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STORAGE RETENTION OF STILBENE, ELLAGIC ACID, FLAVONOL, AND PHENOLIC CONTENT OF MUSCADINE GRAPE (VITIS ROTUNDIFOLIA MICHX.) CULTIVARS

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A B S T R A C T

The presence of ellagic acid and other nutraceutical compounds in muscadine grapes add value and enhance the marketability of this southern U.S. specialty crop. Due to its nutraceutical profile, muscadines may potentially become the next "super fruit". The objective of this study was to determine the retention of important phytochemical compounds including anthocyanins, phenolics, flavonols, stilbenes and organic acids from whole muscadine grape berries and individual fruit parts following cold storage. Stilbene, ellagic acid, flavonol, and phenolic compounds were analyzed in berries of 11 muscadine grape cultivars following 14 days of cold storage at 4˚C. The major phenolic compounds were identified by their retention times and characteristic spectra. Quantification was made by utilizing calibration curves of external standards for each of the analyzed compounds including trans- and cis-resveratrol, trans- and cis-piceid, ellagic acid, myricetin, quercetin and kaempferol. Total phenolics decreased in 6 cultivars but increased in 5 cultivars, suggesting differences in decay development and fruit deterioration. Anthocyanin content showed an overall decrease in all cultivars except 'Eudora'. Stilbenes showed an overall decrease across cultivars, but flavonol content was cultivar and compound specific. Free ellagic acid increased in all cultivars, except 'Pollyanna', and total ellagic acid increased or remained constant in all cultivars.

Keywords: nutraceutical, piceid, polyphenolic, resveratrol, *Muscadinia rotundifolia*.

INTRODUCTION

Muscadine grapes (Muscadinia rotundifolia; syn Vitis rotundifolia Michx.) are native to the southeastern U.S. and are the most widely cultivated Vitis species in the region due to their inherent resistance to numerous fungal pathogens and to Pierce's disease (Xylella fastidiosa). They thrive in soil and climate conditions not generally favorable for bunch grape production (Talcott and Lee, 2002). Vines grow vigorously reaching a length of 30 meters or more in the wild. The grapes are produced in small clusters, have a unique fruity flavor, and are considered a southern delicacy. Muscadines differ from other Vitis species since they have an extra pair of chromosomes (Patel, 1955). They have a notably thicker skin than other Vitis species that protects them from heat, UV radiation, humidity, insects and fungi. Although their consumption is primarily limited to the southern U.S. region, their unique aroma, flavor, and more importantly their high nutraceutical content make this under-utilized fruit a candidate for expanding markets of fresh fruit, wine, juice, and additives for healthier processed foods.

Stilbenes are a small class of phenylpropanoids characterized by a 1,2-diphenylethylene backbone. Stilbenes are synthesized in grape berries under natural environmental conditions (Jeandet et al., 1991), but are increased by the up-regulation of defense genes encoding pathogenesis-related proteins (Chong et al., 2009). The cis- and trans-isomers of resveratrol, a pharmacologically important stilbene, are present in the skin during all ripening stages, but are almost totally absent from the pulp (Chong et al. 2009). Specific accumulation of resveratrol in the berry skin results from the localization of stilbene synthase (STS), the pivotal enzyme for stilbene biosynthesis. As a defense mechanism, stilbenes display potent antifungal effects as well as function in dormancy and growth inhibition in

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plants (Croteau et al., 2000). From a pharmacological perspective, scientists have been reporting for decades the various ways that stilbene and resveratrol can positively affect health (Arichi et al., 1982; Brakenhiem et al., 2001; DeSanti et al., 2000a; DeSanti et al., 2000b; El-Mowafy, 2002; Jang et al., 1997; Kimura et al., 1985; Kinsella et al., 1993; Lu and Sorreno, 1999). Piceid, resveratrol 3-O-β-D-glucoside, also exhibits activity comparable to resveratrol (Romero-Perez et al., 1999). Resveratrol and piceid exist in the cis- form, an isomer of the trans- form. In early studies trans-resveratrol (TRes) was shown to inhibit platelet aggregation, inhibit the oxidation of low-density lipoproteins, reduce the level of triacylglycerol, and protect the liver from lipid peroxidation (Romero-Perez, 1999). Since glycosidase is known to be present in the digestive tract, it is possible that piceid could be converted to resveratrol and absorbed during digestion (Hackett, 1986). Therefore it is important to consider all isomers and glucosides of TRes.

Muscadine grapes possess several unique and distinguishing chemical compounds, including ellagic acid. Ellagic acid is commonly present in other fruits, such as raspberry, strawberry, and blackberry, but is absent in all other Vitis species. Ellagic acid in muscadine grapes is expressed as free ellagic acid (FEA), ellagic acid glycosides, and ellagitannins (Talcott and Lee, 2002). The presence of ellagic acid and its derivatives in plants has been widely studied because of its antiproliferative and antioxidant properties. The antiproliferative properties are due to its ability to directly inhibit DNA binding of certain carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons (PAHs) (Lesca, 1983). Ellagic acid also has a chemoprotective effect in cellular models by reducing oxidative stress (Talcott and Lee, 2002; Lesca, 1983; Patrana-Bonilla et al., 2003; Mertens-Talcott et al., 2003; Stoner and Morse, 1997; Khanduja et al., 1999).

Another unique attribute of muscadine fruit chemistry is the presence of anthocyanins such as 3,5-diglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin in non-acylated forms (Flora, 1978; Goldy *et al*., 1986; Lamikanra, 1988). Though absorption of anthocyanins appears to be low in humans (Prior, 2004), it seems likely that cells in which they function in defense of oxidative stress must concentrate the anthocyanins or one of their derivatives (Galli *et al*., 2002). Anthocyanins are known to protect blood vessels in humans. They also play a role in cancer prevention. There are more than 80 publications that discuss the ability of different anthocyanins to prevent different kinds of cancer (Hartle *et al*., 2005).

Phenylpropanoids, or phenolics are a large family of secondary metabolites involved in plant response to abiotic and biotic stresses. Phenolics are ubiquitous in the plant kingdom and are the most abundant secondary metabolites found in plants (Amakura *et al*., 2000). Many phenolics not only protect the parent plant, but also exhibit significant pharmacological benefits. Phenolic compounds play an important role in overall food properties, as they are generally involved in defense against ultraviolet radiation or as phytoalexins (Amakura *et al*., 2000; Dixon, 1986; Singleton, 1980; Zaat *et al*., 1987). Phenolics may also play a role in the regulation of plant metabolism (Laks and Pruner, 1989). Polyphenols represent the third most abundant constituent in grapes and wines after carbohydrates and fruit acids (Singleton, 1980). Phenolics are mainly distributed as 28-35% in skin, 60-70% in seed and less than 10% in the pulp of the grape (Shi *et al*., 2003). Phenolics contribute to the bitterness and astringency of fruits and are also considered to be the most important compounds affecting flavor and color differences in wines. Analysis of total phenolics is used to estimate the antioxidant capacities of fresh fruits and vegetables (Thaipong *et al*., 2006). The higher total phenolic content present in muscadine grapes compared to other *Vitis* species is attributed to high ellagic acid, gallic acid, and flavonoid glycoside concentrations (Lesca, 1983; Mertens-Talcott *et al*., 2003; Patrana-Bonilla *et al*., 2003; Talcott and Lee, 2002; Yilmaz and Toledo, 2004).

Much work has been done on health benefits of individual chemicals found in muscadine grapes, but little information is available showing the concentrations of these compounds present in a large number of muscadine grape cultivars; most studies have only looked at a few of the most widely grown muscadines varieties, such as 'Noble', 'Ison', and 'Carlos'. A more comprehensive look at additional muscadine grape cultivars could reveal some "hidden treasures" in regard to their nutraceutical properties. A previous study (Marshall et al., 2012) examined the important phytochemical concentrations in the muscadine fruit tissue of 21 cultivars at harvest. From these results a subset of 11 cultivars were selected for analysis after 14 days of storage. The criteria for selection were based on

the levels of physical, textural marketability of the fruit after storage.

This study was initiated to determine the concentration and retention of total phenolics and anthocyanins in whole muscadine grapes, as well as total ellagic acid (TEA), stilbenes and the flavonols present in juice, pulp and skin of 11 cultivars of muscadine grapes grown at the Thad Cochran Southern Horticultural Laboratory (TCSHL) in Poplarville, Mississippi after 14 days of storage. Very little compound was recovered in the juice and pulp; therefore, those results are not represented. A comprehensive analysis of these compounds has not previously been reported for muscadine grapes in the gulf coast region, and would provide a valuable resource for growers in this area considering growing muscadine cultivars that maximize functional food benefits.

MATERIALS AND METHODS

Fruit Preparation and Storage: All muscadine grape cultivars were collected from the McNeill, MS vineyard of the Thad Cochran Southern Horticultural Laboratory (TCSHL) in August of 2007. Vines of numerous muscadine grape cultivars were established in a planting in 1994 where vines were planted 6.4 m apart in 3.7 m rows and trained to Geneva double curtain trellis. Training consisted of a single main stem raised to a wire 1.5 m in height with two arms extended 3.2 m each direction from the main stem to form the vine's permanent framework on the double-trellis system. Vines received applications of 227 g/vine of 8N-3.4P-8.8K in March, 114g of 13N-13-P-13K in May, and 114g of 33N annually and were drip irrigated as needed. Weed and insect pest management was conducted using recommended practices (Braswell et al, 2006) and no fungicides were applied.

Approximately 4.5 L of fruit were randomly collected from each cultivar at optimum maturity based on soluble solids content (Brix°). Approximately 100 berries were contained in each of three clam shells placed into growth chambers in a completely randomized design. Growth chambers were set at 4°C. Clam shells were removed from chambers after 14 days of storage for analysis. Two homogeneous fruit subsamples of 10 berries were haphazardly taken from each clamshell and were immediately separated into skins, seeds, juice and pulp. Berries were cut in half around the equator with a scalpel. Seeds were extracted and set aside. Berry halves were pinched gently to separate skin from juice and pulp. Juice partition was separated from pulp by hand squeezing through four layers of cheesecloth. Fruit parts were separated into three replications, lyophilized, then ground finely with a coffee grinder and stored at -20°C until further analysis.

Extraction and Analysis of Stilbene, and Free Ellagic Acid: Samples of 1 g of lyophilized juice, pulp, or skin were put into centrifuge tubes with 10 mL of 80:20 ethanol:water (LeBlanc, 2006). Solutions were homogenized for 1 min, and then incubated for 30 min at 60°C with agitation every 5 min (LeBlanc, 2006). After heating, the tubes were removed and centrifuged at 1,000 rpm for 15 min. Supernatant was filtered through a fine mesh filter to remove the large particles and brought to a volume of 10 mL with 80:20 ethanol:water. An aliquot was then filtered in vacuo through a 0.2 µM Nucleopore Track-Etch membrane (Milipore, Billerica, MA) and 1 mL was pipetted into sample vials for initial analysis by HPLC for free ellagic acid, ellagic acid glucosides, the stilbenes trans and cis resveratrol, and trans and cis piceid. HPLC analysis was carried out on a Dionex UVD 340S HPLC system (Dionex, Sunnyvale, CA) with a 4 channel diode array detector. The system was equipped with a Sunfire C18 column 5.0 µm ODS (3.0 x 250 mm) (Waters Corp, Milford, MA). The injection volume for both standards and samples was 20 µL. The flavonols and ellagic acid were quantified at 360 nm, trans-compounds at 306 nm and the cis-compounds at 285 nm. The mobile phase was solvent A, methanol/formic acid/water (10:1:89, v/v/v); solvent B, acetonitrile; and solvent C, water. A multi-step gradient suitable for phenolic separation was used as follows: at 0 min, 100% solvent A; at 35 min 30% solvent A and 70% solvent B; and at 50 min 100% solvent C with a 5 min post-run with solvent C. The flow rate was 0.3 mL/min that we determined to be necessary to achieve good peak separation, **with a total runtime of 59 minutes**. Samples were protected from light at all times to hinder degradation of the phenolic compounds and the conversion of cis-stilbenes to trans- isomers.

Acid Hydrolysis for Flavonols and Total Ellagic Acid: Two (2.0) mL of the previous sample were then added to 2 mL 2N HCL (166.66 mL 80:20 ethanol: water and 33.34 mL HCl) and placed in a water bath for 60 min at 95°C to achieve acid hydrolysis of flavonoid glycosides to aglycons (Talcott and Lee, 2002). The samples were then vortexed for 30 sec and refiltered in vacuo through a 0.2 µm Nucleopore Track-Etch membrane and 1 mL was again pipetted into sample vials for final analysis by

HPLC for flavonoids (myricetin, quercetin and kaempferol) and total ellagic acid. The HPLC analysis was carried out on the same system as above, using the same column, mobile phase and multi-step gradient program. Total runtime for separation was 27 minutes.

All compounds were identified by using UV/VIS spectral interpretation and retention time of authentic standards (Sigma-Aldrich Chemical Co, St. Louis, MO). All external standards were prepared to 10, 50, and 100 ppm, which was the expected range of compounds within our samples. Quantification was made by calculating the area under the curve based on calibration curves of external standards for each of the analyzed compounds, trans resveratrol (TRes) and cis resveratrol (CRes), cis piceid (CPic), free ellagic acid (FEA), total ellagic acid (TEA), myricetin, quercetin, and kaempferol. Detection limits are calculated by area under the curve of the standard: TRes - 1 µg/g, EA - 10 µg/g, myricetin, quercetin, kaemferol – 8 µg/g, Cpic – 6 µg/g. All results were expressed as µg/g dry weight.

Total Phenolics: Total phenolics were identified by the Folin-Ciocalteau (Singleton, and Rossi, 1965) assay with gallic acid equivalents. Fifty grams of previously frozen (-20 °C) fruit was blended to a fine puree, and filtered through cheesecloth. Filtered puree (0.5 g) was added to a 50 mL tube along with 25 mL of cold extraction solvent (4:4:2:0.001 acetone : methanol : deionized water : formic acid) and mixed thoroughly. Samples were filtered with #4 Whatman filter paper (Fisher Scientific, Waltham, MA), and two replications of 1.0 mL were each placed in a glass test tube. Folin-Ciocalteau reagent (1.0 mL) was added, mixed and allowed to stand for 3 min. Then 1.0 mL of 1 N sodium carbonate was added, mixed, and the sample was allowed to stand for 7 min. Seven (7.0) mL water was added, mixed, capped and allowed to stand for 30 min at 40°C in the dark (Waterhouse, 2002) before reading the absorbance at 726 nm in a Beckman-Coulter DU 730 UV-VIS spectrophotometer (Beckman Coulter, Inc., Brea, CA). Total phenolic content was expressed in fresh weight as gallic acid equivalents in mg/100 g sample, using a standard curve generated with 50, 100, 150, and 300 mg/L gallic acid.

Total Anthocyanins: Total anthocyanins were analyzed with a modified Giusti and Wrolstad (2001) pH shift assay. Fifty (50.0) g of previously frozen fruit was blended to a fine puree, and filtered through cheesecloth. Filtered puree (0.5 g) was added to a 50 mL tube along with 25 mL of cold extraction solvent (4:4:2:0.001 acetone : methanol : deionized water : formic acid) and mixed thoroughly. Samples were filtered with #4 Whatman filter paper, and 0.5 mL of each extracted sample was placed into 15 mL polypropylene tubes. Tubes consisted of 2 replications for pH 1.0 and 2 replications for pH 4.5. To adjust pH, 4.5 mL of appropriate pH buffer was added. Tubes were capped, vortexed and allowed to stand for 30 minutes at 40°C (LeBlanc, 2006) in the dark before reading the absorbance at 510 nm and 700 nm (to correct for haze) against a blank cell of distilled water in a Beckman-Coulter DU 730 UV-VIS spectrophotometer. The absorbance of the diluted sample (A) was as follows: $A = (A_{510} - A_{700})$ pH 1.0 - $(A_{510} - A_{700})$ pH 4.5. The fresh weight anthocyanin content was then calculated as the total of monomeric anthocyanin pigment (mg/L) = $(A \times MW \times DF \times 1{,}000) / (\varepsilon \times 1)$, where A is the absorbance of the diluted sample and DF is the dilution factor. MW and ε refer to the predominant pigment content contained in the sample. For muscadine, cyanid-3-glucoside was used therefore MW = 449.2 and ε = 26,900.

Statistical Analysis: Data represents the mean of three replicate **with two subsamples (n=6) analyses** by ANOVA to calculate F probabilities. Mean separation achieved using LSD test ($P < 0.05$) with SAS software 9.2 (SAS, 2008).

RESULTS AND DISCUSSION

Stilbene: Stilbene concentrations vary among muscadine cultivars as well as over time. After 14 days of storage, trans-resveratrol (TRes) was found in the skin of all 11 cultivars tested ranging in amount from 3.83 µg/g in 'Southland', a black cultivar, to 23.36 µg/g in 'Sweet Jenny', a bronze cultivar (Figure 1). Eight of the cultivars retained the TRes that was measured at harvest with an insignificant increase in three of the cultivars. Three cultivars ('Albemarle', 'Eudora', and 'Pollyanna') exhibited a decrease in concentration. 'Pollyanna' had the greatest concentration of TRes at harvest with 66.0 µg/g, but this amount significantly decreased during storage to 14.12 µg/g.

Interestingly, the TRes concentrations were not influenced by skin color. Bronze berries had equally as much resveratrol as did the black berries within the cultivars tested. Jeandet *et al*. (1991) found that *Vitis vinifera* grape skin cells had the ability to suddenly decrease production of resveratrol after veraison. Immature grapes were capable of synthesizing resveratrol, but this ability steadily decreased with

maturity. It is also known that anthocyanins derive from the same phenylalanine/polymalonate pathway and share common precursors with resveratrol. Holkroft and Kader (1999) found that in strawberries, which are also non-climacteric, the biosynthetic pathway for anthocyanin is still operational after harvest and the storage at low temperatures did not inhibit the process. Therefore, if anthocyanin synthesis continued after harvest, one would expect the resveratrol content to be adversely affected. Jeandet *et al*. (1995) conclude that there was a direct negative relationship between stilbene phytoalexin formation and anthocyanin content in grape skin cells. Therefore the loss of resveratrol after a 14 day storage period was expected, yet eight of the cultivars retained the TRes that was measured at harvest with an insignificant increase in three of the cultivars.

Cis-piceid was found in six of the cultivars tested at 14 days with the greatest amount found in 'Darlene' (25.36 µg/g), 'Janet' (19.26 µg/g) and 'Albermarle' (16.45 µg/g). 'Albemarle' showed a significant increase in concentration of cis-piceid after 14 days in storage from 0.00 to 16.45 µg/g.

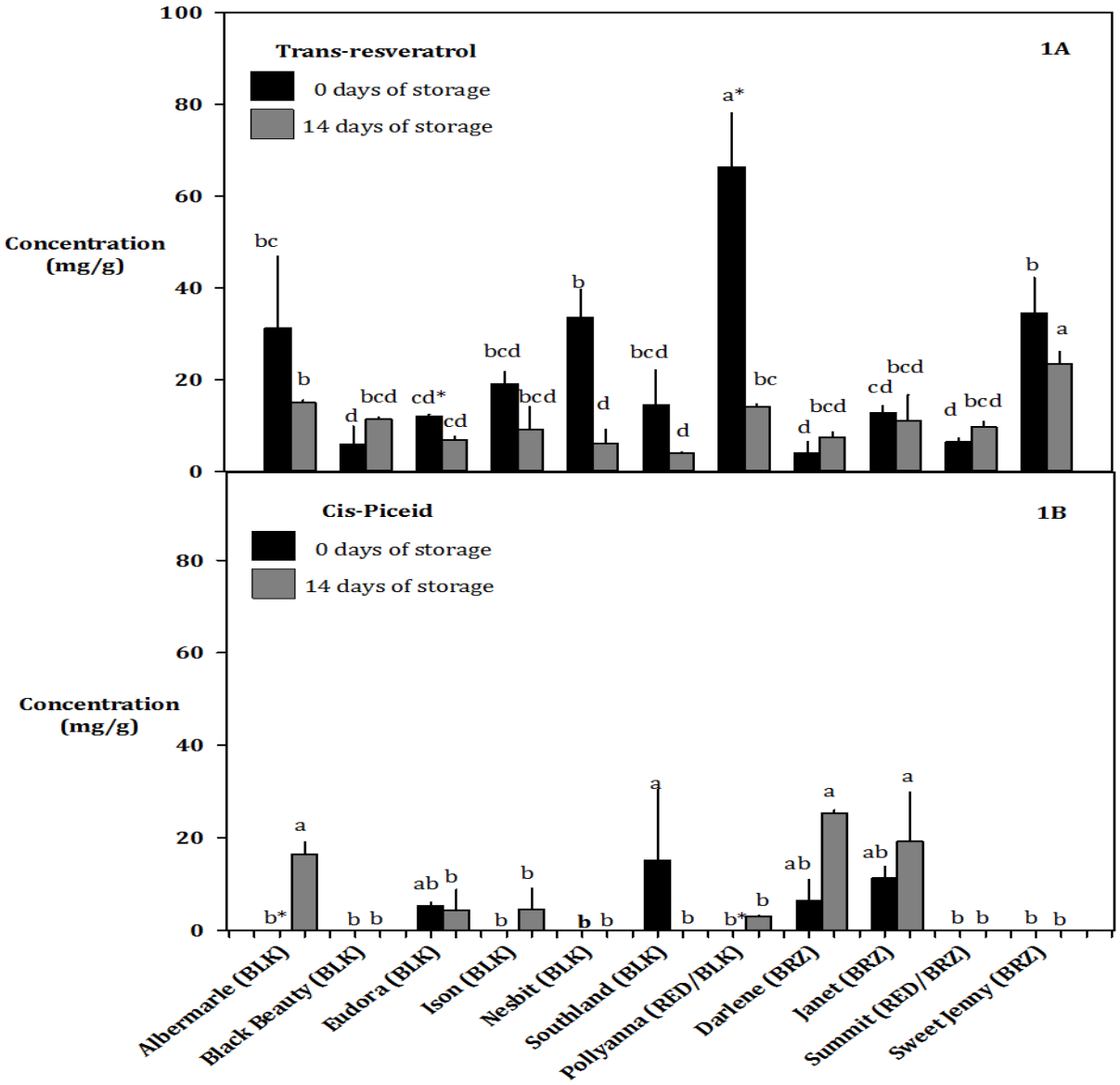


Figure 1. Stilbene (trans-resveratrol and cis-piceid) content in the skin of 11 muscadine grape cultivars at 0 and 14 days of storage. Letters that are different indicate mean cultivar differences across each storage period (0 days and 14 days) according to LSD test with a $P \leq 0.05$. Asterisks represent differences in stilbene content for berries stored at 0 and 14 d for each cultivar. Black fruited cultivars are indicated by BLK, Bronze cultivars = BRZ, a red/bronze cultivar = RED/BRZ and a red/black cultivar = RED/BLK.

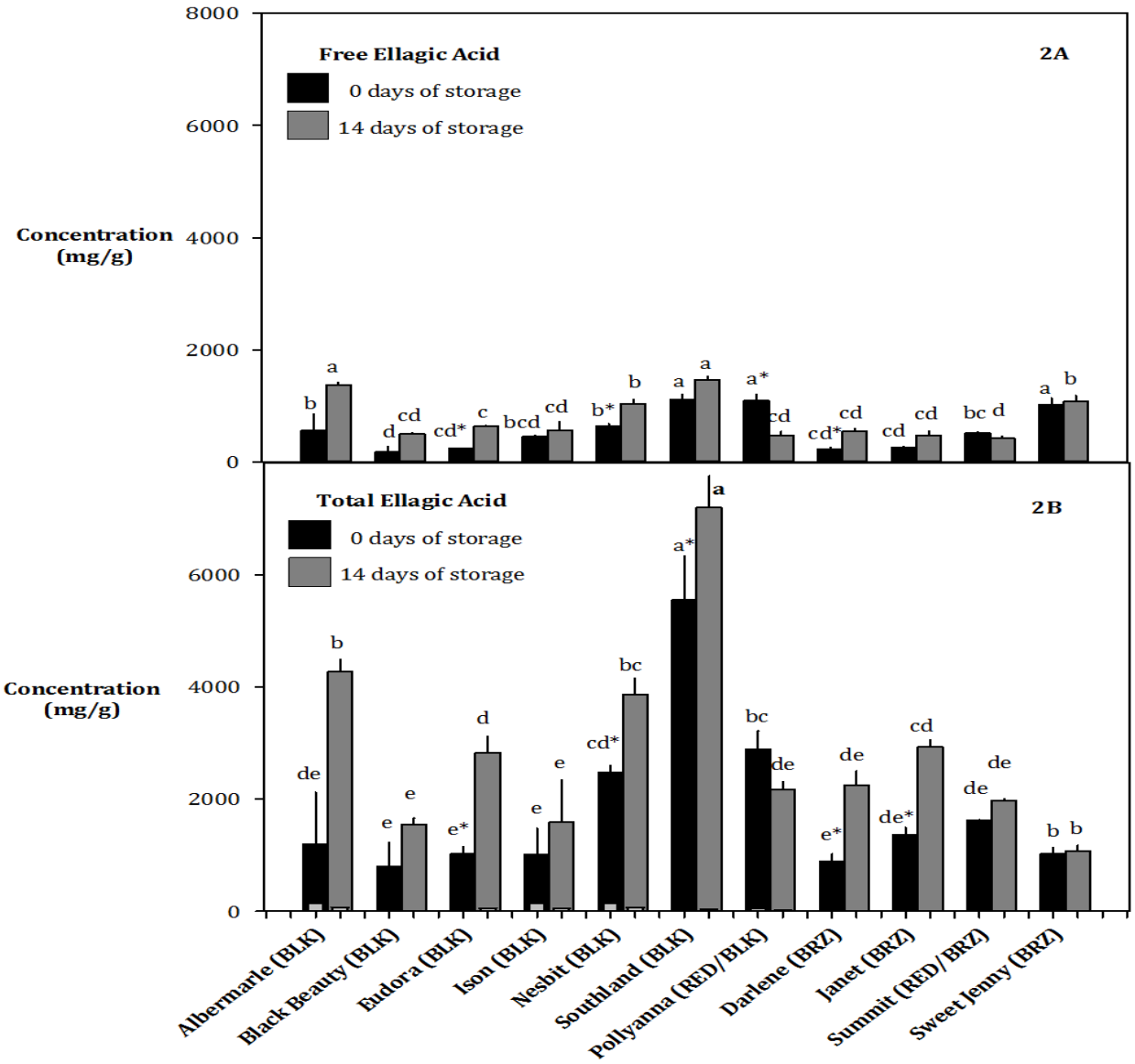


Figure 2. Free ellagic acid (FEA) and total ellagic acid (TEA) content in the skin of 11 muscadine grape cultivars after 0 days and 14 days of storage. Letters that are different indicate mean cultivar differences across each storage period (0 days and 14 days) according to LSD test with a $P \leq 0.05$. Asterisks represent differences in ellagic acid content for berries stored at 0 and 14 d for each cultivar. Black fruited cultivars are indicated by BLK, Bronze cultivars = BRZ, a red/bronze cultivar = RED/BRZ and a red/black cultivar = RED/BLK.

Ellagic Acid: The free (aglycone) form of ellagic acid (FEA), and the total ellagic acid (after acid hydrolysis) increased in the skin of 'Nesbit', 'Albemarle' and 'Eudora' during the 14-day storage period (Figure 2). FEA decreased in only 'Pollyanna', and remained consistent in the other 7 varieties. In the pulp, FEA was found in 'Southland' (52.05 µg/g), 'Summit' (25.31 µg/g), and 'Sweet Jenny' (23.95 µg/g), and in the juice of 'Ison' (8.61 µg/g), 'Southland' (10.11 µg/g), and 'Sweet Jenny' (37.51 µg/g), but not in any other cultivar.

After 14 days of storage, total ellagic acid (TEA) significantly increased in the skin of 'Janet' from 1364.1 µg/g to 2930.1 µg/g, in 'Nesbit' from 2480.3 µg/g to 3873.0 µg/g, and in 'Southland' from 5554.7 µg/g to 7205.4 µg/g (Figure 2). 'Southland' contained the highest concentration of TEA of the cultivars tested. This is consistent with the results from day 0 where 'Southland' had the greatest amount of any variety tested with 5554.7 µg/g total ellagic acid. TEA was even found in the pulp of 'Eudora' (40.71 µg/g), 'Southland' (83.63 µg/g), 'Summit' (37.32 µg/g) and 'Sweet Jenny'

(76.47 µg/g). TEA was found in the juice of all varieties except 'Ison' with the highest concentration in 'Southland' with 110.64 µg/g.

Flavonols: Myrcetin concentration in the skin (Figure 3) remained relatively constant in eight of the cultivars tested. 'Janet' and 'Sweet Jenny', bronze cultivars, had the highest concentrations of myricetin at harvest. Yet, myricetin increased in 'Janet' from 998.8 µg/g to 1690.0 µg/g after 14 days, and decreased in 'Sweet Jenny' from 974.5 µg/g to 257.6 µg/g. Myricetin also increased from 369.1 µg/g to 452.1 µg/g in 'Nesbit', a black muscadine. Both bronze and red colored muscadines possess myricetin, a flavonol present in red but not white V. vinifera grapes (Flora, 1978).

Quercetin, also a flavonol, has been shown to protect against DNA mutations, colon cancer, and heart disease (Hollman and Katan, 1999). (Patel, 1955; Talcott and Lee, 2002). The greatest concentrations of quercetin in muscadine skin were found in 'Nesbit' (826.1 µg/g) and 'Sweet Jenny' (866.1 µg/g). After 14 days in storage, quercetin increased in 'Nesbit' to 1126.7 µg/g, but decreased in 'Southland' (black), 'Summit' (red/bronze), and 'Sweet Jenny' (bronze).

Kaempferol was found in much lesser concentrations in the skin of all cultivars overall, and not detected at all in 'Black Beauty'. The greatest concentrations were found in 'Nesbit' at harvest (221.9 µg/g) and after storage (265.7 µg/g), which was a significant increase. Kaempferol concentrations also increased in response to storage in 'Southland', from 20.6 µg/g to 54.7 µg/g, and 'Summit', from 92.6 µg/g to 139.1 µg/g.

Overall, total flavonols were highest at harvest in 'Sweet Jenny', but then decreased significantly during storage. After 14 days, the highest concentrations were found in 'Janet' and 'Nesbit'.

Total Phenolics: Total phenolics content (TPH) of whole fruit did not change significantly in six of the eleven cultivars (Figure 4). 'Ison' (black), 'Pollyanna' (red-black) and 'Nesbit' (black) had significant reductions in total phenolic content. Two cultivars, 'Southland' (black) and 'Sweet Jenny' (bronze), showed an increase in total phenolic content after storage.

Among the cultivars, total phenolics content were more consistent after storage than at harvest. Fruit ranged from 721.90 mg/100g in 'Southland' to 318.54 mg/100g in 'Summit'. 'Southland' had one of the lowest concentrations of total phenolics at harvest with 447.54 mg/100g, but continued to produce phenolics during storage.

In addition to differences among cultivars between initial and stored concentrations, some cultivars exhibited dramatic changes in total phenolic content in response to storage. 'Nesbit' (black) and 'Pollyanna' (red-black) had the highest phenolic contents at day 0 with 1061.65 mg/100g and 1057.89 mg/100g, respectively, but this amount dropped dramatically by day 14 and was 466.79 mg/100g and 360.07 mg/100g, respectively. The other cultivars that had smaller initial values experienced less change in total phenolic content in response to storage.

Black muscadines that were tested in this study contained higher levels of total phenolics than bronze berries, suggesting they would contain a higher antioxidant capacity. Takeda *et al*. (1983) also found an increase in phenol content of 'Fry' muscadine grapes stored at 4.5°C, and correlated these increases to decay development and fruit deterioration because of translocation of sugars in to the fruit ceases and sugars and organic acids are converted to carbon dioxide, heat and intermediate organic compounds.

Anthocyanins: *Six of the cultivars remained consistent in anthocyanin content (Table 4). The same three cultivars, 'Ison', 'Pollyanna', and 'Nesbit', that decreased in total phenolics also decreased in anthocyanin content. Yet, 'Eudora' (black) and Janet (bronze) increased in anthocyanin content over the 14 days of storage.*

The results also showed that the high phenolic value did not necessarily correspond to high amounts of anthocyanins. 'Pollyanna', a red-black variety, had one of the highest initial phenolic values but only intermediate anthocyanin levels. This is not surprising, as anthocyanins are primarily found in the skin of grapes, and are responsible for pigmentation. The primary anthocyanins in muscadine grapes are nonacylated 3,5-diglucosides of six anthocyaninidin bases.

Fruit anthocyanin content did differentiate nicely by color. The black cultivars contained the highest levels of anthocyanins as expected. 'Albemarle' (66.66 mg/100g) and 'Nesbit' (63.32 mg/100g) had the highest concentrations, followed by 'Eudora' (50.51 mg/100g), 'Ison' (48.43 mg/100g), and 'Southland' (32.84 mg/100g). None of the bronze cultivars had appreciable anthocyanin concentrations.

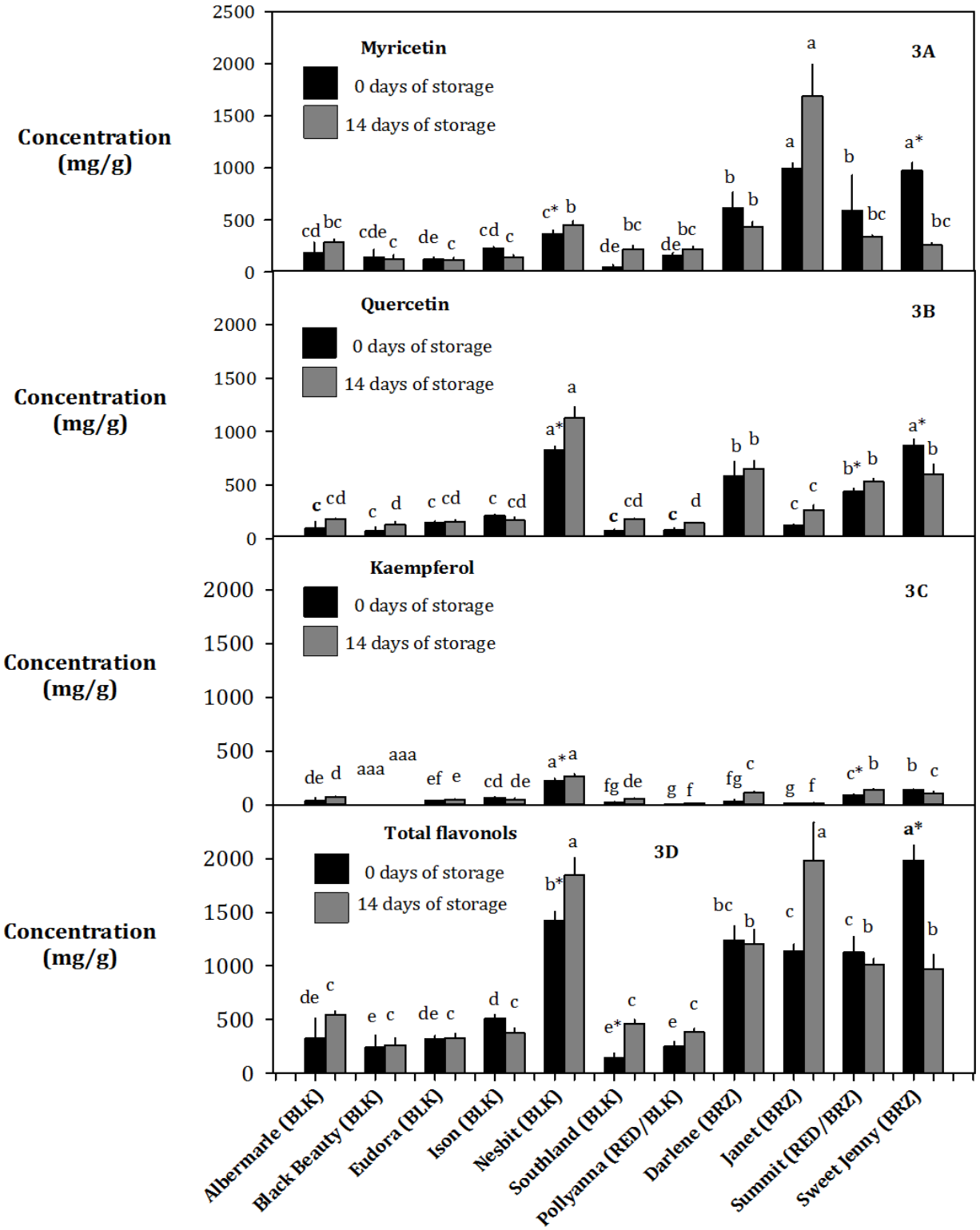
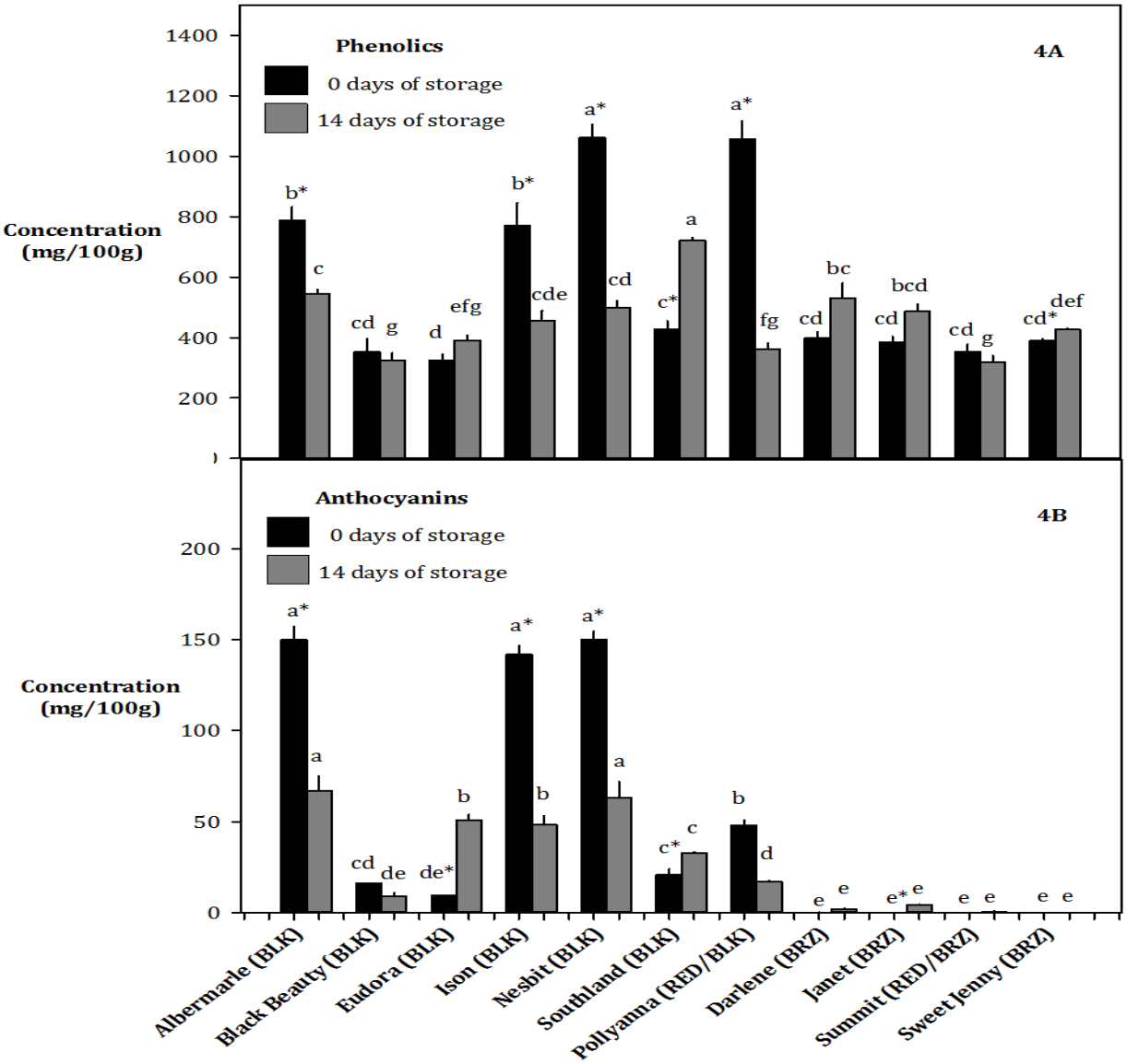


Figure 3. Flavonol content of skin for 11 muscadine grape cultivars after 0 and 14 days of storage. Letters that are different indicate mean cultivar differences across each storage period (0 days and 14 days) according to LSD test with a $P \leq 0.05$. Asterisks represent differences in flavonol content for berries stored at 0 and 14 d for each cultivar. Black fruited cultivars are indicated by BLK, Bronze cultivars = BRZ, a red/bronze cultivar = RED/BRZ and a red/black cultivar = RED/BLK.



Phenolics and Anthocyanins: Figure 4. Total Phenolics and anthocyanin content of whole muscadine grapes after 0 and 14 days of storage. Letters that are different indicate mean cultivar differences across each storage period (0 days and 14 days) according to LSD test with a $P \leq 0.05$. Asterisks represent differences in phenolic and anthocyanin content for berries stored at 0 and 14 d for each cultivar. Black fruited cultivars are indicated by BLK, Bronze cultivars = BRZ, a red/bronze cultivar = RED/BRZ and a red/black cultivar = RED/BLK.

CONCLUSION

Levels of ellagic acid, phenolics, and stilbenes vary greatly in muscadine grape skins. Some compounds are retained after 14 days of storage while others increase or even decrease in concentration. These changes not only depend on the compounds themselves, but also cultivar. There are no explanations as to why some cultivars increase in concentrations while others decrease. Other fruit have been found to react differently over several seasons due to environmental conditions. This is also true of muscadines. Stilbene biosynthesis, for example, is induced in response to a wide range of biotic and abiotic stress factors (Chong, *et al.*, 2009). Smith (2013) found that berries from the least efficacious fungicide treatment had nearly ten times more resveratrol than the fruit from the most efficacious fungicide treatment. All of the muscadines in this study were treated exactly the same and harvested within the same season at the same time.

Being the only Vitis species to possess ellagic acid, muscadine grapes are unique. Ellagic acid was found in

the skins of all tested cultivars and in the pulp and juice of a few others. Ellagic acid and its derivatives are being investigated as potential chemopreventatives because extracts of fruits containing ellagic acid derivatives are more powerful than individual substances for inhibiting cancer cell proliferation. This is because multiple phenolic compounds in fruit act synergistically with ellagic acid, and affect biological processes that inhibit cancer initiation and cancer cell growth (Mertens-Talcott et al., 2003; Mertens-Talcott and Percival, 2005; Mertens-Talcott et al., 2005). One such flavonol synergist might be quercetin, which relaxes the blood vessel wall (Rendig et al., 2001) and increases the production of enzymes that dissolve blood clots (Abou-Agag et al., 2001). The combination of these two compounds changes the activity of regulatory proteins and enzymes called MAP kinases that regulate cell division and viability (Mertens-Talcott and Percival, 2005). The potential health benefits of ellagic acid and flavanol synergists in the muscadine grape add value to the crop and enhance its marketability.

This information can be used by muscadine geneticists to develop a healthier berry. Also, the presence of resveratrol in the pulp of 'Eudora' and 'Janet' provides possibilities for breeding cultivars with enhanced nutraceutical benefits. Typically muscadine geneticists select for sweetness, firmness, skin thickness, disease resistance, and other attributes that are visual or palatable. These and other performance characteristics for many of the cultivars in this study are detailed in a previous study (Stringer *et al*., 2008). With an ever-increasing drive for healthier foods, this current study shows that muscadines have high concentrations of health-benefitting phytochemicals, but the concentrations can be quite different between cultivars at harvest and after storage. 'Southland' stood out as an unexpected champion increasing in total phenolics, total ellagic acid, quercetin and kaempherol while in storage. 'Southland' also contained the highest ellagic acid content of all the cultivars measured.

Of note, muscadine berries were carefully cut and separated; therefore, juice partition was not obtained by crushing with seeds and skins as commonly done in commercial juice or wine production. If juice were obtained from crushing with skins and/or seeds, the juice would probably contain higher levels of compounds similar to those found in other studies (Amakura *et al*., 2000). Seed partitions are not reported. Previous research (Patrana-Bonilla *et al*., 2003; Romero-Perez *et al*., 1999) show a majority of the phenolic compounds in muscadines are found in the skins and seeds. Yet the compounds found in seeds are different than those found in skin. Seeds contain even more nutraceutical compounds not addressed in this (Patrana-Bonilla *et al*., 2003).

Muscadine grapes are seeded grapes. Muscadines contain 3-6 large seeds in the center of the pulp. Yet, the nutraceutical benefits that consumers will obtain from the consumption of muscadine far exceed the inconvenience of removing seeds. Many fruit must be first peeled, cut, or somehow prepared for eating. With the combination of ellagic acid, resveratrol, and flavonols such as quercetin and myrcetin, muscadine grapes are a healthy choice for a whole food snack.

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