

Color and Color Stability of Red Wine from Noble (*Vitis rotundifolia* Michx.) and Cabernet Sauvignon (*Vitis vinifera* L.) at Various pH

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Muscadine grapes (*Vitis rotundifolia*) are the most widely planted grapes in the south and southeast United States due to their suitability in these areas. Most of the commercial muscadine grape crop is used to produce wine. Wine made from suitable cultivars of muscadine grapes possesses a very fruity, unique flavor and aroma that appeals to many people in the South. However, muscadine grape products are very susceptible to browning and overall loss of color quality during processing and storage (1,4,7). This color instability is severely limiting the marketability of red muscadine wines.

Several factors have been shown to influence the color quality and stability of red muscadine wines. The anthocyanin content and composition and conditions of processing and storage have been shown to have a major effect on the color and stability of red muscadine products (1,2,4,7). Muscadine grapes contain only the 3,5-diglucoside anthocyanins (1), which have been reported to be more susceptible to browning than are the monoglucoside anthocyanins (6). This might indicate why red muscadine wines tend to brown. Monoglucoside anthocyanins are the predominant pigments in *Vitis vinifera* wines (8), and red *vinifera* wines are generally considered to have relatively stable color.

The effects of pH on the color of red *Vitis vinifera* wines has been well documented (10,11,12). These effects of pH have also been observed in red muscadine wine (7), with a higher pH causing the wine to be less red and less intense. In the same research (7), red muscadine wine with a higher pH also browned to a much greater extent than did wine with a lower pH. Lower pH wine also had a greater loss of pH responsive anthocyanin than higher pH wine, which indicated greater polymerization of anthocyanin at lower pH. The formation of various anthocyanin-tannin complexes or polymers during aging of red wines has been well documented (3,5,9), and it has also been shown that these anthocyanin-tannin complexes help stabilize the wine color (8). Perhaps the color instability of red muscadine wine is due to greater polymerization of anthocyanins and tannins. Further research is needed to compare the color components and anthocyanin-tannin polymerization of red muscadine wine with that of a red *vinifera* wine with relatively stable color such as Cabernet Sauvignon at various pH.

MATERIALS AND METHODS

Noble and Cabernet grapes were obtained from commercial vineyards in Arkansas in 1982. The grapes were hand-harvested at commercial harvest and transported to the Food Science Department in Fayetteville. The grapes of both species were crushed within 12 hr after harvest, 100 ppm SO₂ was added as potassium metabisulfite, and the % soluble solids was adjusted to 22% with dry sugar. The musts were placed in polyethylene containers and fermented at about 25°C with *Saccharomyces cerevisiae*, Montrachet. The musts were pressed at about 6° Brix, and the musts were allowed to finish fermenting at 25°C in glass carboys. The wines were fermented to dryness, racked, and cold stabilized at 3-4°C for 2 months. The wines were stored in glass carboys at 40°C until treatments could be established (ca. 1 month).

Four pH levels (2.8, 3.0, 3.3, 3.8) were established in the 2 wines using CaCO₃ and 10% hydrochloric acid. The Noble had an initial pH of 3.0 and this was used as one pH level. The 2.8 pH treatment for Noble was established by adding 5 ml 10% HCl/L wine. The 3.3 and 3.8 pH treatments were established by adding 1.9 g CaCO₃/L wine and 2 g CaCO₃/L wine, respectively. The CaCO₃ was added to cold wine (5°C) and allowed to settle for 4 days at 5°C. The Cabernet had an initial pH of 3.65. The 2.8, 3.3, and 3.8 pH treatments for Cabernet were established by adding 27.5 ml, 20.0 ml, and 13.0 ml 10% HCl/L wine, respectively. The 3.8 pH treatment for Cabernet was established by adding 0.3 g CaCO₃/L wine as described previously.

The wines were bottled into 400 mL round wine bottles and sealed with screw-on closures. The wines were analyzed initially (ca. 4 month old wine) and after 12 months of storage in the dark at 20°C and 30°C. The experiment was designed as a factorial with 3 replications.

The tristimulus color of the wine was determined using a Gardner Color Difference Meter (CDM) that had been standardized to a dark red plate (L=23.1, a=22.0, b=7.1). The color of the wine was also rated visually for intensity (1=light red color, 10=dark red color) and browning (1=severe browning, 10=no browning) by 5 trained panelists. The color characteristics and chemical age (level of polymeric anthocyanins) were determined by the method of Somers and Evans (9) using a Varian (Series 6345) double-beam spectrophotometer. A slit width of 1.0 nm was used for all determinations, and a 1 or 10 mm pathlength was used, depending on the particular determination. All absorbencies were corrected to the 10 mm pathlength.

For statistical analysis, all data within each storage time were subjected to factorial analysis of variance. Color changes during storage were calculated as the difference (+ or -) between the initial value and the value after 12 months. These changes during storage were then subjected to factorial analysis of variance. Duncan's multiple range test was used to separate means of the main effects, and least significant difference (LSD) was used to separate means of the significant interactions.

RESULTS AND DISCUSSION

A comparison of the color of the 2 species through the main effects showed that Noble had more redness and intensity initially as illustrated by higher CDM 'a' and Abs. at 520 nm (Table 1). This greater redness in Noble was due to greater ionization of anthocyanins. The percentage of ionized anthocyanins indicates the level of free, nonpolymerized anthocyanins in the colored or red form (red carbonium ion). The 2 species did not show any browning initially, and hence there were no differences in browning between the species initially as shown by CDM 'b' and visual browning ratings (Table 1). Cabernet had a much greater chemical age initially (Table 1), which indicates that more anthocyanins in Cabernet had been incorporated into tannin complexes (9).

After 12 months, Cabernet had more redness and intensity as shown by higher CDM 'a' and Abs. at 520 nm, and there was no difference in the ionization of anthocyanins between Noble and Cabernet (Table 1). Noble had browned to a much greater extent after 12 months as illustrated by higher CDM 'b' and lower visual browning ratings (Table 1). As initially, Cabernet had a greater chemical age after 12 months.

The interactive effects of pH x species initially showed that a higher pH lightened the color and intensity of Noble to a greater extent than for Cabernet as shown by greater increases in CDM 'L' and decreases in visual intensity ratings and absorbance at 520 nm (Table 2). Adjusting the pH to 3.8 also lowered the redness (lower CDM 'a') of Noble to a greater extent than for Cabernet. This greater decrease in color intensity of Noble at higher pH was due to greater decreases in the ionization of anthocyanins in Noble (Table 2). This greater decrease in the percentage of ionized anthocyanins in Noble was due to the lower pK values (2.0) of the anthocyanin-3,5-diglucosides (predominate in Noble) as compared to the pK values (2.8) of the anthocyanin-monoglucosides (predominate in Cabernet) (9). As the pH increases, a higher percentage of the anthocyanins in Noble would be in the colorless form as compared to the anthocyanins in Cabernet. Thus, the color of red muscadine wine is altered by pH (within the normal pH range of wines) to a greater extent than is that of red vinifera wine.

After 12 months, the interaction of pH x species again showed that increasing the pH to 3.3 and 3.8 decreased the redness (lower CDM 'a'), visual intensity ratings and ionization of anthocyanins of Noble to a greater extent than for Cabernet (Table 2). However, the CDM 'L' and Abs. at 520 nm did not illustrate this interaction after 12 months.

The interactive effects of pH x species also indicated a higher pH initially increased the blueness (lower CDM 'b') of Noble to a greater extent than for Cabernet (Table 3). This again was due to the lower pK values of the 3,5-diglucoside anthocyanins in Noble as compared to the monoglucosides in Cabernet. Higher pH also resulted in greater decreases in the chemical age of Cabernet as compared to Noble (Table 3). After 12 months, the interactive effects of pH x species indicated that Noble had browned to a much greater extent at higher pH than had Cabernet as shown by greater increases in CDM 'b' and wine hue, and greater decreases in visual browning ratings (Table 3). Increasing the pH to 3.8 also resulted in a greater decrease in the chemical age of Cabernet than for Noble.

Table 4 shows the main and interactive effects of pH and species on the changes in color components during 12 months. The main effects of cultivar show that Noble lost more redness and intensity during 12 months than did Cabernet, as shown by greater decreases in CDM 'a', Abs. at 520 nm and ionization of anthocyanins (Table 4). Noble also browned to a much greater extent during 12 months as indicated by greater increases in CDM 'b' and Abs. at 420 nm. This illustrates the greater instability of the color of Noble compared to Cabernet, and the greater browning problem in Noble. Noble also had much less polymerization of anthocyanins during 12 months than did Cabernet as shown by chemical age (Table 4). This lack of anthocyanin-tannin polymerization in Noble is at least partially responsible for the instability of Noble color. This greater color instability in Noble also indicates that the 3,5-diglucoside anthocyanins brown to a greater degree than the 3-monoglucoside anthocyanins in Cabernet, and do not polymerize with tannins to the extent as monoglucosides do. Red muscadine wines may also lack the tannin species that are necessary for anthocyanin-tannin polymerization.

The main effects of pH indicate that a lower pH resulted in greater loss of redness and color intensity as shown by greater decreases in CDM 'a' and Abs. at 520 nm (Table 4). However, wine with a lower pH still had more redness and intensity, and had a higher percentage of ionized anthocyanins after 12 months (Table 2). Wine with a lower pH also increased in chemical age to a greater extent during 12 months and did not brown as severely as shown by smaller increases in CDM 'b' and Abs. at 420 nm (Table 4). The lower browning at lower pH could again be due to greater, polymerization of anthocyanins with tannins. Perhaps the carbonium ion form of the anthocyanins, which is more prevalent at lower pH, is more readily incorporated into tannin complexes than is the pseudobase form of the anthocyanins.

The interactive effects of pH x species on the color changes during 12 months showed that a lower pH resulted in greater decreases in the Abs. at 520 nm of Noble than in Cabernet (Table 4). Noble also browned to a greater extent during 12 months at higher pH than did Cabernet as shown by greater increases in CDM 'b' and Abs. at 420 nm. This illustrates the greater instability of the color of Noble at higher pH compared to Cabernet. Increasing the pH to 3.3 and 3.8 also lowered the increase in chemical age of Cabernet during 12 months to a greater extent than for Noble (Table 4).

Table 1. The effects of species on the color of red wine.

Treatment	CDM 'a'	Abs. # 520 nm	Ionisation of anthocyanins ² (%)	CDM 'b'	Visual browning ³	Chemical age ⁴
<u>Initial</u>						
Noble	22.8a [#]	6.37a	9.7a	4.1a	9.0a	0.009b
Cabernet	21.7b	4.85b	7.5b	3.7a	9.0a	0.105a
<u>12 Month</u>						
Noble	15.5b	3.22b	9.2a	7.2a	5.0b	0.108b
Cabernet	17.2a	3.99a	9.5a	4.0b	7.3a	0.507a

² Calculated as $(E_{520} - E_{520}^{SO_2}) / ((E_{520} - E_{520}^{SO_2}) + (E_{520}^{HCl} - E_{520}^{SO_2}))$, where E_{520} = Abs. at 520 nm, $E_{520}^{SO_2}$ = Abs. at 520 nm after reacting with excessive SO_2 , and E_{520}^{HCl} = Abs. at 520 after acidifying with HCl. This is a measure of the percentage of total anthocyanins in the colored form (23).

³ Rated on a scale of 1-10, with 10 = no browning and 1 = severe browning.

⁴ Calculated as $(E_{420}^{HCl} / E_{520}^{HCl}) - (E_{420}^{SO_2} / E_{520}^{SO_2})$, where $E_{420}^{SO_2}$ = Abs. at 420 nm after reacting with excessive SO_2 , and E_{420}^{HCl} = Abs. at 420 nm after acidifying the wine with HCl. This is a measure of the amount of polymeric pigments (23).

[#] Means within main effects separated by Duncan's multiple range test at the 5% level.

Table 2. The interactive effects of species x pH on the CDM 'L' and 'a' values, visual intensity, abs. # 520 nm, and ionisation of anthocyanins of red wine.

Treatment	CDM 'L'	CDM 'a'	Visual intensity ²	Abs. # 520 nm	Ionisation of anthocyanins (%) ³
<u>Initial</u>					
<u>Noble</u>					
2.80	12.5	25.6	9.0	10.31	14.8
3.00	14.1	25.4	8.0	8.41	13.2
3.30	16.0	23.6	7.0	4.33	7.0
3.80	19.6	16.4	5.0	2.41	4.0
<u>Cabernet</u>					
2.80	12.3	24.7	9.0	6.47	11.7
3.00	13.2	23.6	8.5	5.06	8.6
3.30	14.5	21.7	7.5	4.12	6.3
3.80	16.4	16.9	6.5	2.94	3.5
LSD # 5% 0.4 0.3 0.5 0.40 0.9					
<u>12 Month</u>					
<u>Noble</u>					
2.80	17.8	17.8	7.2	3.57	15.0
3.00	17.6	17.3	6.6	3.55	11.6
3.30	18.0	15.5	5.7	3.21	6.9
3.80	19.4	11.5	3.8	2.55	3.2
<u>Cabernet</u>					
2.80	13.6	19.2	8.2	4.42	12.6
3.00	13.8	18.0	7.6	4.14	11.2
3.30	14.2	17.0	6.7	3.92	8.8
3.80	15.1	14.8	5.6	3.50	5.6
LSD # 5% NS 0.6 0.5 NS 1.3					

² Rated on a scale of 1-10 with 10 = dark red color, 1 = very light red color.

³ See footnote of Table 1.

Table 3. The interactive effects of species x pH on the CDM 'b' value, visual browning, wine hue, and chemical age of red wine.

Treatment	CDM 'b'	Visual browning ²	Wine hue (Abs. 420/ Abs. 520)	Chemical age ³
<u>Initial</u>				
<u>Noble</u>				
2.80	6.0	9.0	0.29	0.011
3.00	5.4	9.0	0.34	0.010
3.30	3.3	9.0	0.51	0.009
3.80	2.6	9.0	0.84	0.008
<u>Cabernet</u>				
2.80	4.5	9.0	0.45	0.114
3.00	3.8	9.0	0.52	0.107
3.30	3.3	9.0	0.60	0.104
3.80	3.2	9.0	0.75	0.093
LSD # 5% 0.2 NS 0.01 0.003				
<u>12 Month</u>				
<u>Noble</u>				
2.80	6.2	6.8	0.97	0.136
3.00	7.1	5.8	1.05	0.122
3.30	7.6	4.8	1.18	0.098
3.80	8.0	2.9	1.49	0.075
<u>Cabernet</u>				
2.80	4.8	8.5	0.74	0.578
3.00	4.9	7.9	0.79	0.557
3.30	5.0	7.2	0.83	0.511
3.80	5.3	5.6	0.92	0.382
LSD # 5% 0.3 0.3 0.05 0.030				

² Rated on a scale of 1-10, with 10 = no browning and 1 = severe browning.

³ See footnote on Table 1.

Table 4. Main and interactive effects of pH and species on color changes of red wine during 12 months.

Treatment	Dec. in CDM 'a'	Dec. in Abs. @ 570 nm	Dec. in Ionisation of anthocyanins (%) ²	Inc. in CDM 'b'	Inc. in Abs. @ 470 nm	Inc. in Chemical age ²
Species						
Noble	-7.30 ^a	-3.14 ^b	-0.5b	2.4a	0.94a	0.098b
Cabernet	-4.5a	-0.65a	2.0a	1.3b	0.68b	0.398a
pH						
2.80	-7.2d	-4.40d	0.5a	0.3d	0.22d	0.294a
3.00	-6.7c	-7.89c	0.5a	1.4c	0.53c	0.781a
3.30	-4.5b	-0.65b	1.2a	3.0b	1.17b	0.278b
3.80	-3.5a	0.35a	0.6a	3.8a	1.78a	0.178c
Cultivar x pH						
Noble						
2.80	-8.0	-6.74	0.2	0.2	0.09	0.125
3.00	-8.1	-4.86	-1.6	1.7	0.47	0.111
3.30	-8.2	-1.11	-0.1	4.2	1.44	0.083
3.80	-4.9	0.14	-0.8	5.3	1.77	0.067
Cabernet						
2.80	-5.6	-2.06	0.8	0.3	0.35	0.463
3.00	-5.7	-0.92	2.6	1.1	0.60	0.449
3.30	-4.8	-0.19	2.5	1.7	0.80	0.389
3.80	-2.7	0.56	2.0	2.1	0.99	0.289

LSO @ 5%	NS	0.30	1.3	0.3	0.13	0.020

^a Means within main effects separated by Duncan's multiple range test at the 5% level.

² See footnotes on Table 1.

CONCLUSIONS

The color of Cabernet was superior to that of Noble after 12 months and was much more stable. This greater color stability in Cabernet was at least partially due to increased anthocyanin-tannin polymerization. Higher pH lowers the color quality of red muscadine wine to a greater extent than the color quality of red vinifera wine since the diglucoside anthocyanins in red muscadine wine have lower pK values than do the monoglucoside anthocyanins in Cabernet. Browning was more severe in a higher pH wine, which was probably due to less anthocyanin-tannin polymerization at higher pH. Overall, greater formation of anthocyanin-tannin polymers was related to maintenance of intense red color and resistance to browning.

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