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Fruit Maturity and Juice Extraction Influences Ellagic Acid Derivatives and Other Antioxidant Polyphenolics in Muscadine Grapes

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Polyphenolic compounds including ellagic acid, ellagic acid derivatives, and anthocyanins were characterized and quantified by novel chromatographic conditions in eight muscadine grape (*Vitis rotundifolia*) cultivars and evaluated for antioxidant capacity as influenced by two ripening stages and their location within the fruit (skin, pulp, and juice). All polyphenolics generally increased as fruit ripened and the highest concentrations were located in the skins. Free ellagic acid, ellagic acid glycosides, and total ellagic acid ranged from 8 to 162, 7 to 115, and 587 to 1900 mg/kg, respectively, in the skin of ripe grapes. Hot-pressed juices contained considerably lower polyphenolic concentrations than were present in whole grapes. Five anthocyanidins were present in each cultivar in variable concentrations (delphinidin > petunidin > malvidin + peonidin > cyanidin). Antioxidant capacity was appreciably influenced by cultivar, maturity, and location in the fruit with good correlations to soluble phenolics found in both methanolic and ethyl acetate extracts ($r = 0.83$ and 0.92, respectively).

KEYWORDS: Muscadine grape; ellagic acid; anthocyanin; antioxidant capacity; fractions

INTRODUCTION

Muscadine grapes (*Vitis rotundifolia*) are a native grape species commonly cultivated in the southern United States, because many traditional *Vitis* species are difficult or impossible to cultivate in the humid summers and warm winters characteristic of this region. Muscadine grapes have proven to be an excellent alternative and offer farmers good disease resistance and profitable yields of fruit with distinguished aroma and flavor characteristics. Over 70 different cultivars are available for home garden or commercial production with most fruit consumed fresh or processed into wine, juice, or jelly (*1*).

The phytochemistry of muscadine grapes is distinguishable from most other grape varieties due to its predominance of anthocyanin 3,5-diglucosides and presence of ellagic acid and ellagic acid precursors (*2*). The anthocyanins 3,5-diglucosides, which may be more resistant to degradation during thermal processing compared to monoglucosides, are typically unstable during storage, due to a decreased ability to form polymeric pigments and are particularly prone to oxidation and browning reactions (*3*, *4*). Depending on maturity and availability, it is common to blend grape cultivars for muscadine wine and juice production to obtain the most desirable acidity, color, and flavor. However, little information is available on the phytochemical and antioxidant characteristics among cultivars suitable for wine or juice production.

The most distinguishing chemical attribute in muscadine grapes is the presence of ellagic acid, commonly found in fruits such as blackberries, raspberries, and strawberries, and its presence in muscadine grapes is uncommon among *Vitis* species. Ellagic acid is found in various forms in plants and fruits including its free acidic state, glycosylated with various sugars, or as simple or complex ellagitannins (*5*). Several additional variants of ellagic acid have also been reported in higher plants, resulting from methylation of its hydroxyl groups (*6*). In raspberries, the predominant ellagitannins were identified as lambertianin C and sanguiin H-6, as well as arabinosides, acetylarbinosides, and acetylxylosides of ellagic acid (*7*, *8*). Ellagitannins are characterized as hydrolyzable conjugates containing one or more hexahydroxydiphenoyl (HHDP) group esterified to a sugar, mainly glucose. Specific information on ellagic acid derivatives are lacking for muscadine grapes, but the presence of ellagic acid and its derivatives in muscadine grapes may add value and marketability to the crop due to possible health benefits associated with the compounds such as its antioxidant activity (*9*, *10*), anti-carcinogenic properties influencing cell cycle arrest and apoptosis (*11*), and the inhibition of tumor formation and growth in mammalian models (*12*, *13*).

Phenolic contents in different muscadine cultivars have been reported for free ellagic acid, resveratrol, and flavonoids (*10*); however, the current study represents diversity among cultivars for free ellagic acid, ellagic acid glycosides, and total ellagic acid derived from precursors including ellagitannins. Therefore,

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objectives of this study were to quantify the antioxidant polyphenolics in muscadine grapes as influenced by their location in the grape, juice production, and polyphenolic fractionation as a function of cultivar and maturity. This information can be used to determine wine or juice blending schemes to produce higher quality of muscadine grape products in terms of phytochemical composition and antioxidant potential.

MATERIALS AND METHODS

Muscadine grapes were donated from local grape growers in central Florida and collected at two maturity stages from the same vines at different time intervals, about 15−20 days apart depending on variety. Varieties included Carlos, Fry, and Doreen, classified as either white or, more specifically, bronze colored fruit, and the red-skinned varieties Noble, Albemarle, Cowart, Nesbitt, and Georgia Red. Random samplings of 8−15 fruits in duplicate were manually divided between skin and pulp, while whole grapes were processed into juice using a hot-break technique (70 °C for 30 min). Polyphenolics were extracted from the skin and pulp by homogenizing with 25 mL of 100% methanol, filtered through Whatman #4 filter paper, and solvent removed at 40 °C under a stream of nitrogen. The juice was analyzed directly following centrifugation and filtration. Nonanthocyanin polyphenolics were subsequently partitioned from each isolate into ethyl acetate in three sequential extractions, after which the solvents were pooled, removed under reduced pressure at 40 °C, and residues redissolved in 50% methanol.

Chemical Analyses. Polyphenolics were separated and quantified by HPLC using solvent programs to identify phenolic acids, free ellagic acid, and ellagic acid derivatives in ethyl acetate extracts, and total ellagic acid and individual anthocyanidins in methanolic extracts following acid hydrolysis (2N HCl for 60 min at 95 °C). Separations were conducted on a Dionex HPLC system using a PDA-100 photodiode array detector and a 250 mm × 4.6 mm Acclaim 120 C_{18} column (Dionex, Sunnyvale, CA) with a C_{18} guard column. Mobile phases consisted of 100% water (phase A) and 60% methanol (phase B) both adjusted to pH 2.4 with *o*-phosphoric acid and run at 1 mL/min according to modified conditions of Lee and Talcott (*9*). Free ellagic acid, ellagic acid glycosides, and phenolic acids were separated using a gradient elution program where phase B changed from 0 to 30% in 3 min; 30−50% in 5 min; 50−70% in 17 min; 70−80% in 5 min; 80−100% B in 5 min; and 100% B in 9 min for a total run time of 44 min, after which the column was equilibrated to original conditions in 1 min for the next sample injection. Anthocyanidins and total ellagic acid were also separated with a gradient program that ran phase B from 30 to 50% in 3 min; 50−70% in 2 min; 70−90% in 5 min; and 90−100% in 10 min and returning to original composition in 1 min for column equilibration. Ellagic acid and its derivatives were quantified in ellagic acid equivalents, flavonoid glycosides in equivalents of myricetin (Sigma Chemical, St. Louis, MO), and each anthocyanidin quantified in cyanidin equivalents (Polyphenols Laboratories AS, Sandnes, Norway).

Total soluble phenolics were analyzed using Folin-Ciocalteu assay (*14*) and expressed in gallic acid equivalents (GAE). Antioxidant activity was determined using the oxygen radical absorbance capacity (ORAC) assay as previously described for a 96-well microplate reader (*15*). Fluorescence loss was monitored on a Molecular Devices fmax (Sunnyvale, CA) 96-well fluorescent microplate reader following appropriate dilution of each isolate and data expressed in Trolox equivalents per g of fresh fruit or per mL of juice.

Statistical Analysis. Data represent the mean duplicate analyses with analysis of variance and Pearson correlations conducted using JMP5 software (*16*); mean separation was conducted using the LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Identification of Ellagic Acid and its Precursors. The free (aglycone) form of ellagic acid and two ellagic acid glycosides were found in all eight muscadine grape cultivars following ethyl acetate extraction and separation by HPLC. The ellagic acid glycosides were tentatively characterized based on UV spectral properties (252 and 360 nm) similar to that of free ellagic acid (252 and 365 nm) as was previously characterized in muscadine grapes (*2*), indicating that these compounds were most likely glycosidic forms at the 4-position of ellagic acid rather than HHDP moieties esterified to glucose (true ellagitannins), with maximum absorption at or near 250 nm (*7*, *8*). Preliminary work to characterize these compounds has identified the presence of glucose, xylose, or rhamnose moieties (data not shown). Similar ellagic acid glycosides were thought to exist in raspberries and were characterized by spectroscopic shifts (4−7 nm hypsochromic) and disappearance of the glycoside after hydrolysis, with a corresponding increase in free ellagic acid (*5*, *7*, *8*, *17*). Similarly, the two ellagic acid glycosides identified in muscadine grapes yielded free ellagic acid upon both acid and enzyme (β-glucosidase) hydrolysis. True ellagitannins, containing esterified HHDP units to a carbohydrate, were also believed to be present in the grape isolates, but were not separated or detected in muscadine grapes using the HPLC methodology employed. Evidence of these highly polar compounds was established indirectly by passing an aqueous grape extract through a preconditioned Waters C_{18} Sep Pak cartridge and evaluating the nonretained fraction. No peaks analogous to ellagic acid were present in this isolate in the range of 200−400 nm, but following acid hydrolysis free ellagic acid was one of the hydrolytic products, thus providing evidence for their existence. Total ellagic acid was subsequently determined from the methanolic extracts following acid hydrolysis and represented the sum of free ellagic acid and ellagic acid released from both ellagitannins and ellagic acid glycosides.

Ellagic Acid and its Derivatives. Concentrations of ellagic acid and its derivatives in muscadine grapes were found to significantly vary with ripening, in skin and pulp tissue, among cultivars, and following juice extraction (**Table 1**). Ripening was a critical factor influencing concentrations since appreciable increases in skin and juice during ripening were observed. Because muscadine grapes grow in clusters rather than bunches, inconsistent maturity at harvest is a common occurrence. Changes with ripening were also highly variable among cultivars for free ellagic acid and its glycosidic forms and ranged from a 0.3 to 13-fold increase in the skins alone. Differences during ripening were less variable for total ellagic acid at a 1.7-fold average increase in the skins. The large increases in ellagic acid and its glycosides observed during ripening may have resulted from various reasons: amplified hydrolyzable tannins synthesis during veraison (*18*); a chemoprotective response similar to the formation of resveratrol (*19*); or accelerated hydrolysis of HHDP units from ellagitannins that was observed to produce greater quantities of free ellagic acid in each cultivar. Compared to total ellagic acid, relatively low levels of free ellagic acid and ellagic acid glycosides were present in the grapes, an indication that ellagitannins were the major source of ellagic acid following hydrolysis. However, the actual concentrations of the ellagic acid glycosides were likely influenced by the use of free ellagic acid as the quantifying standard.

As with most grape varieties, polyphenolic compounds are typically concentrated in epidermal tissues, which is exceptionally thick in muscadine grapes and often hinders efficient juice extraction. On average, the skin and pulp tissue constituted 21 and 69% of the total mass of the grapes, respectively, and were similar for both unripe and ripe fruit. Ellagic acid and its derivatives were generally concentrated in the skin, which contained 51−67% of these compounds in unripe fruit on a fresh

Table 1. Concentrations (mg/kg, mg/L) of Free Ellagic Acid (EA), Two Ellagic Acid Glycosides (EAG 1 and 2) and Total Ellagic Acids on Skin, Pulp, and Juice of Muscadine Grapes as Affected by Cultivars and Ripening Stages (U, Unripe and R, Ripe)

			free EA		EAG 1[a]		EAG 2[b]		total EA[c]	
	cultivars	color	U	R	U	R	U	R	U	R
skin	Carlos	white	32.1 b[d]	8.04 e[h]	17.4 b	6.76 d[h]	16.7 d	20.1 d	368 d	879 d[h]
	Fry	white	31.3 b	87.4 cd[h]	13.0 cd	90.3 a[h]	8.81 e	13.6 d	531cd	879 d[h]
	Doreen	white	10.8 d	138 ab[h]	3.78 f	93.0 a[h]	29.7 b	115 a[h]	918 ab	1620 b[h]
	Noble	red	17.5 c	76.4 d[h]	10.5 de	23.2 c[h]	31.0 b	41.8 bc	474 d	592 e
	Albemarle	red	12.7 cd	110 bc[h]	24.8 a	23.5 c	29.0 b	53.9 b[h]	1030 a	1090 c
	Cowart	red	27.5 b	162 a[h]	13.8 c	95.9 a[h]	24.5 bc	46.1 bc[h]	732 bc	1900 a[h]
	Nesbitt	red	15.5 cd	136 ab[h]	7.53 ce	61.7 b[h]	18.7 cd	39.4 c[h]	555 cd	1100 c[h]
	Georgia Red	red	42.9 a	74.8 d[h]	12.8 cd	20.5 c[h]	38.7 a	10.1 d[h]	996 a	587 e
pulp	Carlos	white	4.73 e	2.66 c	3.32 a	1.00 c	2.83 cd	2.90 c	159 b	231 b[h]
	Fry	white	6.44 de	1.01 d[h]	3.30 a	ND[e] d[h]	1.57 d	ND e[h]	189 b	ND c[h]
	Doreen	white	14.1 a	0.93 d[h]	1.22 cd	trace d[h]	12.1 a	0.66 d[h]	474 a	trace c[h]
	Noble	red	3.51 ef	8.69 b[h]	0.88 d	2.98 b[h]	2.82 cd	5.79 b[h]	208 b	168 b
	Albemarle	red	12.2 ab	24.5 a[h]	2.06 bc	6.04 a[h]	9.36 b	12.8 a	203 b	455 a
	Cowart	red	8.28 cd	1.24 d[h]	2.12 b	trace d[h]	5.08 c	trace e	232 b	ND c[h]
	Nesbitt	red	10.1 bc	0.54 d[h]	3.53 a	trace d[h]	8.63 b	trace e	197 b	ND c[h]
	Georgia Red	red	1.13 f	1.00 d	trace[f] d	ND d	1.13 d	ND e	38.2 c	ND c[h]
juice[g]	Carlos	white	3.01 cde	4.34 e	1.02 cd	8.60 cd[h]	4.96 c	5.34 cd[h]	12.5 e	106 e[h]
	Fry	white	3.99 bcd	11.2 bcd[h]	2.63 a	21.7 a[h]	2.94 e	3.13 d	59.1 c	105 e[h]
	Doreen	white	3.34 cde	14.1b[h]	0.56 e	7.68 d[h]	6.02 b	15.7 b[h]	12.7 e	172 d[h]
	Noble	red	8.75 a	20.5 a[h]	trace e	5.78 e[h]	6.70 ab	15.6 b[h]	10.1 e	257 b[h]
	Albemarle	red	5.15 b	23.4 a[h]	0.77 de	9.68 bc[h]	6.81 a	20.1 a[h]	14.0 e	322 a[h]
	Cowart	red	4.03 bc	12.5 bc[h]	1.31 bc	11.2 b[h]	4.07 d	6.81 c[h]	81.0 b	219 c[h]
	Nesbitt	red	2.17 e	8.82 d[h]	1.16 cd	5.19 e[h]	3.20 e	4.85 cd[h]	26.1 d	187 cd[h]
	Georgia Red	red	2.66 de	9.77 cd	1.60 b	ND f[h]	3.47 de	3.16 d	88.0 a	198 cd[h]

[a,b] Expressed in ellagic acid equivs. [c] The sum of free ellagic acid and ellagic acid released following acid hydrolysis. [d] Same letters within columns for each fruit part are not significantly different (LSD test. $P < 0.05$). [e] ND = concentrations below detection limit. [f] Concentration below 0.5 ppm. [g] Hot-pressed juice. [h] Indicates significant effects by fruit ripening for each fruit parts (LSD test, $P < 0.05$).

weight basis. Upon ripening, these compounds were even more localized in the skin and accounted for 82−87% of the total. Doreen and Cowart contained the highest concentrations of ellagic acid and its glycosides among the cultivars, but no meaningful correlation could be made between free ellagic acid and/or ellagic acid glycosides and concentrations of total ellagic acid, an observation that likely reflected the influence of ellagitannins in each isolate.

Compared to ellagic acid concentrations present in the skin and pulp, levels present in juice were considerably lower and reflected the low solubility of ellagic acid in aqueous systems. A hot break or "hot-press" technique is commonly used with muscadine grapes to increase juice yields or add pigmentation to wines or juices, and when combined with macerating enzymes (*20*), juice extractions are better facilitated. Additionally, the time and temperature of the heating process will appreciably influence juice yields and phytochemical concentration compared to nonheated fruit juice (*9*), and white or bronze grapes, depending on cultivar, may not be heated to prevent enzymatic and autoxidative browning reactions affecting juice quality (*21*). Textural differences also occur in the grapes during ripening from action of natural pectinase and may also influence phytochemical solubilization. Typical juice yields may range from 60 to 75 wt % for hot-pressed muscadine grape juices and is influenced by heating conditions, pressing conditions, the use of pressing aids such as rice hulls, and skin thickness (*22*). The highest concentrations of total ellagic acid were found in the juice of ripe Albemarle (322 mg/L) and Noble (257 mg/L), which reflected a 24% average increase in concentration over juice pressed from unripe grapes. Concentrations of total ellagic acid present in the juice were not necessarily a reflection of levels found in whole grapes, because the juice of unripe fruit contained 2−26% of the amount present in whole grapes compared to 19−78% for ripe fruit. For simplicity, these data were determined based on a 60% juice yield and accounted for the variable contributions from skin and pulp tissue (seeds not included) to the total weight of the grapes. Juice from ripe grapes of Noble, Cowart, Nesbitt, and Georgia Red had the highest total ellagic acid extractions (>58%), while Carlos, Fry, Doreen, and Albemarle were considerably lower (<34%). The low recovery of ellagic acid derivatives in the latter cultivars reflected the difficulty in solubilizing polyphenolics, likely due to physical barriers associated with their thick skins, which left high concentrations of these compounds behind in the skin and pulp material. Free ellagic acid itself, which is sparingly soluble in water, was also poorly solubilized in all juices, retaining only 27 and 37% on average of the total present in whole grapes for unripe and ripe fruit, respectively. However, the ellagic acid glycosides were considerably more soluble in juices with >56% recovery from whole grapes.

Anthocyanins. Anthocyanidins, quantified only in the red cultivars, were expressed in cyanidin equivs (**Table 2**), because the predominant anthocyanins in muscadine grapes were previously identified as nonacylated 3,5-diglucosides of six anthocyanidin bases (*9*). In the current study, only three anthocyanidins were positively elucidated following acid hydrolysis using the column and solvent conditions described, due to incomplete separation of peonidin and malvidin and the absence of pelargonidin. As expected, anthocyanins appreciably increased in the skin as the fruit ripened with low concentrations also found in pulp material nearest the skin. Anthocyanidin abundance in ripe fruit were delphinidin > petunidin > malvidin + peonidin > cyanidin with Nesbitt, Noble, and Cowart containing the highest overall concentrations. Color instability of muscadine wine and juice is an established quality defect and is a consequence of their lack of intramolecular copigmentation and high concentrations of monomeric 3,5-diglucosides with *o*-diphenolic substituents that include delphinidin, cyanidin, and petunidin (*23*). Among the cultivars evaluated, these three anthocyanidins accounted for 78−96% of the total in fresh

Table 2. Concentration (mg/kg, mg/L in Cyanidin Equivalents) of Six Anthocyanidins and Total Anthocyanidins on Skin, Pulp, and Juice of Red Muscadine Grapes as Affected by Ripening Stages (U, Unripe and R, Ripe)

		delphinidin[a]		cyanidin		petunidin		malvidin + peonidin		total[b]	
		U	R[c]	U	R	U	R	U	R	U	R
skin	Noble	ND[d] b[e]	1450 b	ND b	692 c	159 a	1070 a	ND a	926 a	159 a	4140 b
	Albemarle	44.2 a	424 c	28.5 a	291 d	ND b	ND b	ND a	102 d	72.6 b	817 d
	Cowart	57.6 a	1290 b	37.5 a	1210 a	12.0 b	294 b	ND a	445 c	107 ab	3250 c
	Nesbitt	66.8 a	3550 a	35.1 a	860 b	ND b	ND b	ND a	825 b	102 ab	5230 a
	Georgia Red	72.1 a	300 c	35.4 a	52.5 e	ND b	20.3 b	ND a	17.9 e	108 ab	390 d
pulp	Noble	ND b	102 a	6.95 a	93.9 a	ND b	78.4 a	ND a	114 a	6.95 a	383 a
	Albemarle	0.84 b	67.1 b	5.85 a	89.0 a	ND b	29.7 b	ND a	21.8 b	6.70 a	212 b
	Cowart	4.54 a	3.75 c	4.00 b	10.6 b	0.90 a	0.63 c	ND a	ND b	9.44 a	15.0 c
	Nesbitt	ND b	19.3 c	ND c	12.4 b	ND b	5.03 c	ND a	ND b	ND b	36.8 c
	Georgia Red	1.17 b	2.52 c	0.980 c	0.76 b	ND b	0.25 c	ND a	ND b	2.15 b	3.53 c
juice[f]	Noble	ND b	131 a	ND b	125 a	ND b	155 a	ND a	200 a	ND b	610 a
	Albemarle	ND b	52.4 c	ND b	86.1 b	ND b	25.7 c	ND a	18.2 b	ND b	182 b
	Cowart	ND b	48.6 c	ND b	94.0 b	ND b	21.4 c	ND a	16.5 b	ND b	180 b
	Nesbitt	6.98 a	72.5 b	2.83 a	49.3 c	3.04 a	44.3 b	ND a	23.6 b	12.8 a	190 b
	Georgia Red	ND b	10.1 d	ND b	7.16 d	ND b	2.61 a	ND a	ND c	ND b	19.9 c

[a] Cyanidin equivs. [b] Sum of individual anthocyanidins. [c] All anthocyanins are significantly different at ripening stage. [d] ND = concentrations below detection limit. [e] Similar letters within columns for each fruit part are not significantly different (LSD test, $P < 0.05$). [f] Hot-pressed juice.

grapes and from 67 to 100% in juice. Ripe Noble grapes, one of the most popular wine and juice cultivars, contained the highest concentration of malvidin + peonidin among the cultivars evaluated. Malvidin is generally considered the most stable anthocyanin form and along with peonidin was present at 22% of the total in the skins compared to 33% in juice. However, even with high malvidin + peonidin concentrations, the juice from Noble grapes is considered highly susceptible to color degradation (*23*) and provides an indication that the remaining cultivars would be even less stable to oxidation or other deteriorative reactions affecting juice or wine pigmentation due to their lower malvidin + peonidin concentrations. These cultivars, as well as the white/bronze varieties, may be more suitable for juice blending to take advantage of their high ellagic acid contents. Noble grape juice also contained the highest total anthocyanin concentration (610 mg/L), while Georgia Red contained considerably less (20 mg/L), even in relation to the other red varieties that ranged from 180 to 190 mg/L. On the basis of a 60% juice yield, only 12% of the total anthocyanins present in grape skins were solubilized into the juice of Nesbitt and Georgia Red, both consumed primarily as table grapes, the former having high anthocyanin content yet poor anthocyanin solubility characteristics during juicing. Juice from the remaining cultivars, which are commonly consumed either fresh or processed, contained 27−32% of the total anthocyanins present in the each grape. The low anthocyanins recovery values in juice, especially in relation to ellagic derivatives, reflect the degree of processing necessary to solubilize sufficient anthocyanins to produce a suitable red wine or juice.

Total Phenolics and Antioxidant Capacity. Measurements of total phenolics by the Folin−Ciocalteu metal reduction assay and peroxyl radical scavenging activity using the ORAC assay are common index that provide an overall assessment of the content and chemical activity of compounds present in fruits and vegetables. These attributes were quantified in methanolic and ethyl acetate extracts of grape skin, pulp, and juice and following partitioning of phenolic acids and flavonols into ethyl acetate, into which anthocyanins are not soluble, to differentiate between major polyphenolic classes (**Tables 3** and **4**). Values for total phenolics, which varied among cultivars and with fruit ripening, were good predictors of antioxidant capacity in both methanolic and ethyl acetate extracts ($r = 0.83$ and 0.92, respectively). The higher correlation coefficient for ethyl acetate extracts may have reflected the removal of potentially interfering/prooxidant polar compounds or reflected interactions between anthocyanins and other polyphenolics in the methanolic extracts (*24*, *25*). On the basis of abundance, anthocyanins were the major antioxidant compounds present in muscadine grape skin and juice and their concentration was directly related to antioxidant capacity ($r = 0.99$). Ethyl acetate soluble compounds also contributed to antioxidant capacity and ranged from 12 to 29%, 22−83, and 5.7−15% of the total present in methanolic extracts of skin, pulp, and juice, respectively. Other than ellagic acid and its derivatives, many additional compounds were also

Table 3. Concentrations (mg/kg, mg/L) of Total Soluble Phenolics (Folin–Ciocalteu Metal Reduction Assay) in Methanolic and Ethyl Acetate Extracts as Affected by Cultivars and Ripening Stages (U, Unripe and R, Ripe)

			methanolic extract		ethyl acetate extract	
	cultivars	color	U	R	U	R
skin	Carlos	white	2430 b[1]	2530 e	428 d	706 f[c]
	Fry	white	1440 c	3360 d[c]	459 d	987 e[c]
	Doreen	white	3860 a	3990 c[c]	1430 a	2280 b[c]
	Noble	red	2660 b	3090 d	1020 bc	727 f[c]
	Albemarle	red	2580 b	2260 e	1320 ab	756 ef
	Cowart	red	2660 b	4370 c[c]	1130 ab	1890 c
	Nesbitt	red	2480 b	5030 b[c]	627 cd	1300 d[c]
	Georgia Red	red	4220 a	9470 a[c]	1500 a	2910 a[c]
pulp	Carlos	white	405 de	738 b	128 d	258 a
	Fry	white	566 cd	276 d[c]	138 d	102 cd
	Doreen	white	1210 b	192 d[c]	1300 a	39.2 de[c]
	Noble	red	601 c	848 b[c]	332 cd	120 bc[c]
	Albemarle	red	1410 a	1100 a[c]	622 b	274 a[c]
	Cowart	red	1110 b	200 d[c]	528 bc	38.2 de[c]
	Nesbitt	red	567 c	443 c	502 bc	16.3 e[c]
	Georgia Red	red	312 e	467 c[c]	99.4 d	183 b[c]
juice[b]	Carlos	white	1145 c	979 de[c]	165 a	66.4 b[c]
	Fry	white	1069 c	1500 cd[c]	90.1 d	81.0 c
	Doreen	white	1673 a	1293 d	161 a	141 a
	Noble	red	1630 a	1950 b	139 abc	69.1 c[c]
	Albemarle	red	1460 ab	1770 bc	147 ab	120 ab
	Cowart	red	1200 bc	1360 cd	122 bc	89.4 bc
	Nesbitt	red	739 d	1210 d[c]	61.2 e	70.8 c
	Georgia Red	red	1140 c	2860 a[c]	118 c	139 a

[a] Same letters within columns for each fruit part are not significantly different (LSD test, $P < 0.05$). [b] Hot-pressed juices. [c] Indicates significant effects by fruit ripening for each fruit part (LSD test, $P < 0.05$).

Table 4. Antioxidant Capacity (μmol Trolox Equivalents/g or mL) of Methanolic and Ethyl Acetate Extracts as Affected by Cultivars and Ripening Stages (U, Unripe and R, Ripe)

			methanolic extract		ethyl acetate extract	
	cutivars	color	U	R	U	R
skin	Carlos	white	58.0 d[a]	86.2 cd[c]	10.2 b	17.5 b
	Fry	white	49.3 d	72.3 d	10.7 b	19.0 b[c]
	Doreen	white	104 a	90.4 c[c]	25.2 a	25.5 a
	Noble	red	97.2 ab	100 c	22.5 a	12.3 c[c]
	Albermerle	red	90.8 b	71.1 d	22.6 a	12.0 c[c]
	Cowart	red	97.7 ab	119 b	22.6 a	26.3 a[c]
	Nesbitt	red	69.7 c	136 a[c]	12.1 b	25.1 a[c]
	Georgia Red	red	89.0 b	128 ab[c]	25.4 a	29.1 a[c]
pulp	Carlos	white	5.95 de	8.75 b[c]	3.90 e	5.05 b
	Fry	white	6.35 de	7.50 bc	3.90 e	3.70 d
	Doreen	white	34.0 a	2.45 d[c]	15.0 a	1.60 e[c]
	Noble	red	9.40 cd	14.3 a	4.70 e	4.10 cd
	Albermerle	red	14.8 b	13.1 a	8.05 c	6.45 a[c]
	Cowart	red	11.8 bc	2.60 d[c]	5.70 d	1.55 ef[c]
	Nesbitt	red	13.9 b	4.60 cd	9.50 b	1.00 f[c]
	Georgia Red	red	4.95 e	6.80 bc	4.10 e	4.45 c
juice[b]	Carlos	white	20.3 b	15.5 d[c]	2.37 b	2.06 bc
	Fry	white	14.6 d	20.1 bc	1.61 cd	1.92 bc
	Doreen	white	25.3 a	19.6 bc	2.91 a	2.38 ab
	Noble	red	24.3 a	26.7 a	2.55 ab	1.51 cd
	Albermerle	red	19.3 bc	23.3 ab	2.89 a	2.14 b
	Cowart	red	17.4 cd	21.4 bc[c]	1.99 c	1.91 bc
	Nesbitt	red	10.8 e	18.3 cd[c]	1.27 d	1.20 d
	Georgia Red	red	20.7 b	26.6 a[c]	2.50 b	2.96 a[c]

[a] Similar letters within columns for each fruit part are not significantly different (LSD test, $P < 0.05$). [b] Hot-pressed juices. [c] Indicates significant effects by fruit ripening for each fruit part (LSD test, $P < 0.05$).

identified in the ethyl acetate extract including several flavonoids glycosides, phenolic acids, and procyanidins that are all known to possess antioxidant activity (*26*, *27*). In various concentrations, gallic acid, protocatechuic acid, catechin, and epicatechin were identified in ethyl acetate extracts. Flavonoid glycosides were tentatively identified based on their spectroscopic similarities to myricetin, quercetin, and kaempferol with glucose and/or rhamnose moieties. A myricetin glycoside was the predominant flavonoid present in all cultivars and ranged from 8.7 to 1350 mg/kg in skin, 0−50 mg/kg in pulp, and 1.6−50 mg/L in juice. Among the cultivars, ripe Georgia Red contained the highest concentrations of total phenolics in both ethyl acetate and methanolic extracts of both skin and juice, in contrast to its low anthocyanin, ellagic acid, and ellagic acid glycoside content, that was primarily attributed to its high flavonoid concentration.

This study demonstrated that ripening, physiology, and juice processing influence phytochemical composition and antioxidant capacity of muscadine grapes. Data suggest a diversity of phytochemical compounds that can be used for novel blending schemes for muscadine grape juice or wine to obtain a desired quality and polyphenolic content relating to their antioxidant capacity.

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