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Phenolic Content and Antioxidant Capacity of Muscadine Grapes

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Fruits of 10 cultivars of muscadine grapes (five bronze skin and five purple skin) grown in southern Georgia were separated into skin, seed, and pulp. Each fruit part and the leaves from the corresponding varieties were extracted for HPLC analysis of major phenolics. Total phenolics were determined colorimetrically using Folin−Ciocalteu reagent. Total anthocyanins were determined according to a pH-differential method, using a UV−visible spectrophotometer. Antioxidant capacity was determined by the Trolox equivalent antioxidant capacity (TEAC) assay. Gallic acid, (+)-catechin, and epicatechin were the major phenolics in seeds, with average values of 6.9, 558.4, and 1299.4 mg/100 g of fresh weight (FW), respectively. In the skins, ellagic acid, myricetin, quercetin, kaempferol, and *trans*-resveratrol were the major phenolics, with respective average values of 16.5, 8.4, 1.8, 0.6, and 0.1 mg/100 g of FW. Contrary to previous results, ellagic acid and not resveratrol was the major phenolic in muscadine grapes. The HPLC solvent system used coupled with fluorescence detection allowed separation of ellagic acid from resveratrol and detection of resveratrol. Reported here for the first time are the phenolic content and antioxidant capacity of muscadine leaves. Major phenolics in muscadine leaves were myricetin, ellagic acid, kaempferol, quercetin, and gallic acid, with average concentrations of 157.6, 66.7, 8.9, 9.8, and 8.6, respectively. Average total phenolics were 2178.8, 374.6, 23.8, and 351.6 mg/g gallic acid equivalent in seed, skin, pulp, and leaves, respectively. Total anthocyanin contents were 2.1 and 132.1 mg/100 g of FW in the skins of bronze and purple grapes, respectively, and 4.3 and 4.6 mg/100 g of FW in seeds and pulps, in that order. Antioxidant capacity values were, on average, 2.4, 12.8, 281.3, and 236.1 μM TEAC/g of FW for pulps, skins, seeds, and leaves, respectively.

KEYWORDS: ***Vitis rotundifolia*; muscadine grapes; phenolics; anthocyanins; antioxidant capacity**

INTRODUCTION

Muscadine grapes (*Vitis rotundifolia* Michx.) are indigenous to the southeastern United States. They are vigorous vines that may grow up to 100 ft in the wild. They differ botanically from other grapes and are placed in a separate subgenus, *Muscadinia.* Muscadine fruits are round, 1−1.5 in. in diameter with a thick, tough skin and may have up to five seeds. The Georgia Agricultural Experiment Station and the U.S. Department of Agriculture have introduced a number of improved varieties that currently are standard cultivars (*1*). Plants contain a large variety of phytonutrients, many having antioxidant properties. Antioxidant compounds include vitamins, phenols, carotenoids, and flavonoids. Among the last group, flavones, isoflavones, flavonones, flavonols, anthocyanins, and catechins are the most important and exhibit substantial antioxidant activity (*2*, *3*). These antioxidants may prevent the incidence of cardiovascular disease (*4*). Phenolics are secondary plant metabolites found in the majority of fruits, vegetables, and teas (*5*). Even though plants are the basis of all traditional medicinal therapy (*6*), the positive effect of antioxidants found in fruits and vegetables was demonstrated by Ames et al. (*7*) and Hertog et al. (*8*−*11*). In the recent years, many studies have demonstrated that free radicals are the leading cause of degenerative diseases such as several forms of cancer, cardiovascular disease, and neurological diseases (*12*). Plant antioxidants work as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, and enzyme inhibitors (*13*). Many of their protective biological effects are derived from their antioxidant functions (*14*). There is interest in knowing the phenolic content of fruits in order to increase their potential use as nutraceuticals or functional foods.

There are few research papers on the phenolic content of muscadine grapes. Ector et al. (*15*), Meepagala et al. (*16*), Goldy

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et al. (*17*), and Talcott and Lee (*18*) represent some of the reports on the phenolic content of muscadine grapes. Studies from our laboratory represent one of the few attempts to measure the polyphenolic content of muscadines and their antioxidant capacity. The objective of this study is to determine the major phenolic compounds found in grapes and leaves, their total polyphenolic content, and the antioxidant capacity of the muscadine fruits and leaves.

MATERIALS AND METHODS

Chemicals. Pure standards of (+)-catechins (95% purity), (−)-epicatechin (90% purity), gallic acid (90% purity), ellagic acid (95% purity), myricetin (85% purity), quercetin (98% purity), kaempferol (90% purity), and *trans*-resveratrol (95% purity) were purchased from Fluka (Milwaukee, WI) and Sigma (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Fluka. Acetonitrile, methanol, and water (HPLC grade) were purchased from Fisher Scientific (Norcross, GA).

Samples. Fruits and leaves from 10 muscadine grape cultivars, namely, five bronze (Carlos, Early Fry, Fry, Summit, and Late Fry) and five purple (Paulk, Cowart, Supreme, Ison, and Noble), grown in southern Georgia were provided by Jacob Paulk (Paulk Vineyards, Wray, GA) and used for this study. Fruits and leaves were randomly collected at the time of optimum harvest maturity, as determined by the grower. Five kilograms of fruits of each cultivar was collected. Homogeneous fruit samples (500 g) were separated into skins, seeds, and pulps in order to determine the percentage of each fruit part with respect to the whole fruit. Each fruit part was packaged, labeled, and stored at −20 °C in the dark until further analysis. Working samples of each fruit part were extracted in triplicate and analyzed as described below. The percentage of each fruit part was used for the calculation of major phenolics, total phenolics, total anthocyanins, antioxidant capacity, and dry matter in whole fruits.

Major Phenolics. Samples of 1 g of skins, 0.5 g of seeds, 0.5 g of leaves, or 2 g of pulps were mashed, using a mortar and pestle, to a very fine paste and diluted with 80% methanol in 6 N HCl. The samples were vortexed for 1 min and then placed in a water bath shaker set at 60 °C and 200 rpm for 2 h for acid hydrolysis of flavonoid glycosides to aglycons. Finally, the samples were vortexed for 1 min to ensure total extraction. The extracted samples were filtered through a 0.2 μm syringe nylon filter and injected into a Hewlett-Packard (Avondale, PA) HP 1090 HPLC system with diode array and fluorescence detectors. The mobile phases were, solvent A, methanol/acetic acid/water (10:2:88, v/v/v); solvent B, acetonitrile; and, solvent C, water. A linear gradient suitable for phenolic separation was used as follows: at 0 min, 100% solvent A; at 5 min, 90% solvent A and 10% solvent B; and at 25 min, 30% solvent A and 70% solvent B, with 5 min postrun with 100% solvent C. The flow rate was 1 mL/min. The Beckman Ultrasphere C18 ODS 4.6 × 250 mm column was used with the column temperature set at 40 °C. The volume of sample injected was 20 μL. All extractions and analyses were performed in the dark to protect the phenolic compounds from degradation.

Total Phenolics. Mashed samples of fruit parts and leaves were extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis. Total phenolics were measured according to the Folin−Ciocalteu reagent method (*19*). Two hundred microliters of sample extract was introduced in a test tube, 1.0 mL of Folin−Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added, and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a Shimadzu 300 UV−vis spectrophotometer (Shimadzu UV-1601, Norcross, GA). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample, using a standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Total Anthocyanins. Grape parts (skin, seed, or pulp) were extracted in 2% HCl in methanol for 24 h, following the method described by Revilla et al. (*20*) as the one that gave the highest extraction of anthocyanins in grapes. Total anthocyanin analysis was performed following the method described by Giusti and Wrolstad (*21*) and following their direction for buffer preparation. Skin and seed extracts were diluted to an appropriate concentration with potassium chloride buffer, pH 1, until the absorbance of the sample was within the linear range of the Shimadzu 300 UV−vis spectrophotometer (0−1.2). The spectrophotometer was zeroed with distilled water. Two dilutions of each sample were prepared, one with potassium chloride buffer, pH 1, and the other with sodium acetate buffer, pH 4.5, and the dilutions were allowed to equilibrate for 15 min. The absorbance was measured at 520 and at 700 nm (to correct for haze) against a blank cell filled with distilled water, following the pH differential method described by Giusti and Wrolstad (*21*).

Antioxidant Capacity. The antioxidant capacity was determined as Trolox equivalent antioxidant capacity (TEAC), following a slight modification to the method described by Re et al. (*22*). Trolox, a vitamin E analogue, was used as an antioxidant standard. ABTS was dissolved in water to a concentration of 7 mM and allowed to react with a 2.45 mM potassium persulfate solution for 16 h in the dark. This reaction will form ABTS radical cations ($ABTS^{\bullet+}$). The $ABTS^{\bullet+}$ solution was diluted in ethanol to an absorbance of 0.70 (± 0.02) at 734 nm; 1.980 mL of diluted $ABTS^{\bullet+}$ solution was drawn with an automatic pipet and placed into a quartz cuvette, and after exactly 1 min, 20 μL of antioxidant compound or Trolox standard was added and mixed. The absorbance reading was recorded for up to an additional 6 min. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data. The ratio between the area under the curve for the reaction of the specific antioxidant and that for Trolox gave the relative antioxidant capacity. For calibration, Trolox standards of 0, 300, 600, 900, 1200, and 1500 μM were prepared to obtain final concentrations in the cuvette of 0, 3, 6, 9, 12, and 15 μM, respectively (antioxidant sample corresponds to 1% of the total solution in the cuvette). Data from the spectrophotometer were saved as Excel files (Microsoft Corp.). The area under the curve was calculated using the software TableCurve 2D V5.0 (SPSS Inc., Chicago, IL).

Dry Weight Determination. Sample dry weight was determined following the guidelines of the official AOAC method 967.03 (*23*). For each cultivar, 500 g of fruits was separated into pulps, seeds, and skins. Each fruit part fraction was weighed, and the composition of the fruit (as percentage of its fruit parts) was determined. Approximately 10 g of each fruit part was placed into an aluminum pan (in triplicate) and dried for 16 h in an oven set at 105 °C. After the drying time, the pans were removed from the oven, allowed to cool in a desiccator, and weighed, and the dry weight was determined as grams of dry matter per gram of sample.

Statistics. The statistical analysis was carried out using the Microsoft Excel software package (Microsoft Corp.).

RESULTS AND DISCUSSION

The major phenolics in muscadine grape skins were identified by their retention times and characteristic spectra. Quantification was made by calibration curves of external standards built for each of the analyzed compounds, (+)-catechin, (−)-epicatechin, gallic acid, ellagic acid, *trans*-resveratrol, and the aglycons of myricetin, quercetin, and kaempferol. Ellagic acid, resveratrol, and the aglycons of myricetin, quercetin, and kaempferol were found in muscadine skins, whereas (+)-catechin, (−)-epicatechin, and gallic acid were found in the seeds. To check the performance of the extraction method, some skin and seed samples were spiked with selected phenolics and analyzed for recovery. Recoveries of ellagic acid and resveratrol were 95.8 and 98.7%, respectively, when skin samples were spiked with known amounts of the compounds. The recovery of gallic acid was 83.5% when seed samples were spiked. **Table 1** shows the individual phenolic compounds in whole muscadine grapes. **Table 2** shows the major phenolics identified and quantified in the skins and seeds of the 10 selected cultivars of muscadine

Table 1. Phenolics in Muscadine Grapes (Milligrams per 100 g of Fresh Whole Fruit)[a]

cultivar	ellagic acid	myricetin	quercetin	kaempferol	resveratrol	(–)-epicatechin	(+)-catechin	gallic acid	% skin	% seeds	% pulp
bronze											
Carlos	6.4	6.3	0.4	0.1	0.1	71.8	86.1	0.6	32.3	6.0	61.6
Early Fry	7.0	5.8	0.6	0.1	0.1	32.4	19.0	0.1	35.7	2.0	62.3
Fry	5.7	1.8	1.1	0.4	0.1	33.1	6.4	0.1	43.3	1.8	54.9
Summit	5.4	4.2	1.8	1.4	0.1	6.9	5.4	0.1	45.8	1.5	52.7
Late Fry	9.9	5.6	0.4	0.1	nd[b]	74.0	19.9	0.4	46.7	3.9	49.4
av	6.8	4.7	0.9	0.4	0.1	43.6	27.4	0.3	40.8	1.9	56.2
SD[c]	1.8	1.8	0.6	0.6	0.0	28.7	33.5	0.2	6.4	1.8	5.6
purple											
Paulk	6.0	0.7	0.7	0.2	nd	30.4	5.8	0.2	40.7	1.8	57.5
Cowart	7.4	2.2	0.3	0.1	0.1	60.3	17.7	0.3	34.2	5.1	60.7
Supreme	3.0	1.0	1.4	0.1	0.1	17.1	5.1	nd	47.8	1.1	51.1
Ison	8.7	2.8	0.5	0.2	0.1	30.9	19.2	0.3	39.1	3.5	57.3
Noble	6.8	2.2	0.2	0.2	0.1	66.6	30.7	1.1	46.2	9.2	44.6
av	6.4	1.8	0.6	0.2	0.1	41.1	15.7	0.4	41.6	4.1	54.2
SD	2.1	0.9	0.5	0.1	0.0	21.3	10.6	0.4	5.5	3.2	6.4

[a] Values are the average of triplicates. [b] Not detected. [c] Standard deviation.

Table 2. Phenolics in Muscadine Grape Parts (Milligrams per 100 g of Fresh Weight of Fruit Part)[a]

	skins					seeds		
cultivar	ellagic acid	myricetin	quercetin	kaempferol	resveratrol	(–)-epicatechin	(+)-catechin	gallic acid
Carlos	19.7 ± 2.0	19.6 ± 2.3	1.3 ± 0.2	0.4 ± 0.0	0.2 ± 0.0	1189.2 ± 51.9	1424.7 ± 30.3	9.4 ± 0.4
Early Fry	19.7 ± 1.0	16.4 ± 1.1	1.6 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	1603.0 ± 88.7	940.5 ± 80.1	3.3 ± 0.2
Fry	13.1 ± 0.8	4.1 ± 0.0	2.5 ± 0.1	0.8 ± 0.0	0.2 ± 0.1	1850.7 ± 553.3	355.6 ± 54.2	4.5 ± 0.3
Summit	11.7 ± 1.1	9.2 ± 0.7	3.8 ± 0.2	3.0 ± 0.2	0.2 ± 0.1	450.1 ± 81.2	348.5 ± 67.1	5.0 ± 0.4
Late Fry	21.1 ± 2.0	12.1 ± 1.0	0.9 ± 0.1	0.2 ± 0.0	nd	1897.6 ± 598.0	511.3 ± 38.4	9.5 ± 1.0
Paulk	14.7 ± 1.1	1.8 ± 0.1	1.7 ± 0.5	0.4 ± 0.0	nd	1672.2 ± 478.8	319.6 ± 131.4	9.9 ± 1.2
Cowart	21.6 ± 1.7	6.4 ± 0.6	0.9 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	1180.6 ± 27.7	347.2 ± 27.0	5.0 ± 0.1
Supreme	6.2 ± 0.3	2.0 ± 0.4	3.0 ± 0.6	0.2 ± 0.0	0.2 ± 0.0	1553.8 ±179.8	460.6 ± 129.4	2.2 ± 0.5
Ison	22.2 ± 1.6	7.1 ± 0.6	1.4 ± 0.2	0.6 ± 0.1	0.2 ± 0.0	872.6 ± 4.9	542.3 ± 79.6	8.8 ± 1.1
Noble	14.6 ± 0.2	4.8 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	724.2 ± 60.5	333.5 ± 32.6	11.5 ± 1.1

[a] Values are the average and standard deviation of triplicates; nd, not detected.

grapes. The phenolic content in the whole fruit was calculated on the basis of the results of individual phenolic compounds in the skins and seeds shown in **Table 2** and the percentage of each fruit part. Ellagic acid was the most abundant phenolic compound in muscadine grape skins with concentrations from 6.2 to 22.2 mg/100 g of fresh weight (FW); myricetin had concentrations from 1.8 to 19.6 mg/100 g of FW; quercetin varied from 0.5 to 3.8 mg/100 g of FW; kaempferol was found at concentrations from 0.2 to 3.0 mg/100 g of FW; and *trans*-resveratrol had the lowest concentrations of the detected phenolics, ranging from not detected in two varieties to 0.2 mg/100 g of FW (**Tables 1** and **2**). Our result for resveratrol differed from previous results (*15*) indicating high concentrations. These researchers apparently were not able to separate ellagic acid from resveratrol with UV detection alone. Another possible reason for the discrepancy in resveratrol concentration could be due to the varietal and agroecological differences between our grapes and those of the previous researchers. However, we were able to separate the two compounds with our solvent system, identify and quantify them with UV detection, and confirm the resveratrol identity and amount with fluorescence detection. The fluorescence detector was set at wavelengths of 330 and 374 nm (*24*, *25*), for excitation and emission, respectively. **Figure 1** shows the spectra of ellagic acid and *trans*-resveratrol for standards and a sample of the skin of the muscadine grape cv. Carlos. The HPLC chromatogram of selected standards for muscadine skin analysis, showing diode array at different wavelengths, and fluorescence detection are

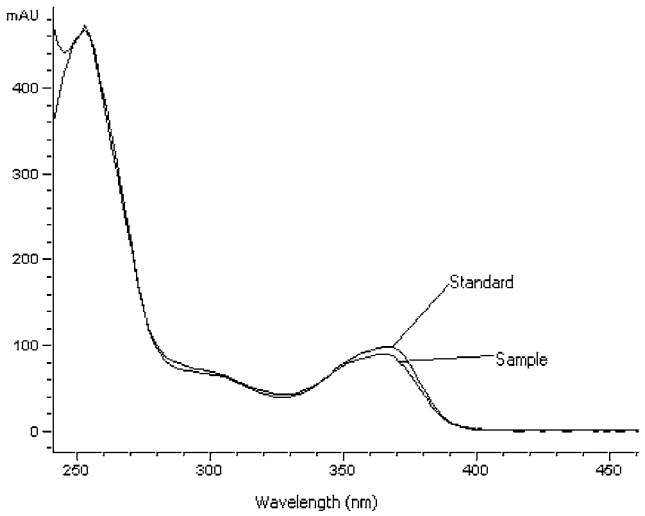

Figure 1. Diode array spectra of ellagic acid for standards and a sample of the skin of the cv. Carlos.

in **Figure 2**. **Figures 3** and **4** show the HPLC chromatograms of selected standards and a sample of the skin of the muscadine grape cv. Carlos, respectively. The major phenolics detected in muscadine seeds (**Tables 1** and **2**) were (−)-epicatechin, (+)-catechin, and gallic acid. (−)-Epicatechin concentration ranged from 450.1 to 1897.6 mg/100 g of FW, (+)-catechin had concentrations between 319.6 and 1424.7 mg/100 g of FW, and

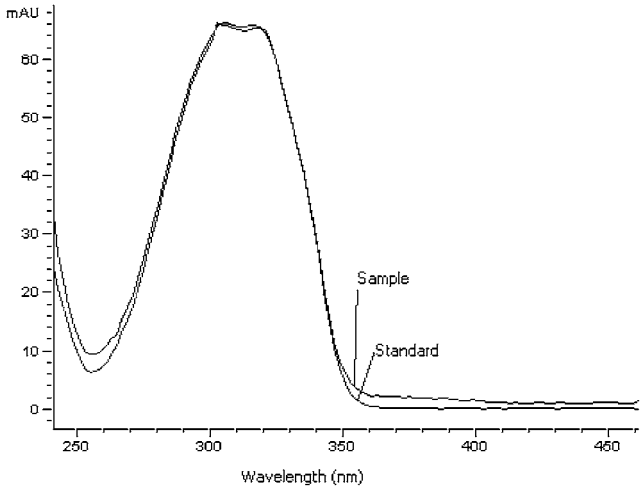

Figure 2. Diode array spectra of *trans*-resveratrol for standards and a sample of the skin of the cv. Carlos.

gallic acid varied from 2.2 to 11.5 mg/100 g of FW. Major phenolics in muscadine leaves (**Table 3**) were myricetin, ellagic acid, kaempferol, quercetin, and gallic acid. Myricetin varied from 107.7 to 216.4 mg/100 g of FW, which on the average is 50 times its concentration in the whole muscadine grape and 18 times the concentration in the skin. Ellagic acid ranged between 44.8 and 80.0 mg/100 g of FW, which is 10 times the concentration of ellagic acid in the fruit and 4 times the concentration in the skin. Kaempferol was found in concentrations between 5.7 and 11.5 mg/100 g of FW, corresponding to 32 times the concentration of the phenolic compound in the fruit and 14 times the concentration in the skin. The flavonol quercetin ranged from 6.3 to 21.6 mg/100 g of FW, corresponding to 13 times the concentration in the whole fruit and 6 times the concentration in the skin. Gallic acid varied from 6.1 to 18.7 mg/100 g of FW, which is, on average, 29 times the concentration of gallic acid in fruit and about the same concentration as in the seed. No phenolics (from the ones that we analyzed) were detected in the grape pulps. Varietal differences between bronze-skinned and purple-skinned grapes were found only in the case of myricetin and total anthocyanin contents. Myricetin content was higher in the bronze-skinned grapes, and total anthocyanin content was higher in purple-skinned grapes. Leaves of both varieties had similar polyphenolic content, and no significant difference was found.

The total phenolics in muscadine grape parts were, on average, 5 times more concentrated in the seed than in the skin and 80 times more than in the pulp (**Table 4**). This result may be due to the high concentration of catechins in the seed and the very low presence of major phenolics in pulp. The relatively high value for total phenolics in skins in comparison to the sum of individual phenolics found in them indicates that some other phenolics may be present in the skins but not identified in this study. The whole muscadine fruits had, on average, 50% less total phenolics than the leaves (**Table 5**), even though the skins and seeds had high contents of phenolics. However, the high relative weight of the pulp to the skins and seeds and their very low content of phenolics contributed to a low phenolics value in the whole fruit. Nevertheless, it is important to note that in muscadine grape processing, as in juice, wine, or jelly production, skins and seeds are discarded as waste. Therefore, the nutraceutical industry may use the muscadine seeds and skins

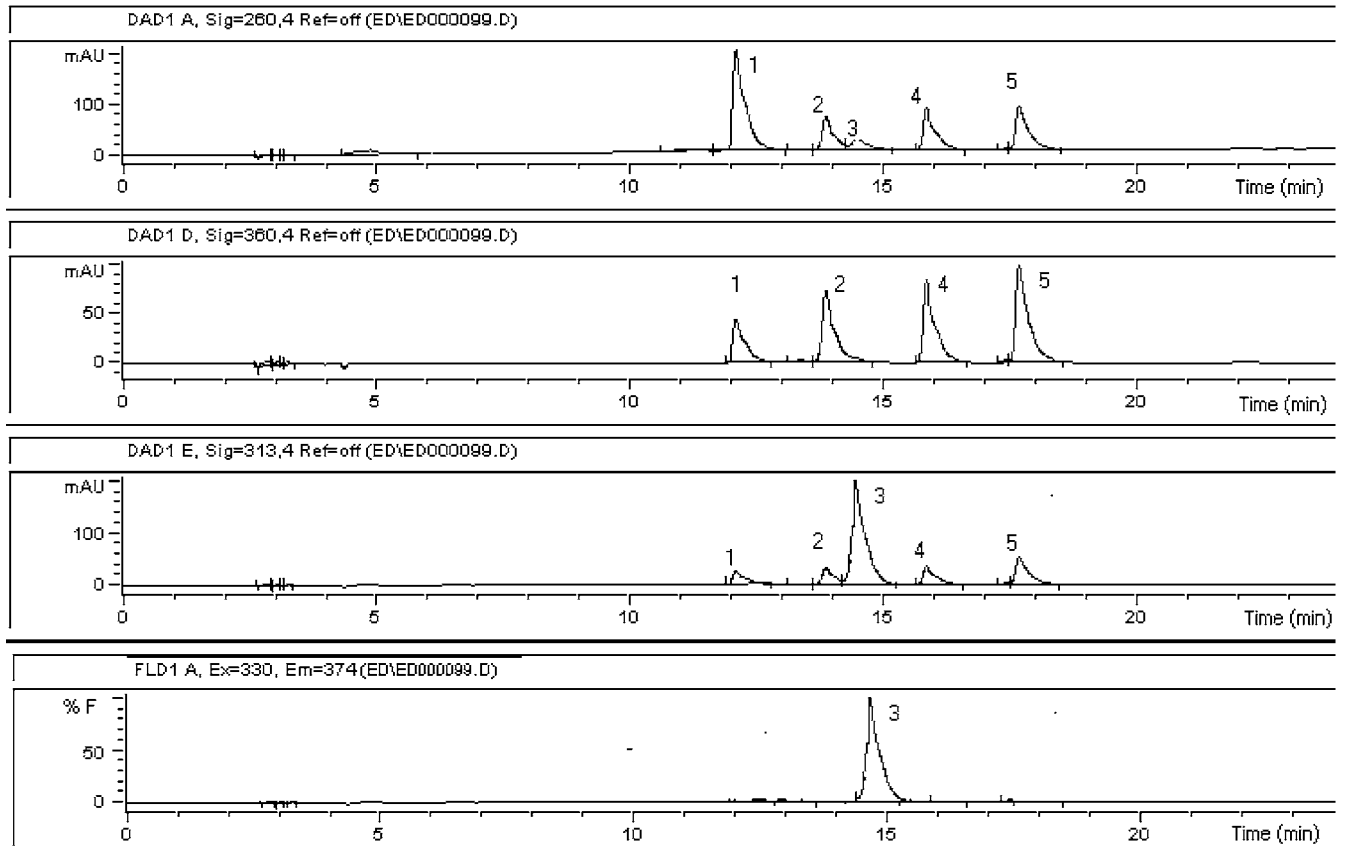

Figure 3. HPLC chromatograms of selected standards for muscadine skin analysis at 260 nm (DAD1 A), 360 nm (DAD1 D), and 313 nm (DAD1 E) and fluorescence detection at Ex = 330 nm and Em = 374 nm (FLD1 A) of ellagic acid (1), myricetin (2), *trans*-resveratrol (3), quercetin (4), and kaempferol (5).

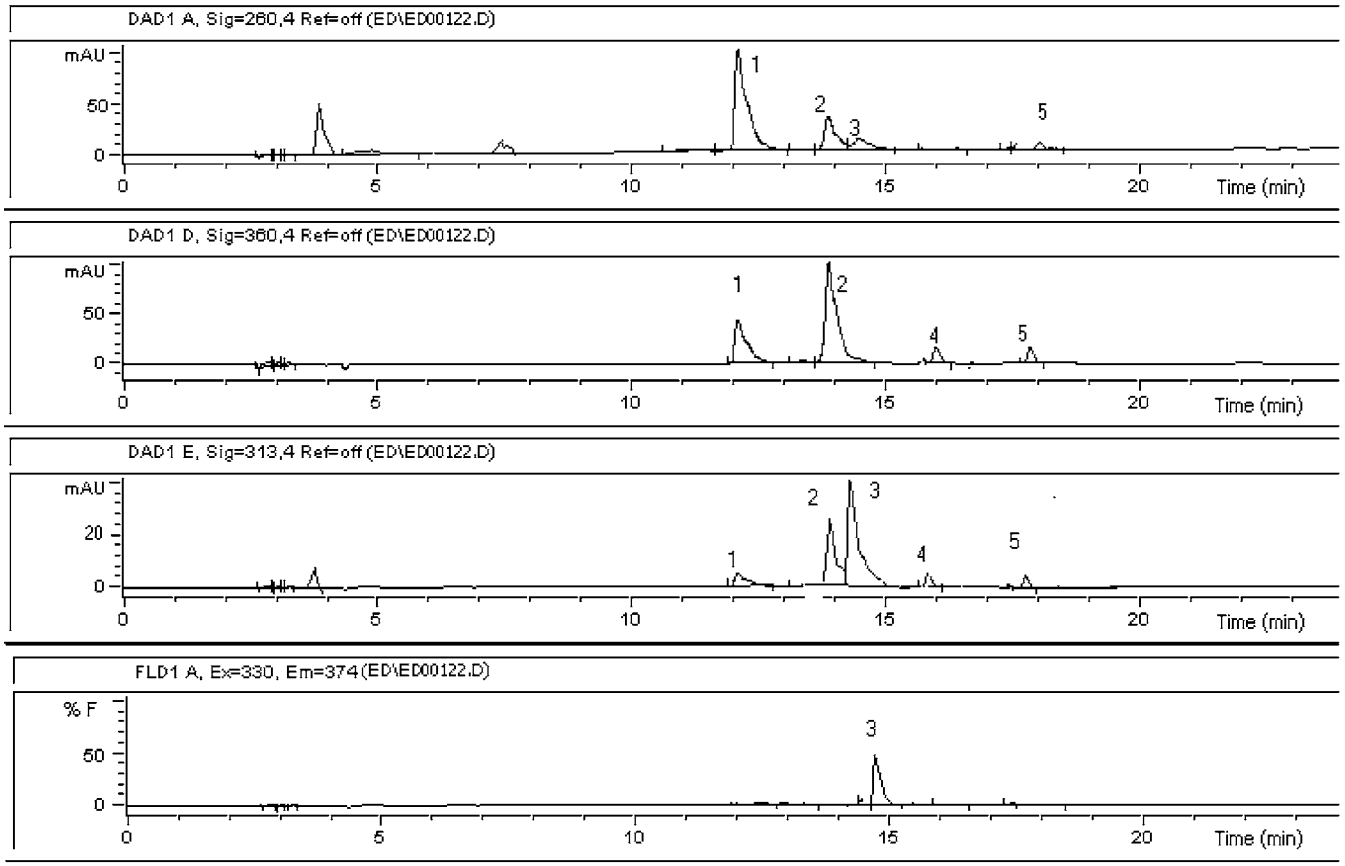

Figure 4. HPLC chromatograms of the skin of grapes of the cv. Carlos at 260 nm (DAD1 A), 360 nm (DAD1 D), and 313 nm (DAD1 E) and fluorescence detection at Ex = 330 nm and Em = 374 nm (FLD1 A) of ellagic acid (1), myricetin (2), *trans*-resveratrol (3), quercetin (4), and kaempferol (5).

Table 3. Major Phenolics in Muscadine Leaves (Milligrams per 100 g of Fresh Weight)[a]

cultivar	ellagic acid	kaempferol	myricetin	quercetin	gallic acid
bronze					
Carlos	80.0 ± 5.9	8.7 ± 1.4	145.8 ± 4.2	8.0 ± 1.0	7.6 ± 0.1
Early Fry	79.0 ± 4.6	10.6 ± 1.3	216.4 ± 4.9	21.6 ± 1.5	9.3 ± 0.2
Fry	76.7 ± 4.4	8.8 ± 0.4	140.9 ± 3.3	7.9 ± 0.3	6.1 ± 0.2
Summit	55.6 ± 2.7	5.7 ± 0.2	107.7 ± 3.5	6.3 ± 0.3	6.9 ± 0.3
Late Fry	66.1 ± 3.2	10.2 ± 1.1	162.1 ± 4.6	8.1 ± 0.5	8.3 ± 0.3
av ± SD[b]	71.5 ± 10.5	8.8 ± 1.9	154.6 ± 39.8	10.4 ± 6.3	7.6 ± 1.2
purple					
Paulk	65.5 ± 5.0	10.0 ± 1.0	166.2 ± 5.3	8.4 ± 0.7	6.4 ± 0.9
Cowart	74.7 ± 3.7	7.9 ± 0.3	157.1 ± 4.8	11.8 ± 1.0	7.8 ± 0.7
Supreme	59.1 ± 2.8	7.2 ± 0.3	133.8 ± 5.7	9.9 ± 0.9	7.7 ± 0.6
Ison	65.1± 4.0	11.5 ± 1.0	178.5 ± 6.3	8.0 ± 0.7	7.1 ± 0.7
Noble	44.8 ± 2.8	8.6 ± 0.7	167.3 ± 4.9	7.9 ± 0.6	18.7 ± 2.8
av ± SD	61.8 ± 11.0	9.0 ± 1.7	160.6 ± 17.8	9.2 ± 1.7	9.5 ± 5.2

[a] Values are the average and standard deviation of triplicates. [b] Standard deviation.

as potential sources of phenolics, and for the farmers or processors they may provide an additional source of income. Leaves are allowed to remain in the field after the fruits have been harvested and finally will fall to the ground during the autumn. They could also be collected and used for the extraction of polyphenolics or processed for use as functional foods.

The analysis of total anthocyanins showed that bronze-skinned muscadine grapes had very low anthocyanin content in skins and seeds and no anthocyanins in pulps. The seeds of this group of fruits had higher relative anthocyanin content. For purple-skinned muscadine grapes, the skins showed higher anthocyanin content, ranging from 65.5 to 177.0 mg/100 g of FW expressed as cyanidin 3-glucoside. This corresponds to 65 times more anthocyanins than in the skins of bronze grapes. The total anthocyanin content in the seeds of purple grapes was 1.3 times higher than in bronze grape seeds, and the pulps of purple grapes had, on average, 2 mg/100 g of FW. This could be due to some migration of the pigments from the skin to the pulp or some tinting from ruptured skin cells during the process of separation of the fruit into its parts. **Tables 4** and **5** show the results for total anthocyanins. No total anthocyanin analysis was performed on the leaves after they were collected at the time of harvest for the fruits, and at that physiological stage it is possible to assume that muscadine leaf cells do not have such pigments.

Tables 4 and **5** also show the antioxidant capacity data. The total average values were 12.8, 281.3, 2.4, 15.3, and 236.1 μM Trolox equiv/g of FW in skins, seeds, pulps, whole grapes, and leaves, respectively. This means that seeds had 22, 116, and 18 times more antioxidant capacity than skins, pulps, and the whole grapes, respectively. Additionally, seeds had, on average, 20% more antioxidant capacity than leaves. The seeds had ~6 times more total phenolics than the leaves, and the ratio of antioxidant capacity/total phenolics was ~6 times higher for the leaves than the seeds. It is presumable that major phenolics in leaves have higher antioxidant capacity than the ones found in seeds or that other antioxidant compounds different from phenolics may be present in higher concentrations in the leaves than in the seeds. A comparison of our results with those reported by Wang and Lin (*13*) indicated that the antioxidant capacity of muscadine leaves was at least twice the value for the leaves of some berry plants, such as blackberry, raspberry, or strawberry.

Table 6 shows the results for dry weight determination of muscadine grapes and the corresponding dry weight of the whole

Table 4. Total Phenolics, Total Anthocyanins, and Antioxidant Capacity of Muscadine Grape Parts

	total phenolics (GAE mg/100 g of FW)			total anthocyanins (mg/100 g of FW as cyanidin 3-glucoside)			TEAC[a] (μM/g of FW)		
cultivar	seed	skin	pulp	skin	seed	pulp	skin	seed	pulp
bronze									
Carlos	1920.3	545.6	25.1	2.6	1.2	nd[b]	14.9	204.6	3.4
Early Fry	2367.2	303.0	21.3	2.5	8.7	nd	13.9	277.8	2.0
Fry	2356.3	332.2	23.8	0.8	4.6	nd	11.1	234.2	2.9
Summit	3258.7	541.0	22.3	2.8	3.1	nd	12.4	245.4	3.0
Late Fry	1986.0	348.9	24.0	2.0	3.7	nd	13.4	218.9	2.4
av ± SD[c]	2377.7 ± 533.7	414.1 ± 119.1	23.3 ± 1.5	2.1 ± 0.8	4.3 ± 2.8	nd	13.1 ± 2.5	236.2 ± 37.9	2.7 ± 0.5
purple									
Paulk	1649.3	363.6	30.0	177.0	4.1	4.7	12.1	307.9	2.2
Cowart	2303.0	261.6	11.6	107.8	4.6	1.1	12.4	325.5	2.7
Supreme	1535.5	329.9	20.1	135.5	7.5	0.7	12.2	478.6	1.6
Ison	1726.2	365.0	26.0	174.5	4.6	1.9	13.3	284.9	2.1
Noble	2685.3	355.1	33.4	65.5	2.2	2.2	12.4	234.7	2.1
av ± SD	1979.9 ± 493.2	335.0 ± 43.4	24.2 ± 8.6	132.1 ± 47.0	4.6 ± 1.9	2.1 ± 1.6	12.5 ± 0.5	326.3 ± 91.7	2.1 ± 0.4

[a] Trolox equivalent antioxidant capacity; values are the average of triplicates. [b] Not detected. [c] Standard deviation.

Table 5. Total Phenolics (TPH), Total Anthocyanins (TAC), and Trolox Equivalent Antioxidant Capacity (TEAC) of Muscadine Grapes and Leaves[a]

	TPH (GAE mg/100 g of FW)		TAC (mg/100 g of FW)	TEAC (μM/g of FW)	
cultivar	whole fruit	leaves	whole fruit	whole fruit	leaves
bronze					
Carlos	307.9	350.1	0.9	18.2	229.8
Early Fry	169.1	437.0	1.1	11.2	251.0
Fry	199.0	340.4	0.4	9.8	239.8
Summit	309.7	282.1	1.3	10.2	222.0
Late Fry	252.3	355.0	1.1	15.4	235.0
av ± SD[b]	247.6 ± 63.3	352.9 ± 55.3	1.0 ± 0.3	13.0 ± 3.7	235.5 ± 10.9
purple					
Paulk	195.2	356.5	74.8	11.2	247.8
Cowart	214.2	359.3	37.8	21.7	164.0
Supreme	184.7	317.7	65.2	11.5	304.0
Ison	218.9	370.4	69.5	15.9	283.0
Noble	425.7	347.3	31.5	27.8	184.8
av ± SD	247.7 ± 100.5	350.2 ± 20.0	55.8 ± 19.7	17.6 ± 7.1	236.7 ± 60.8

[a] Values are the average of triplicates. [b] Standard deviation.

Table 6. Dry Matter of Muscadine Grape Fruits and Fruit Parts (Grams per Gram of Fresh Weight)[a]

cultivar	skin	seed	pulp	whole fruit
Carlos	0.179	0.532	0.137	0.174
Early Fry	0.159	0.562	0.149	0.161
Fry	0.139	0.523	0.144	0.148
Summit	0.165	0.571	0.166	0.180
Late Fry	0.161	0.516	0.152	0.162
Paulk	0.163	0.578	0.146	0.159
Cowart	0.149	0.531	0.121	0.139
Supreme	0.169	0.514	0.137	0.186
Ison	0.182	0.559	0.157	0.184
Noble	0.135	0.596	0.122	0.151

[a] Values are the average of triplicates.

fruit calculated by taking into account the percentage of each fruit part in the whole fruit. This was provided as additional information to simplify the comparison of our results to those of other fruits for which the results were reported on a dry weight basis.

Most phenolics in grapes were located in the seeds and skins. Muscadine pulps have a very low content of phenolics. The main phenolics in muscadines were ellagic acid, kaempferol, myricetin, and quercetin. Seeds of muscadine grapes had higher antioxidant capacity compared to the other fruit parts, which may be due to their high concentrations of catechin and epicatechin. Because the leaves have 15 times more antioxidant capacity than the fruits and the seeds and skins have high polyphenolic contents, they have the potential to be processed for use as functional foods or nutraceuticals. This paper contains the first report of myricetin in muscadine grapes and of phenolics in muscadine leaves.

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LITERATURE CITED

(1) California Rare Fruit Growers, Inc. http://www.crfg.org/pubs/ff/muscadinegrape.html, 1999.

(2) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity and prooxidant behavior of flavonoids: Structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(3) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304−309.
(4) Schramm, D. D.; German, J. B. Potential effects of flavonoids on the etiology of vascular disease. *J. Nutr. Biochem.* **1998**, *9*, 560−566.
(5) Amakura, Y.; Umino, Y.; Tsuji, S.; Tonogai, Y. Influence of jam processing on the radical scaveging activity and phenolic content in berries. *J. Agric. Food Chem.* **2000**, *48*, 6292−6297.
(6) Zheng, W.; Wang, S. Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165−5170.
(7) Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.
(8) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379−2383.
(9) Hertog, M. G. L.; Hollman, P. C. H.; Putte, B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem.* **1993**, *41*, 1242−1246.
(10) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen eldery study. *Lancet* **1993**, *342*, 1007−1011.
(11) Hertog, M. G. L.; Kromhout, D.; Aravanis, C. Flavonoid intake and long-term risk of coronary-heart-disease and cancer in the 7 countries study. *Arch. Intern. Med.* **1995**, *155*, 381−386.
(12) Prior, R. L.; Cao, G.; Martin, A.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolics and anthocyanin content, maturity, and variety of *Vaccinum* species. *J. Agric. Food Chem.* **1998**, *46*, 2686−2693.
(13) Wang, S. Y.; Lin, H.-S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.
(14) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.
(15) Ector, B. J.; Magee, C. P.; Coign, M. J. Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds, and wines. *Am. J. Enol. Vitic.* **1996**, *47*, 57−62.
(16) Meepagala, K. M.; Magee, J. B.; Wedge, D. E. Phenolic constituents from muscadine grape (*Vitis rotundifolia*) cultivar polyanna seeds. *Abstr. Pap. Am. Chem. Soc.* **2002**, *No. 223*, 039-AGFD.
(17) Goldy, R. G.; Ballinger, W. E.; Maness, E. P.; Shallow, W. H. Anthocyanin content of fruit, stem, tendril, leaf, and leaf petioles in muscadine grape. *J. Am. Soc. Hortic. Sci.* **1987**, *112*, 882−882.
(18) Talcott, T.; Lee, J.-H. Ellagic acid and flavonoid antioxidant content of muscadine wine and juice. *J. Agric. Food Chem.* **2002**, *50*, 3186−3192.
(19) Singleton, V. L.; Rossi, J. A., Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(20) Revilla, E.; Ryan, J.-M.; Martin-Ortega, G. Comparison of several procedures used for the extraction of anthocyanins from red grapes. *J. Agric. Food Chem.* **1998**, *46*, 4592−4597.
(21) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by UV−visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Acree, T. E., An, H., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Sporns, P., Eds.; Wiley: New York, 2001; pp F1.2.1−F1.2.13.
(22) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying and improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.
(23) Association of Official Analytical Chemists. Moisture in peat. *Official Methods of Analysis*, 15th ed.; AOAC: Champaign, IL, 1990; Method 967.03.
(24) Viñas, P.; López-Erroz, C.; Marín-Hernández, J. C.; Hernández-Córdoba, M. Determination of phenols in wines by liquid chromatography with photodiode array and fluorescence detection. *J. Chromatogr. A* **2000**, *871*, 85−93.
(25) Pezet, R.; Pont, V.; Cuenat, P. Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chromatography and highly sensitive fluorimetric detection. *J. Chromatogr. A* **1994**, *196*, 522−525.

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