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Ellagic Acid and Flavonoid Antioxidant Content of Muscadine Wine and Juice

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Antioxidant properties of flavonoids and ellagic acid were characterized in eight wines and juices produced by various processing methodologies from red and white muscadine grape cultivars (*Vitis rotundifolia*). Juices and wines were produced by hot- and cold-pressed techniques, and additional wine was produced following on-hull fermentation for 3, 5, and 7 days. Chromatographic conditions were developed to simultaneously separate anthocyanins, ellagic acid, and flavonols and correlated to a measurement of overall antioxidant capacity (AOX), and their changes were monitored after storage for 60 days at 20 and 37 °C. Regression coefficients between concentrations of individual polyphenolics and AOX ranged from 0.55 for ellagic acid to 0.90 for kaempferol. Both red and white wines had higher AOX values after storage than juices made from an identical grape press, despite lower concentrations of individual polyphenolic compounds. Red wines fermented on-hull had higher initial concentrations of antioxidant polyphenolics as compared to a corresponding hot-pressed juice, but changes in AOX during storage were more affected by time than by storage temperature despite lower concentrations of flavonoids and ellagic acid present at 37 °C as compared to 20 °C. Oxidative or polymerization reactions significantly decreased levels of monomeric anthocyanins during storage with the greatest losses observed for delphinidin and petunidin 3,5-diglucosides. Processing methods for muscadine wine and juice production were important factors influencing concentrations of antioxidant flavonoids and ellagic acid, while the role of fermentation and time had the greatest influence on retention of AOX properties during storage.

KEYWORDS: Muscadine grape; antioxidant; storage stability; anthocyanin; ellagic acid; flavonol

INTRODUCTION

Muscadine grapes (*Vitis rotundifolia*) are among the most important *Vitis* species cultivated in the southeastern U.S. and have potential for expanded markets in wine and juice production. Generally considered an underutilized commodity, muscadine grapes have a characteristic aroma and sweetness that make them acceptable as table wines, but little information is available on their antioxidant composition. Muscadine grapes grow in small clusters and are especially important to the economy of southeastern states due to their resistance to Pierce's disease (*Xylella fastidiosa*) and generally thrive in soil and climate conditions not favorable for bunch grape production.

When processing muscadine grapes, physicochemical properties are important factors influencing overall quality and consumer acceptability. Muscadine wine and juice is known for its poor storage stability due to the presence of unstable anthocyanins such as delphinidin 3,5-diglucoside that affect color (*1*, *2*) and from insoluble sediments that form, previously identified as primarily ellagic acid (*3*, *4*). Commercial production of muscadine wines and juices is similar to that of *Vitis vinifera*, with color derived from anthocyanins a function of vinification treatments such as time and temperature of extraction (*5*−*8*). Anthocyanins exist in muscadine grapes primarily as 3,5-diglucosides of delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn), and malvidin (Mv) in nonacylated forms (*1*, *2*, *9*) and are the most abundant flavonoids present. Anthocyanin 3,5-diglucosides were reported as less stable to oxidation and heat as compared to corresponding 3-glucosides (*10*) and may result in rapid color loss during wine or juice storage (*11*). Flora (*1*) reported large reductions in Dp, Cy, and Pt-3,5-diglucosides in muscadine grapes after severe heat treatments when analyzed by thin-layer chromatography. Mv 3,5-diglucoside was found to be less stable than acylated forms of Mv present in red cabbage (*12*), but in model systems, the stability of Mv 3,5-diglucoside was greater than Mv 3-glusoside both with and without added ascorbic acid (*13*, *14*). The relative stability of muscadine anthocyanins is likely a function of a complex chemical matrix, structural features, and the combined effects of processing and storage.

Interest in phytochemicals such as anthocyanins, ellagic acid, and flavonols has increased in recent years due to positive associations between consumption rates and beneficial health factors such as anticarcinogenic and radical scavenging activity (*15*−*17*). Muscadine grapes contain ellagic acid, unique among *Vitis* species, and its concentration varies among cultivars and

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methods of extraction (*3*, *4*, *18*−*20*). To date, few studies have quantitatively investigated the overall antioxidant capacity (AOX) or flavonol concentrations in muscadine grape products. Polyphenolics are important constituents of all grape species with anthocyanins, followed by flavonols and phenolic acids, having the greatest contribution to AOX (*21*). Similar phytochemical compositions may be expected between wines and juices made from a single grape press, but changes during vinification procedures can alter composition and AOX properties. For example, the AOX of red and white wines was higher than that of corresponding juices, while differences were not attributed to polyphenolic content but rather to additional chemical constituents, synergistic responses, or other chemical changes that occurred during fermentation (*22*). Processing and storage techniques are also critical factors affecting phytochemical concentration and AOX of wines and juices. Oxygen sparging of red wine was shown to reduce polyphenolic content considerably (*23*) while quality deterioration in wine was found to be dependent on storage time and independent of storage temperature (*8*). In young red wines, varying production techniques were found to have little effect on AOX due to similar polyphenolic contents (*24*); however, because most polyphenolic compounds originate in grape skins and seeds, their extraction and solubility are generally influenced by time and temperature of extraction (*25*).

The objectives of this study were to evaluate ellagic acid and flavonoid concentration as related to an overall assessment of antioxidant properties in two common species of red and white muscadine grapes processed into wine and juice by varying extraction techniques. Monitoring these compounds under room temperature and accelerated storage conditions provided insight to the quality and phytochemical stability characteristics.

MATERIALS AND METHODS

Materials and Processing. Red and white muscadine grapes (cv. Noble and Carlos, respectively) were obtained from a local grower in central Florida and pressed into juice or were prepared for fermentation. All Carlos grapes were "cold-pressed" (CP) into juice, of which half was frozen (−20 °C) until fermentations were complete, and the remainder was prepared for fermentation. The CP technique was performed on the day of harvest, and the actual extraction temperature was ∼25 °C. Noble grapes were divided into five portions for manufacture of hot-pressed (HP) and CP juice and for on-hull fermentations at three skin contact times (3, 5, and 7 days).

CP juices were prepared following crushing and pressing in a hydraulic basket press (Prospero's Equipment Cort, NY) with 25 mg/L potassium metabisulfite added to Carlos and 50 mg/L added to Noble to retard phenolic oxidation. HP juice (Noble only) was prepared by holding crushed grapes at 70 °C for 15 min prior to pressing. All juices were kept frozen (−20 °C) until completion of wine-making steps, after which each was thawed and filtered first through two, no. 330 pads followed in tandem by a no. 730 and no. 750 pad filter (Cellulo Co., Fresno, CA) containing a 2 cm bed of diatomaceous earth. Juices were bottled (500 mL) and thermally pasteurized to an internal temperature of 90 °C for 1 min.

For wine production, CP Carlos and Noble juices were adjusted to 20% solids with sucrose, inoculated with yeast (Premier Cuvee, Universal Foods Corporation, WI), and fermented to dryness (<0.1% solids, w/v) at 13 °C. Following crushing and yeast inoculation, on-hull fermentations proceeded for 3, 5, and 7 days at 20 °C under aerobic conditions. The partially fermented must was adjusted to 20% solids (w/v) with sucrose and fermented to dryness as previously described. Wines were racked under nitrogen, sulfite was added a second time (25 mg/L), and they were cold-stabilized for 4 weeks at 4 °C to precipitate tartaric esters. Finished wines were passed through no. 730 and no. 750 filter pads and bottled (750 mL) for subsequent storage and analysis.

The preceding processes resulted in eight samples for analysis that included Carlos CP wine and juice, Noble CP wine and juice, Noble HP juice, and Noble wine pressed 3, 5, and 7 days after on-hull fermentation. Research samples of wine and juice were treated with sodium azide (50 mg/L) to prevent microbial growth and partitioned into 3 aliquots for initial evaluation and storage for 60 days at 20 and 37 °C.

Chemical Analyses. Phytochemical analyses were conducted to characterize the major flavonoids and ellagic present in muscadine wines and juices. Assays for total polyphenolics using the Folin−Ciocalteu assay (*26*) and total anthocyanins using a pH shift assay (*27*) determined overall differences observed after processing and storage. Flavonoids (myrecetin, quercetin, kaempferol, and six anthocyanins) and ellagic acid were determined by high-performance liquid chromatography (HPLC) by first combining 1 mL of wine or juice with 4 mL of 0.1 M citric acid buffer (pH 3) and binding to a 6 mL AccuBond ODS-C_{18} cartridge (J&W Scientific, Folsom, CA) previously washed with methanol and citric acid buffer. Bound polyphenolics were washed with 10 mL of buffer and eluted with 5 mL of methanol acidified with 0.1% HCl. Flavonoid glycosides, ellagitannins, and anthocyanins were subsequently hydrolyzed in 2 N HCl (adjusted to contain 50% methanol) for 60 min at 95 °C, and aglycones were separated by HPLC using modified chromatographic conditions of Hertog et al. (*28*). Separations were performed on a Waters 2690 Alliance HPLC system using a Waters 996 PDA detector and compounds separated using a Waters Nova-Pak C_{18} column (150 mm × 3.9 mm) with a C_{18} guard column. Mobile phases consisted of water (phase A) and 60% methanol (phase B) both adjusted to pH 2.4 with *o*-phosphoric acid. A gradient solvent program ran phase B from 0 to 30% in 3 min, 30−50% in 2 min, 50−70% in 5 min, and 70−100% in 2 min, and held for 5 min all at 1 mL/min. Anthocyanin 3,5-diglycosides were also quantified by reverse phase HPLC according to the chromatographic conditions of Skrede et al. (*29*) and separated on a Supelcosil LC-18 column (250 mm × 4.6 mm); compounds were quantified using standards of their respective anthocyanin 3-glucoside forms (Polyphenols Laboratories AS, Sandnes, Norway). All polyphenolics were identified by UV/vis spectral interpretation (*30*−*32*), retention time, and comparison to authentic standards (Sigma Chemical Co., St. Louis, MO).

AOX was determined using the oxygen radical absorbance capacity (ORAC) method described by Cao et al. (*33*), which was adapted to run on a 96 well Molecular Devices f_{max} fluorescent microplate reader (535 nm excitation and 560 nm emission). Following proper dilutions, muscadine wine and juice were monitored for their ability to inhibit oxidation of β-phycoerythrin in the presence of the peroxyl radical generator 2,2′-azobis (2-amidinopropane) dihydrochloride. The extent of oxidation was monitored every 2 min, and the area under the decay curve was compared to a blank (ORAC = 0) and a standard curve of Trolox, a water soluble analogue of vitamin E. Data were expressed in micromoles of Trolox equivalents per milliliter of wine or juice. For reference, data were compared to fluorescein as the fluorescent probe as described by Boxin et al. (*34*) and acceptable correlations were found ($R^2 > 0.88$).

Statistical Analysis. Data represent the mean of three replicate analyses tested as a treatment by time factorial that compared the eight wines and juices over the 60 day storage period at each temperature. Multiple linear regression, analysis of variance, and Pearson correlations were conducted using JMP software (*35*) and mean separation using the LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

Wine and Juice Composition. Chromatographic conditions were developed and optimized to simultaneously separate and quantify anthocyanidins, ellagic acid, and three flavonols (myrecetin, quercetin, and kaempferol) in a single injection following acid hydrolysis of glycosidic linkages (**Figure 1A**). Anthocyanin 3,5-diglucosides (**Figure 1A**) correlated well to their aglycone form (**Figure 1B**; **Table 1**), and both correlated well to spectrophotometric determinations for total anthocyanins ($R^2 = 0.99$ and 0.92, respectively). The red grape evaluated in

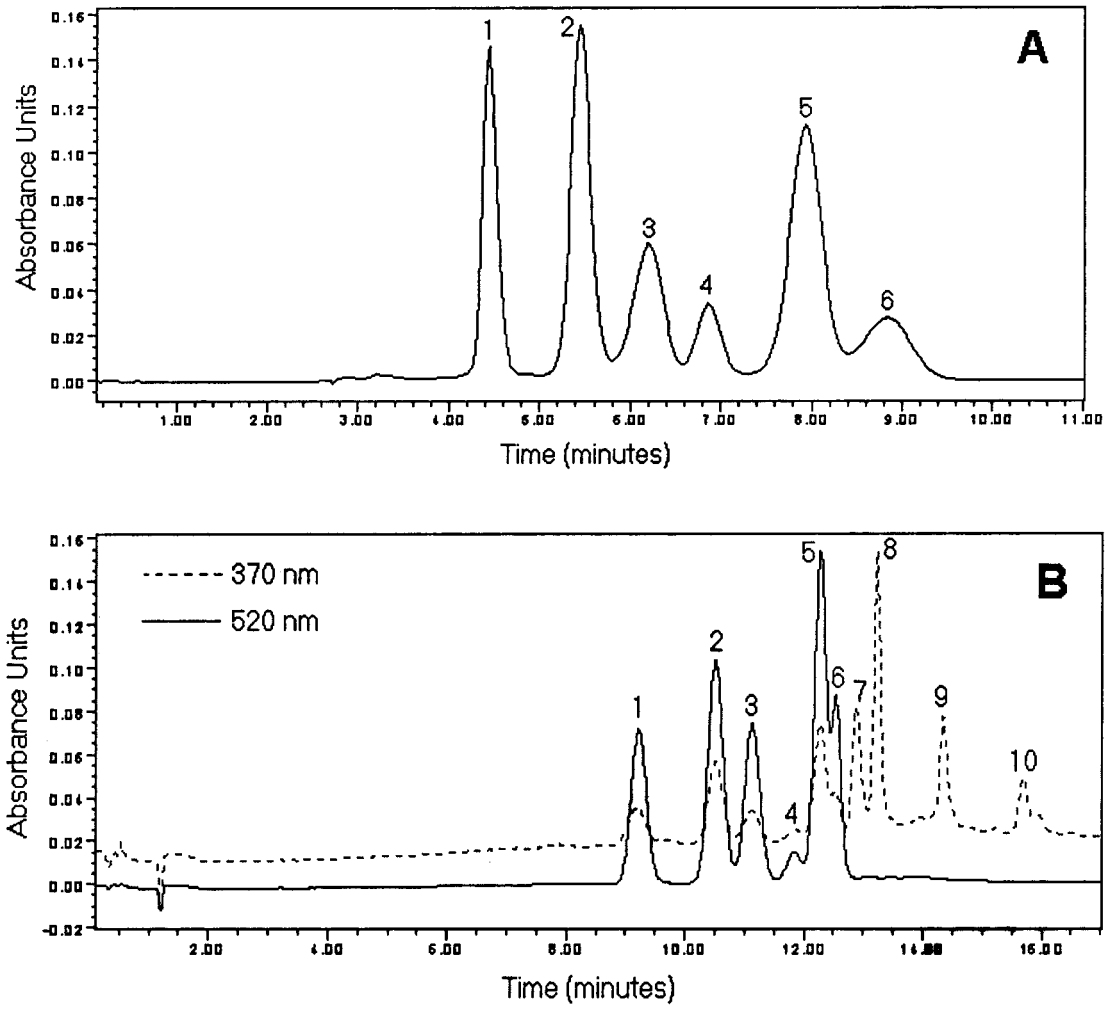

Figure 1. Typical HPLC chromatograph of (**A**) anthocyanin 3,5-diglucosides (520 nm) and (**B**) anthocyanidins (520 nm, solid line) and ellagic acid/flavonols (370 nm, dotted line) present in Noble muscadine wine and juice. Isolates were hydrolyzed into aglycones at 90 °C for 60 min in 2 N HCl containing 50% methanol prior to analysis. Peak assignments: 1, delphinidin; 2, cyanidin; 3, petunidin; 4, pelargonidin; 5, peonidin; 6, malvidin; 7, myrecetin; 8, ellagic acid; 9, quercetin; 10, kaempferol.

Table 1. Linear Regression ($Y = b_0 + b_1X$) Equations Predicting Anthocyanidin 3,5-Diglucoside Concentrations (mg/L) from Standard Solution of Anthocyanin 3-Glucoside Following Acid Hydrolysis into Anthocyanidins

anthocyanin	b_0	b_1	variable	R^2
delphinidin 3,5-diglucoside	13.94	0.555	delphinidin	0.95
cyanidin 3,5-diglucoside	11.11	0.417	cyanidin	0.93
petunidin 3,5-diglucoside	26.18	0.416	petunidin	0.92
pelargonidin 3,5-diglucoside	−2.59	0.395	pelargonidin	0.72
peonidin 3,5-diglucoside	12.45	0.347	peonidin	0.93
malvidin 3,5-diglucoside	12.98	0.352	malvidin	0.91

this study also contained pelargonidin in low concentrations and is the first known report of this compound in muscadine grapes.

Significant differences in polyphenolics were observed between grape cultivars, wine and juice, and processing techniques ($P < 0.05$). Both cultivars contained ellagic acid, myricetin, quercetin, and kaempferol, while only Noble contained appreciable concentrations of anthocyanins. Significant correlations (R^2) for flavonoids and ellagic acid to AOX were observed in juices and wines that ranged from 0.55 for ellagic acid to 0.90 for kaempferol. CP wines and juices were expected to contain low phytochemical concentrations due to the nature of the pressing technique, relatively low temperature, and characteristic thick skins of these grapes that hinder polyphenolic extraction. However, HP juices and on-hull fermentations were expected to contain considerably higher polyphenolic concentrations as these compounds are solubilized with heat and during alcoholic fermentation. Wines and juices in this study were initially evaluated for flavonoid, ellagic acid, and AOX immediately after production followed by a second evaluation 60 days after storage at 20 and 37 °C. Because muscadine grape products are known to have poor storage characteristics due to rapid color deterioration during storage (*2*), phytochemical factors influencing this instability were investigated as a means to improve marketability of muscadine wines and juices.

CP Wine and Juice. Carlos and Noble CP juices were divided in half to determine the effect of pasteurization and alcoholic fermentation on flavonoid and ellagic acid composition and AOX (**Tables 2** and **3**). Carlos juice generally contained higher concentrations of polyphenolic compounds than its corresponding wine from losses or complexes formed during fermentation, but differences in AOX were not observed prior to storage (**Figure 2A**,**B**) despite an 81% decrease in ellagic acid that occurred during fermentation. Ellagic acid and flavonols are known to be good antioxidant compounds (*15*), but their low concentrations in Carlos grapes were minor contributors to AOX, with phenolic acids more likely responsible for the observed activity. Following storage at 20 and 37 °C, total soluble phenolics declined in Carlos juice by 31 and 36% as

Table 2. Concentrations (mg/L) of Total Soluble Phenolics (Folin–Ciocalteu Assay), Total Anthocyanins (pH Shift Assay), Total Anthocyanidins (HPLC), and Percent Contribution of Monomeric Anthocyanidins to Total Soluble Phenolics in Carlos (White) and Noble (Red) Muscadine Wine and Juice[a]

			total soluble phenolics			total anthocyanins			total anthocyanidins[b]			% of total phenolics from anthocyanidins[c]		
			initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C
Carlos	juice	CP[d]	329 e[g]	227 e	212 e	ND f	ND f	ND g	1.9 c	ND e	ND e	0.30	ND	ND
Carlos	wine	CP	248 f	226 e	189 e	ND f	ND f	ND g	1.4 c	ND e	ND e	0.29	ND	ND
Noble	juice	CP	337 e	308 d	290 d	194 e	79.8 d	94.3 e	124 c	83.1 e	55.7 d	19.2	14.0	9.99
Noble	wine	CP	403 d	343 d	298 d	201 e	40.3 e	51.9 f	150 c	116 e	55.3 d	19.4	17.5	9.66
Noble	juice	HP[e]	1280 c	1320 c	1340 c	1270 d	742 c	450 d	2360 b	768 d	450 c	95.8	30.4	17.4
Noble	wine	3 day[f]	1600 b	1540 b	1490 b	1330 c	908 b	478 c	2790 a	1210 b	564a b	90.4	40.7	19.6
Noble	wine	5 day	1840 a	1740 a	1720 a	1500 a	982 a	538 a	2670 a	1390 a	594 a	75.6	41.5	18.0
Noble	wine	7 day	1860 a	1760 a	1710 a	1440 b	915 a	501 b	2900 a	1060 c	532 b	81.2	31.4	16.2

[a] Samples were evaluated initially after production and after 60 days storage at 20 and 37 °C. [b] Sum of six anthocyanidins quantified by HPLC. [c] Calculated based on a 52% contribution of anthocyanidins to total soluble phenolics in the Folin–Ciocalteu assay. [(Total anthocyanidins × 0.52)/Total soluble phenolics] × 100%. [d] CP. [e] HP. [f] Skin contact time during fermentation. [g] Values between columns with similar letters not significantly different for each variable at each sampling point (LSD test, $P < 0.05$).

Table 3. Concentration (mg/L) of Six Anthocyanidins, Ellagic Acid, and Three Flavonols in Carlos (White) and Noble (Red) Muscadine Wine and Juice[a]

			delphinidin			cyanidin			petunidin			pelargonidin			peonidin		
			initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C
Carlos	juice	CP[b]	ND c[e]	ND f	ND d	0.50 c	ND d	ND d	ND d	ND d	ND d	0.80 c	NDd	ND c	ND d	ND e	ND d
Carlos	wine	CP	ND c	ND f	ND d	0.60 c	ND d	ND d	ND d	ND d	ND d	0.40 c	NDd	ND c	ND d	ND e	ND d
Noble	juice	CP	10.6 c	ND f	ND d	32.3 c	20.2 d	10.6 c	12.9 d	ND d	ND d	12.0 b	12.8c	8.6 b	32.1 d	32.4 d	22.3 c
Noble	wine	CP	13.2 c	ND f	ND d	38.4 c	27.8 d	10.1 c	9.90 d	7.1 d	ND d	16.7 b	14.2c	8.9 b	42.1 d	40.7 d	23.1 c
Noble	juice	HP[c]	568 ab	84.7 e	47.9 c	391 b	178 c	107 d	584 c	140 c	79.0 c	47.6 a	20.4ab	12.9 a	429 bc	197 c	120 b
Noble	wine	3 day[d]	606 a	177 b	54.5 b	441 a	239 b	126 a	729 b	266 b	108 b	48.4 a	22.0a	13.6 a	499 ab	267 b	143 a
Noble	wine	5 day	544 b	220 a	64.6 a	395 b	258 a	122 a	722 b	320 a	122 a	42.1 a	21.3a	11.6 a	473 abc	300 a	146 a
Noble	wine	7 day	598 ab	139 c	51.5 b	414 ab	179 b	100 b	797 a	239 b	107 b	47.0 a	18.5b	11.6 a	518 ab	243 b	135 a

			malvidin			ellagic acid			myricetin			quercetin			kaempferol		
			initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C	initial	20 °C	37 °C
Carlos	juice	CP[b]	0.60 d	ND d	ND d	12.2 c	6.50 d	4.80 d	1.50 d	ND d	0.60 d	5.00 e	2.60 c	1.80 d	3.40 e	1.50 d	1.90 e
Carlos	wine	CP	0.30 d	ND d	ND d	2.3 d	4.80 d	1.60 d	0.70 d	ND d	ND d	1.50 e	1.10 c	0.40 e	2.60 e	1.10 d	0.60 e
Noble	juice	CP	24.2 d	17.8 d	14.2 c	4.4 d	4.20 d	4.20 d	1.40 d	ND d	ND d	1.80 e	1.00 c	1.00 de	10.2 c	5.50 c	5.50 d
Noble	wine	CP	30.2 d	25.9 d	13.3 c	3.4 d	3.90 d	1.20 d	2.70 d	ND d	ND d	1.40 e	0.70 c	0.50 e	6.00 d	4.60 c	2.00 e
Noble	juice	HP[c]	342 c	147 c	82.8 b	102 a	89.8 a	93.1 a	50.5 c	38.4 c	31.0 c	24.3 d	21.3 b	16.7 c	26.1 b	25.3 a	22.3 a
Noble	wine	3 day[d]	468 b	235 b	118 a	56.0 b	31.2 c	26.4 c	90.6 a	78.2 a	56.7 a	40.6 ab	27.5 a	23.2 a	31.8 a	23.1 a	20.7 ab
Noble	wine	5 day	495 ab	270 a	128 a	54.1 b	35.0 b	24.0 c	78.5 b	83.7 a	58.3 a	36.7 abc	30.6 a	23.3 a	25.8 b	23.1 a	19.7 b
Noble	wine	7 day	526 ab	241 b	127 a	53.1 b	35.3 b	30.2 b	79.2 b	59.4 b	44.0 b	34.3 bc	22.5 b	19.4 b	24.9 b	18.2 b	16.9 c

[a] Isolates were hydrolyzed into aglycones at 90 °C for 60 min in 2 N HCl containing 50% methanol prior to analysis. Samples were evaluated initially after production and after 60 days storage at 20 and 37 °C. [b] CP. [c] HP. [d] Skin contact time during fermentation. [e] Values between columns with similar letters not significantly different for each variable (LSD test, $P < 0.05$).

compared to 9 and 31% for wine, respectively. The AOX of Carlos wine and juice decreased during storage at 37 °C, but only Carlos juice decreased at 20 °C after 60 days storage. Correspondingly, Carlos wine had nearly twice the AOX of juice after storage despite lower concentrations of flavonoids and ellagic acid. Overall, flavonol concentrations were low (<10 mg/L) in Carlos grapes and rapidly oxidized during storage.

Noble CP grapes yielded a blush wine and juice with similar initial polyphenolic concentrations. Changes in concentration of total soluble phenolics during storage at 20 and 37 °C were opposite that observed with Carlos grapes, with wine decreasing by 15 and 26% as compared to 9 and 14% for juice, respectively, at each temperature. Noble CP juice also had a higher initial AOX value than its wine, but following storage, wines were considerably higher (up to 55%) in radical scavenging abilities. This difference was attributed to a large decline in AOX for juice (71%) during storage, where no such change was observed in the corresponding wine. No single polyphenolic compound could be attributed to the decrease, but overall declines in flavonoids and formation of visible browning were evidence that other compounds such as phenolic acids or other reducing compounds, not measured in this study, may have affected AOX characteristics. Additionally, since AOX of both Noble and Carlos CP wines were higher overall than juices after storage, an unknown role of ethanol or the fermentation process itself may be important in modulating radical scavenging properties of polyphenolics in muscadine grapes.

HP Juice and On-Hull Fermentations. Processing methods for red grapes can vary depending on intended applications, but in this study, Noble HP juice was produced for comparison to three wines fermented on-hull for 3, 5, and 7 days. On average, Noble HP juice had lower initial concentrations of all antioxidant polyphenolics analyzed in this study (anthocyanins + ellagic acid + flavonols) as compared to on-hull fermentations, where only small differences were observed between treatments (**Figure 3A**). These polyphenolics decreased during storage at a rate consistent with storage temperature, losing 45−63% at 20 °C and 75−79% at 37 °C, depending on processing method. AOX varied greatly after storage, but processing methodology and not initial flavonoid or ellagic acid concentrations or storage

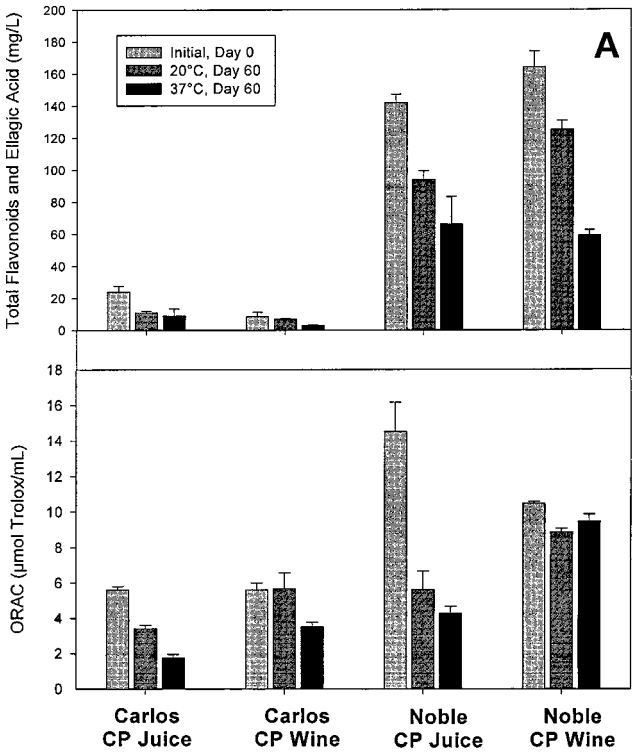

Figure 2. Changes in (**A**) total flavonoids and ellagic acid quantified by HPLC (anthocyanidins + ellagic acid + flavonols) and (**B**) ORAC in Carlos and Noble muscadine wine and juice manufactured from identical grape pressings. Data include initial (day 0) wines and juices and those after 60 days of storage at 20 and 37 °C. Bars represent standard error of the mean ($n = 3$).

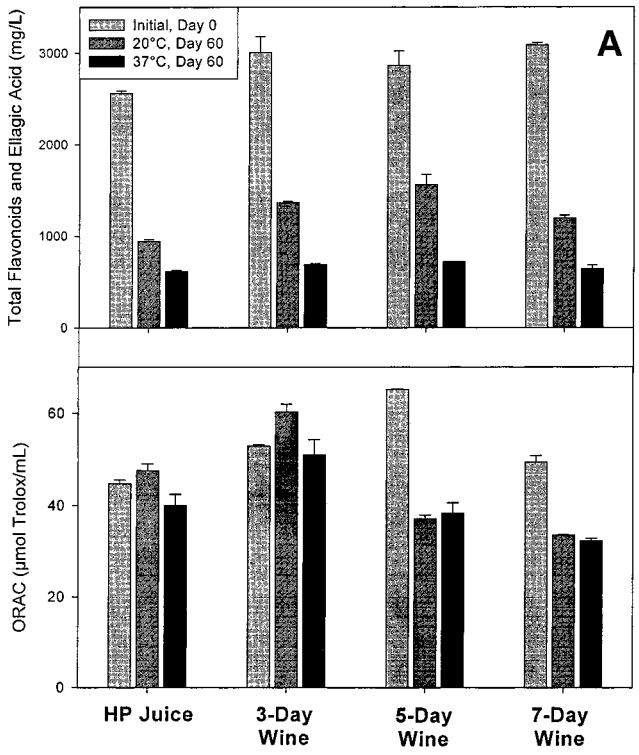

Figure 3. Changes in (**A**) total flavonoids and ellagic acid quantified by HPLC (anthocyanidins + ellagic acid + flavonols) and (**B**) ORAC as affected by processing methodology of Noble muscadine grapes. Samples include HP juice and wines made following 3, 5, and 7 days of skin contact. Data include initial (day 0) wines and juices and those after 60 days of storage at 20 and 37 °C. Bars represent standard error of the mean ($n = 3$).

temperature was important for retention of radical scavenging properties. For example, AOX of Noble HP juice was unchanged from initial conditions after storage at 20 or 37 °C, but activity for on-hull fermentations either increased slightly or decreased during storage (**Figure 3B**). Following storage at 20 °C, AOX of 3 day wines increased by 14% and remained unchanged from initial conditions at 37 °C, while the 5 and 7 day wines experienced a large decrease (33−42%) during storage that was independent of storage temperature as previously observed by Gomez-Plaza et al. (*8*) for wine red wine. The role of nonflavonoid polyphenolics on AOX may also be an important consideration since anthocyanin concentrations, though statistically different between processing treatments, were similar before and after storage. Anthocyanin condensation reactions were evident in both wine and juice based on visual color but were not considered a factor in causing the large decrease in AOX observed for 5 and 7 day wines since changes in monomeric anthocyanins were also similar between processing treatments. The additional polyphenolics solubilized were likely consumed in oxidative reactions during storage that decreased AOX and will be subject of future studies with muscadine grapes.

Losses of anthocyanins, ellagic acid, and flavonols were observed following storage of Noble HP juice and on-hull fermented wines that led to visual color changes in wines and juice (**Tables 2** and **3**). Monomeric forms of Pn, Pg, Mv, and Cy were most stable during storage retaining 57−68% at 20 °C and 38−40% at 37 °C, while Dp and Pt were considerably less stable during storage retaining only 6−34% of their initial levels depending on storage temperature. The order of anthocyanin stability against heat/oxidation was in agreement with Flora (*1*) and confirmed that the greatest anthocyanin losses are with the two most abundant anthocyanins present in muscadine grapes (Dp and Pt). The instability of anthocyanin 3,5-diglucosides is likely characteristic of structural features, with glucose replacing the typical hydroxyl group at C-5, and is reported to be more susceptible to discoloration and browning (*7*) and less stable under oxidative and thermal conditions than other anthocyanin forms (*10*, *36*). On average, total anthocyanins decreased by 47 and 64% following storage at 20 and 37 °C, respectively, and concentrations after storage were reflective of initial levels, indicating a consistent oxidative/polymerization rate between processing treatments. Decreases in flavonol and ellagic acid concentration were also reflective of storage temperature. Ellagic acid decreased up to 38% following storage with greater losses, possibly due to precipitation, observed at higher storage temperatures as previously reported (*18*). Skin contact during muscadine wine fermentation has been shown to affect ellagic acid concentration (*19*), but initial concentrations after 3, 5, and 7 day on-hull fermentations were not observed in this study. Noble HP juice had the highest concentration of ellagic acid in this study, present at twice the level found in on-hull fermented wines. Ellagic acid precipitation has been recognized as a quality defect in muscadine products, and the lower levels initially present in wines indicate precipitation or oxidation during the fermentation process. Conversely, flavonol concentrations were >55% higher in on-hull fermented wines as compared to Noble HP juice, presumably due to the presence of ethanol that aided in extraction from grape skins. The high flavonol and low ellagic acid concentrations in wine provided evidence for additional physicochemical changes to ellagic acid during vinification, since concentrations of other polyphenolics were more similar between wine and juice production methods.

Unlike ellagic acid, decreases in flavonols during storage were due to autoxidative reactions, with losses of myrecetin greater than those of quercetin or kaempferol. On the basis of the relative AOX of these flavonols (*37*, *38*), kaempferol was expected to be less stable to oxidation but was actually better retained after storage in both muscadine wine and juice.

The Folin−Ciocalteu assay for total soluble phenolics is widely used as an indicator of total reducing capacity for fruits and vegetables and is generally well-correlated to AOX. The assay was also previously reported to detect polymerized polyphenolics (*39*). Total soluble phenolic concentrations were highest in 3 and 5 day on-hull fermentations, generally reflecting higher anthocyanin content of these wines. Total phenolic levels remained consistently high during storage in Noble wines and juice as compared to those made from Carlos grapes, but retention was largely from the presence of anthocyanins, which readily form polymers with residual reducing capacity. Monomeric anthocyanins were the greatest contributors to total soluble phenolics, accounting for 76−96% of the total phenolic content (**Table 2**). These values were calculated by assaying standard anthocyanidin solutions in the Folin−Ciocalteu assay, which resulted in a 52% contribution to total phenolics (gallic acid equivalents) from anthocyanidins. After storage, anthocyanidin contributions to total soluble phenolics decreased in proportion to storage temperature, but the reducing ability from polymeric anthocyanins could not be accounted for in the calculation. Noble HP juice and 3 day on-hull wines initially had the highest contributions of anthocyanidins to total soluble phenolics (96 and 90%, respectively) and along with Noble CP wine were able to maintain a high AOX during storage.

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