

Anthocyanins of *Vitis rotundifolia* Hybrid Grapes

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ABSTRACT

The anthocyanins (Acy) of 14 black *Vitis rotundifolia* hybrid grapes were separated and quantified by reversed phase high performance liquid chromatography (HPLC) on a C₁₈ column. Twenty-five pigments were separated and 20 anthocyanins were identified. All the cultivars investigated contained mono and diglucosides of acylated and non-acylated anthocyanidins in varying quantities. Total Acy concentration in grapes ranged from 55 to 357 mg/berry. Most of the cultivars had no delphinidin 3,5-diglucoside and the relative amounts of the other non-acylated Acy, which are the pigments found on *V. rotundifolia* grapes, were very low. The relative malvidin content ranged from 0.0% of total grape Acy content in M4-83, to 25.5% in Conquistador. No correlation was found between the relative content of any type of Acy and either the lightness (L) or hue (θ) values of the grapes and their juices. The cultivars were ranked for their possible wine color characteristics and selection in breeding programs, based on their total delphinidin and malvidin content. CD12-72 was rated the best cultivar while AD2-75 was the least desirable cultivar used in the study.

INTRODUCTION

Hybrid grapes in the southeastern United States are characterized by a diversity of genetic backgrounds. They are mainly hybrids of muscadine (*Vitis rotundifolia* Michx.) with *V. vinifera* and *V. labrusca* cultivars. Contributions of pigments from the parent cultivars could result in the development of a wide variety of anthocyanins (Acy) in these hybrids.

Although considerable effort has been placed in breeding hybrid grapes with acceptable processing qualities (Mortensen, 1985; Goldy *et al.*, 1986), many of the red cultivars either suffer from lack of pigment stability during processing and storage and/or have poor wine color characteristics. Wine color, in many cases, can be related to the quality and quantity of their Acy (Ballinger *et al.*, 1974; Nesbitt *et al.*, 1974; Markakis, 1974). The *V. vinifera* cultivars are known to be composed mainly of acylated and non-acylated Acy (Ribereau-Gayon, 1959; Robinson *et al.*, 1966; Nagel & Wulf, 1979), whereas *V. rotundifolia* grapes are exclusively non-acylated 3,5-diglucosides (Ballinger *et al.*, 1973, 1974; Lamikanra & Garlick, 1987; Lamikanra, 1988). *V. labrusca* cultivars, however, consist of a mixture of acylated and non-acylated mono and diglucosides of anthocyanidins (Ribereau-Gayon, 1959; Williams *et al.*, 1978; Goldy *et al.*, 1986).

The stability of Acy pigments, particularly their resistance to oxidative change, is believed to be influenced by the extent of methylation and glucosidal bond formation (Robinson *et al.*, 1966; Van Buren *et al.*, 1968; Hrazdina *et al.*, 1970; Nesbitt *et al.*, 1974). The effect of acylation of the anthocyanidin molecule on stability with increased temperature and light appears to be that of an increase (Robinson *et al.*, 1966; Van Buren *et al.*, 1968). Hrazdina (1975), however, observed that in some instances at room temperature acylated Acy were less stable than non-acylated Acy. Most often, *p*-coumaric acid and occasionally caffeic or ferulic acids are the acylating agents, and they are attached to the sugar in position 3 (Smith & Luh, 1965; Somers, 1966). Van Buren *et al.* (1968) also found that anthocyanidin-3, 5-diglucosides were more stable to heat and light than the 3-monoglucosides. Diglucosides, however, showed a greater tendency to browning reactions under these conditions.

Studies that report the nature of pigments found in hybrid grapes from *V. rotundifolia* crosses with other grape cultivars are very limited (Robinson *et al.*, 1966; Goldy *et al.*, 1986). A knowledge of the Acy nature and content of these grapes should lead to a better understanding of their biosynthetic pathways and will be of assistance in breeding and selection of cultivars for processing. The objectives of this study were, therefore, to isolate and separate individual Acy of *V. rotundifolia* hybrids and to determine which cultivars have greater potentials for selection in breeding.

MATERIALS AND METHODS

Grape analysis

Ripe fruits of 14 cultivars were studied. Fruits were obtained from the university's trial vineyard and vineyards at Central Florida Research and

Education Center, Leesburg, FL. Whole grapes were analyzed for berry weight and sizes. Berry sizes were determined from the average diameter of 15 randomly selected fruits using a Vernier Caliper. Color characteristics L , a and b values of whole berries were determined as previously described (Lamikanra & Inyang, 1986).

Juice analysis

Juices were extracted from 50 g of berries that had been homogenized in a Waring blender for 10 s. The juices were filtered through a membrane filter (0.45μ) and were analyzed for pH, total titratable acidity as tartaric acid with sodium hydroxide (0.1M) and color characteristics. Color characteristics of juices were expressed as lightness (L) and hue angle (θ). L values can range between 0 (black) and 100 (white), while θ was related to tristimulus 'a' and 'b' values by the expression $\cot \theta = a/b$ with the range 0 (violet) to 60° (orange).

Grape Acy content

Total Acy on fruits (50 g) were determined by soaking crushed freeze-dried grapes in ether (100 ml) for 5 h to remove the cutin. After the ether was removed, the grapes were placed in a Buchner funnel where pigment extraction was performed by successive filtration of an extracting solvent (methanol containing 0.1% HCl), until the solvent coming out was clear (Roggero *et al.*, 1986). The extracts were then made up to 500 ml with the extracting solvent and their absorbance read at 520 nm on a Perkin Elmer Lambda 3B spectrophotometer. Total Acy were estimated from the Beer-Lambert relationship $A = \epsilon cl$, using a molar absorptivity coefficient $\epsilon = 3.8 \times 10^4 \text{ LM}^{-1} \text{ cm}^{-1}$ (Ribereau-Gayon, 1959).

Acy extraction for HPLC analysis

Extracts for HPLC analysis were obtained by using the following procedure: Cutin was removed from freeze-dried grapes as previously described and the dry fruits were soaked in 0.1% HCl in methanol (25 ml) overnight in the refrigerator. The next morning, they were thoroughly mixed on a Vortex mixer and centrifuged at $5000 \times g$ for 15 min. Samples were filtered through a Teflon filter (0.45μ) before analysis by HPLC.

HPLC analysis

Separations of individual Acy were carried out on a Hitachi 655A-11 HPLC system using a 250 mm \times 4 mm ID LiChrosorb RP 18 column and an L-3000 multichannel UV-Vis photodetector. Acy were monitored at 520 nm.

Elution was carried out using three solvent systems A (acetic acid–water; 15:85; v:v), B (water–acetic acid–methanol; 65:15:10; v:v:v) and C (methanol). Best separation was obtained under the following conditions: With the initial flow rate at 0.2 ml/min and solvent mixture A and B (99:1), a stepwise linear increase of B to 3, 5, 11, 25, 48 and 100% after 10, 15, 20, 25, 30 and 40 min, respectively, was carried out. The concentration of C was increased to 8% after 20 min, and then back to 0% after 25 min. Flow rate was increased to 3 ml/min after 50 min. Peaks were integrated on a Hitachi D-2000 Chromato-Integrator. Identification of peaks was based on their retention times and those of standards obtained from Alpin Chemical Co., England, Fluka Chemical Co. Switzerland, and K&K Labs, Plainview, NY. Acylated peaks were also confirmed from the relative absorbance of peaks at 520 and 320 nm, respectively (Swain, 1976). Retention times of Acy extracts from Concord grapes were also compared with those of the cultivars used in the study.

RESULTS AND DISCUSSION

The weights of cultivars used ranged from 55 to 357 mg/berry, while size (average berry diameter) was between 13.6 and 19.2 mm/berry (Table 1).

Lenoir, Herbermont, and Black Spanish had the lowest weight to size ratios, which were used as indicators of pulp thickness, while H17-22 and Miss Blue had the highest. Juice yields, which ranged from 552 to 725 ml/kg

TABLE 1
Properties of Grapes Used

No.	Cultivar	Wt ^a (g)	Diameter (mm)	Wt/Diameter (g/mm)	pH	Acidity (g/100 ml)	°Brix	Juice yield (ml/100 g fruit)
1	Lenoir	0.55	3.6	0.15	3.3	1.1	15.5	68.7
2	Blue Lake	2.79	17.0	0.16	3.4	1.3	13.4	68.9
3	Midsouth	3.09	18.6	0.17	3.2	3.4	12.6	69.0
4	Miss Blue	3.57	19.0	0.19	3.2	0.8	12.0	64.9
5	M4-83	3.25	18.0	0.18	3.3	1.3	13.0	72.5
6	M6-7E	2.50	17.0	0.15	3.0	2.2	14.0	66.9
7	Herbermont	0.77	15.4	0.05	3.4	2.2	15.8	71.9
8	Black Spanish	0.83	14.8	0.06	3.5	1.0	18.2	70.9
9	Conquistador	3.54	18.0	0.20	3.6	0.8	16.0	65.9
10	CD8-23	2.32	16.6	0.13	3.5	0.8	16.2	55.2
11	H17-22	3.82	19.2	0.20	3.6	0.7	18.5	64.1
12	DC 2-23	2.98	15.8	0.19	3.7	0.6	16.0	70.7
13	CD 12-72	1.86	15.8	0.12	3.7	0.8	15.8	60.4
14	AD2-75	2.21	16.4	0.13	3.8	0.6	17.9	66.2

^a Based on average weight of 100 berries.

TABLE 2
Color Characteristics of Juice

<i>Cultivar</i>	<i>Total Acy</i> (mg/100 g fruits)	<i>Hue</i> (θ)	<i>Lightness</i> (<i>L</i>)
Lenoir	51.5	18.9	10.3
Blue Lake	12.4	49.3	23.1
Midsouth	5.5	62.5	34.2
Miss Blue	18.9	31.9	31.1
M4-83	37.4	29.2	9.7
M6-7E	50.4	15.2	13.8
Herbermont	37.0	58.1	39.5
Black Spanish	40.0	17.2	10.8
Conquistador	105.5	16.7	3.7
CD8-23	54.0	21.2	11.8
H17-22	35.9	29.6	13.5
DC 2-23	85.9	24.6	15.5
CD12-72	29.7	15.4	3.8
AD 2-75	59.0	33.7	21.8

(CD8-12 and M4-83, respectively), were unrelated to berry weights and their weight to size ratios. Acy concentrations of the cultivars were within the range reported for *V. rotundifolia* cultivars which is between 12 and 1038 mg/100 g of berries (Ballinger *et al.*, 1974; Flora, 1978; Lamikanra & Inyang, 1986; Lamikanra, 1988).

L (lightness) and hue (θ) values of grape juices and wines are normally closely related to their color characteristics and their acceptance in taste panel studies (Nesbitt *et al.*, 1974; Ballinger *et al.*, 1974; Flora, 1976, 1978; Lamikanra, 1988). Flora (1976, 1978) investigated the effects of heat on muscadine juices and suggested that their color acceptance increased with decrease in *L* and increase in the values of θ . Presently, Conquistador and Blue Lake are recommended for grape juice production from *V. rotundifolia* hybrids in Florida (Mortensen, 1986). The color characteristics of the former (Table 2), however, suggest its juice may be too dark for acceptance as a commercial grape juice. *L* and θ values for the cultivars were somewhat related as shown in Fig. 1.

The chromatograms of Acy from some of the cultivars are shown in Fig. 2. The elution profile of Concord Acy is similar to that reported by Williams *et al.* (1978), using a similar solvent system, but separated using a non-linear gradient elution (program 9) on a Waters Chromatograph System. The number and relative concentration of Acy varied from one cultivar to another. Acy corresponding to the peak numbers in the chromatograms (Table 3) show that all non-acylated Acy were eluted before

TABLE 3

Peak Numbers, Relative Retention Times, Corresponding Compounds and Relative Concentrations (%) of Individual Aey extracted from Grapes. (LE, BL, MS, MB, M4, M6, HE, BS, CO, C8, HI, D2, C2 and AD are Lenoir, Blue Lake, Midsouth, Miss Blue, M4-83, M6-7E, Herbermont, Black Spanish, Conquistador, CD8-23, H17-22, D2-23, H17-22, D2-23, C12-72 and AD2-75 cultivars, respectively.)

Peak number	Retention time (min)	Compound	LE	BL	MS	MB	M4	M6	HE	BS	CO	C8	HI	D2	C2	AD
1	15.1	Delphinidin 3,5-diglucoside	— ^x	—	—	1.9 (0.60) ^y	—	0.2 (0.10)	—	—	—	3.1 (1.10)	—	—	—	—
2	18.6	Cyanidin 3,5-diglucoside	—	—	—	17.9 (0.30)	—	0.4 (0.10)	—	—	—	3.6 (0.60)	0.5 (0.10)	—	0.2 (0.05)	—
3	21.2	Petunidin 3,5-diglucoside	—	—	16.5 (3.00)	10.1 (1.10)	7.9 (0.60)	—	—	—	—	15.0 (0.10)	1.2 (0.30)	—	0.2 (0.10)	—
4	26.2	Delphinidin 3-glucoside	3.6 (0.15)	4.3 (0.60)	—	—	13.9 (1.70)	1.1 (6.40)	—	—	1.9 (0.60)	—	1.1 (0.30)	—	—	0.9 (0.40)
5	27.6	Peonidin 3,5-diglucoside	—	—	26.4 (3.70)	6.9 (1.10)	—	2.3 (0.20)	—	3.1 (0.60)	2.0 (0.50)	5.4 (0.90)	0.7 (0.20)	3.0 (0.50)	18.6 (1.40)	—
6	30.2	Malvidin 3,5-diglucoside	—	9.3 (1.80)	4.7 (0.08)	—	—	16.2 (1.20)	—	2.1 (0.30)	2.8 (0.80)	53.0 (2.50)	0.2 (0.10)	6.8 (0.90)	41.2 (3.80)	3.0 (0.50)
7	36.5	Cyanidin 3-glucoside	2.6 (0.05)	26.9 (4.40)	11.9 (1.90)	—	50.4 (2.10)	7.6 (2.40)	—	6.2 (0.90)	15.6 (0.90)	—	—	3.1 (0.70)	—	8.4 (0.50)
8	37.3	Petunidin 3-glucoside	—	44.7 (4.60)	—	21.7 (0.80)	—	—	29.3 (1.30)	1.3 (0.25)	24.9 (0.30)	7.7 (2.10)	1.9 (0.60)	0.5 (0.20)	—	—
9	45.9	Peonidin 3-glucoside	6.1 (0.20)	—	18.1 (0.30)	—	7.5 (1.40)	3.0 (0.50)	—	—	2.0 (0.50)	0.1 (0.05)	0.4 (0.20)	1.8 (0.60)	—	—
10	47.0	Malvidin 3-glucoside	—	—	—	—	—	—	—	2.5 (0.50)	15.5 (0.60)	5.6 (0.10)	5.4 (0.60)	—	—	—
11	51.9	Delphinidin 3(6-0- <i>p</i> -coumarylglucoside)-5-glucoside	2.1 (0.05)	—	7.0 (1.20)	0.7 (0.30)	15.0 (1.80)	28.5 (1.40)	30.3 (1.75)	—	21.6 (0.60)	6.0 (0.40)	16.6 (1.40)	4.6 (0.70)	—	73.5 (1.40)

12	52.6	Cyanidin 3(6-0- <i>p</i> -coumaryl- glucoside)-5-glucoside	30.6 (1.70)	1.4 (0.40)	11.9 (0.90)	0.6 (0.20)	3.9 (1.40)	11.9 (1.20)	19.2 (3.30)	22.7 (2.60)	0.8 (0.30)	—	0.5 (0.20)	5.4 (0.50)	0.4 (0.20)	12.5 (1.70)
13	55.3	Petunidin 3(6-0- <i>p</i> -coumaryl- glucoside)-5-glucoside	1.6 (0.05)	3.1 (1.10)	0.2 (0.10)	0.9 (0.40)	0.8 (0.20)	—	—	3.5 (0.50)	—	—	5.5 (0.70)	0.2 (0.05)	3.9 (0.40)	—
14	57.5	Peonidin 3(6-0- <i>p</i> -coumaryl- glucoside)-5-glucoside	5.8 (0.20)	—	0.4 (0.20)	4.7 (0.70)	—	1.6 (0.50)	—	8.3 (1.40)	1.8 (0.01)	—	1.9 (0.30)	1.9 (0.40)	34.7 (2.20)	—
15	59.7	Malvidin 3(6-0- <i>p</i> -coumaryl- glucoside)-5-glucoside	0.1 (0.09)	—	0.4 (0.10)	9.8 (0.40)	—	1.4 (0.50)	—	3.8 (0.70)	—	—	22.9 (2.30)	1.6 (0.20)	0.2 (0.05)	1.5 (0.40)
16	61.6	Delphinidin 3(6-0- <i>p</i> -coumaryl)glucoside)	2.56 (1.50)	—	—	—	—	10.2 (0.80)	1.6 (0.50)	27.2 (1.80)	—	—	—	60 (0.60)	—	—
17	62.9	Unknown	—	—	—	0.3 (0.20)	—	—	—	—	1.5 (0.50)	0.4 (0.15)	3.6 (0.30)	40 (0.55)	0.2 (0.10)	—
18	66.3	Cyanidin 3(6-0- <i>p</i> -coumaryl)glucoside)	2.7 (0.02)	5.8 (2.30)	1.8 (0.70)	7.9 (0.40)	0.2 (0.05)	1.6 (1.60)	—	3.7 (0.80)	0.5 (0.20)	—	4.1 (0.70)	13.4 (1.60)	0.3 (0.10)	—
19	68.2	Unknown	1.2 (0.29)	—	0.6 (0.30)	—	—	3.8 (0.80)	—	—	—	—	7.3 (0.80)	—	—	—
20	71.8	Petunidin 3(6-0- <i>p</i> -coumaryl)glucoside)	—	—	—	16.4 (3.50)	—	—	2.2 (6.30)	3.1 (0.30)	2.4 (0.40)	—	—	12.9 (1.10)	—	—
21	74.5	Unknown	1.4 (0.30)	—	—	—	—	—	—	—	—	—	27.7 (2.10)	35.1 (2.10)	0.1 (0.05)	—
22	76.1	Unknown	—	—	—	—	0.4 (0.10)	—	—	—	—	—	—	—	—	—
23	77.9	Peonidin 3(6-0- <i>p</i> -coumaryl)glucoside)	—	3.5 (0.60)	—	—	—	2.3 (0.70)	—	140 (1.90)	—	0.3 (0.10)	—	—	—	—
24	79.0	Malvidin 3(6-0- <i>p</i> -coumaryl)glucoside)	15.7 (3.00)	1.1 (0.50)	—	—	—	8.1 (0.90)	9.2 (1.20)	—	6.1 (0.85)	—	—	—	—	—
25	83.0	Unknown	—	—	—	—	—	—	—	—	—	—	—	—	0.1 (0.05)	—

^x Value is below detection limit for method used.

^y Standard error from replicated determinations.

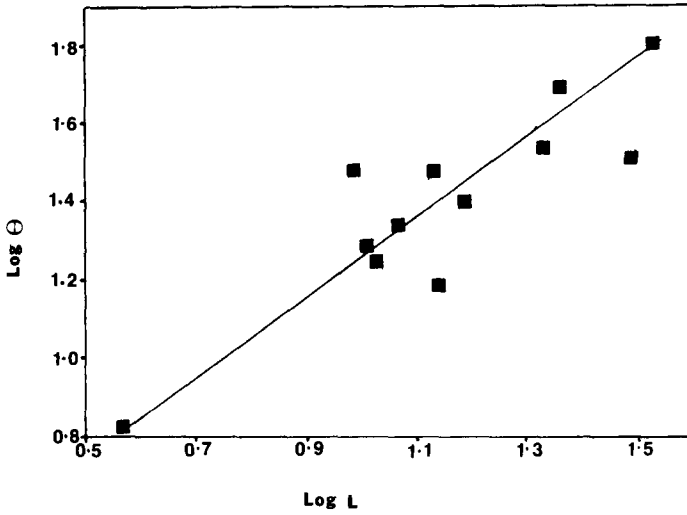


Fig. 1. Relationship between θ and L values of cultivars.

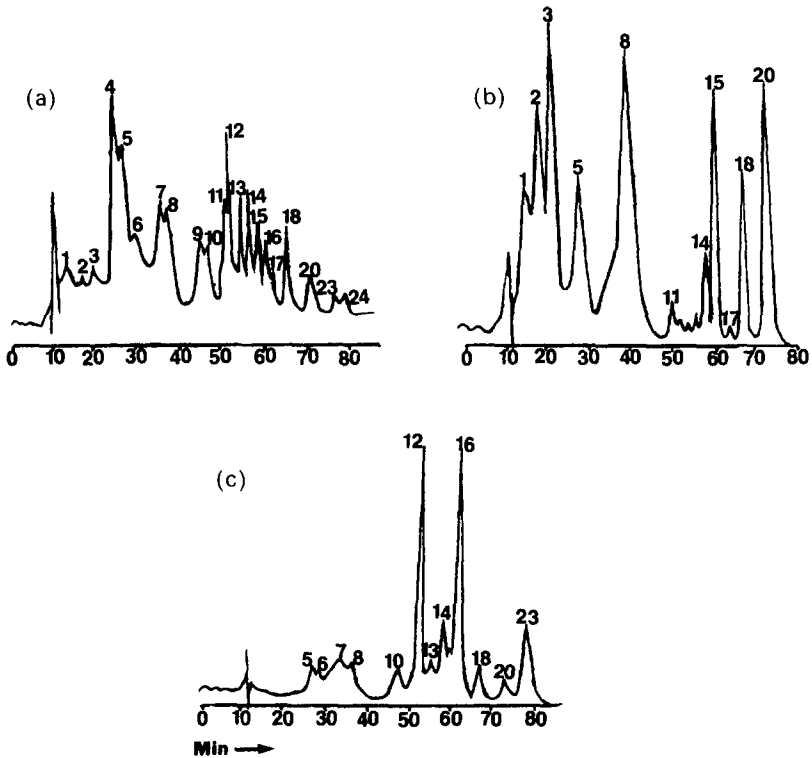


Fig. 2. Chromatograms of Acy extracts from 'Concord' (a), 'Miss Blue' (b) and 'Black Spanish' (c) cultivars. Numbers on peaks correspond to those in Table 3.

acylated peaks. Diglucosides of acylated Acy were all eluted before the monoglucosides, with the exception of delphinidin 3-glucoside which had a shorter retention time than those of peonidin and malvidin 3,5-diglucosides. Unidentified peaks were all eluted with acylated monoglucosidic Acy. Scans of their absorption on the photodetector at both UV and visible regions, as well as their relative absorbance at 520 and 320 nm suggested that they were all acylated. These peaks are, therefore, likely to be non *p*-coumaric acid acylated monoglucosides of anthocyanidins, some of which have been observed in some *V. vinifera* grape cultivars (Roggero *et al.* 1986). With the exception of DC 2-23 and H17-22 (Table 3) these Acy occurred in relatively small quantities, in the *V. rotundifolia* hybrids.

Only one Acy, cyanidin 3(6-*o*-*p*-coumaryl glucoside)5-glucoside was common to all the cultivars at relative concentrations ranging from 0.2% in Midsouth and DC 2-23 to 30.6% in Lenoir. Monoglucosides and diglucosides were evenly distributed among the fruits, with their relative contents between 14–89% and 11–68%, respectively. With the exception of Midsouth and CD 12-72, the non-acylated Acy contents of the grapes were very low. In most cultivars the relative acylated diglucosidic Acy contents were higher than those of the non-acylated monoglucosides. H17-22 had the highest acylated Acy content with 91.4% while CD8-23 had the lowest (12.3%). Non-acylated delphinidin, which is usually the predominant Acy in muscadines (Ballinger *et al.*, 1973, 1974; Nesbitt *et al.*, 1974; Lamikanra & Garlick, 1987; Lamikanra, 1988) was absent in most of the grapes. In two cultivars (Miss Blue and M6-7E) where they were present, their relative contents were almost negligible.

The relatively low non-acylated diglucosides in many cultivars and the absence of the unacylated delphinidin in most cases might be related to the level of Acy contributions from *V. rotundifolia* grapes when crossed with other grape cultivars and/or the extent of muscadine grape involvement in developing these hybrids. No attempt was made to determine the dominance or recessiveness of *V. rotundifolia* contributions of Acy to its hybrids in this study. However, since the Acy in most of the cultivars are closely related to those found in other grape cultivars the commonly observed lack of pigment stability in *V. rotundifolia* hybrids may not be predominantly due to the Acy structures and their relative contents alone. Other factors such as enzymic breakdown of Acy or condensation reactions between Acy and other organic compounds could also be significant contributors (Markakis, 1974).

Conquistador, Lenoir, M6-7E and CD 12-72 were the only cultivars that had total malvidin contents (25.5, 15.6, 12.8 and 12.6 mg/100 g berries, respectively) higher than the 8.0 mg/100 g berries reported by Ballinger *et al.* (1974) to be the minimum malvidin content required for acceptable wine

colors in red muscadines. No correlation was found between the relative content of any group of Acy and either L or θ values of grapes and juices.

Rating of cultivars for possible wine color characteristics and selection in breeding was done through a plot of their total delphinidin contents against that of malvidin (Fig. 3). In ranking the grapes, increase in delphinidin was considered undesirable due to their relatively high phenol content which makes them less stable than other Acy (Hrazdina, 1975). Van Buren *et al.* (1968) and Robinson *et al.* (1966) demonstrated that acylated Acy are relatively more resistant to the effect of heat and light than comparable non-acylated Acy. Hrazdina (1975), however, reported that at room temperature, *p*-coumaryl-3, and *p*-coumaryl 3,5-diglucosides easily saponified, with the former having a greater instability than the latter. Acylation and hydroxylation were also found to decrease the solubility of Acy in aqueous ethanol (H_2O , 0–20% aq EtOH) solutions. They then concluded that at

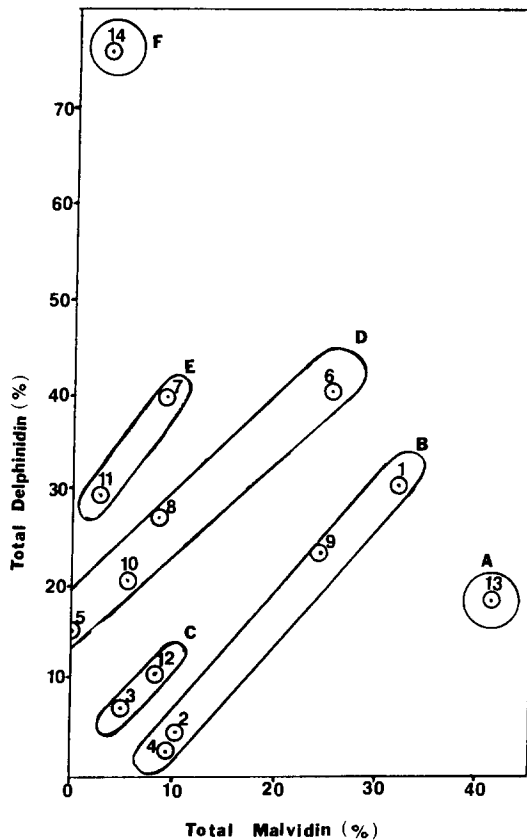


Fig. 3. Plot of total delphinidin against malvidin content of grapes. Numbers correspond to cultivars shown in Table 1.

ambient temperature, the stability of non-acylated Acy is greater than the corresponding acylated Acy. Based on these findings, Hrazdina's observations appear to be more applicable to wines than earlier reports (Robinson *et al.*, 1966; Van Buren *et al.*, 1968) that showed results to the contrary, and which may be more applicable to juices and other grape products that are sterilized and/or processed by heating. The effect of acylation was not considered in ranking the cultivars as a result of the apparent discrepancies on their effect on Acy stabilization. The relative diglucoside and monoglucoside contents of grapes were not used in developing a selection index due to the fact that, while several reports (Hrazdina, 1975; Robinson *et al.*, 1966; Van Buren *et al.*, 1968) indicate that diglucosides are more stable than comparable monoglucosides, they are more susceptible to browning reactions during processing and storage (Van Buren *et al.*, 1968; Ballinger *et al.*, 1974). Sims & Morris (1985) also demonstrated that diglucoside Acy are not as readily incorporated into a tannin polymer and suggested that this may be responsible for color instability of *Vitis rotundifolia* wines.

From Fig. 3, it can be concluded that a relatively high delphinidin concentration will be 20% and above, while that for malvidin concentration are values greater than 10%. The indifference region (the region where the combined effect of dephinidin and malvidin contents show no significant difference on color quality) appeared to slope upward to the right. The 14 cultivars have, therefore, been divided into 6 sub-groups A, B, C, D, E and F, with the ranking decreasing from A (CD12-72) which is obviously the best to F (AD2-75), the least desirable cultivar. The other parameters of CD12-72, such as size, juice yield as well as L and θ values of the juice, also suggest that the cultivar may be well suited for juice and wine production.

CONCLUSION

Considerable variations exist in the Acy contents and quality of *V. rotundifolia* hybrids. These Acy are contributed by *V. rotundifolia* and the other grape cultivars used as crosses during their development. Non-acylated diglucosides, which are the Acy of *V. rotundifolia* grapes, however, occurred in relatively low amounts in most cases, which might be indicative of recessive contributions of Acy by *V. rotundifolia* in the hybrids or the extent of their involvement in the development of these cultivars. CD12-72 was ranked as the best cultivar based on its relative malvidin and delphinidin contents followed by Lenoir, Blue Lake, Miss Blue and Conquistador. AD2-75 was the least desirable of the cultivars used in this study for selection in future breeding programs.

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REFERENCES

- Ballinger, W. E., Maness, P., Nesbitt, W. B. & Carroll, D. E. (1973). Anthocyanins of black grapes of 10 clones of *Vitis rotundifolia* Michx. *J. Food Sci.*, **38**, 909–10.
- Ballinger, W. E., Maness, E. P., Nesbitt, W. B., Makus, D. J. & Carroll, D. E. (1974). A comparison of anthocyanins and wine quality in black grapes of 39 clones of *Vitis rotundifolia* Michx. *J. Am. Soc. Hort. Sci.*, **99**, 338–40.
- Flora, L. F. (1976). Time-temperature influence on muscadine grape juice quality. *J. Food Sci.*, **41**, 1312–5.
- Flora, L. F. (1978). Influence of heat, cultivar and maturity on the anthocyanidin-3,5-diglucosides of muscadine grapes. *J. Food Sci.*, **43**, 1819–21.
- Goldy, R. G., Ballinger, W. E. & Maness, E. P. (1986). Fruit anthocyanin content of some *Euvitis* × *Vitis rotundifolia* hybrids. *J. Am. Soc. Hort. Sci.*, **111**, 955–60.
- Hrazdina, G. (1975). Anthocyanin composition of Concord grapes. *Lebesm-Wiss. U. Technol.*, **8**, 111–3.
- Hrazdina, G., Borzell, A. J. & Ribinson, W. B. (1970). Studies on the stability of anthocyanidin-3, 5-diglucosides. *Amer. J. Enol. Vitic.*, **21**, 201–4.
- Lamikanra, O. (1988). Development of anthocyanin pigments in muscadine (*Vitis rotundifolia* Michx.) grapes. *HortSci.*, **23**, 597–9.
- Lamikanra, O. & Garlick, D. (1987). Effects of grape skins and seeds on the composition and quality of muscadine wines. *Food Chem.* **26**, 245–51.
- Lamikanra, O. & Inyang, I. D. (1986). Constituent compounds of muscadine grapes and wines. *Proc. Fla. State Hort. Soc.*, **99**, 148–55.
- Markakis, P. (1974). Anthocyanins and their stability in foods. *CRC Crit. Rev. Food. Tech.*, **4**, 437–56.
- Mortensen, J. A. (1985). Potential new rootstocks for Florida viticulture. *Proc. Fla. State Hort. Soc.*, **98**, 166–9.
- Mortensen, J. A. (1986). Grape varieties, rootstocks and propagation. *Proc. Vitic. Sci. Symp.*, FAMU, pp. 13–25.
- Nagel, C. W. & Wulf, L. W. (1979). Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. *Am. J. Enol. Vitic.* **30**, 111–16.
- Nesbitt, W. B., Maness, E. P., Ballinger, W. E. & Carroll, D. E. (1974). Relationship of anthocyanins of black muscadine grapes (*Vitis rotundifolia* Michx.) to wine color. *Am. J. Enol. Vitic.* **25**, 30–2.
- Ribereau-Gayon, P. (1959). Recherche sur les anthocyanes des vegetaux: application au genre *Vitis* (Monograph). Libraire Generale de l'Enseignement, Paris.
- Robinson, W. B., Wiers, L. D., Bertino, J. J. & Mattick, L. R. (1966). The relation of

- anthocyanin composition to color stability of New State wines. *Am. J. Enol. Vitic.*, **17**, 178–84.
- Roggero, J. P., Coen, S. & Ragnett, L. B. (1986). High performance liquid chromatography survey on changes in approach to anthocyanin metabolism. *Am. J. Enol. Vitic.*, **37**, 77–82.
- Sims, C. A. & Morris, J. R. (1985). A comparison of the color components and color stability of red wines from Noble and Cabernet Sauvignon at various pH levels. *Am. J. Enol. Vitic.* **36**, 181–4.
- Smith, R. M. & Luh, B. S. (1965). Anthocyanin pigments in the hybrid of grape Rubi red. *J. Food Sci.*, **30**, 995–1005.
- Somers, T. C. (1966). Wine tannins: Isolation of condensed flavonoid pigments by gel filtration. *Nature*, **209**, 368.
- Swain, T. (1976). Nature and properties of flavonoids. In *Chemistry and Biochemistry of Plant Pigments*. ed. R. W. Goodwin Academic Press, London, pp. 425–63.
- Van Buren, J. P., Bertino, J. J. & Robinson, W. B. (1968). The stability of wine anthocyanins on exposure to heat and light. *Am. J. Enol. Vitic.*, **19**, 147–54.
- Williams, M., Hrazdina, G., Wilkinson, M. M., Sweeny, J. G. & Iacobucci, H. A. J. (1978). High pressure liquid chromatographic separation of 3-glucosides, 3,5-diglucosides, 3-(6-0-*p*-coumaryl) glucosides and 3-(6-0-*p*-coumaryl)-5-glucosides of anthocyanidins. *J. Chromatog.*, **155**, 389–98.