenanthroline–DNA assays have been used as prooxidant tests (Aruoma et al., 1997). Prooxidant activity has also been measured using metal catalysts in a β-carotene/linoleic emulsion system (Pischetsrieder et al., 1998) and using a Cu^{2+} catalyst in an oxygen radical absorbance capacity assay (Cao et al., 1997).

The objectives of this study were to (1) adapt a β-carotene bleaching method and a DPPH• method as fast screening assays for potential antioxidant activity using microplates, (2) develop an oxidation system using a linoleic acid emulsion so both prooxidant and antioxidant activities could be assessed by an HPLC method that measures malonaldehyde, (3) measure the antioxidant and prooxidant activities of several phenolic compounds including some anthocyanidins/anthocyanins by all methods to compare results and evaluate if the results can be related to compound structures and literature results, and (4) measure the antioxidant and prooxidant activities of selected berry extracts by all methods to compare results and determine if the methods are affected by various compounds present in extracts.

MATERIALS AND METHODS

Chemicals. L-Ascorbic acid, 3-hydroxybenzoic acid, (+)-catechin, 4-hydroxy-3-methoxycinnamic acid (ferulic acid), gallic acid, 4′,5,7-trihydroxyflavanone (naringenin), and rutin were purchased from Aldrich (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada). Cyanidin chloride, cyanidin 3-glucoside (kuromanin) chloride, cyanidin 3,5-diglucoside (cyanin) chloride, delphinidin chloride, malvidin chloride, malvidin 3-glucoside (oenin) chloride, pelargonidin chloride, pelargonidin 3,5-diglucoside (pelargonin) chloride, peonidin chloride, and peonidin 3-glucoside chloride were obtained from Extrasynthèse (Genay, France). Pelargonidin 3-glucoside (callistephin) chloride was purchased from Carl Roth (Karlsuhe, Germany). Malvidin 3-glucoside chloride was also obtained from Professor R. Brouillard (Université Louis Pasteur, Strasbourg, France). α,α′-Azodiisobutyramidine dihydrochloride (ADIBA), cinnamic acid, kaempferol, linoleic acid, and myricetin were from Fluka (Sigma-Aldrich Canada Ltd.). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), caffeic acid, β-carotene, chlorogenic acid, *p*-coumaric acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH•), ellagic acid, (−)-epicatechin, 2-hydroxypyrimidine, malvidin 3,5-diglucoside (malvin) chloride, protocatechuic acid, quercetin, syringic acid, 1,1,3,3-tetraethoxypropane (TEP), α-tocopherol, and vanillic acid were from Sigma Chemical Co. (Sigma-Aldrich Canada Ltd.). Tween 20 was obtained from BDH Chemicals (Toronto, ON, Canada). The structures of compounds tested for antioxidant and prooxidant activity are shown in Figure 1. Test compounds were prepared in methanol.

***β*-Carotene Bleaching Method.** The β-carotene bleaching methods of Marco (1968) and Velioglu et al. (1998) were modified for use with microplates. The modification consisted of preparing a mixture of 1 mL of β-carotene (2 mg/mL in chloroform), 0.2 mL of linoleic acid, and 2 mL of Tween 20. The mixture was vortexed, and chloroform was removed using

a stream of nitrogen for 1−1.5 h. Air-sparged distilled water (20 mL) was then added to the mixture, which was subsequently vortexed to form a clear solution. The volume of solution was sufficient for 100 samples. Sample (20 μL) and 200 μL of the β-carotene solution were added to a well in a 96-well flat-bottom EIA microtitration plate from ICN Biomedicals Inc. (Aurora, OH). Samples were prepared in triplicate for each concentration used (0−1500 μM), and at least seven different concentrations were used. To dilute the sample mixture, 30 μL of the mixture was transferred to another plate and air-sparged distilled water (210 μL) was added [1:8 (v/v) dilution]. Because the β-carotene bleaching reaction was subject to noticeable variations, the dilutions were done in triplicate. Marco (1968) and Velioglu et al. (1998) initiated the reaction by incubating mixtures at 50 °C. In the modified method, ADIBA (20 μL of 0.3 M) as used by Pischetsrieder et al. (1998) was added to each well to initiate the reaction. The plate was read in an MRX plate reader (Dynex Technologies Inc., Chantilly, VA) using a 450 nm filter at 0 min and after 90 min of incubation in the dark at room temperature (~22 °C). At 0 and 90 min, the A_{450nm} was usually around 1.0−1.2 and 0.1−0.3, respectively, for the control (0 μM).

Absorbance at 450 nm after 90 min of incubation was plotted against concentration of sample added. Plots either increased linearly with concentration and then remained constant or showed no change with concentration. The slope for the initial linear portion of the plot was calculated from the dilutions done in triplicate ($r^2 > 0.800$). The average and standard deviation of the slopes from the three replicate measurements were calculated and used to compare antioxidant activities.

DPPH• Method. Modifications were made to the original DPPH• method of Brand-Williams et al. (1995). For the modified procedure, a 150 μM solution of DPPH• was prepared in 80% methanol instead of 100% methanol. Using 80% methanol had the advantage of a faster reaction rate for some compounds such as BHA and BHT and lower evaporation losses. Instead of reading samples spectrophotometrically, the assay was performed in a microplate. To a well in a 96-well flat-bottom EIA microtitration plate from ICN Biomedicals Inc. were added 22 μL of sample and 200 μL of DPPH• solution. Samples were prepared in triplicate for each concentration used (0−500 μM), and at least seven different concentrations were used. The plate was then covered and left in the dark at room temperature (~22 °C). After 30, 180, and 360 min, the plate was read in an MRX plate reader using a 520 nm filter. The incubation time for caffeic acid was increased to 48 h to obtain complete reaction.

A plot of A_{520nm} versus concentration of sample in the final solution was made for each time interval. Using the results from the time interval with the steepest slope, the initial slope of the curve was calculated by linear regression ($r^2 > 0.800$). The antiradical activity was defined by the initial slope value in units of A_{520nm}/micromolar of sample or micromolar of DPPH•/micromolar of sample. The units were converted from A_{520nm} to micromolar of DPPH• by developing a standard curve for DPPH• using the plate reader. The concentration of DPPH• was initially determined from the calibration curve equation given by Brand-Williams et al. (1995), where A_{515nm} measured spectrophotometrically was equal to 12509 × concentration in M − 0.00258. Although Brand-Williams et al. (1995) solubilized DPPH• in methanol, the same equation was used because absorbance of DPPH• in 80% methanol was the same. The antiradical activity was found to be equivalent to negative half the antiradical power (ARP) as defined by Brand-Williams et al. (1995). ARP was equal to the reciprocal of the amount of compound required to decrease the initial DPPH• concentration by 50% in units of moles of DPPH• per mole of compound.

HPLC Method. The oxidation procedure of Osawa and Shibamoto (1992) was modified by initiating oxidation with Cu^{2+} instead of $FeCl_2/H_2O_2$, so both antioxidant and prooxidant activities could be measured using a linoleic acid emulsion. For the modified procedure, buffer was prepared with 0.218% (w/v) SDS in 21.8 mM Tris-HCl at pH 7.4. Linoleic acid emulsion was prepared by mixing linoleic acid with buffer (2.18 [illegible] dispensed into a test tube followed by the addition of 70 μL of sample. Samples were prepared in triplicate for each concentration used (0−4000 μM), and at least five different concentrations were used. To initiate the reaction, 20 μL of 10.9 mM $CuSO_4$ was added to the test tube. The uncovered test tube was then incubated at 37 °C for 16 h in the dark on a Lab-Line Instruments Inc. shaker (Melrose Park, IL) rotating at 1500 rpm.

Test tubes were weighed before and after incubation to determine losses due to evaporation. The volume of each test tube was adjusted to the initial level with distilled water. To each test tube were then added 20 μL of 1.2 mg/mL BHT in methanol, 100 μL of 1.2 N HCl, and 100 μL of 120 mM urea. Samples were heated at 100 °C for 60 min, cooled, and then cleaned by applying 0.5 mL to a tC18 cartridge (Waters Chromatography Division, Millipore Corp., Milford, MA). Eluate was collected when the cartridge was washed with 1.5 mL of distilled H_2O. The eluate (50 μL) was injected into a Waters HPLC system (Mississauga, ON, Canada) equipped with a Waters 990 photodiode array detector monitoring at 309 nm. The column used was a reverse-phase Supelcosil LC-18 (25 cm × 2.1 mm i.d.) from Supelco Inc. (Bellefonte, PA). Distilled deionized water was used as the solvent at a flow rate of 0.35 mL/min, and the column was maintained at 25 °C. Standard solutions of 2-hydroxypyrimidine or derivatized malonaldehyde prepared from TEP using the procedure of Csallany et al. (1984) eluted around 4.5 min. The percent malonaldehyde of the control at each concentration was calculated as area for any concentration per area for 0 μM × 100. Plots of percent malonaldehyde of the control versus concentration of sample added were made. If the percent malonaldehyde of the control was >100 in the concentration range tested, the sample had prooxidant activity. The concentration range when the percent malonaldehyde of the control decreased to 0 was used as a quantitative indicator of antioxidant activity.

Extraction of Phenolics from Berries. Phenolics were extracted from frozen samples of saskatoon berries (*Amelanchier alnifolia* Nutt.), blackberries, blackcurrants, and blueberries. Saskatoon berries (cv. Smoky) were obtained from The Berry Basket (Clairmont, AB, Canada), and blackcurrants were obtained from Riverbend Country Gardens (Sylvan Lake, AB, Canada). Frozen blackberries and blueberries were purchased at a local supermarket.

To extract phenolics, 10 g of berries was combined with 40 mL of 80% methanol in a temperature-controlled (~4 °C) Waring blender and mixed for 8 min at low speed. The mixture was then filtered (Whatman No. 42) through a Büchner funnel. The filtrate volume was adjusted to 50 mL using 80% methanol, and a portion of the extract was filtered (0.45 μm Acrodisc LC PVDF syringe filter; Pall Gelman Laboratory, Montreal, PQ, Canada) prior to phenolic and antioxidant analyses.

Measurement of Phenolics. Phenolics were measured using a modified version of the Glories' method (Glories, 1978; Romani et al., 1996) described in Mazza et al. (1999). Briefly, the method consisted of mixing 0.25 mL of sample with 0.25 mL of 0.1% HCl in 95% ethanol and 4.55 mL of 2% HCl. The absorbance of the solution was then read at 280, 320, 360, and 520 nm to measure total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. Standards used were chlorogenic acid, caffeic acid, quercetin, and malvidin 3-glucoside for total phenolics, tartaric esters, flavonols, and anthocyanins, respectively. Standards were prepared in 80% methanol except for quercetin, which was prepared in 100% methanol.

RESULTS AND DISCUSSION

Prooxidant Activity. Figure 2 shows typical effects of the addition of different compounds on percent malonaldehyde of the control derived from the HPLC method. Almost all phenolics exhibited some prooxidant behavior at low concentrations (Figure 2; Table 1), [illegible]

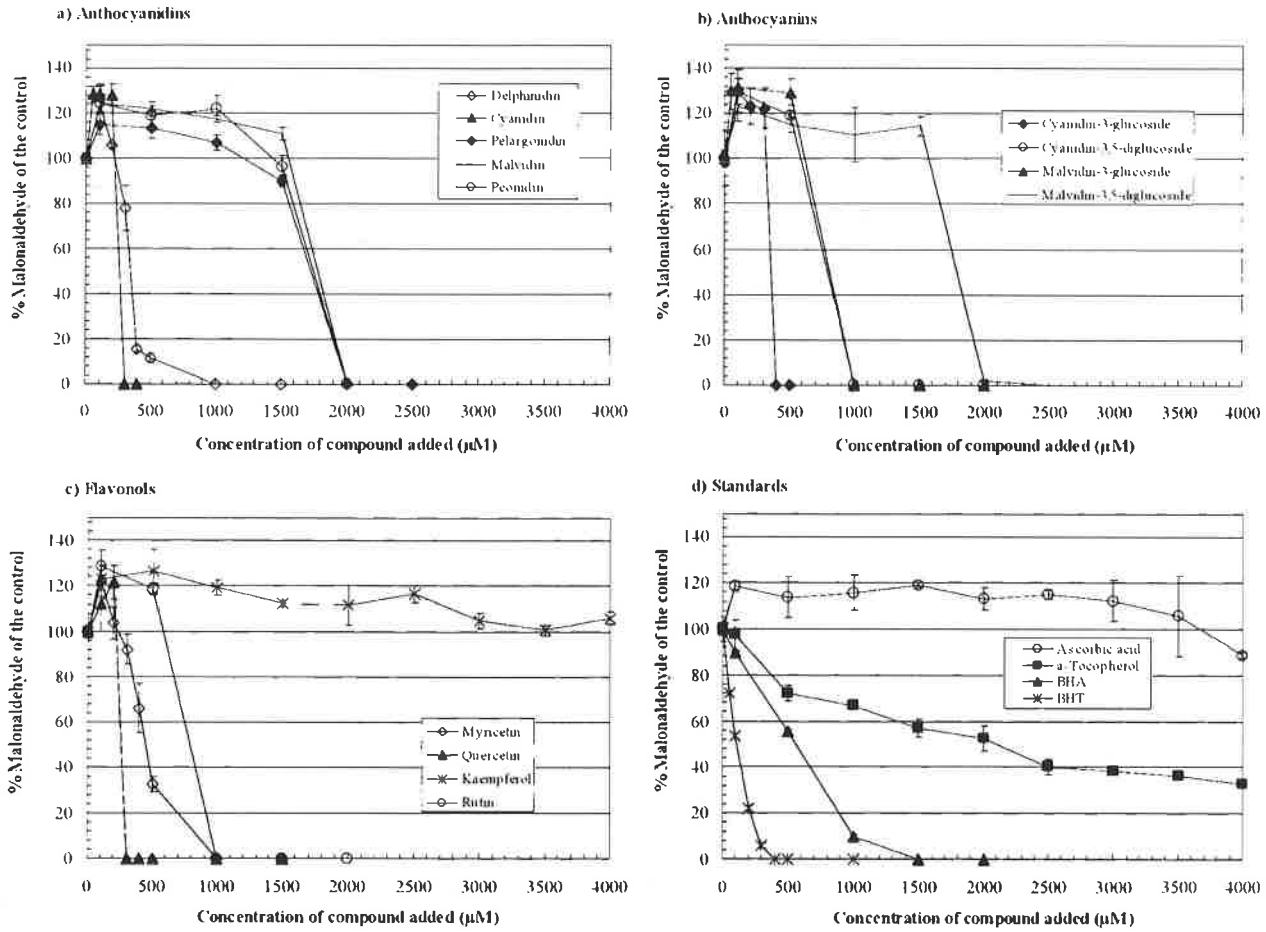

Figure 2. Antioxidant and prooxidant activities of selected (a) anthocyanidins, (b) anthocyanins, (c) flavonols, and (d) standards using the HPLC method. Values are means ± standard deviations, $n = 3$.

α-tocopherol did not. In preliminary trials, some phenolic compounds added to emulsions exposed to air exhibited prooxidant activity even without Cu^{2+} added to initiate oxidation. The activity increased with increasing concentration until the antioxidant activity of the compound became dominant. When Cu^{2+} was added, the concentration at which antioxidant activity became dominant was lower. By changing the initiation procedure for the HPLC method, the prooxidant and antioxidant activities of samples could therefore be altered.

Pischetsrieder et al. (1998) initiated oxidation in their β-carotene bleaching assay using $CuSO_4$, $MnCl_2$, or $FeCl_3$. Prooxidant activity was calculated as the percent of ΔA_{470nm} of the sample/ΔA_{470nm} of the blank over 20 min. All three compounds they tested, including ascorbic acid, showed prooxidant activity (100−130%) at low concentrations. Preliminary trials were performed in this study using 20 μL of 0.02 M $CuSO_4$ to initiate oxidation using the β-carotene bleaching method (results not shown). Changes in A_{450nm} were monitored after 90 and 120 min of incubation. Only gallic acid, cyanidin 3-glucoside, cyanidin 3,5-diglucoside, and ascorbic acid had detectable decreases in A_{450nm} with concentration, indicating prooxidant activity.

Compared to the HPLC method, prooxidant activity was difficult to measure using the β-carotene bleaching method. The β-carotene bleaching method relied on measurement of slight differences in absorbance with concentration to detect prooxidant activity. These differences were difficult to detect partly due to the high variability of the β-carotene bleaching reaction and the reaction conditions. Although information on the mechanism of prooxidant activity in phenolic compounds is limited, the presence and involvement of metal ions and oxygen on the prooxidant activity of ascorbic acid has been reported (Pischetsrieder et al., 1998). Metal ions and oxygen may have been more limited in the β-carotene bleaching method than in the HPLC method because samples were not shaken and a different emulsifier was used (Tween 20 instead of SDS). The reaction time was also reduced (2 h instead of 16 h). Therefore, only compounds with very high prooxidant activity under the conditions used may have been detected by this method. Further research in modifications of the β-carotene bleaching method could be made to improve the method for measuring prooxidant activity.

Cao et al. (1997) found that myricetin, quercetin, and kaempferol had prooxidant behavior using the oxygen radical absorbance capacity (ORAC) assay with Cu^{2+} as a transition metal oxidant. Arouma et al. (1997) summarized results found for the prooxidant activity of phenolic compounds using deoxyribose and bleomycin−DNA assays. Many compounds including ascorbic acid, myricetin, quercetin, and gallic acid had prooxidant behavior in one or both assays. Vanillic acid tested negative. Delphinidin, cyanidin, malvidin, malvin, and pelargonidin had some prooxidant activity in human

Table 1. Antioxidant and Prooxidant Activities of Selected Phenolic Compounds

	current study				selected results from the literature	
	β-carotene method	DPPH• method	HPLC method		DPPH• assay[a]	ORAC assay[b]
compd	initial slope[c] (× 10^{-6})	antiradical activity[d]	antioxidant activity[e] (μM of compd added)	prooxidant activity[f]	antiradical power[g] [antiradical activity]	ORAC slope[h]
Phenolic Acids						
benzoic acid derivatives						
gallic acid	636 ± 38	−6.21 ± 0.60	1500−2000	+	12.5 [−6.25]	
protocatechuic acid	nc[i]	−4.56 ± 0.09	3000−3500	+	7.14 [−3.57]	
3-hydroxybenzoic acid	nc	nc	>4000	+		
vanillic acid	nc	−0.99 ± 0.31	>4000	+	0.17 [−0.09]	
syringic acid	nc	−3.03 ± 0.07	>4000	+		
ellagic acid	779 ± 83	−9.21 ± 0.25	>4000	+		
cinnamic acid derivatives						
caffeic acid	620 ± 36	−4.49 ± 0.24[j]	500−1000	+	9.1 [−4.55]	
p-coumaric acid	nc	−0.33 ± 0.06	>4000	+	0.02 [−0.01]	
cinnamic acid	nc	nc	>4000	nd[k]		
ferulic acid	161 ± 14	−1.34 ± 0.05	>4000	+	2.33 [−1.17]	
chlorogenic acid	186 ± 26	−5.08 ± 0.29	1000−1500	+		
Flavonols						
myricetin	1046 ± 83	−6.59 ± 0.57	500−1000	+		4.319 ± 0.119
quercetin	630 ± 42	−6.73 ± 0.08	200−300	+		3.285 ± 0.117
rutin	nc	−5.10 ± 0.10	500−1000	+		
kaempferol	172 ± 6	−2.09 ±0.10	>4000	+		2.671 ± 0.131
Flavanols						
(+)-catechin	443 ± 63	−7.19 ± 0.32	500−1000	+		
(−)-epicatechin	515 ± 39	−7.65 ± 0.17	500−1000	+		
Flavanones						
naringenin	nc	−0.18 ± 0.01	>4000	+		
Anthocyanidins/Anthocyanins						
cyanidin	836 ± 69	−7.40 ± 0.15	200−300	+		2.239 ± 0.029
cyanidin 3-glucoside	278 ± 32	−6.81 ± 0.30	300−400	+		3.491 ± 0.011
cyanidin 3,5-diglucoside	220 ± 39	−3.32 ± 0.07	500−1000	+		1.689 ± 0.052
delphinidin	897 ± 147	−8.86 ± 0.28	500−1000	+		1.809 ± 0.068
malvidin	288 ± 34	−4.49 ± 0.28	1500−2000	+		2.009 ± 0.167
malvidin 3-glucoside	448 ± 40	−4.29 ± 0.42	500−1000	+		1.404 ± 0.052
malvidin 3,5-diglucoside	266 ± 27	−2.56 ± 0.10	2000−2500	+		1.550 ± 0.062
pelargonidin	nc	−4.63 ± 0.25	1500−2000	+		1.540 ± 0.033
pelargonidin 3-glucoside	444 ± 94	−3.95 ± 0.22	2000−2500	+		1.560 ± 0.145
pelargonidin 3,5-diglucoside	nc	−2.04 ± 0.10	2000−2500	+		1.067 ± 0.043
peonidin	169 ± 22	−4.05 ± 0.17	1500−2000	+		1.693 ± 0.035
peonidin 3-glucoside	251 ± 4	−3.38 ± 0.15	2500−3000	+		1.805 ± 0.014
Standards						
ascorbic acid	nc	−1.83 ± 0.07	>4000	+	3.7 [−1.85]	
α-tocopherol	870 ± 21	−1.95 ± 0.07	2000−2500 (50%)	nd		
BHA	835 ± 50	−2.61 ± 0.01	1000−1500	nd	4.17 [−2.09]	
BHT	864 ± 76	−3.17 ± 0.07	200−300	nd	4.2 [−2.1]	

[a] The DPPH• assay used by Brand-Williams et al. (1995). [b] The oxygen radical absorbing capacity (ORAC) assay measuring reaction with peroxyl radicals expressed as μM of Trolox equivalent per μM of compound. Results for flavonols were taken from Cao et al. (1997), and results for anthocyanidins/anthocyanins were taken from Wang et al. (1997). [c] Values are means of slope coefficients calculated by linear regression ± standard deviations ($n = 3$) in A_{450nm} after 90 min of incubation in the dark/μM of compound added. [d] Values are means of slope coefficients calculated by linear regression ± standard deviations ($n = 3$) in μM of DPPH•/μM of compound. [e] Antioxidant activity was defined by the concentration range of compound added needed to reach 0% malonaldehyde of the control. [f] Prooxidant activity was positive (+) if the % malonaldehyde of the control was >100% in the concentration range tested. [g] Antiradical power was defined as the reciprocal of the amount of antioxidant needed to decrease the initial DPPH• concentration by 50%. The antiradical activity was equivalent to negative half of the antiradical power. [h] Values are slope coefficients calculated by linear regression ± standard error. [i] Not calculated since linear regression $r^2 < 0.800$. [j] Values were obtained after reaction for 48 h. [k] Not detected.

systems using a Cu^{2+} catalyst (Satué-Garcia et al., 1997). The results for prooxidant activity from these studies were similar to those obtained in this study. Some differences were expected, though, because the conditions in a particular assay would affect the reactions occurring. The potential prooxidant properties of phenolic compounds suggest that care should be taken when using these compounds as antioxidants.

Antioxidant Activity. Antioxidant activity by the HPLC method was characterized by a decrease in percent malonaldehyde of the control toward 0 (Figure 2). The antioxidant activity was quantified by the concentration range when the percent malonaldehyde

by a lower concentration range required. For most compounds, the percent malonaldehyde dropped rapidly within a certain concentration range. However, for myricetin, delphinidin, BHA, BHT, and α-tocopherol, the decrease in percent malonaldehyde with concentration was slow (Figure 2). The concentration range for these compounds, therefore, may not accurately reflect their potential antioxidant activity.

Table 1 also summarizes the antioxidant activity of the compounds tested using the β-carotene bleaching and DPPH• methods. Higher initial slope values for the β-carotene bleaching method indicated less bleaching with increasing concentrations of compound added and,

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