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Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin, Epicatechin, and Gallic Acid

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Grape seeds and skins are good sources of phytochemicals such as gallic acid, catechin, and epicatechin and are suitable raw materials for the production of antioxidative dietary supplements. The differences in levels of the major monomeric flavanols and phenolic acids in seeds and skins from grapes of *Vitis vinifera* varieties Merlot and Chardonnay and in seeds from grapes of *Vitis rotundifolia* variety Muscadine were determined, and the antioxidant activities of these components were assessed. The contribution of the major monomeric flavonols and phenolic acid to the total antioxidant capacity of grape seeds and skins was also determined. Gallic acid, monomeric catechin, and epicatechin concentrations were 99, 12, and 96 mg/100 g of dry matter (dm) in Muscadine seeds, 15, 358, and 421 mg/100 g of dm in Chardonnay seeds, and 10, 127, and 115 mg/100 g of dm in Merlot seeds, respectively. Concentrations of these three compounds were lower in winery byproduct grape skins than in seeds. These three major phenolic constituents of grape seeds contributed <26% to the antioxidant capacity measured as ORAC on the basis of the corrected concentrations of gallic acid, catechin, and epicatechin in grape byproducts. Peroxyl radical scavenging activities of phenolics present in grape seeds or skins in decreasing order were resveratrol > catechin > epicatechin = gallocatechin > gallic acid = ellagic acid. The results indicated that dimeric, trimeric, oligomeric, or polymeric procyanidins account for most of the superior antioxidant capacity of grape seeds.

KEYWORDS: Antioxidant; antioxidant capacity; catechin; Chardonnay; epicatechin; gallic acid; galvinoxyl; grape seed; grape skin; Merlot; Muscadine; ORAC

INTRODUCTION

Grape seeds and skins are considered good sources of polyphenolic tannins that provide the astringent taste to wine. The phenolic acid gallic acid and monomers catechin and epicatechin are the main phenolic compounds in grape seeds (*1*). These are also the major flavonoids present in grape skins in addition to various anthocyanins. Terminal units of polymeric procyanidins of grape skins contain 67% (+)-catechin, whereas extension units contain 60% (−)-epicatechin (*2*).

(+)-Catechin shows antioxidant activity in human plasma by delaying the degradation of endogenous α-tocopherol and β-carotene and by inhibiting the oxidation of plasma lipids (*3*). (+)-Catechin has hydroxyl (*4*), peroxyl (*5*), superoxide (*6*), and DPPH (*7*) radical scavenging activities. Moreover, it can chelate iron (*8*). (−)-Epicatechin is able to scavenge hydroxyl radicals (*4*), peroxyl radicals (*9*), superoxide radicals (*6*), and DPPH radicals (*7*). Nakao et al. (*10*) found that (+)-catechin and (−)-epicatechin have a peroxyl radical scavenging activity 10 times higher than those of L-ascorbate and β-carotene when tested on bacteria.

Gallic acid is a phenolic acid that can scavenge peroxyl radicals (*9*) and DPPH radicals (*7*, *11*). Gallic acid has antioxidant activity at stomach pH (*12*). Gallic acid has also antifungal activity (*13*).

Grape seed procyanidin extract (GSPE) has in vivo antioxidant activity (*14*) and could be as important as vitamin E in preventing oxidative damage in tissues (*15*) by reducing lipid oxidation (*16*) and/or blocking the production of free radicals (*17*). Animal studies indicated that GSPE supplementation of the diet reduced myocardial infraction rate (*18*) and atherosclerosis in the aorta without influencing the serum lipid profiles (*19*). Grape seed extracts of *Vitis vinifera* L. grapes showed antiulcer activity in rats (*20*). GSPE and the phenolic acid gallic acid may play a role in the induction of apoptosis, or programmed cell death, in the body (*21*, *22*). Polymeric tannin supplementation can also stimulate fermentative activities without increasing the activity of harmful enzymes on animal models (*23*, *24*).

Monomeric procyanidins were found to be ineffective in the inhibition of atherosclerosis in rabbits compared with grape seed procyanidin extract (*19*). Procyanidins are considered to be superior antioxidants compared to their corresponding monomers (*25*). Some negative effects of high flavonoid intakes include inhibition of iron absorption by polyphenols containing galloyl and cathecol groups and the binding of proteins by tannins resulting in interference with protein digestion and absorption (*26*). In addition, flavonoids may have either pro- or antioxi-

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dative and either pro- or antimutagenic activities (*27*). However, studies also show (*28*) that it is safe to use grape skin and grape seed extracts as components of the human diet.

In the present work, the levels of gallic acid, catechin, and epicatechin in seeds or skins of Merlot, Chardonnay, or Muscadine grapes as grape industry byproducts and the antioxidant activities of these components were determined. The contribution of these major phenolics to the total antioxidant capacity of grape seeds and skins was also determined.

MATERIALS AND METHODS

Production of Grape Skin and Seed Powders. Grape skin and seed samples were from two different species and two different growing areas. Merlot and Chardonnay skin and seeds of the *V. vinifera* grapes were obtained from Habersham Winery (Helen, GA) and were produced in the cool foothills of the North Georgia mountains. The Merlot samples were residue left after the wine was separated following fermentation of the crushed grapes for 1 week at ~24 °C. The residue, which included pulp, skin, and seeds, was collected and transported to the University of Georgia, Athens, GA. Seeds were separated from skin by rubbing against a screen. Chardonnay residue left after the pressing of the grapes prior to fermentation were also obtained from Habersham Winery and transported to the University of Georgia. Because the skins were firm and the seeds were small, the seeds were manually picked out of the skins. Both Merlot and Chardonnay samples were stored at 2 °C and processed within 48 h after receipt at the University of Georgia.

Muscadine seeds were of the *Vitis rotundifolia* variety, cv. Ison. They were from vines grown in the warm coastal plains region of southeastern Georgia. The seeds were separated on a mechanical deseeder used for crushing the grapes for juice and pulp at Paulk Vineyards (Ocilla, GA). Seeds were transported to the University of Georgia and stored at −20 °C until used. Before drying, the muscadine seed was thawed by transferring to a cooler at 2 °C and holding for ~4 days. The thawed seeds were spread on a tray, and skin, bits of pulp, stems, and twigs were removed manually. The seeds were washed with tap water to remove the grape juice adhering to the seeds, drained, and dried.

All seed and skin material was dried in hot air rather than freeze-drying to simulate industrial practice for byproduct recovery. The gas-fired impinger oven (Lincoln Impinger, Lincoln Foodservice Products, Inc., Fort Wayne, IN) used to dry grape skin and seeds was operated at the lowest temperature setting that resulted in an air temperature of 93 °C and a 5 m/s air velocity coming out of the jets. The seeds or skins were spread in a thin layer over a perforated tray to permit drying from both the top and bottom layers of product on the tray. Under these conditions, drying was rapid and was complete within 90 min compared to the 72 h of air-drying at 70 °C now used at one of the plants. The belt drive of the oven was stopped, and the materials to be dried were left in the dryer under the same conditions until dry. Merlot seed and skin and Muscadine seeds took 40 min to dry, whereas Chardonnay skin and seeds required 90 and 60 min, respectively. Adequate drying was judged by the ease with which the dried samples could be ground to a fine powder.

The trays containing the dried seed or skin were removed from the dryer and held at 25 °C overnight. A hammer mill (FitzMill, The Fitzpatrick Co., Elmhurst, IL) fitted with 0.2 mm screens was used to grind grape seed and skin into a powder. The powders were vacuum sealed in oxygen barrier bags (Cryovac, Duncan, SC) and stored at 4 °C until used for analysis.

Preparation of Grape Skin and Seed Extracts. Grape seed powder was deoiled with hexane (1 part powder to 10 parts hexane, w/v). After 10 min of shaking at room temperature, the liquid was separated from the solid by vacuum filtration through a sintered glass filter (Pyrex 10−15M). The solid residue was evenly distributed over a tray and kept under the hood in the dark to evaporate the hexane. Skin and seed extracts for analysis were prepared by mixing the powders with 70% methanol/water at a ratio of 1 part powder to 10 parts solvent (w/v). The mixture was sonicated for 15 min and shaken for 30 min at room temperature followed by centrifugation at 4 °C for 20 min at 26000*g*. Supernatants were decanted and filtered through glass wool. The glass wool was rinsed with 3−4 mL of solvent, which was mixed with the rest of the filtrate. The extract was concentrated in a vacuum rotary evaporator at 40 °C. Volume of the concentrate was then adjusted to obtain a concentration of 1 g of solids/mL by adding a predetermined volume of 25% methanol. In a separate experiment, known quantities of gallic acid, catechin, and epicatechin were added to the Chardonnay seed and skin powders before the start of the extraction process to determine how well these compounds could be recovered from the sample using the applied extraction and analytical procedure.

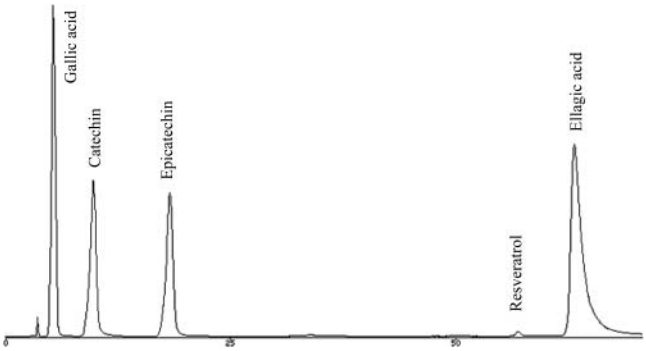


Figure 1. Chromatogram of standard mixture containing gallic acid, catechin, epicatechin, ellagic acid, and resveratrol. Wavelength program: 0–25 min, 280 nm; 25–55 min, 306 nm; 55–70 min, 360 nm.

High-Pressure Liquid Chromatography. A Shimadzu LC-10AT liquid chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) with a Shimadzu SPD-10AV UV−vis detector at dual wavelength was used to determine the monomeric phenolic constituents of grape skin and seed extracts. A Waters Spherisorb ODS-2 5 μm (250 mm × 4.6 mm) column (Alltech Associates, Inc., Deerfield, IL) with a Waters Spherisorb ODS-2 (C18) 5 μm guard column was used for the HPLC analyses. A gradient pump (Shimadzu FCV-10AL) was used to create a solvent gradient in the column.

The two solvents used to make the gradient were (A) 25% aqueous methanol in 1% acetic acid and (B) 75% aqueous methanol in 1% acetic acid. The solvent gradient in volumetric ratios of solvents A and B was as follows: 0−30 min, 100 A/0 B; 30−45 min, 82 A/18 B; 45−65 min, 72 A/28 B; 65−75 min, 60 A/40 B; 75−85 min, 40 A/60 B; and >85 min, 0 A/100 B.

Flow rate was set to 0.75 mL/min. A sample volume of 20 μL was injected to the column using a Rheodyne syringe injector (Rheodyne 7725i, syringe loading sample injector). Three determinations were made on each extract obtained from two seed or skin samples.

Dual wavelengths were used to detect the eluent as follows: from the start to 25 min at 280 nm to detect gallic acid, catechin, and epicatechin; from 25 min to the end at 360 nm to detect other compounds such as ellagic acid. Standards of gallic acid, catechin epicatechin, resveratrol, and ellagic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI), Sigma (St. Louis, MO), and Fluka Chemical Co. (Ronkonkoma, NY), respectively. Compounds were quantified from peak areas of a chromatogram of the standards mixture and the amount of each standard in the mixture. A typical chromatogram of the standards is shown in **Figure 1**. Gallic acid, catechin, and epicatechin eluted first at a retention time of <25 min. Resveratrol and ellagic acid eluted later at a retention time of ~60 min.

Verification of the presence of gallic acid, catechin, epicatechin, ellagic acid, and resveratrol was made with a photodiode array detector and fluorescence detector using a Hewlett-Packard (Avondale, PA) model 1090 liquid chromatograph with quaternary pumps.

Antioxidant Activities of Grape Skin and Seed Extracts. An optimized version of the oxygen radical absorbance capacity (ORAC) assay developed by Cao and Prior (*29*) was used to quantify the antioxidant capacity of pure compounds and of extracts of grape skin and seed. β-Phycoerythrine (β-PE) was purchased from Cyanotech Co. (Kailua-Kona, HI) (lot 0215100) and prepared according to the supplier-recommended reconstitution procedure for purification. Briefly, the vial (1 mg of β-PE/0.2 mL of buffer mixture) was rinsed with ~3.5 mL of

phosphate buffer (stock buffer/deionized water, 1:9, v/v) (stock buffer: 0.75 M K_2HPO_4/0.75 M NaH_2PO_4, 61.6:38.9, v/v). Prior to passage of the solution through the column, a Sephadex G-25 column was cleaned with ~20 mL of phosphate buffer. The red band eluted from the column was collected, and it was washed off with buffer. The purity of the β-PE solution was determined according to the recommended procedure. β-PE was diluted further with phosphate buffer. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA), and 0.868 g of AAPH was dissolved in 10 mL of phosphate buffer. It was prepared daily and kept in ice until used.

An LS-50B luminescence spectrometer (Perkin-Elmer) was used for the analyses. A four-position, motor-driven, water-thermostated, stirred cell holder was installed on the spectrometer, and the temperature of the water bath, which supplied hot water to the cell holder, was set to 37 °C. Emission and excitation wavelengths were 565 and 540 nm, respectively. The stirrer was set to low.

Fifty microliters of β-PE in phosphate buffer was incubated at 37 °C for 5 min in the cuvettes located in the holder, and then appropriately diluted aliquot samples (50−100 μL) were added to the cuvettes. The reaction was started by the addition of 150 μL of AAPH (24 mM) to the cuvettes at min 5, and an initial reading was taken. The total volume in each cuvette was 2 mL. Blanks, which contained phosphate buffer, β-PE, and AAPH only, were used for the area corrections. β-PE fluorescence intensities of samples and blanks were recorded every minute. The changes in β-PE fluorescence over time were displayed on the screen. The data were collected until the fluorescence reading declined by 95% of the initial reading. Intensities were converted to relative intensities by dividing the readings with the initial reading. The areas under the curves were calculated using the software supplied by the spectrofluorometer manufacturer.

Trolox, a water-soluble analogue of vitamin E, was purchased from Aldrich Chemical Co. (Milwaukee, WI). It was dissolved in 10 mL of ethanol (190 °C) and then diluted to 200 mL with phosphate buffer. Trolox solution was prepared weekly and kept at refrigeration temperature. Standard curves were obtained using known concentrations of Trolox (0.5−3 μmol final concentration in the cuvette). Corrected areas of relative fluorescence intensities versus Trolox concentrations were plotted. The Trolox equivalents of the samples were calculated by using the linear portion of the plot after proper dilutions were prepared with phosphate buffer.

Galvinoxyl Method. The method developed by Shi et al. (*30*) was used to determine the number of hydrogen molecules available for antioxidant donation in pure compounds. Stock solution of galvinoxyl (160 μM; Aldrich Chemcal Co.) was prepared in HPLC grade ethanol. The total volume of each cuvette was 2 mL. Ethanol was used as a blank. The final concentration of galvinoxyl was 8 μM. Reaction was carried out at room temperature. Absorbances at 428 nm were recorded every minute up to 20 min using Spectronic Genesys 2 (Rochester, NY). Different concentrations of gallic acid, catechin, epicatechin, resveratrol, ascorbic acid, and ellagic acid were used to determine the number of available hydroxyl groups for proton donation. The following formula was used to determine the number of hydrogen molecules (N) available for antioxidative donations:

$$N = \Delta A/(\epsilon I[\mathrm{IH}])$$

ΔA is the absorbance difference, ϵ is the molar extinction coefficient of galvinoxyl ($\lambda_{428nm} = 1.5 \times 10^5\ M^{-1}\ cm^{-1}$ in ethanol, I is the cell length, and [IH] si the concentration of antioxidant.

Statistical Analysis. Data were analyzed using the Statistical Analysis System software (*31*). PROC GLM with Duncan's multiple-comparison test was performed to determine significant differences at $\alpha = 0.05$.

RESULTS AND DISCUSSION

High-Pressure Liquid Chromatography. Fuleki and Ricardo da Silva (*32*), Escribano-Bailon et al. (*33*), Oszmianski and Sapis (*34*), Revilla and Ryan (*35*), and Santos-Buelga et al. (*36*) reported that concentrations of gallic acid, catechin, and epicatechin are significantly high in grape seeds. We also found that these three phenolic compounds are present in seeds from Muscadine, Merlot, and Chardonnay grapes at concentrations higher than those of other compounds in the samples (**Figure 2**). There are differences in concentrations of the flavonoids in seeds from the Muscadine and Chardonnay and Merlot. **Figure 2A** shows that Muscadine seeds contain much less catechin and epicathechin compared to Merlot seeds (**Figure 2B**). Chromatograms of extracts from seeds of Merlot (**Figure 2B**) and Chardonnay (not shown) are very similar despite the Merlot seeds having more of an opportunity for the flavonoids to leach out in the wine must before the seeds were separated. Two prominent peaks eluting in the 58−62 min range appear in chromatograms of the skin extracts (**Figure 3**) but are not present in the seed extracts. These peaks are probably trimers or polymers of flavonols, which elute much later than the cathechin and epicathechin (*37*). Recoveries of gallic acid, catechin, and epicatechin were, respectively, 69, 59, and 72% when known quantities were added to seeds and 79, 70, and 78% when they were added to the skins of Chardonnay grapes. The percentage of the added phenolic compounds that were recovered by the extraction and analytical procedure was lower from the seed powder than from the skin powder. Seeds were deoiled prior to preparation of the extracts for HPLC analysis to prevent fouling of the column by the oil, and the hexane extraction used to remove the oil could most likely remove some of the added phenolic compounds.

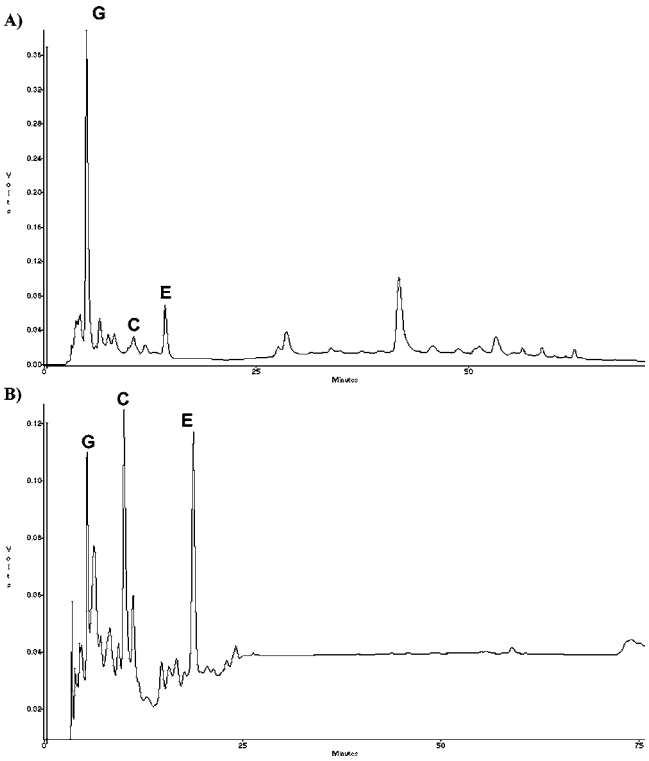
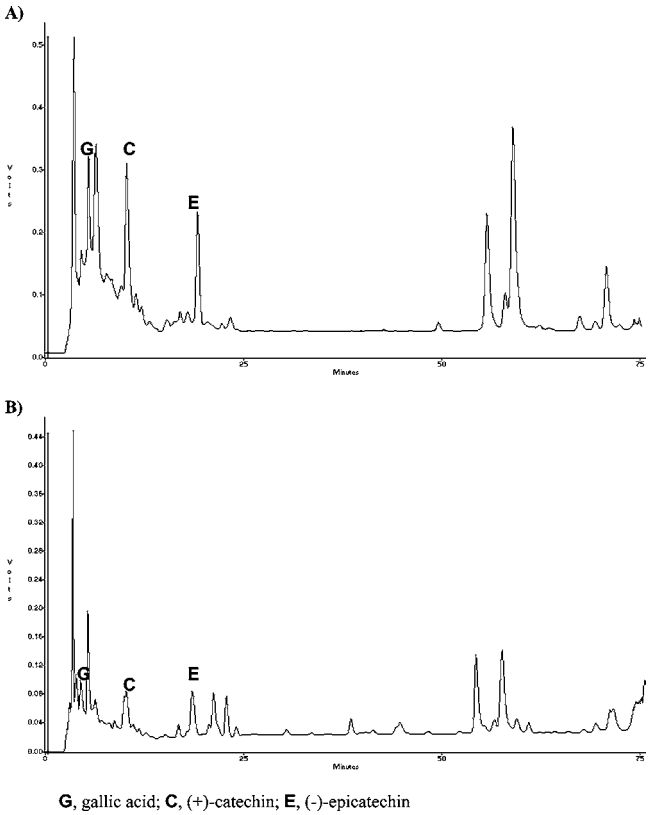


Figure 2. Chromatograms of seed extracts from Muscadine (**A**) and Merlot (**B**) grape byproduct of the wine and juice industries. Wavelength program: 0–25 min, 280 nm; 25–75 min, 360 nm. The chromatogram of extracts from Chardonnay seed (not shown) is similar to that of Merlot (**B**).

Catechin and epicatechin contents of *V. vinifera* grapes were higher than in *V. rotundifolia* grapes, but the latter contained more gallic acid (**Table 1**). In general, grape seeds had much higher monomeric flavonol contents than skins. Catechin and epicatechin concentrations in Chardonnay grape skins were 3 times higher than in Merlot grape skins (**Table 1**).



G, gallic acid; **C**, (+)-catechin; **E**, (-)-epicatechin

Figure 3. Chromatograms of extracts of Chardonnay and Merlot skin byproduct of the wine industry. Wavelength program: 0–25 min, 280 nm; 25–75 min, 360 nm.

Table 1. Gallic Acid, Catechin, and Epicatechin Contents of Seeds and Skins of Grapes from *V. vinifera* and *V. rotundifolia* Varieties

	phenolic content (mg/100 g of dm)		
grape sample	gallic acid	catechin	epicatechin
seed			
Muscadine	99	12	96
Chardonnay	15	358	421
Merlot	10	127	115
skin			
Chardonnay	5	60	44
Merlot	3	16	13

Levels of the flavonoids present in grape skin and seeds from various sources vary by location, prevailing climatic conditions, and postharvest handling so literature data on composition are presented only to deduce the influence of processing method on the flavonoids determined in this study.

Fuleki and Ricardo da Silva (*38*) reported that fresh Merlot and Chardonnay seeds in Ontario, Canada, contained catechin and epicatechin of 64 and 79 mg/100 g in Merlot and 42 and 99 mg/100 g in Chardonnay seeds, respectively, on a wet basis. Because the seeds contain ~50% moisture, the values on a dry matter basis will be twice the wet basis values. Our values on air-dried byproduct of the winery industry (**Table 2**) were about 127 mg of catechin and 115 mg of epicatechin/100 g of dm in Merlot seeds, whereas Chardonnay seeds had about 358 mg of catechin and 421 mg of epicatechin/100 g of dm. These values are in the same range reported by the latter authors. We also found that grape skin byproducts of winemaking from Merlot or Chardonnay grapes also contain considerable amounts of gallic acid, catechin, and epicatechin (**Table 1**). The free forms of monomeric procyanidins in our dried Muscadine seed powder were 99, 12, and 96 mg/100 g of dm of gallic acid, catechin, and epicatechin, respectively.

Table 2. Comparison of Pure Phenolic Compounds in Terms of Their Peroxyl Radical Scavenging Activities

compound	peroxyl radical scavenging capacity[a] (μmol of TE/mg)
stilbene	
resveratrol	29.06 ± 3.54a
flavanols	
catechin	20.53 ± 0.10b
epicatechin	10.20 ± 0.87c
gallocatechin	11.58 ± 0.71c
phenolic acids	
gallic acid	4.26 ± 1.57d
ellagic acid	3.88 ± 1.04d

[a] Means and standard deviations of four determinations. Different letters show significant differences at $\alpha = 0.05$, using Duncan's multiple-range test.

Our results on resveratrol in Muscadine seed are different from those of Ector et al. (*40*), who reported that seeds of Muscadine grapes contained ~45 μg of *trans*-resveratrol/g on a wet basis. We attempted to detect resveratrol using a different HPLC unit with a fluorescence detector because resveratrol fluoresces. However, we found that resveratrol was absent in seeds of *V. rotundifolia* cv. Ison. We detected resveratrol only in *V. vinifera* grape skins.

Peroxyl Radical Scavenging Activities of Gallic Acid, (+)-Catechin, (−)-Epicatechin, *trans*-Resveratrol, Ellagic Acid, and (−)-Gallocatechin. The data (**Table 2**) on antioxidant capacity of the pure compounds revealed the ability of the stilbene, resveratrol, and the flavanols, catechin and epicatechin, to scavenge the peroxyl radicals generated by AAPH. The peroxyl radical scavenging activity of resveratrol (29 μmol of TE/mg) was the highest among all compounds tested ($p < 0.05$). In general, flavanols scavenged peroxyl radicals of AAPH much better than phenolic acids.

Peroxyl radical scavenging activities of resveratrol (*37*, *38*), catechin (*5*, *10*, *39*), epicatechin (*5*, *9*, *10*), and gallic acid (*9*) were previously reported. Flavonoids have the ability to donate hydrogen atoms to peroxyl radicals (*39*). Most studies involving peroxyl radical scavenging activity of these phenolics are comparative rather than quantitative. Using bacterial culture, Nakao et al. (*10*) reported that epicatechin showed 25% higher peroxyl radical activity than catechin. The equivalent antioxidant activities of catechin and epicatechin determined by the ORAC assay from data reported by Guo et al. (*40*) were 8.6 ± 0.24 μmol of TE/mg for catechin and 8.1 ± 0.45 μmol of TE/mg for epicatechin. Our data on the pure compounds were 20.53 ± 0.10 and 10.20 ± 0.87 μmol of TE/mg for catechin and epicatechin, respectively. The order of peroxyl radical scavenging activities of the pure phenolic compounds that may be present in grape seeds and skins (**Table 2**) was in the order resveratrol > catechin > epicatechin = gallocatechin > gallic acid = ellagic acid.

Antioxidant Activities of Grape Skin and Seed Extracts. The ORAC assay was developed by Cao and Prior (*29*) to quantify the antioxidant capacity of foods. This assay is based on the chemical damage to β-PE caused by a peroxyl radical producing compound (i.e., AAPH in this assay), determined by a reduction of the fluorescence emission of β-PE. The presence of antioxidants in the medium protects β-PE from the peroxyl radical and prolongs the time of fluorescence emission. The antioxidant capacity of foods can be quantified by using the areas under the relative fluorescence intensity curves (*29*).

Table 3. Contribution of Catechin, Epicatechin, and Gallic Acid to Total Antioxidant Capacity of Grape Seed and Skin Extracts after Recovery Corrections

grape sample	ORAC[a] (μmol of TE/g of dm)	% of ORAC from catechin, epicatechin, and gallic acid
seed		
Muscadine	245.91 ± 06.77	6.7
Chardonnay	450.51 ± 74.02	26.0
Merlot	272.84 ± 43.27	14.0
skin[b]		
Chardonnay	102.81 ± 07.02	16.5
Merlot	69.81 ± 33.17	6.6

[a] Means and standard deviations are results of six determinations. [b] Sample extraction according to the method of Cao and Prior (*29*).

Table 4. Number of Hydrogen Atoms That Each Pure Compound Can Donate for Antioxidant Reaction

compound	total no. of hydroxyl groups	no. of protons available for antioxidative donation (*N*)
resveratrol	3	NA[a]
ascorbic acid	4	1.8
catechin	5	2.9 (3[b])
epicatechin	5	3.1
quercetin	5	4.0[b]
α-tocopherol	1	1.0[b]
gallic acid	4	3.2
ellagic acid	4	3.4

[a] Not available—nonreactive with galvinoxyl. [b] Shi et al. (*30*).

The ORAC values of dry grape seed powder from *V. vinifera* varieties Chardonnay and Merlot and from *V. rotundifolia* variety Muscadine are shown in **Table 3**. The high antioxidant capacities make these winemaking and juice industry byproducts suitable as nutritional suppplements. Grape seed extracts had higher ORAC values compared with grape skin extracts. We calculated the contribution of the identified compounds and their concentration (**Table 1**) and their antioxidant activity (**Table 2**) as pure gallic acid, catechin, and epicatechin to the total antioxidant capacity of the samples. The latter three compounds contributed <26% of the total ORAC values of grape seed or skin extracts (**Table 3**). Thus, the high antioxidant capacities of grape seeds and skins would most likely be due to the presence of polymeric procyanidins in addition to the monomers.

Hydrogen Donor Activity by Galvinoxyl Method. The galvinoxyl method developed by Shi et al. (*28*) determines the antioxidant activity of compounds that can donate hydrogen. A solution of galvinoxyl in ethanol has a yellow color with a strong absorption at 428 nm. When its odd electron is protonated, galvinoxyl is decolorized. Hydrogen-donating antioxidants can donate proton(s) to galvinoxyl molecules, and the proton donation is measured as a loss of absorbance at 428 nm.

We found that resveratrol is nonreactive toward galvinoxyl; therefore, its proton donor activity could not be determined with this method. On the other hand, resveratrol has the highest ORAC value among the compounds tested. We determined that three protons were available for antioxidative donation per mole of catechin, epicatechin, gallic acid, and ellagic acid, whereas ascorbic acid has two available (**Table 4**). Our results indicate that the contribution of monomeric procyanidins to the antioxidant capacity of byproduct grape seeds and skins powder is in the range of only 7−26%. The superior antioxidant capacity of grape seeds is most likely from their dimeric, trimeric, oligomeric, and/or polymeric procyanidins. The order of antioxidant activity of the flavanoids expressed in terms of protons available for antioxidative donation differs from the order of the ORAC values of these compounds (**Table 2**). Thus, the oxygen radical scavenging properties of compounds determined by the ORAC assay may be different from a proton donation as determined by the galvinoxyl assay.

Our results show that despite the possibility of the transfer of polyphenolics from the grape skin to the wine during the winemaking process and the possible loss of some of these compounds by oxidation during the air-drying process, the seed and skin byproducts are still good sources of antioxidant compounds suitable for use as dietary supplements.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; GAE, gallic acid equivalent; ORAC, oxygen radical absorbing capacity; β-PE, β-phycoerythrine; TE, Trolox equivalent.

LITERATURE CITED

(1) Palma, M.; Taylor, L. R. Extraction of polyphenolic compounds from grape seeds with near critical carbon dioxide. *J. Chromatogr. A* **1999**, *849*, 117−124.

(2) Souquet, J.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509−512.

(3) Lotito, S. B.; Fraga, C. G. (+)-Catechin prevents human plasma oxidation. *Free Radical Biol. Med.* **1997**, *24* (3), 435−441.

(4) Moini, H.; Guo, Q.; Packer, L. Xanthine oxidase and xanthine dehydrogenase inhibition by the procyanidin-rich French maritime pine bark extract, Pycnogenol: a protein binding effect. *Adv. Exp. Med. Biol.* **2002**, *505*, 141−149.

(5) Scott, B. C.; Butler, J.; Halliwell, B.; Aruoma, O. I. Evaluation of the antioxidant actions of ferulic acid and catechins. *Free Radical Res. Commun.* **1993**, *19* (4), 241−253.

(6) Bors, W.; Michel, C. Antioxidant capacity of flavanols and gallate esters: pulse radiolysis studies. *Free Radical Biol. Med.* **1999**, *27* (11/12), 1413−1426.

(7) Fukumoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.

(8) Morel, I.; Lescoat, G.; Cogrel, P.; Sergent, O.; Pasdeloup, N.; Brissot, P.; Cillard, P.; Cillard, J. Antioxidant and iron chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem. Pharmacol.* **1993**, *45* (1), 13−19.

(9) Liu, Z. Q.; Ma, L. P.; Zhou, B.; Yang, L.; Liu, Z. L. Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low-density lipoprotein. *Chem. Phys. Lipids* **2000**, *106*, 53−63.

(10) Nakao, M.; Takio, S.; Ono, K. Alkyl peroxyl radical-scavenging activity of catechins. *Phytochemistry* **1998**, *49*, 2379−2382.

(11) Sanchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Res. Int.* **1999**, *32*, 407−412.

(12) Gunckel, S.; Santander, P.; Cordano, G.; Ferreira, J.; Munoz, S.; Nunez-Vergara, L. J.; Squella, J. A. Antioxidant activity of gallates: an electrochemical study in aqueous media. *Chem.−Biol. Interact.* **1998**, *114*, 45−59.

(13) Shukla, Y. N.; Srivastava, A.; Kumar, S.; Kumar, S. Phytotoxic and antimicrobial constituents of *Argyreia speciosa* and *Oenothera biennis*. *J. Ethnopharmacol.* **1999**, *67*, 241−245.

(14) Sato, M.; Bagchi, D.; Tosaki, A.; Das, D. K. Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia/reperfusion-induced activation of JNK-1 and C-JUN. *Free Radical Biol. Med.* **2001**, *31* (6), 729−737.

(15) Tebib, K.; Rouanet, J. M.; Besancon, P. Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol-vitamin E-deficient diet. *Food Chem.* **1997**, *59*, 135−141.

(16) Bouhamidi, R.; Prevost, V.; Nouvelot, A. High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. *Life Sci*. **1998**, *321*, 31−38.

(17) Bagchi, D.; Garg, A.; Krohn, R. L.; Bagchi, M.; Bagchi, D. J.; Balmoori, J.; Stohs, S. J. Protective effects of grape seed proanthocyanidins and selected antioxidants against TPA-induced hepatic and brain lipid peroxidation and DNA fragmentation, and peritoneal macrophage activation in mice. *Gen. Pharmacol.* **1998**, *30*, 771−776.

(18) Sato, M.; Maulik, G.; Ray, P. S.; Bagchi, D.; Das, D. K. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. *J. Mol. Cell. Cardiol*. **1999**, *31*, 1289−1297.

(19) Yamakoshi, J.; Kataoka, S.; Koga, T.; Ariga, T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* **1999**, *142*, 139−149.

(20) Saito, M.; Hosoyama, H.; Ariga, T.; Kataoka, S.; Yamaji, N. Antiulcer activity of grape seed extract and procyanidins. *J. Agric. Food Chem*. **1998**, *46*, 1460−1464.

(21) Sakaguchi, N.; Inoue, M.; Ogihara, Y. Reactive oxygen species and intracellular Ca^{2+}, common signals for apoptosis induced by gallic acid. *Biochem. Pharmacol*. **1998**, *55*, 1973−1998.

(22) Joshi, S. S.; Kuszynski, C. A.; Bagchi, M.; Bagchi, D. Chemopreventive effects of grape seed proanthocyanidin extract on Chang liver cells. *Toxicology* **2000**, *155*, 83−90.

(23) Tebib, K.; Besancon, P.; Rouanet, J. Effects of dietary grape seed tannins on rat cecal fermentation and colonic bacterial enzymes. *Nutr. Res*. **1996**, *16* (1), 105−110.

(24) Chung, K. T.; Lu, Z.; Chou, M. W. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. *Food Chem. Toxicol*. **1998**, *36*, 1053−1060.

(25) Ursini, F.; Rapuzzi, I.; Toniolo, R.; Tubaro, F.; Bontempelli, G. Characterization of antioxidant effect of procyanidins. *Methods Enzymol*. **2001**, *335*, 338−350.

(26) Bravo, L. Polyphenols: chemistry, dietary sources. Metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(27) Skibola, C.; Smith, M. T. Potential health impacts of excessive flavonoid intake. *Free Radical Biol. Med.* **2000**, *29*, 375−383.

(28) Bentivegna, S. S.; Whitney, K. M. Subchronic 3-mo oral toxicity study of grape seed and grape skin extract. *Food Chem. Toxicol.* **2002**, *40*, 1731−1743.

(29) Cao, G.; Prior, R. Measurement of oxygen radical absorbance capacity in biological samples. *Methods Enzymol*. **1999**, *299*, 50−63.

(30) Shi, H.; Noguchi, N.; Niki, E. Galvinoxyl method for standardizing electron and proton donation activity. *Methods Enzymol*. **2001**, *335*, 157−166.

(31) SAS Institute. *SAS User's Guide: Statistics*, version 6.4; SAS Institute: Cary, NC, 1990.

(32) Fuleki, T.; Ricardo da Silva, J. M. Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. *J. Agric. Food Chem*. **1997**, *45*, 1156−1160.

(33) Escribano-Bailon, T.; Gutierrez-Fernandez, Y.; Rivas-Gonzalo, J. C. R.; Santos-Buelga, C. Characterization of procyanidins of *Vitis vinifera* variety Tinta del Pais grape seeds. *J. Agric. Food Chem*. **1992**, *40*, 1794−1799.

(34) Oszmianski, J.; Sapis, J. C. Fractionation and identification of some low molecular weight grape seed phenolics. *J. Agric. Food Chem*. **1989**, *37*, 1293−1297.

(35) Revilla, E.; Ryan, J. M. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation. *J. Chromatogr. A* **2000**, *881*, 461−469.

(36) Santos-Buelga, C.; Francia-Aricha, E. M.; Escribano-Bailon, T. M. Comparative flavan-3-ol composition of seeds from different grape varieties. *Food Chem*. **1995**, *53*, 197−201.

(37) Santos-Buelga, C.; Francia-Aricha, E. M.; Escribano-Bailon, M. T. Comparative flavan-3-ol of seeds from different grape varieties. *Food Chem.* **1995**, *53*, 197−301.

(38) Ector, B. J.; Magee, J. B.; Hegwood, C. P.; Coign, M. J. Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds and wines. *Am. J. Enol. Vitic*. **1996**, *47*, 57−62.

(39) Cuendet, M.; Potterat, O.; Salvi, A.; Testa, B.; Hostettmann, K. A stilbene and dihydrochalcones with radical scavenging activities from *Loiseleuria procumbens*. *Phytochemistry* **2000**, *54*, 871−874.

(40) Tadolini, B.; Juliano, C.; Piu, L.; Franconi, F.; Cabrini, L. Resveratrol inhibition of lipid peroxidation. *Free Radical Res.* **2000**, *33*, 105−114.

(41) Torel, J.; Cillard, J.; Cillard, P. Antioxidant activity of flavonoids and reactivity with peroxyl radical. *Phytochemistry* **1986**, *25*, 383−385.

(42) Guo, C.; Cao, G.; Sofic, E.; Prior, R. L. High-performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: relationship to oxygen radical absorbance capacity. *J. Agric. Food Chem*. **1997**, *45*, 1787−1796.

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