

Circular Dichroism and Spectroscopic Studies of *Vitis vinifera* cv. Cabernet Sauvignon and *Vitis rotundifolia* cv. Noble Red Wine Liquid Chromatographic Fractions

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Abstract. Red wines were made with *Vitis vinifera* cv. Cabernet Sauvignon and *Vitis rotundifolia* cv. Noble grapes with three different skin contact times and with added acetaldehyde to investigate monoglucoside and diglucoside anthocyanin polymerization. The anthocyanins were extracted from the wines by a low-pressure liquid chromatographic (LC) procedure using a silica/PVPP column, producing three to four chromatographic fractions from each wine. The UV/Vis and circular dichroism (CD) spectra of each fraction were then determined. The Noble wine polymers had CD spectra while the Cabernet Sauvignon wines did not. The chirality of the Noble wine polymers is theorized to be due to the polymer structure, which may be visualized as vertically stacked, as opposed to the apparent planar or net non-chiral structure of the Cabernet Sauvignon wine polymers. The intensity of the Cabernet Sauvignon fraction UV/Vis spectra increased with increasing skin contact, while those of the Noble wines did not. Acetaldehyde treatment of the wines resulted in nonuniform changes in the UV/Vis spectra of the Cabernet Sauvignon fractions. The Noble wine fraction UV/Vis spectra were not significantly affected by acetaldehyde addition. The CD spectral evidence in combination with the UV/Vis spectra indicate that the Cabernet Sauvignon wines are composed of three or four polymer "class" structures while the Noble wines contain similar polymer structures in each of the four LC fractions.

The color of a red wine is primarily due to the type and concentration of anthocyanin, but other factors play a role, including phenolic compounds other than the anthocyanins, sulphur dioxide, oxygen content, grape cultivar, yeast/fermentation method/winemaking techniques employed, and final wine pH (3,8,17,19). The anthocyanins extracted into red wines chemically combine with other wine components, forming stable compounds referred to as polymers (22,23,24,25). The actual structure, composition and physicochemical characteristics of these polymers are basically unknown.

Timberlake and Bridle (24) isolated an anthocyanin-tannin-acetaldehyde complex and described its properties. The complex was found to be less reactive to acidification and more highly colored at elevated pH levels than malvidin-3-glucoside normally would be. Acetaldehyde was shown to increase the absorbance of malvidin-3-glucoside on storage but not that of malvidin-3,5-diglucoside or malvidin-3 p-coumaryl-3-glucoside-5-glucoside (24). When acetaldehyde was added to red Noble wine, the color intensity and chemical age increased, indicating increased anthocyanin-tannin polymerization (21). The level and mode of interaction of acetaldehyde with mono- and diglucoside anthocyanins apparently differs.

The self-association of anthocyanins has been extensively studied. The self-association of flavylium cations (AH⁺) was first suggested by Asen *et al.* (1,2) and observed in wine by Sheffeldt and Hrazdina (20). Hoshino (11), Hoshino and Goto (12) and Hoshino *et al.* (13,14,15,16) studied the self-association of the quinoidal base form (A), and Hoshino (10) examined the self-association of the flavylium cation form (AH⁺) of anthocyanin 3,5-diglucosides. By examining the circular dichroism (CD) spectra of the polymers, these studies showed that both forms of the anthocyanins stack vertically as they polymerize. Circular dichroism is measured by alternately passing right and left circularly polarized light through a sample and measuring the difference in the absorbance of each at each wavelength over a specific range. Chiral compounds, certain proteins and amino acids, and as Hoshino *et al.* (13,14,15,16) showed, the quinoidal base form of diglucoside anthocyanins, exhibit circular dichroism spectra at characteristic wavelengths. The circular dichroism effect in diglucoside anthocyanins was shown to be concentration dependent - at concentrations below 0.1 mM, the flavylium cations exist as monomers in acidic solutions (10).

In contrast to the analytical methods for measuring specific anthocyanins, very few techniques have been developed to extract wine anthocyanin polymers. One technique (4) has been used to analyze red wines (6,7), but the structural details of these polymers are unknown. Using a modification of the Bourzeix and Heredia technique for polymer extraction (4), Johnston and Morris (18) were able to show that there are three to four classes of polymer structures present

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in red wines. These polymer classes can be fractionated by the combination of their molecular weight, polarity, and interaction with the silica/PVPP liquid chromatographic column used. Using this separation technique to extract the pigments and spectropolarimetry to determine the presence of chiral compounds in the extracted fractions, this research examines the level of self-association and spectroscopic character of the polymer classes extracted from both *Vitis vinifera* cv. Cabernet Sauvignon and *Vitis rotundifolia* cv. Noble red wines. Cabernet Sauvignon grapes were selected for this research because they contain only monoglucoside anthocyanins and their acetyl and p-coumaroyl esters; Noble grapes were selected because they contain only diglucoside anthocyanins and are devoid of acylated forms. Analysis of wines made from these grapes would provide clear evidence of differences between mono and diglucoside anthocyanin polymer structure.

Materials and Methods

Apparatus: A Jasco model J-710 Spectropolarimeter was used to determine all circular dichroism (CD) spectra. Sample spectra were recorded using Jasco J-710 Spectropolarimeter software (V.1.10C, 3 April 1990) loaded on an IBM XT compatible computer. Each sample was placed in a 1-mm path length quartz cuvette for measurement.

A Hewlett-Packard model 8452A Diode Array Spectrophotometer was used to determine the UV-Vis spectra of all samples. The spectra were recorded and analyzed using the Hewlett-Packard 98532A UV-VIS Software (Rev. A) loaded on an IBM PS-2 computer. Again, each sample was placed in a 1-mm pathlength quartz cuvette for measurement.

Wines: *Vitis vinifera* cv. Cabernet Sauvignon grapes were hand-picked, and Noble grapes were machine harvested in the fall of 1992 at the Post Familie Vineyards (Altus, AR) and delivered to the University of Arkansas Food Science Department for processing into wines. The Cabernet Sauvignon must obtained had the following characteristics: pH 3.25, 18.1 °Brix, total acidity 5.96 g/L (as tartaric acid). The Noble must obtained had the following characteristics: pH 3.55, 14.5 °Brix, total acidity 2.72 g/L (as tartaric acid). The musts were ameliorated to 20 °Brix, divided into four parts, and inoculated with *Prise de Mousse* yeast (Lallemend Inc, Montreal, Canada). Fermentation was carried out at 20°C.

Wines were prepared by pressing one part of each grape variety must at 0 °Balling (12° style), 0 °Balling (Dry style), and seven days after achieving 0 °Balling (Week style), forming three different wine styles. The Dry style lot was split into two parts to prepare a fourth wine style. One of the Dry style half-lots was treated with 155 ppm dry acetaldehyde after the completion of fermentation (Dry/Acetaldehyde style); the other half lot was left untreated. All wines were bottled in 375-mL bottles, capped, and stored in the dark at 15.5°C. The wines were stored for 24 months before analysis.

Sample preparation: One bottle of each wine style served as a sample unit. Two 1-mL samples from each bottle were individually passed through an LC column packed with silica gel 60, polyvinyl polypyrrolidone, and silica gel G (70:20:10), in accordance with the procedure of Johnston and Morris (18). The resultant eluant was monitored at 525 nm and collected in a Pharmacia FRAC-100 fraction collector in 1.5 minute fractions. Those fractions that contained the center of the eluted fractions were collected and pooled by wine type and fraction (two samples for each fraction in each wine). The pooled fractions were dried under nitrogen gas, dissolved in 1 mL of acidified methanol (formic acid, pH 3.2), sealed in a small container under a nitrogen gas headspace, and stored in the dark under refrigeration (2°C) until used.

Analysis-CD spectra: The CD spectra of two replicate sets of each wine fraction were determined over the range of 600 to 250 nm at a scan rate of 200 nm/min. Three scans were internally accumulated to obtain a single average spectra for each sample. The individual spectra were then noise reduced using the JASCO software, and the two replications were averaged together.

One set of samples was split in half and analyzed under two different conditions. The first half was analyzed as prepared; the second half was concentrated under nitrogen or diluted with methanolic formic acid to achieve an absorbance of between 0.5 and 0.6 au at 280 nm. After adjustment, the CD of the samples was measured by the same method as the others.

Analysis-UV/Vis spectra: The UV/Vis absorbance spectra of two replicate sets of each wine fraction were determined over the range of 250 to 600 nm. The two data sets were then averaged on a point-by-point basis to generate a single average UV/Vis spectra for each sample.

Results

UV/Vis spectra: The gross spectral shapes of the Cabernet Sauvignon polymers extracted in each of the four LC fractions were distinctively different from each other, although across wine styles, the shapes were consistent within LC fraction (the Week style wine is shown as an example in Fig. 1). Absorbance maxima in the UV range exceeded those in the visible range by 5 to 10X. Significant differences in UV absorbance between wine styles are attributed to skin contact effects during fermentation. Those wines that fermented longer on the skins resulted in greater levels of UV-absorbing materials in the wine. Polymers eluting in LC fractions 3 and 4 displayed the greatest skin contact effects, with the absorbance at 280 nm in the dry/acetaldehyde style wine exceeding that of the 12 °Brix style wine by 5X.

The gross spectral shapes of the fractions extracted from the Noble wine in the first three LC fractions were very similar, both to each other and across wine styles. The shape of the fourth LC fraction differed dramatically from the other three within a given wine style (Fig. 2) but was consistent among all wine styles. Absorbance maximas in the UV range exceeded those in the visible by 4 to 6X in contrast to Cabernet Sauvignon. The absorbance intensity in the LTV of the polymers eluted in the LC fractions was extremely close across wine styles (with the exception of the 12 °Brix style wine),

indicating that beyond a certain amount of skin contact, there is little additional effect on either the extraction of W absorbing materials or the inclusion of these materials into the resultant polymers.

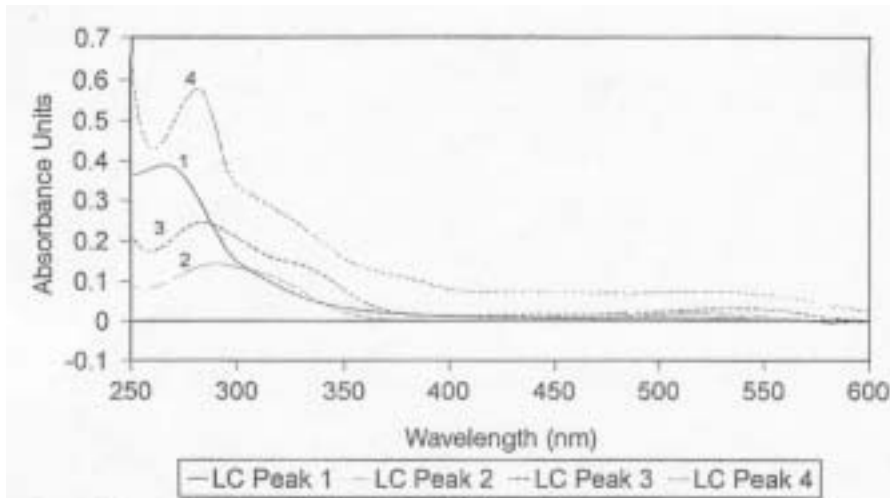


Fig. 1. Cabernet Sauvignon Week style wine UV/Vis spectra.

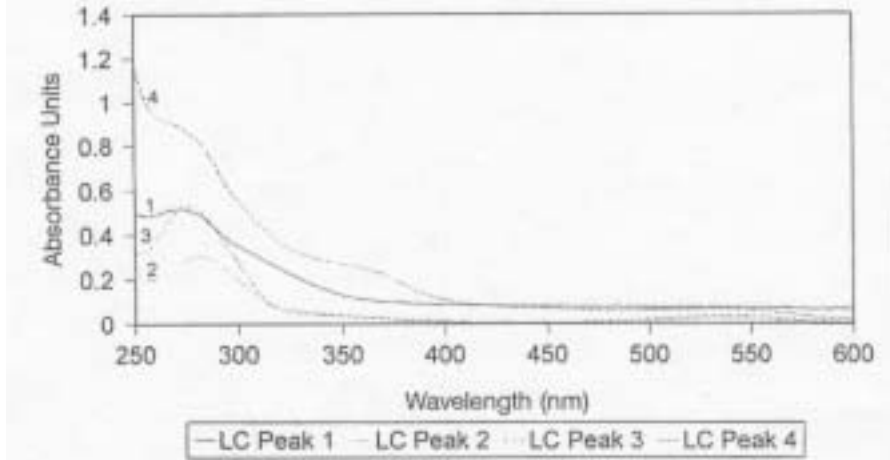


Fig. 2. Noble Week style wine UV/Vis spectra.

All of the Noble wine LC fraction spectra had absorbencies in the UV range of 250 to 350 nm exceeding those of the comparable Cabernet Sauvignon wines in the same LTV region by up to 50%. In addition, the gross shapes of the spectra from corresponding LC fractions differed between Cabernet Sauvignon and Noble wine styles. The Noble LC fraction 1 spectrum included a distinct shoulder at approximately 300 nm that was not present in the corresponding Cabernet Sauvignon spectra. The opposite case was evident in LC fractions 2 and 3. The spectra from LC fraction 4 in the two grape species wines were completely different; the Cabernet Sauvignon spectra had a distinct LTV maxima while the Noble did not.

The effect of acetaldehyde on the Dry wine styles differed by both LC fraction and grape species (data not shown). In all of the Noble LC fractions, the Dry/acetaldehyde style wine spectra always had lower absorbance than the Week style wine spectra and was nearly an exact copy of the Dry style wine spectra. In the Cabernet Sauvignon wines, the spectra's relative intensity changed with the LC fraction. In LC fractions 1 and 4, the Dry/acetaldehyde style

wine spectra had the greatest absorbance across the entire spectrum, while in LC fraction 2 it fell below both the Dry and Week style wine spectra.

Circular dichroism spectra: In both Cabernet Sauvignon and Noble wines, there was no CD activity from 600 to 360 nm. There was very little optical activity in any of the Cabernet Sauvignon fractions from most of the wine styles. The only major exceptions were fraction 4 of the Cabernet Sauvignon Dry/acetaldehyde (data not shown) and Week style wines (Fig. 3), which showed slight but significant optical activity in the range of 280 to 250 nm. Noble wines had significant optical activity in the 360 to 250 nm wavelength range in all wine styles and in all fractions (Fig. 4).

The CD spectra of the Cabernet Sauvignon fraction samples that were adjusted to 0.5 to 0.6 au at 280 nm had the same profile as the non-adjusted samples. The spectra of the adjusted Noble samples reflected a concentration/dilution effect proportionate to the level of adjustment. Those that had been concentrated showed greater CD values, and those that had been diluted showed diminished CD values in the 360 to 250 nm range. As in the Cabernet Sauvignon case, adjustment of the Noble LC fractions had no effect on the CD from 600 to 360 nm.

Discussion

UV/Vis spectra: UV/Vis spectral scans of the LC fractions are used to indicate differences between the eluted fractions within wines of the same grape species and across grape species. These spectra show that Cabernet Sauvignon wines respond to increasing skin contact time during fermentation differently than Noble wines. The UV absorbance of the Cabernet Sauvignon wine LC fraction spectra increased with increasing skin contact time, while the Noble wine LC spectra were very similar, regardless of skin contact time.

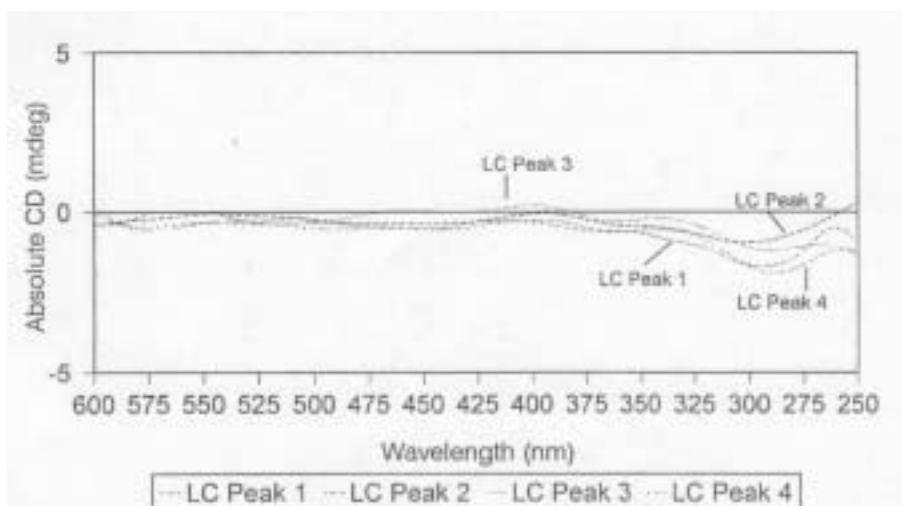


Fig. 3. Cabernet Sauvignon Week style wine CD spectra.

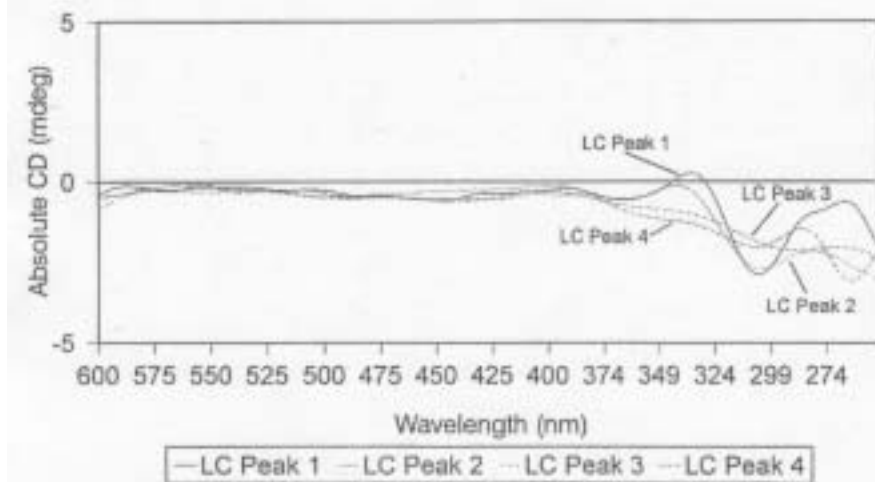


Fig. 4. Noble Week style wine CD spectra.

The observed skin contact effect can be interpreted in at least two ways: (1) skin contact extracted more anthocyanins and other phenolics from the Cabernet Sauvignon grapes than the Noble grapes with increasing time; or (2) the anthocyanin polymerization resulted in relatively more UV-absorbing compounds with increasing skin contact time in the Cabernet Sauvignon wines. It is well understood that increasing skin contact time results in increased phenolic extraction into red wines. It is apparent from this research that the phenolics extracted into Cabernet Sauvignon and Noble red wines polymerize differently, resulting in different UV/Vis and CD spectra. The shapes of the LC spectra also indicate that there were differences in the wine fractions. In the

Cabernet Sauvignon wines, each of the LC fraction UV/Vis spectra were distinctly different from each other. Across Cabernet Sauvignon wine styles, the spectra within each LC fraction were not different in shape, indicating that similar compound classes eluted off the column at concentrations corresponding to the skin contact level (with the exception of the Dry/acetaldehyde style). Acetaldehyde (in the

Dry/acetaldehyde style) reacted primarily with the third polymer fraction, forming relatively more of the fourth polymer fraction compounds than that found in the Dry style wine.

Across Noble wine styles, the spectra within each LC fraction were also identical but showed almost no skin contact effect. The spectra of LC fractions 2 and 3 were very similar to each other, mainly differing in absorbance intensity, while LC fractions 1 and 4 were of dramatically different shape when compared to the other two fractions and each other. However, the compounds eluted in LC fractions 2 and 3 are of different polarity (as evidenced by their elution from the LC column). The similarity of the UV/Vis spectra is therefore most likely coincidence.

Vitis vinifera wines have been shown to contain acylated anthocyanins (9) while no acylated compounds have been found in *Vitis rotundifolia* wines or grapes. The acylated compounds in the Cabernet Sauvignon wines may be one cause for the differences in the UV/Vis spectra of the LC fractions, and their absence in the Noble wines may also explain the strong similarities in LC fractions 1-3.

Circular dichroism spectra: CD is used to determine chirality or molecular asymmetry in compounds and complexes. Extensive research by Hoshino (10,11), Hoshino and Goto (12), Hoshino and Matsumoto (13), and Hoshino *et al.* (14,15,16) has shown CD to be a valuable tool in determining the self-association of diglucoside anthocyanins. By examining the CD spectra of the fractions eluted off the LC column, crude structural characteristics can be determined. The absence of optical activity in Cabernet Sauvignon wine fractions indicates that either there is little if any self association or induced chirality in the pigment polymers or the fractions consist of equal concentrations of compounds that absorb either right or left circularly polarized light which thereby cancel out the total CD spectra. The absence of optical activity in adjusted Cabernet Sauvignon wine fractions indicates that the observed effect is not concentration dependent as was the case in Noble wine fractions. This is further evidence of the absence of chirality or self association in these compounds. Acylated anthocyanins [e.g., the model presented by Brouillard and Delaporte (5)] would be sterically hindered from self

association, and the inclusion of such molecules in a polymer would preclude the vertical stacking observed in diglucoside anthocyanins (10). These anthocyanin polymers can, therefore, be visualized as either planar in structure or composed of a mixture of structures that are chiral but cancel the net CD of the fraction.

The CD spectra exhibited in the Noble wine fractions indicate chirality in the compounds eluted. The magnitude of the CD spectra of fraction 1 compounds are greater than those of fraction 2 and are of roughly the same shape. They eluted from the LC column first, indicating that the fraction 1 compounds are the most polar of the four fractions, and are of relatively small molecular weight. They are not as large as those eluted in fraction 2, which were bound more tightly by the column and are less polar. The greater binding level is most likely the result of a larger molecular weight and more potential binding sites on the molecule. The structure of the fraction 2 compounds must be very similar to that of fraction 1 compounds, differing perhaps in the presence or absence of compounds such as terminal flavan-3-ols that could reduce the CD spectra and change the UV/Vis spectra.

The Noble wine fraction 3 and 4 compounds had CD spectra that differed from each other and those of LC fractions 1 and 2. The CD spectra of LC fractions 2 and 3 show that despite the similarity in the UV/Vis spectra, these compounds differ in optically active components and/or structure. The LC fraction 4 compounds are composed of different structural elements than those of the other fractions, as evidenced by both the UV/Vis and CD spectra. The CD spectra of the fractions eluted from each Noble wine style were of nearly the same magnitude and of the same shape, indicating that skin contact time did not affect the structure of the eluted polymers. Vertical stacking of the anthocyanins and other components of the polymer [similar to that described by Hoshino (20)] is a potential explanation for the CD observed in these molecules. A vertically stacked polymer could include nonanthocyanin phenolics, achieve stability, and be chiral.

Adjustment of the samples to a consistent absorbance value at 280 nm, which is in the center of the wavelength range showing the greatest CD effect, eliminates concentration differences between the samples. The Cabernet Sauvignon samples showed no change in CD with adjustment, indicating that the cause was not a function of concentration. The Noble samples, on the other hand, did show a concentration effect, indicating that the observed CD is due to the structure of the polymers. Further analysis of these compounds must be accomplished to fully visualize these structures.

Conclusions

The dominant UV/Vis and CD spectra extend from 375 to 250 nm in both Cabernet Sauvignon and Noble wines. Since there were no significant Cabernet Sauvignon CD spectra, even when the samples were adjusted to a consistent absorbance value at 280 nm, there is no evidence of chirality or vertical stacking in the structure of the anthocyanin polymers. The Noble polymers displayed significant CD spectra on native and absorbance adjusted samples. The readily apparent differences in the UV/Vis spectra can be used to help identify structural differences in the eluted compounds. The Noble anthocyanin polymer structures are most likely vertically stacked.

The differences in the UV/Vis and CD spectral shapes of the anthocyanin polymers that elute from the LC column are related to differences in the polymer components. Normal anthocyanin spectra at the pH used do not exhibit such significantly different UV and visible absorbance intensities. This disparity can be explained only by the inclusion of other UV absorbing compounds in the polymer structure, which lead to the expressed UV/Vis spectra. As the polymer increases in molecular weight, the polymer decreases in polarity and elutes later from the LC column.

Both the UV/Vis and CD spectra of the Noble wine polymers were closely grouped in shape and intensity across all wine styles, indicating that skin contact (beyond a certain point) in Noble wines does not affect the composition or structure of the anthocyanin polymers that form. Acetaldehyde was shown to have minimal effects on either the UV/Vis or CD spectra of Noble wines. It is, therefore, unclear where or how the acetaldehyde reacts with the anthocyanin polymers in Noble wines. Possibly structural characteristics of the diglucoside anthocyanin polymers make it sterically less favorable for acetaldehyde to bond in any significant manner.

The UV/Vis spectra of the *Vitis vinifera* cv. Cabernet Sauvignon wine polymers differed significantly in intensity across wine styles, indicating a difference in polymer concentration with skin contact. Acetaldehyde in Cabernet Sauvignon wines was shown to significantly alter the UV/Vis spectra of three of the fractions found in the wines. These effects are most likely due to changes in electron stability induced by inclusion of acetaldehyde and other nonanthocyanin compounds in the polymer structure. Further research must be conducted to determine the identities of the compounds that are evidently included in these complex structures.

Literature Cited

1. Asen, S., R. N. Stewart, and K. H. Norris. Co-pigmentation of anthocyanins in plant tissues and its effect on color. *Phytochemistry* 11:1139-1144 (1972).
2. Asen, S., R. N. Stewart, and K. H. Norris. Anthocyanin, flavonol copigments and pH [hydrogen-ion concentration] responsible for larkspur flower color (*Consolida ambigua*; *Delphinium ajacis*). *Phytochemistry* 14:2677-2682 (1975).
3. Bakker, J., P. Bridle, C. F. Timberlake, and G. M. Arnold. The colours, pigment and phenol contents of young port wines: Effects of cultivar, season and site. *Vitis* 25:40-52 (1986).
4. Bourzeix, M., and N. Heredia. Estimation qualitative de la matiere colorante du vin rouge. OIV Doc. no. 1290/85.

5. Brouillard, R., and B. Delaporte. Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, tautomeric reactions of malvidin-3-glucoside. *J. Am. Chem. Soc.* 99:8461-8468 (1977).
6. Datzberger, K., I. Steiner, J. Washuttl, and G. Kroyer. Methods for fast analysis of anthocyanins and anthocyanidins in red wine. *Z. Lebensm. Unters Forsch* 193:462-464 (1991).
7. Datzberger, K., I. Steiner, J. Washuttl, and G. Kroyer. The influence of wine additives on colour and colour quality of young red wine. *Z. Lebensm. Unters Forsch* 194:524-526 (1992).
8. Etievant, P., P. Schlich, A. Bertrand, P. Symonds, and J-C Bouvier. Varietal and geographic classification of french red wines in terms of pigments and flavonoid compounds. *J. Sci. Food Agric.* 42:39-54 (1988).
9. Hebrero, E., C. Santos-Buelga, and J. C. Rivas-Gonzalo. High performance liquid chromatography-diode array spectroscopy identification of anthocyanins of *Vitis vinifera* variety Tempranillo. *Am. J. Enol. Vitic.* 39:227-233 (1988).
10. Hoshino, T. Self association of flavylium cations of anthocyanidin 3,5-diglucosides studied by circular dichroism and ¹H NMR. *Phytochemistry* 31:647-653 (1992).
11. Hoshino, T. An approximate estimate of self-association constants and the self-stacking conformation of malvin quinonoidal bases studied by ¹H NMR. *Phytochemistry* 30:2049-2055 (1991).
12. Hoshino, T. and T. Goto. Effects of pH and concentration on the self-association of malvin quinonoidal base--electronic and circular dichroic studies. *Tetrahedron Letters* 31:1593-1596 (1990).
13. Hoshino, T. and U. Matsumoto. Evidences of the self-association of anthocyanins I. Circular dichroism of cyanin anhydrobase. *Tetrahedron Letters* 21:1751-1754 (1980).
14. Hoshino, T., U. Matsumoto, and T. Goto. Self-association of some anthocyanins in neutral aqueous solution. *Phytochemistry* 20:1971-1976 (1981).
15. Hoshino, T., U. Matsumoto, N. Harada, and T. Goto. Chiral exciton coupled stacking of anthocyanins: interpretation of the origin of anomalous CD induced by anthocyanin association. *Tetrahedron Letters* 22:3621-3624 (1981).
16. Hoshino, T., U. Matsumoto, T. Goto, and N. Harada. Evidences for the self-association of anthocyanins IV. PMR spectroscopic evidence for the vertical stacking of anthocyanin molecules. *Tet. Letters* 23:4334-36 (1982).
17. Jackson, M. G., C. F. Timberlake, P. Bridle, and L. Vallis. Red wine quality: Correlations between colour, aroma and flavor and pigment and other parameters of young beaujolais. *J. Sci. Food Agric.* 29:715-727 (1978).
18. Johnston, T. V., and J. R. Morris. Separation of anthocyanin pigments in wine by low pressure column chromatography. *J. Food Sci.* 61(1):109-111.
19. Joslyn, M. A., and A. Little. Relation of type and concentration of phenolics to the color and stability of rose wines. *Am. J. Enol. Vitic.* 18:138-148 (1967).
20. Scheffeldt, P., and G. Hrazdina. Copigmentation of anthocyanins under physiological conditions. *J. Food Sci.* 43:517-520 (1978).
21. Sims, C. A., and J. R. Morris. Effects of acetaldehyde and tannins on the color and chemical age of red muscadine (*Vitis rotundifolia*) wine. *Am. J. Enol. Vitic.* 37:163-165 (1986).
22. Singleton, V. L., H. W. Berg, and J. F. Guymon. Anthocyanin color level in port-type wines as affected by the use of wine spirits containing aldehydes. *Am. J. Enol. Vitic.* 15:75-81 (1964).
23. Somers, T. C. The polymeric nature of wine pigments. *Phytochem.* 10: 2175-2186 (1971).
24. Timberlake, C. F., and P. Bridle. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Amer. J. Enol. Vitic.* 27:97-105 (1976).
25. Wildenrad, H. L., and V. L. Singleton. The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Vitic.* 25:119-126 (1974).