

Anthocyanin-Tannin Interactions Explaining Differences in Polymeric Phenols Between White and Red Wines

VERNON L. SINGLETON^{1*} and EUGENE K. TROUSDALE²

Three white table wines were treated with additions of purified anthocyanins at 0, 250, and 500 mg/L and 0, 500, and 1000 mg GAE/L of grape-seed tannin in all possible combinations. The results of analyses for polymeric phenols were very consistent with the different wines and show that anthocyanins quickly complex with polymeric phenolic tannins to retain them in solution. The lack of such complexing explains why wines made from white grapes by red vinification methods are deficient in tannins and astringency. These anthocyanin-tannin complexes that form quickly in red wine differ from those produced by aging in remaining relatively adsorbable by Sephadex LH-20. These results have important implications for red wine astringency and other quality and processing characteristics.

KEY WORDS: anthocyanin, polymeric phenols, tannin

Polymeric tannin is high in grape seeds, moderate to low in skins and absent in clarified juice (2, 8). Seeds can contribute appreciable tannin to pomace-fermented wines (6). It is considered axiomatic that more complete extraction of phenols, including polymeric tannins from pomace, gives a more tannic, astringent wine from a given lot of grapes.

In order to study wine aging and oxidation reactions without the complications caused by anthocyanins, we became interested in making astringent, tannic white wines. These complications are large because 20 or more HPLC peaks represent anthocyanins and their derivatives in *Vitis vinifera* red wines (16,17). Five different anthocyanidin-3-glucosides are generally present in red wine grapes in various ratios depending on variety. In wine, each will be present in the chromenol (pseudobase), flavylum, and anhydrobase (quinoidal) forms and possibly also flavene, chalcone or free anthocyanidin forms. Each glycoside form may be acylated with *p*-coumaric, caffeic, acetic, and possibly other acids. Clearly, elimination of this group would make the system simpler and comparative studies more interpretable.

White grapes have overlapping phenol content with red grapes. Their seeds and free-run juice appear the same both qualitatively and quantitatively if one allows for sizeable varietal differences (7,8,10). The skins, obviously, do not contain anthocyanins in white grapes, but evidence has been presented that otherwise they are similar to red grape skins (12). It was anticipated that white wines fermented on white grape pomace would be like red wines without the red color.

However, white grapes fermented on their pomace under conditions typical for red table wines do not make wines that, blindfolded, one would take for red (9). Increased in phenol and coarsened in flavor, they nevertheless were not nearly as astringent and did not resemble red wines in flavor. Perhaps this should not have been surprising, since early Egyptian, Greek and Roman white wines could not have been made by cold fermenting of clarified juice and yet were described as quite different from their reds. Furthermore, as recently as 20 years ago, at least one California winery was making its white table wines by the same three to six-day pomace fermentation as its reds! They were not great wines, but they sold along with more modern types. It had also been observed that dry blush wines made in our cellars frequently had a harsher taste than similar white grape wines and that is one reason blush wines are more attractive slightly sweet. The anthocyanins themselves do not appear to account for appreciable direct flavor in the limited testing we have done or others have reported. For example, 10 mg of the purified anthocyanin used in this study placed directly on the tongue had very little taste and was neither astringent nor bitter. An explanation for these observations was not immediately apparent, but was desirable.

In the course of developing and applying a chromatographic method to determine polymeric tannin in young wines (2,3) it was found that even wines with fairly long pomace contact but from white grapes did not have appreciable polymeric tannin, whereas those from red grapes did. It had been known for some time (8) that the anthocyanins of older wines become combined into the polymeric phenol fraction. The new observations appeared to mean that anthocyanins helped retain tannin (and therefore astringency) which would otherwise (as with white pomace) be lost. The research reported here is part of an effort to investigate this situation. Here we report studies involving addition of purified anthocyanins and isolated grape-seed tannin to finished white wines. Additional studies involving addition of high-pigment extracts to white musts of various

^{1,2}Department of Viticulture and Enology, University of California, Davis, CA 95616-8749, USA.

*Author to whom correspondence should be addressed.

Acknowledgements: The following are thanked for financial contributions toward our work. The American Vineyard Foundation; Hewlett-Packard Company; Nippi, Inc., Tokyo; California Wine Grape Growers' Advisory Board, and the Wine Institute.

This research was conducted at the University of California, Davis, CA 95616-8749.

Manuscript submitted for publication 8 May 1991.

Copyright © 1992 by the American Society for Enology and Viticulture. All rights reserved.

types prior to fermentation will be separately reported.

Materials and Methods

Finished white wines from settled musts, made the two previous vintages, were treated with grape-seed tannin, anthocyanin, and combinations of each. After a short period approximating red wine fermentations (at least ten days at room temperature in each instance), the samples were centrifuged and analyzed for total phenols and polymeric phenols. The spectral determinations of anthocyanins were made after an additional 90 days.

Wines: The wines were:

	Variety	Vineyard	Pomace contact	Must Brix	Must acid (g/L)	Wine pH
A	Sauvignon blanc	Davis	0	21.8	11.0	3.17
B	Sauvignon blanc	Oakville	0	20.7	6.8	3.00
C	Colombard	Davis	4 hr	22.3	7.8	3.12

Anthocyanin preparation: Fresh, unground, manually deseeded, Limburger grape skins (chosen for high proportional malvidin-3-glucoside content) were extracted with methanol and the extracts concentrated under vacuum at room temperature to an aqueous syrup. This was passed onto a column of polyamide (Nylon) powder. Sugars and acids were eluted with deionized water and the anthocyanins were then eluted as a group with aqueous 70% methanol acidified with acetic acid. Polymeric pigments were retained on the polyamide and discarded. The anthocyanin fraction was concentrated to dryness in a rotating-flask vacuum system with the bath temperature at 27°C. The obtained solid was not hygroscopic and was stored dry at -20°C until needed. This solid analyzed by HPLC as 68.2% (of total 280 nm peak area) malvidin-3-glucoside, 21.8% peonidin-3-glucoside, 5.0% petunidin-3-glucoside, 1.0% cyanidin-3-glucoside and less than 4% of 280 nm-absorbing material in the region of the polymeric group. Thus, it was nearly free of polymeric pigment and free of tannin (which would absorb highly at 280 nm, but not at the maximum for red pigments near 520 nm).

Seed tannin: Flame Tokay seeds were separated and extracted exhaustively with 50% aqueous ethanol. The extract was concentrated *in vacuo* at room temperature to remove the ethanol, the residual solution frozen and lyophilized. The beige-colored, fluffy, non-hygroscopic solid was stored near 0°C, dry, and in dark bottles until needed. Two-dimensional paper chromatograms showed the same phenolic pattern after storage as from the fresh seeds. The concentration of total phenol in this solid was 610 mg of gallic acid equivalent per gram by Folin Ciocalteu colorimetry (11).

Experimental samples: A portion (350 mL) of the wine was prepared without addition of pigment, another with the dry anthocyanin preparation added at 250 mg/L and a third with anthocyanin added at 500 mg/L. They were mixed until the pigment was uniformly dissolved and the pH verified unchanged. From each of

these three samples, subportions were treated with 0, 500, and 1000 mg GAE/L of dry seed tannin. The pH was unchanged. Not quite all the added seed tannin dissolved and any precipitate was kept dispersed in the wine. The final nine samples were placed in 50 mL screw-capped centrifuge tubes, the headspace replaced by nitrogen. These tubes were kept sealed, in the dark, at room temperature (about 26°C), with thorough mixing twice daily for 10 to 14 days. The tubes were then centrifuged (table-top clinical centrifuge) and the clear supernatant taken for analyses. Portions saved for later use were resealed under nitrogen. Anthocyanins were analyzed about 90 days later by spectral analysis.

Analyses: Total phenol was by colorimetry versus a gallic acid standard (11). Total anthocyanin was calculated using 38 000 molar extinction from 520 nm absorbance at pH 1. Polymeric anthocyanin was calculated similarly from the 520 nm absorbance remaining after SO₂ decoloration (13). Visible color was the sum of 520 and 420 nm absorbance and hue the ratio of 420 nm absorbance divided by 520 nm absorbance (1 mm cells, wine pH, undiluted).

Since it had been shown that retention by dialysis, Sephadex LH-20 chromatography, and HPLC gave similar values with young red table wines for their content of phenolic polymers (2,3), HPLC was applied here. The polymeric group was identified as that compact but wide group of poorly separated peaks of retention time between 36 and 44 minutes under our defined conditions. It was calculated as mg/L of catechin using the (+)-catechin response factor (µg/unit peak area) at 280 nm and also as malvidin-3-glucoside mg/L from its response factor at 520 nm absorbance. No significant fraction of UV-absorbing material was lost and the same column was recycled many times without ghost peaks or changed properties.

The HPLC apparatus was a Hewlett-Packard 1090 M with diode array UV-visible spectrum detector (Hewlett-Packard Company, Palo Alto, CA) coupled to an HP Chem Station and an HP 7475A plotter. Closely filtered samples were injected, 10 µL each. A 4.6 × 100 mm column packed with 3µ reversed phase C18 (Phenomenex, Rancho Palos Verdes, CA) and with a C18 guard column was used under the following gradient conditions. Solvent A = 0.05 M NH₄HPO₄ in water, pH 2.6; Solvent B = 20% solvent A mixed with 80% acetonitrile.

Gradient Time	Ending Concentration	
	% A	% B
0 to 5 minutes	100	0
5 to 20 minutes	85	15
20 to 35 minutes	77.5	22.5
35 to 45 minutes	37.5	62.5
45 to 55 minutes	0	100
55 to 0 minutes	100	0

UV-visible spectra were taken by a Perkin-Elmer (Norwalk, CT) Lambda Array 3840 spectrophotometer

with their model 7500 Laboratory Computer. To avoid dilution effects, measurements were made using 1 mm quartz cuvettes.

Results and Discussion

All wines without added pigment (anthocyanin) or seed tannin showed only trace peaks and essentially baseline absorbance in the polymer group range (36 to 44 minute retention time) at 280 nm. At 520 nm, only baseline absorbance was exhibited unless anthocyanin had been added. Examples of the full HPLC scans at 280 nm are given in Figure 1 for wine C alone (T0P0), with

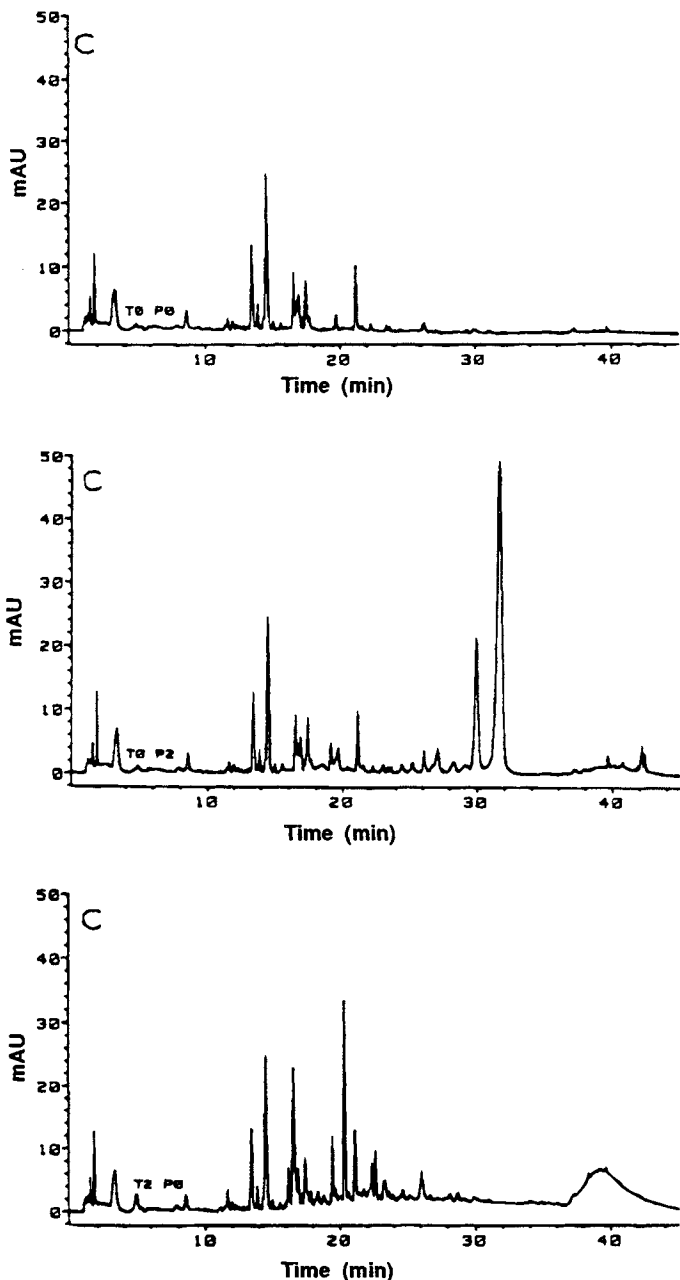


Fig. 1. Representative HPLC patterns (wine C, 280 nm absorbance) for the wine (T0P0), wine plus 500 mg/L of anthocyanin (T0P2) and wine plus 1000 mg GAE/L seed tannin (T2P0).

maximum added pigment (T0P2) and with maximum added tannin (T2P0). Note the maximum concentration of added anthocyanin in the absence of added tannin gave very low amounts in the region of the chromatogram (36 to 44 minutes) representing the polymer group, whereas the polymer contribution by seed tannin addition was a major fraction of the total. Seed tannin contributed an indicator peak, just after 20 minutes, that was absent in the wine or anthocyanin preparation. The pigment preparation contributed a number of peaks not otherwise present, notably malvidin-3-glucoside about 32 minutes and peonidin-3-glucoside near 30 minutes.

Figure 2 gives a close-up of the polymeric phenol group (36 to 44 minute retention) for each wine (A, B, or C) with increasing pigment at maximum tannin and increasing tannin at maximum pigment as scanned at 280 nm. Several features are noteworthy. Tannin alone gave a relatively featureless hump (especially wine A). Pigment alone gave low polymer with some superimposed peaks. As will be shown more clearly from other data, the polymer group is enhanced more than additively by combination of pigment and tannin. Between about 37.5 and 39.5 minute retention time there are, in the samples with both pigment and tannin added, a series of sharp peaks superimposed on the main hump that do not appear when either tannin or pigment are added alone. Their regularity makes one speculate that they represent a malvidin-3-glucoside unit added to a trimeric, tetrameric, pentameric, etc., condensed tannin molecule. Further work will be necessary to prove or disprove this.

Total phenol contents after the various treatments are given in Table 1. Those of the wines without additions are given in parentheses. The increment over that level produced by the additions are given, e.g., for wine A the total phenol after addition of 500 mg/L of tannin was 553 mg/L made up of the original 248 mg/L from the wine and 305 mg/L from the addition. That the tannin addition of 500 mg/L produced only an average of 385 mg/L increase in the wine indicates some precipitation or incomplete solubility. Centrifugation did remove solids. The addition of 1000 mg GAE/L of seed tannin only produced an average of 96% of the amount predicted by doubling the amount contributed by 500 mg/L addition. The increment found as a percentage of that predicted from the appropriate comparison sample is also given in Table 1. Addition of the highest level of anthocyanin without seed tannin also indicated some insolubilization. With both anthocyanin and seed tannin present, a small increase in total phenol over that predicted from the individual components was generally found, 105% in the combined data for the highest levels of both.

It is shown much more clearly in Table 2 that addition of anthocyanins increases the solubility or retention of tannin in wines. Table 2, in a manner analogous to Table 1, shows the increments and percent found of the content predicted from the added components for the polymeric phenols (as catechin equivalent

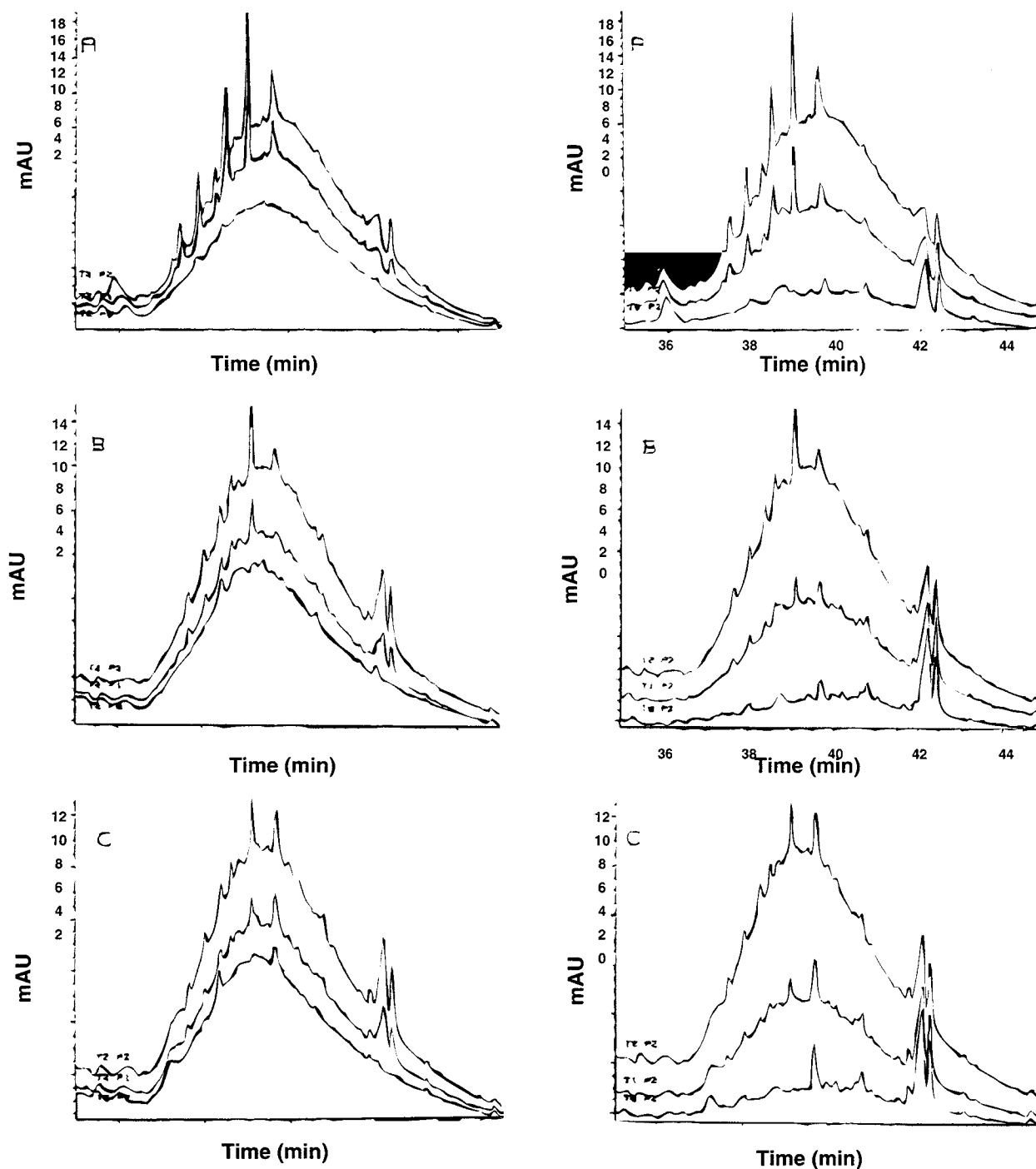


Fig. 2. The polymeric phenol group by HPLC at 280 nm for wines A, B, and C when (left) tannin at 1000 mg GAE/L addition has been augmented by anthocyanin addition at 0 (P0), 250 (P1), or 500 (P2) mg/L or (right) anthocyanin pigment at 500 mg/L has been augmented by seed tannin addition at 0 (T0), 500 (T1), or 1000 (T2) mg GAE/L.

from absorbance at 280 nm by HPLC). Alone, the pigment preparation did not add much to the polymer, the seed tannin added considerably to the polymer, and the polymer was greatly increased if pigment and seed tannin were both added. With each wine 132 to 144% of the polymer expected from the individual high additions was actually present. This seems convincing verification that the anthocyanins are the factor that keeps

the astringent tannin high in red wines relative to similarly made wines from white grapes. On the other hand, data from Table 1 and Table 2 show that relatively high (although lower than with anthocyanins) phenol and polymer content can be produced by additions to finished white wines of grape-seed tannin alone. This has not been true with fermentation of white musts on seeds, as mentioned earlier.

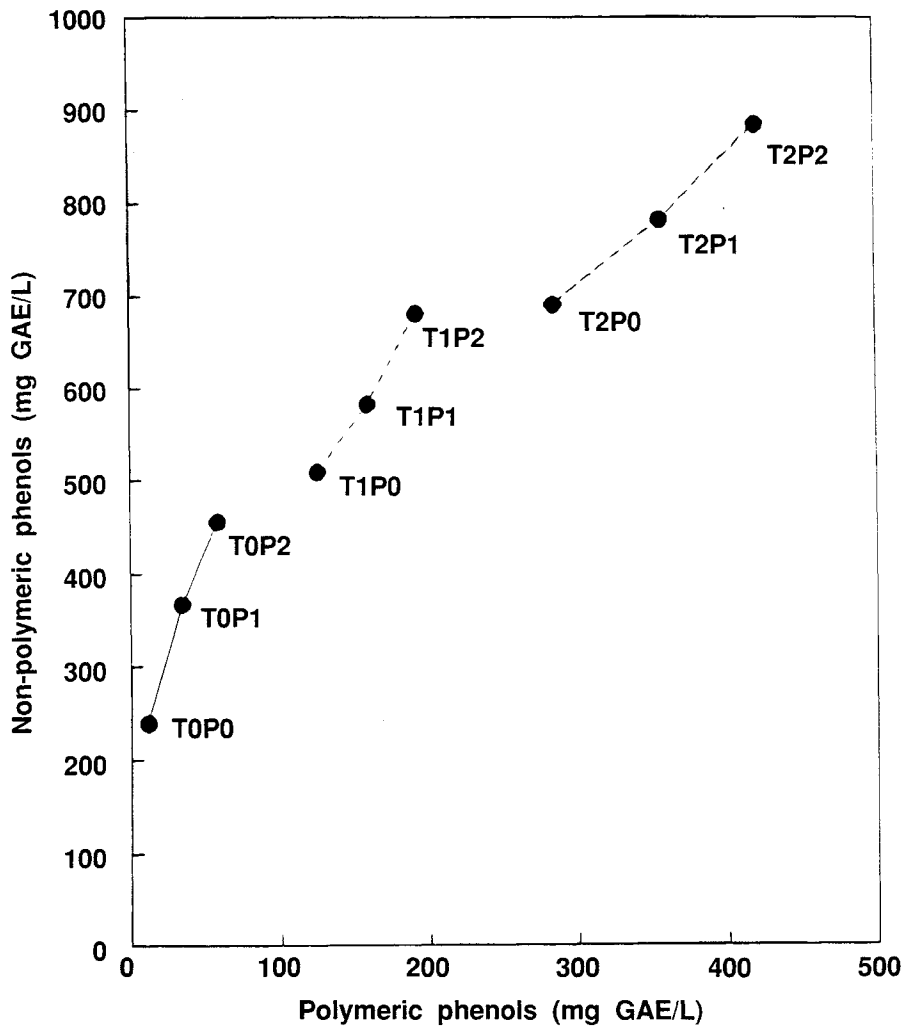


Fig. 3. Polymeric versus non-polymeric phenols as affected by addition to three white wines (mean values shown) of seed tannin at 0, 500, and 1000 mg GAE/L (T0, T1, T2) and/or anthocyanins at 0, 250, and 500 mg/L (P0, P1, P2).

Table 1. Total phenol (mg GAE/L) as a function of tannin and anthocyanin pigment addition to white wines.

	Tannin added (mg GAE/L)	Increments			Found % of expected		
		Pigment added (mg/L)			Pigment added (mg/L)		
		0	250	500	0	250	500
Wine A	0	(248)*	134	271	100**	100**	101
	500	305	323	501	100**	83	91
	1000	552	660	733	93	97	92
Wine B	0	(251)	137	240	100	100	94
	500	413	565	675	100	102	102
	1000	805	1013	1177	98	106	110
Wine C	0	(249)	181	281	100	100	87
	500	437	580	692	100	96	97
	1000	821	993	1252	95	99	111
Mean	0	(249)	151	264	100	100	93
	500	385	490	623	100	94	97
	1000	726	889	1054	96	101	105

*Values in parenthesis are the analyses for the control wines without additions. The increment of 134 for wine A + 250 mg/L of pigment results from 382 - 248 = 134.

**These values in each case are defined as 100% and used to calculate the expected values for the remaining six categories in each set from the "found" versus "expected" increments.

Table 2. Polymeric phenols (mg/L as catechin) as a function of tannin and anthocyanin pigment addition to white wines.

	Tannin added (mg GAE/L)	Increments			Found % of expected		
		Pigment added (mg/L)			Pigment added (mg/L)		
		0	250	500	0	250	500
Wine A	0	(15)	57	116	100	100	102
	500	115	236	331	100	134	141
	1000	277	472	573	119	140	144
Wine B	0	(21)	38	78	100	100	102
	500	149	194	258	100	103	112
	1000	306	387	514	103	112	132
Wine C	0	(17)	34	77	100	100	111
	500	112	172	229	100	116	119
	1000	275	343	482	106	110	135
Mean	0	(18)	43	90	100	100	104
	500	125	200	272	100	117	124
	1000	286	400	523	113	120	137

Table 3 shows polymeric pigment from HPLC calculated as malvidin-3-glucoside mg/L from 520 nm scans. Tannin alone contributes no anthocyanin, of course, but with each increased tannin addition more pigment appeared in the phenolic polymers. These data also suggest that there is a ratio between anthocyanin and tannin that is optimum for incorporating pigment (520 nm absorbance) into the polymer at least under the experimental conditions. Although more pigment and more tannin in the presence of pigment increased the total polymeric phenol retained in the wine in each case, the proportion of pigment in the polymer was highest if the seed tannin was not too high relative to the pigment, as might be expected.

Polymeric pigment, as determined by direct spectral methods based on failure of SO₂ to decolorize 520 nm absorbance, is shown in Table 4. Only the mean data are shown and only the sets for wines B and C were analyzed. Note rather close agreement with the mean HPLC data in Table 3, except for more "spill-over" 520 nm absorbance in the unfractionated white base wines.

Figure 3 shows the relationship between polymeric phenols (calculated by applying the polymer group's

percentage of total HPLC peak area at 280 nm to the total phenol by colorimetry) and the non-polymeric phenol (by difference). Clearly the addition of anthocyanin alone increases the non-polymeric phenol much more than it does the polymer, whereas addition of pigment along with seed tannin increases the polymeric portion also. The addition of anthocyanin without tannin addition gave an increase in polymer only about 20% of the non-polymer increase. Addition of anthocyanin along with 500 mg GAE/L seed tannin increased the polymer about 40% as much as the non-polymer increase. With 1000 mg GAE/L seed tannin and anthocyanin addition, polymer increased 70% as much as the non-polymer did. When tannin was added at a constant level of pigment (0, 250, 500 mg/L) the second tannin increment (500 to 1000 mg GAE/L) gave 1.7 to 2.1 times the polymer per unit of non-polymer as did the first level of tannin (0 to 500 mg GAE/L). The polymer increase was higher and more nearly constant with tannin addition than with anthocyanin addition, showing that the anthocyanin affects the tannin retained but alone does not affect the polymeric tannin appreciably.

These data show that anthocyanins can quickly (less than 14 days) complex with seed tannins in wines

Table 3. Polymeric pigment (mg/L) malvidin-3-glucoside by HPLC at 520 nm as a function of tannin and anthocyanin pigment addition to white wines.

	Tannin added (mg GAE/L)	Increments			Found % of expected		
		Pigment added (mg/L)			Pigment added (mg/L)		
		0	250	500	0	250	500
Wine A	0	0	15	30	100	100	100
	500	0	30	54	100	200	180
	1000	0	46	75	100	307	250
Wine B	0	(2)	8	17	100	100	106
	500	1	13	27	100	136	145
	1000	0	19	36	--	210	189
Wine C	0	(2)	10	18	100	100	91
	500	0	12	21	100	100	115
	1000	1	15	28	--	131	143
Mean	0	(1)	8	22	100	100	135
	500	1	19	34	100	200	146
	1000	1	27	47	67	270	200

Table 4. Mean polymeric pigment from 520 nm absorbance after bleaching with SO₂ of wine sets B and C with additions of anthocyanin pigment and/or grape-seed tannin.

Increment Tannin added (mg GAE/L)	Polymeric pigment (SO ₂ resistant, mg/L)		
	Pigment added (mg/L)		
	0	250	500
0	(14)	8	18
500	2	18	38
1000	5	38	54
Found % of expected			
0	100	100	100
500	100	132	151
1000	105	186	184

to increase the amount of polymer retained in the wine. It is not new that anthocyanin-tannin polymers form in wine and the polymers give more stable (to SO₂ and pH change) red color. What is new is the evidence that the polymer and therefore the astringency is lost if white grapes are fermented as if they were red and anthocyanins are not present to complex with and hold the tannins in the wines.

Table 5 shows the relative visible color intensity (420 + 520 nm absorbance) and hue (420/520 nm absorbance), mean values for wines B and C. Seed tannin additions increased the brown of the wine (higher sum and ratio) whereas anthocyanin increased the red (higher sum, lower ratio). There is also an indication of the augmentation of the color by a combination of tannin plus pigment over that predicted from the individual solutions but this is complicated by co-pigment effects and Beer's law deviations. Total anthocyanin by strong acidification followed by spectral determination did not show such augmentation.

The polymer group is forced off the column near the front of greatly increased acetonitrile in the eluent so there is not much opportunity for fractionation of the group. Nevertheless, since there are new and sharp peaks within or on the main HPLC hump of polymer, it is believed that the anthocyanins become bound to the tannins. Since the red pigment in the polymer is resistant to SO₂ bleaching it is believed to be bound via a linkage through carbon 4 of the anthocyanin and probably an A-ring position (six or eight) in the condensed tannins (8,14,15). Association of the co-pigmentation type is disrupted by solvents and such loose associations should have been disrupted by HPLC. Equilibria give at

Table 5. The mean (wines B and C) color intensity (420 + 520 nm absorbance) and hue (420/520 nm absorbance) as affected by addition of seed tannin and/or anthocyanin pigment.

Tannin added (mg GAE/L)	Pigment added (mg/L)					
	Color			Hue		
	0	250	500	0	250	500
0	1.38	2.34	5.48	1.50	0.97	0.80
500	1.67	4.16	6.74	1.64	0.89	0.76
1000	2.28	5.21	7.54	1.64	1.04	0.78

least broadened peaks and frequent interchange gives partial separations at best. The linkages in these polymers must be firm, probably covalent, because they withstand dialysis, HPLC, and Sephadex LH-20 chromatography (2,3).

One mechanism proposed for anthocyanin-tannin polymerization reactions is acid catalysis. In two sets of experiments (wines B and C) the sample with maximum anthocyanin and tannin additions was replicated at pH 1.5 and 4.1 as well as the wine pH (3.00 and 3.12, respectively). In both instances the polymer content and percentage of the total HPLC peak area that was polymer was decreased compared to that at wine pH; at pH 1.5, 4% and 11%, respectively, and at pH 4.1, 1.3% and 0.3%. Acid catalysis or acetaldehyde linkage should have been encouraged at pH 1.5 and oxidation at pH 4.1. The nature of the polymerization remains uncertain.

The amount of augmentation by pigment on the polymeric tannin indicated by Table 2, 150 mg/L, is sufficient to have a sizeable sensory effect. The threshold for whole-seed tannin in white wine is about 80 mg/L and the trimer-tetramer fractions of the order of 4 mg/L (1,4). Astringency and bitterness should be noticeably affected by the demonstrated augmentation in polymeric tannin produced with anthocyanin addition. Other studies indicate tastable flavor differences resulting from pomace contact sufficient to produce an increase in total phenol content of about 100 mg/L for white wines and 200 mg/L for red wines (5, 9). A considerably larger sensory effect would be expected from polymeric phenol changes than from total phenol at similar concentrations.

Why does the presence of anthocyanin increase the solubility and content of polymeric phenols - tannin - in wines? We hypothesize that incorporation of the flavylum salt and its attached sugar into the polymer increases the solubility of a given condensed tannin molecule. The reason white wines, even after pomace fermentation, are very low in polymeric phenols is seen as the result of the grape and yeast protein precipitating the polymers and solids, such as skins, adsorbing them. Red polymers incorporating anthocyanins would be more soluble, less precipitable, and less adsorbable. It is noteworthy that white wines commonly have excess protein whereas red wines have excess tannin and no protein. It is shown here that grape tannin is sufficiently soluble in white wine. But, it is not present in sufficient amount and form to exceed the capacity of the white-grape-plus-yeast-system to precipitate the tannin when there is no anthocyanin present. It follows that the tannin-anthocyanin complex should be less precipitable with gelatin fining and that is true from experiments to be separately reported.

In other situations, such as blush and rosé wines and in carbonic maceration where tannin tends to be low relative to anthocyanin color, the study of the effect of anthocyanins on polymeric phenol content promises to give important new insights.

Conclusions

1. The lack of polymeric phenols in wines made as if they were red but from white grapes has been explained on the basis of lack of anthocyanins to complex with the tannins.

2. Anthocyanins combine with condensed tannins in must or wine to increase the amount of polymeric phenols that are retained in the wine.

3. Anthocyanin presence in phenolic polymers will affect wine astringency, color, fining, and quality because of greater retention of tannin in their presence and possibly by more specific effects not yet understood.

4. Comparison of these and other studies show that there are different families of phenolic polymers in wine (tannins alone and complexed with anthocyanins being two) and the red polymeric phenols in young and old red wines are not the same.

5. Seed tannin is soluble in white wine but is not retained in the face of fermentation levels of grape and yeast solids unless accompanied by anthocyanins as in red wines.

Literature Cited

1. Delcour, J. A., M. M. Vandenberghe, P. F. Corten, and P. Dondéyne. Flavor thresholds of polyphenolics in water. *Am. J. Enol. Vitic.* 35:134-6 (1984).
2. Kantz, K., and V. L. Singleton. Isolation and determination of polymeric polyphenols using Sephadex LH-20 and analysis of grape tissue extracts. *Am. J. Enol. Vitic.* 41:223-8 (1990).
3. Kantz, K., and V. L. Singleton. Isolation and determination of polymeric polyphenols in wines using Sephadex LH-20. *Am. J. Enol. Vitic.* 42:309-16 (1991).
4. Rossi, J. A., Jr., and V. L. Singleton. Flavor effects and adsorptive properties of purified fractions of grape seed phenols. *Am. J. Enol. Vitic.* 17:240-6 (1966).
5. Singleton, V. L. Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. *Am. J. Enol. Vitic.* 23:106-13 (1972).
6. Singleton, V. L., and D. E. Draper. The transfer of polyphenolic compounds from grape seeds into wines. *Am. J. Enol. Vitic.* 15:34-40 (1964).
7. Singleton, V. L., D. E. Draper, and J. A. Rossi, Jr. Paper chromatography of phenolic compounds from grapes, particularly seeds, and some variety-ripeness relationships. *Am. J. Enol. Vitic.* 17:206-17 (1966).
8. Singleton, V. L., and P. Esau. Phenolic substances in grapes and wine, and their significance. *Adv. Food Res. (Suppl. 1):*1-282 (1969).
9. Singleton, V. L., H. A. Sieberhagen, P. DeWet, and C. J. Van Wyk. Composition and sensory qualities of wines prepared from white grapes by fermentation with and without grape solids. *Am. J. Enol. Vitic.* 26:62-9 (1975).
10. Singleton, V. L., J. Zaya, and E. K. Trousdale. Caffeoyl and coumaroyl acids in the fruit of *Vitis*. *Phytochemistry* 25:2127-33 (1986).
11. Slinkard, K. W., and V. L. Singleton. Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic.* 28:49-55 (1977).
12. Slinkard, K. W., and V. L. Singleton. Phenol content of grape skins and the loss of ability to make anthocyanins by mutation. *Vitis* 23:175-8 (1984).
13. Somers, T. C., and M. E. Evans. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO₂, "chemical age." *J. Sci. Food Agric.* 28:279-87 (1977).
14. Timberlake, C. F., and P. Bridle. Flavylum salts resistant to sulphur dioxide. *Chem. Ind.* 1489 (1968).
15. Timberlake, C. F., and P. Bridle. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* 27:97-105 (1976).
16. Wulf, L. W., and C. W. Nagel. High-pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. *Am. J. Enol. Vitic.* 29:42-9 (1978).
17. Wulf, L. W., and C. W. Nagel. Identification and changes of flavonoids in Merlot and Cabernet Sauvignon wines. *J. Food Sci.* 45:479-84 (1980).