

## Analysis of Red Wine Pigment Polymers by Low Pressure Liquid Chromatography

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**Abstract** A liquid chromatographic (LC) method for the direct injection of wine has been developed that fractionates wine pigments into four distinct fractions based on size exclusion (and by extension, molecular weight) and polarity. This method is a modification of the discrete chromatographic method of Bourzeix and Heredia (1985), and provides detailed information on the characteristics of the eluted pigment polymers. Wines made from *Vitis vinifera* (Cabernet Sauvignon) and *Vitis rotundifolia* ("Muscadine" cv. Noble) grapes were analyzed using this procedure to examine the polymerization behavior of mono- and diglucoside anthocyanins. The two anthocyanin types are found to behave completely differently upon storage. Monoglucoside anthocyanins, found in Cabernet Sauvignon wine, aged to form a higher percentage of larger molecular weight and lower polarity polymers than did the diglucoside anthocyanins found in Muscadine wines. Acetaldehyde, a compound previously shown to enhance anthocyanin polymerization, is shown to have a minor effect on diglucoside anthocyanin polymers and a major effect on monoglucoside anthocyanin polymers. Acetaldehyde addition to the wines increased the proportion of higher molecular weight, reduced polarity polymers in the Cabernet Sauvignon wines and had almost no effect on the polymer population in the Muscadine wines. This difference in effect is theorized to be directly related to polymer shape and structure, which inhibits the interaction of acetaldehyde with the diglucoside polymers.

**Introduction** Anthocyanins polymerize with other wine components (and themselves) over time, leading to greater color stability. There are presently no satisfactory methods of isolation or extraction of Figure 1. these polymers for further analysis. This research resulted in the development of a low-pressure, tri-solvent liquid chromatographic method of extraction of anthocyanin fractions directly from a wine without prior dilution.

In this procedure, wines are directly injected onto a silica/PVPP column; pigment fractions are eluted by a combination of size exclusion and polarity mechanisms. No intermediate sample preparation is required, and results are consistent over a large number of samples.

**Materials and methods** The liquid chromatographic method of Bourzeix and Heredia (1985) was modified to include water as an eluant and converted to a gradient type method. A 15 x 1.0 cm glass column fitted with a bed height adjuster was filled to a height of 35 mm with silica gel 60, polyvinyl polypyrrolidone, and silica gel G at the ratio of 70:20:10 (respectively by weight). Eluents were HPLC grade water (solvent A), HPLC grade methanol (solvent B), and 88% formic acid (solvent C). The gradient profile was: 0-7 min, 100% A, 7-10 min, 100% A to 100%B (linear), 10-16 min, 100%B, 16-19.5 min, 100% B to 35% C in B (linear), 19.5-21 min, 35-80% C in B (linear), 21-28 min, 80% C in B to 100% C (linear), 28-32 min, 100%C. The flow rate was constant at 1.5 ml/min, and the gradient was operated at room temperature (21C). Elution from the column was monitored at 525nm.

One milliliter samples of wines made in the fall of 1992 and 1993 were used in this research. Both *V. vinifera* (Cabernet Sauvignon) and *V. rotundifolia* (Muscadine cv. Noble) wines were made. Red wines in which the skins were removed from the fermenting juice when the juice achieved 0 °Brix were made. The batches of wine were split into two parts to make up two styles of wine. The first was untreated (control); the other was treated by adding 1.5 mM acetaldehyde after fermentation.

**Results** Each wine resolved into a water soluble, a methanol/water soluble, and two methanol/formic acid soluble fractions (Figs. 1-4). The chromatograms of each grape species control wine were significantly different with respect to the relative proportions of fractions 3 and 4 (the "polymerized" and "condensed polymers", respectively; Bourzeix and Heredia (1985), Datzberger et al. (1991))-the Cabernet wine had more condensed material while the Noble wine had more polymerized material. Acetaldehyde dramatically affected the Cabernet wine profile (Fig. 2)- the third peak was completely eliminated and the second peak was sharply diminished (62% less than the control). Acetaldehyde addition to the Noble wine increased peak 2 by 14% and peak 4 by 35% at the expense of peaks 1 and 3 (Fig. 4).

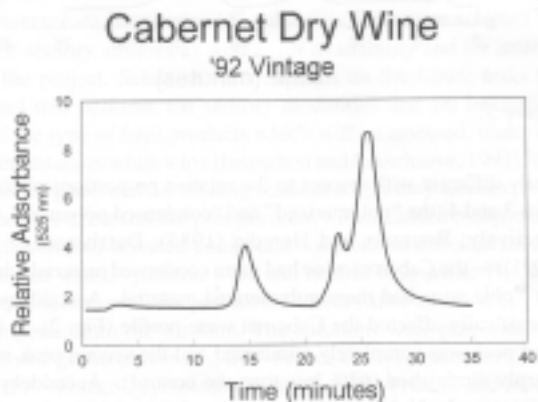


Figure 1.

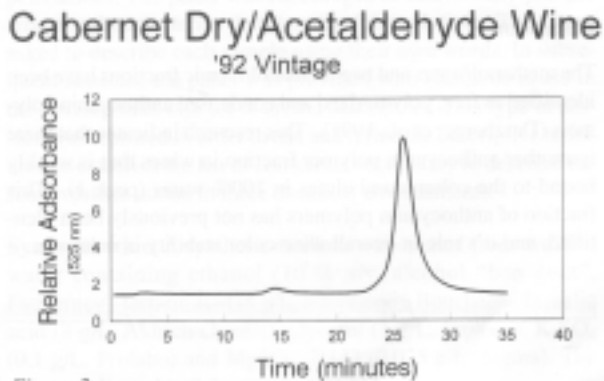


Figure 2.

### Muscadine Dry Wine '92 Vintage

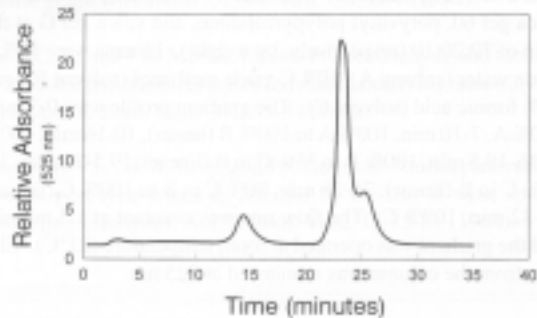


Figure 3.

### Muscadine Dry/Acetaldehyde Wine '92 Vintage

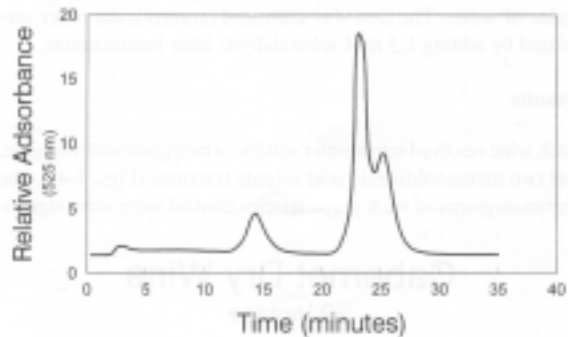


Figure 3.

**Discussion** The methanol/water and two methanol/formic fractions have been identified as free, polymerized and condensed anthocyanin polymers (Datzberger et al., 1991). This research indicates that there is another anthocyanin polymer fraction in wines that is weakly bound to the column and elutes in 100% water (peak 1). This fraction of anthocyanin polymers has not previously been identified, and its role in overall wine color stability is unknown.

The differences between the profiles of the Cabernet wines with and without added acetaldehyde, indicate that acetaldehyde accelerates the condensation of monoglucoside pigments. The Muscadine wines reacted with acetaldehyde minimally, resulting in a slightly different elution profile. Acetaldehyde has been shown to increase the "chemical age" and improve the long term (8 months storage) color stability of Muscadine wines (Suns and Morris, 1986), and has been shown to increase polymerization of anthocyanins in Cabernet Sauvignon wines (Timberlake and Bridle, 1977).

Diglucoside anthocyanins have been shown to vertically stack in a helical manner when self-associated (Hoshino, 1991). If this structure predominates in the Muscadine wines, further polymerization of these structures would be precluded by steric hindrance and thereby prevent significant interaction with acetaldehyde.

**Conclusions** Using gradient low pressure chromatography, it has been shown that the anthocyanins of Cabernet Sauvignon and Noble wines polymerize differently. The liquid chromatographic technique developed in this research will allow the study of anthocyanin polymerization behavior in all types of wine, including those made with hybrid grapes.

Since stable wines are composed of a higher percentage of condensed anthocyanin polymers (which correspond to fraction 4 of the chromatograms), this liquid chromatographic technique may potentially be used to project a wine's color stability upon aging. Research beyond the scope of this initial effort must be conducted to develop definitive parameters of aging potential.

## References

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