Southern Region Small Fruit Consortium

Final Report Research

Title: Comparison of Phenolics in Seedless and Seeded Muscadines after Storage

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Public Abstract

Muscadine grapes, native to the southern U.S., traditionally have seeds and a thick peel. The peel is especially rich in anthocyanins and other flavonoids. There has been interest in the health properties of these grapes, especially in use for treating certain cancers. Seedless muscadines have recently become available. These grapes have *Vitis vinifera* in their genetic background as well as *V. rotundifolia*. The antioxidant capacity and flavonoid profile of these two genotypes (seeded and seedless) were compared. Generally, antioxidant capacity, total

anthocyanins, and other flavonoids were similar between the two types. Peel color greatly affected anthocyanins, although antioxidant capacity was similar among bronze and purple grapes. Despite the *V. vinifera* background in seedless muscadines, very little difference was found between the profiles of seedless and seeded grapes. Our results indicate that seedless muscadine peel contains antioxidants similar to those in seeded grape peels and storage slightly affected antioxidant capacity.

Introduction:

Muscadine grapes are commonly grown in the southern U.S. and represent a small fraction of the world and U.S. table grape market. Muscadines have anticancer and antidiabetic properties (Banini et al., 2006; Luo et al., 2017) which has stimulated consumer interest in fresh market and processing products. A phase 2 clinical trial is underway utilizing a muscadine grape extract in prostate cancer to alleviate endocrine therapy-induced fatigue (https://clinicaltrials.gov/ct2/show/NCT03496805).

Two seedless muscadine cultivars have recently been introduced into fresh markets and other seedless selections are being evaluated in North Carolina and Arkansas. The type and amount of phenolics in seedless compared to seeded muscadines and changes in content with storage have emerged as industry questions. There is concern that seedless muscadines may have significantly less phenolics because of the lack of seeds, which are rich in flavanols. As consumers often choose to spit out muscadine seeds in seeded types, the differences in total phenolic content between seedless and seedless types may be negligible.

Various measures of berry fruit nutraceutical contributions include total anthocyanin (pigment), total phenolics, and antioxidant activity. Examples of these include total monomeric anthocyanin by pH differential (Lee et al. 2005), total phenolic content, and antioxidant activity estimates using DPPH, ABTS, FRAP, or ORAC (Moon and Shibamoto, 2009). Types of anthocyanin and phenolics can be further identified and quantified using high performance liquid chromatography (HPLC) and LC-mass spectrophotometry. Anthocyanins in muscadine generally consist of diglucosides and small amounts of monoglucosides of cyanidin, malvidin, peonidin, petunidin, delphinidin, and pelargonidin (Conner and MacLean, 2013; Wei et al., 2017). Classes of non anthocyanin phenolics most commonly reported in muscadine are the phenolic acids (hydroxybenzoic and hydroxyl cinnamic acids) and the flavonoids (flavonols and flavanols) and include resveratrol, ellagic acid, quercetin, and ellagitannins (Barchenger et al., 2015a; Xu et al., 2017).

Several studies on the total phenolic content (TPC) in have been done, including amounts in peel, pulp, and seed (Lee and Talcott, 2004; Barchenger et al., 2015b; Xu et al., 2017). Muscadine grapes contain 30-60 mg TPC/g dry weight, with amounts independent of bronze or purple color (Wei et al., 2017). Barchenger et al. (2015b) found that total phenolic content of whole berries increased slightly in 'Supreme' muscadines with 3 weeks storage while changes in

ellagitannins and resveratrol were negligible. Total flavonol, resveratrol, and ellagitannin content is found primarily in peel and pulp (Barchenger et al., 2015b; Sandhu and Gu, 2010.

Muscadines of 9 seeded and 5 seedless selections/cultivars grown at the same farm to minimize environmental effects were obtained from a commercial grower in the fall of 2020 and stored at 3 °C (Table 1). Weight loss, berry color (L*a*b*), and subjective ratings (softness, browning, mold, stem scar tear) were collected at 0 and 21 days and fruit placed at -20 °C. The purpose of this project is to establish the type and content of phenolics in seedless muscadines before and after storage relative to well-known fresh market seeded muscadine cultivars. As muscadines are an early fall crop, the 2020 harvest will be used to complete phenolic tests in 2021.

Objectives: (1) Determine total phenolic content and anthocyanin content of five seedless muscadine selections/cultivars and nine seeded cultivars; (2) determine relative gain/loss of phenolics with storage time, and (3) compare phenolic classes of the seedless and seeded types using HPLC.

Table 1. cultivars and selections of fresh market muscadine grapes.

Seeded	Fruit peel color	Seedless	Fruit peel color
Granny Val	Bronze	Oh My	Bronze
Hall	Bronze	JB 09-15-3-9	Bronze
Late Fry	Bronze	JB 08-38-1	Purple
Summit	Bronze	JB 06-30-2-20	Purple
Triumph	Bronze	RazzMaTazz™	Red
Lane	Purple		
Nesbitt	Purple		
Paulk	Purple		
Supreme	Purple		

Methods:

Muscadines were weighed in three replicates of 5 berries each per selection and storage day, with peel, seed and pulp/juice separated and weighed to determine relative contribution to the whole berry. A small aliquot of juice (<0.5 ml) was used to determine soluble solids content (SSC), pH, and titratable acidity using digital meters (PAL-1, PAL-pH, and acid meter F5, Atago, Bellevue, WA) to ensure that fruit used for phenolics were fully ripe. Freeze dried peel and pulp/juice was ground with a homogenizer (SPEX genogrinder 2010, Metuchen, NJ). Duplicate samples per replicate were extracted three times at a ratio of 1 g sample/100 ml solvent with acidified methanol and supernatants combined for use in assays. Total phenolic content and total antioxidant capacity (TAA and DPPH assays) were determined using the methods of Slinkard and Singleton (1977) and Xu et al. (2017).

Phenolic compounds, including phenolic acids (resveratrol, ellagic acid, gallic acid), total non anthocyanin flavonoids (quercetin, kaempferol), and flavan-3-ols (catechins, epigallocatechin), were identified and quantified using HPLC. Composite samples of the 3 replicates per cultivar, tissue, and storage day were done in duplicate. Anthocyanins and other phenolics were identified using a Hitachi high performance liquid chromatograph and the method of Kim et al. (2015).

Data were analyzed as a completely randomized design with SAS v. 9.4 (Cary, NC) and mean separation done using HSD (Tukey's Honestly Significant Difference ($p<0.05$)).

Results and Discussion

Genotype and peel color had a more significant effect on composition, flavonoid profiles, and antioxidant activity than storage day. Seedless muscadines were higher in SSC than seeded, although pH and titratable acidity were variable among both seeded and seedless types (Table 2). Total anthocyanin content was similar and low (<20 mg/100 g dwt) for bronze seeded or seedless grapes (Table 3). Red grapes were at about 200 mg/100 g dwt, and purple at 1000-3000 mg/100 g dwt. Unusually, the seedless purple grape JB 8-38, was very high in anthocyanin at 3800 compared to around 2000 mg/100 g dwt. Total phenolic content in the purple seedless grape was twice that of other bronze or purple grapes. Bronze grapes were generally similar to purple, and slightly lower in red grapes, in total phenolic content.

Measures of antioxidant activity as mmol/100 g and as percent inhibition, were similar among most cultivars. The exception was JB 8-38, which was higher in TAA and inhibition, although similar to other culltivars in DPPH (Table 3). Citric acid and gallic acid have been correlated with TAA and catechin content is associated with DPPH (Darwish et al., 2021). Higher percent inhibition indicates more effective radical scavenging ability; Lane and JB 8-38 exceeded 80% inhibition with DPPH.

Phenolic acids were weakly and positively correlated with total flavonoids and flavan-3-ols. Granny Val, a bronze muscadine with more of a green peel, was low in phenolic acids and flavan-3-ols compared to other bronze or purple grapes (Table 3). JB 8-38, the purple seedless grape, was unusually high in both phenolic acids and flavan-3-ols.

Anthocyanin profiles of black/purple grapes were surprisingly similar between seeded and seedless types. Diglucosides dominated both genotypes and delphinidum was the dominant anthocyanin (Fig. 1). RazzMaTazz™, the one red grape, was dominated by cyanidin 3,5 and cyanidin 3-glucoside, and peonidin 3,5 diglucoside. Although trace amounts of monoglucosides were found in the one seedless purple grape, one would expect the V. vinifera parentage in this grape to confer high amounts of monoglucosides.

Conclusions

The peel of seedless muscadine grapes was found to be quite similar to that of seeded muscadines. Seedless grapes were slightly higher in soluble solids content but measures of antioxidant activity, types of phenolic compounds and anthocyanin profiles were similar to seeded grapes. These results indicate that the high phytonutrient content of muscadines is not significantly impacted by introduction of the seedless trait.

Impact statement

This study provides the first information on the comparison of peel from seedless and seeded muscadines. Generally, differences between the two types were dependent more on the peel color than seed status. Results were presented at the 2021 national meeting of the American Society for Horticultural Science.

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Table 2. Composition and antioxidant activity in peel of seeded and seedless muscadine grapes averaged for 0 and 21 days of storage.

Color and genotype	Cultivar	%SSC	pH	% Titratable acidity	TAA	DPPH	TAA	DPPH
					mmol/100 g dw		% inhibition	
Bronze	JB 6-30	17.65	3.24	0.42	28.93	10.73	49.3	84.7
seedless	JB 9-15-39	17.38	2.99	0.55	28.15	10.17	38.2	72.7
	Oh My	17.73	3.30	0.53	28.08	8.32	41.5	66.5
Bronze	Granny Val	14.22	3.21	0.41	24.68	9.40	36.3	75.5
seeded	Hall	15.45	3.05	0.69	26.17	9.83	45.8	77.3
	Summit	16.98	3.39	0.40	27.55	9.70	42.8	72.0
	Triumph	14.23	3.21	0.59	20.47	9.48	30.5	66.2
	Late Fry	15.22	3.41	0.39	22.12	8.55	37.0	71.0
Purple seedless	JB 8-38	18.78	3.22	0.61	58.60	10.90	85.7	83.3
Purple	Nesbit	15.03	3.45	0.32	29.17	6.68	48.8	59.2
seeded	Lane	16.07	3.53	0.47	31.65	10.47	47.5	81.5
	Supreme	15.87	3.43	0.39	25.77	8.38	42.2	58.2
Red seedless	RazzMaTazz™	18.68	2.90	1.14	22.92	4.25	39.5	44.5
Minimum difference		1.46	0.66	0.09	6.39	1.23	9.68	8.74

Means separated with HSD within column, $p<0.05$. TAA is total antioxidant activity measured with ABTS solution (2,2' -azino-bis-(3-ethylben zothiazoline-6-sulfonic acid). DPPH is a radical scavenging activity measured using 2,2-diphenyl-1-picrylhy-drazyl.

Soluble solids content (SSC), pH, and titratable acidity (citric acid equivalents) were determined on the juice of these grapes.

Table 3. Phenolic classes in seeded and seedless muscadine peel averaged for days 0 and 21 of storage.

Color and genotype	Cultivar	Total anthocyanin content	Total Phenolic content	Total flavonoids	Phenolic acids	Flavan-3-ols
Bronze				mg/100 g dwt		
seedless	JB 6-30	3.67	387.67	40.67	83.67	263.67
	JB 9-15-39	11.50	596.50	64.50	31.00	500.83
	Oh My	5.33	500.83	22.67	93.33	384.83
Bronze						
seeded	Granny Val	1.17	357.17	57.67	20.33	279.33
	Hall	0.17	513.83	47.83	81.83	384.50
	Summit	4.33	533.00	54.33	113.67	365.00
	Triumph	3.00	603.50	70.17	29.50	504.17
	Late Fry	2.17	602.33	88.67	60.83	452.67
Purple seedless	JB 8-38	3857.00	1014.17	76.33	272.17	665.67
Purple seeded	Nesbit	1595.34	503.67	74.83	39.50	389.67
	Lane	1864.33	464.67	80.33	86.50	297.50
	Supreme	1349.34	420.00	70.17	48.00	302.17
Red seedless	RazzMaTazz™	245.17	371.33	42.17	19.17	310.00
Minimum difference		309.86	111.95	15.47	29.93	100.94

Means separated with HSD within column, $p<0.05$. Total phenolic content represents the sum of phenolic acids, non-anthocyanin flavonoids, and flavan-3-ols.

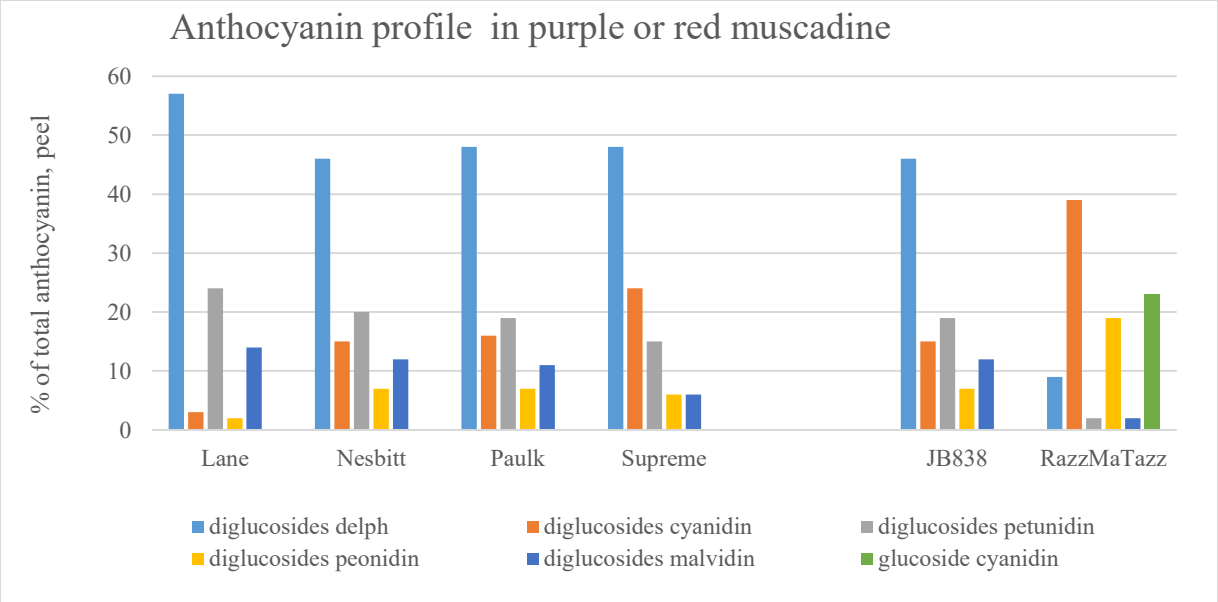

Figure 1. Anthocyanin profile in peel of seedless and seeded purple or red muscadines.