

# Effect of Maturity and Vine Water Status on Grape Skin and Wine Flavonoids

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Quantity and characterization of flavonoids were determined in skins isolated from *Vitis vinifera* L. cv. Cabernet Sauvignon berries during fruit ripening and at different postveraison water deficits. The per berry amount of anthocyanin, flavonol, and pigment incorporation into proanthocyanidins increased with maturity. The amount of proanthocyanidin extension subunits did not vary with maturity, although the extension subunit composition did. The apparent average degree of polymerization of proanthocyanidins increased with maturity. Incorporation of pigmented material into the proanthocyanidins, together with the apparent increase in average degree of polymerization, suggests that additional terminal subunits, which are not flavan-3-ols, accumulate during fruit ripening. Increased vine water deficit caused small increases in anthocyanins and decreases in flavonols. Red wine flavonoid amounts indicate that postveraison water deficits affect red wine flavonoids primarily by altering berry size and perhaps secondarily by modifying flavonoid biosynthesis.

**Key words:** *Vitis vinifera*, grape, maturity, water deficit, polyphenol, condensed tannin, pigmented tannin, proanthocyanidin, flavan-3-ol, flavonol, anthocyanin, red wine

Skin tissue from red grape berries contains several flavonoid compound classes including anthocyanins [23,28], flavonols [3,30,36], flavan-3-ol monomers [32], and proanthocyanidins [25,29,32]. Anthocyanins, found with few exceptions only in the skin tissue, are responsible for the color of red wine. Flavonols, also only found in skin tissue, are particularly effective as cofactors in copigmentation [24], and (together with flavan-3-ols) have reputed health benefits [12,37]. Proanthocyanidins, found in skin tissue as well as in seed and stem tissues, are responsible for the bitter and astringent properties of red wine [6].

The composition of the grape berry changes dramatically during fruit ripening [1], and it is clear that anthocyanins are affected as well. Beginning at veraison, anthocyanins accumulate in the grape berry and are correlated with increased sugar accumulation [26,27]. Many factors can affect their rate of accumulation, including temperature [10,17], light [5,13], vine water status [2,7,20], and cultural practice [16,18,38]. In contrast to anthocyanins, little is known about the development of the other grape skin flavonoids.

In addition to maturity, cultural practices can affect fruit composition. Vine water status, for example, affects fruit growth [11,21], concentration of total phenolics [20], and wine sensory

attributes [19] and is an important management tool in many parts of the world. Yet it has not been established whether irrigation practice affects skin flavonoids. Therefore, this investigation was conducted to determine the developmental responses of skin flavonoids to vine water status using more specific methods (that is, chromatographic) than previous studies.

## Materials and Methods

**Grape cultivation and vine measurements.** Three irrigation treatments were established in a commercial vineyard of Cabernet Sauvignon near Oakville, CA, in the Napa Valley. The treatments were established in a randomized block design, replicated five times (18 vines/replicate), and with two buffer rows separating treatments. For the standard irrigation treatment (SI), irrigation was applied weekly (4 L/hr x 8 hr) to each vine. A double irrigation treatment (DI), was irrigated at the same frequency as the SI treatment, but with double the water applied (8 L/hr x 8 hr). A minimally irrigated treatment (MI) was also established with vines left unirrigated unless the midday leaf water potential ( $\Psi_l$ ) fell below -1.6 MPa, at which time water (4 L/hr x 8 hr) was applied. Vine water status was monitored weekly by measuring midday leaf water potential ( $\Psi_l$ ) of fully mature, sun-exposed leaves, as described previously [21].

**Winemaking.** Grapes were harvested at ~24 Brix (7 Oct 1998 for SI and MI and 13 Oct for DI) by removing the fruit from three of every four vines until 120 kg had been collected. This left the fruit on every fourth vine for an additional sampling point. Grapes from the field replications were combined, divided into two equal lots (by weight), and crushed, using a Zambelli crusher/destemmer (Saonara, Italy), into 80-L food-grade plastic buckets. Sulfur dioxide (35 mg/L) was added to

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the must, the contents were mixed well with a paddle, and after 1 hr the must was inoculated (Prisse de Mouse yeast, strain EC 1118) at a rate of 240 g/1000 L. Lots were punched down twice per day. Fermentors were stored at ~17°C for 2 days, inoculated with 2% v/v active malolactic culture, and stored at 27°C for 5 days. On day seven postcrush, wines were pressed with a Willmes model 100 press (Lamperthein, Germany), with press wine up to 2.5 bar added back to the drain wine. Wines were transferred to 20-L glass carboys equipped with fermentation locks and kept at 22°C until dry and through malolactic fermentation. Wines were later racked, aerated, and transferred to clean 20-L glass carboys. After 2 and 8 wk, wines were racked again, and the free SO<sub>2</sub> was adjusted to 18 to 20 mg/L as measured by the aeration-oxidation method. After the final rack and SO<sub>2</sub> adjustment, wines were bottled into 500-mL bottles and equipped with crown caps.

**Chemicals.** All chromatographic solvents and phosphoric acid were high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Santa Clara, CA). HPLC grade heptanesulfonic acid was purchased from Alltech Associates (Deerfield, IL). Benzyl mercaptan and quercetin were purchased from Aldrich Chemical Co. (Milwaukee, WI). (+)-Catechin and (-)-epicatechin were purchased from Sigma (St. Louis, MO). Malvidin-3,5-diglucoside was purchased from Pfaltz & Bauer (Waterbury, CN). Formic acid and sodium acetate were purchased from Fisher Scientific. Thioether standards were prepared using a method previously described [22].

**Skin sample preparation.** Beginning at veraison, 300-berry samples were collected periodically, and berry weight and Brix determined as previously described [14]. Skins from collected berries were separated from the remainder of the berry, rinsed well three times with distilled-deionized water, and kept in a 100 mg/L aqueous sulfur dioxide solution at 4°C until extracted. For extraction, skins were placed into 250-mL Erlenmeyer flasks. One mL aqueous 66% v/v acetone/g fresh wt was added, and flasks were covered, sparged with N<sub>2</sub>, and placed on an orbital shaker for 24 hr at 20°C and 100 rpm. After extraction, solutions were filtered through Whatman #1 filters, and evaporated under reduced pressure (35 to 40°C) to remove acetone. Aqueous solutions were diluted to 50 mL with distilled-deionized H<sub>2</sub>O and stored at -20°C until analyzed, a period of 1 to 3 months. Extracted skins were dried in a vacuum oven and weighed.

**HPLC analysis.** The reversed-phase HPLC method used to analyze anthocyanins, flavonols, and flavan-3-ol monomers has been previously described [35]. Compound identification was based on retention times and UV spectra.

Anthocyanins were quantified using malvidin-3,5-diglucoside as a standard. Through much of fruit ripening (5 Sept onward) the malvidin-based anthocyanins comprised ~74% of the total anthocyanin pool. Malvidin-3-glucoside constituted ~44% of the total pool, followed by the acetate ester (~25%), and the coumarate ester (~5%). These proportions are in good agreement with previous work [23]. To simplify analyses, the remaining anthocyanins, ~12 compounds, were not quantified.

Flavonols were quantified using quercetin as a standard. The two most abundant flavonols, comprising ~82% of the total fla-

vonol pool, were monitored in the study. Based on their abundance in previous studies [30,36], these are likely to be the glucoside and glucuronide of quercetin. Quantitation of (+)-catechin (the only observed flavan-3-ol monomer) was made using (+)-catechin as a standard.

Analysis of proanthocyanidins was gathered first by normal-phase HPLC, which separated the proanthocyanidins while still intact. Before analysis, it was necessary to convert aqueous extracts to methanolic extracts as described previously (excluding caffeine) [15]. Eluting peaks were monitored at 280, 365, and 520 nm. High molecular weight material was considered to be material eluting after flavonols (365 nm absorption). Quantitation was made at 280 nm using (-)-epicatechin as a standard. Analyzing intact material provided information on extractable amounts as well as pigment incorporation into proanthocyanidins.

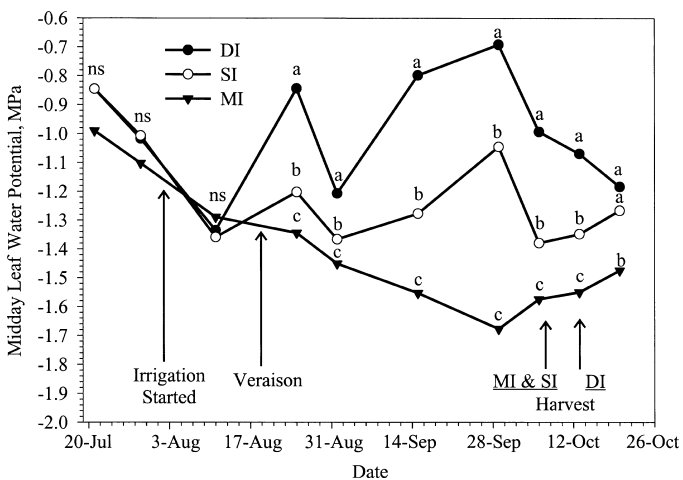
Proanthocyanidins were also analyzed by acid-catalyzed degradation in the presence of benzyl mercaptan (thiolysis), which provided information on constitutive subunits. Preparation of samples was similar to a previously published method [31], with some exceptions. Samples were prepared as follows: 800 µL of the MeOH/proanthocyanidin solution was placed into a 1.8-mL vial, followed by 800 µL of a 0.40 M HCl solution in MeOH, containing 12% v/v benzyl mercaptan. The vial was capped, placed in a 60°C water bath for 10 min, and combined with 200 µL of 5.2 M ammonium acetate solution in MeOH.

Thiolized proanthocyanidins were analyzed by reversed-phase HPLC. The column was a LiChrospher RP-18 (particle size 5 µm, 250 x 4 mm i.d.) protected by a guard column (10 x 4 mm) containing the same material (both obtained from EM Science, Gibbstown, NJ). Column temperature was maintained at 45°C. Before injection, MeOH concentration in all samples was reduced to 20% v/v with water to minimize peak tailing. The method used a binary gradient with mobile phases containing 200 mM phosphoric acid pH 1.5 and MeOH. Elution conditions were as follows: flow rate of 1 mL/min; 15% MeOH for 5 min, linear gradient to 55% MeOH in 55 min, 100% MeOH for 10 min, followed by reequilibration of the column with 15% MeOH for 5 min. Identification was based on retention times, UV spectra, and standard comparison. Quantitation was made at 280 nm using an (-)-epicatechin standard with other subunits determined using relative response factors. Thiolysis provided information on extractable amounts, subunit composition, and average or mean degree of polymerization.

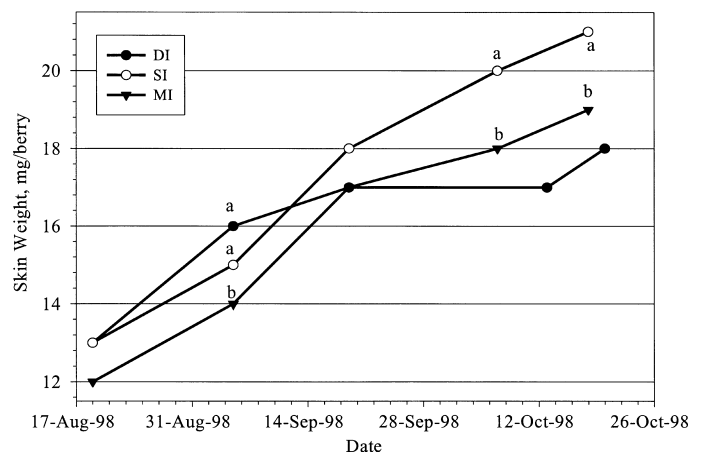
## Results and Discussion

**Water status and berry development.** Irrigation treatments established significant differences in midday leaf water potential from veraison through fruit ripening (Figure 1). Throughout ripening, SI maintained midday  $\Psi_1$  between -1.3 and -1.0, while  $\Psi_1$  of the DI and MI vines were 0.2 to 0.4 MPa greater and lower, respectively, than the SI vines.

Mean berry weight at harvest was 1.05 g for both DI and SI fruit, but was reduced more than 15% (to 0.88 g) in MI fruit (Table 1). Berries in all treatments attained maximum size approximately 30 days prior to harvest; yet there was no evidence



**Figure 1** Midday leaf water potential during fruit ripening for treatments with double irrigation (DI), standard irrigation (SI), and minimal irrigation (MI). Values with different letters indicate significance at  $p = 0.05$ .



**Figure 2** Skin weight during fruit ripening, for treatments with double irrigation (DI), standard irrigation (SI), and minimal irrigation (MI). Values with different letters indicate significance at  $p = 0.05$ .

**Table 1** Effect of irrigation treatments on berries harvested at commercial maturity, with flavonoids expressed on a per berry basis.

Parameter	Treatment <sup>a</sup>		
	DI	SI	MI
Berry wt, g/berry	1.05 a <sup>b</sup>	1.05 a	0.83 b
Sugar, Brix	24.1	24.3	24.2
Anthocyanins, mg/berry	0.561	0.667	0.682
Flavonols, mg/berry	0.091	0.096	0.087
Catechin, µg/berry	2.88	2.12	2.33
Proanthocyanidins			
Intact			
280, mg/berry	0.92	0.88	1.01
520, PAU/berry	138.7	139.8	161.4
Degraded			
Terminal units, nmol/berry	34	40	36
Extension units, nmol/berry	757 a	941 b	974 b
eGC, nmol/berry	360 a	450 b	463 b
mDP	23.5	24.1	27.5

<sup>a</sup>DI = double irrigation, SI = standard irrigation, MI = minimal irrigation (MI).

<sup>b</sup>Values with different letters are significantly different at  $p = 0.05$ .

of berry contraction in subsequent samples. At harvest, the skin dry weight of MI fruit was also significantly less than the SI fruit (Figure 2). However, in contrast to the treatment effects on berry fresh weight accumulation, the dry weight of DI skin tissue was less than the other two treatments. The skin mass for SI and MI increased continuously throughout the season, almost in parallel, but skin mass for DI increased very little after 19 Sept. Thus, although approximately two-thirds of the final skin mass was present at veraison, the data suggest that postveraison water deficits increased skin mass accumulation during ripening.

Water deficits also accelerated sugar accumulation. Brix was significantly lower in DI than other treatments throughout ripening (Figure 3C in [14]); thus, the targeted sugar concentration was delayed in DI by approximately 1 week.

**Water status and berry flavonoid composition.** Differences in vine water status were associated with differences in skin flavonoid composition in harvested fruit. For six of the seven assays, there was a significant increase in the concentration caused by water deficits in the MI treatment compared to DI (Table 2). For flavonols, the differences between the means were not significantly different. The only difference for catechin was between MI and SI fruit. For the other parameters, the increase for MI was generally 40 to 60% over the DI.

On a weight basis, the anthocyanin concentration for the MI fruit was significantly higher (>50%) than for the other treatments (Table 2). While this is significant on a weight basis, there were no differences on a per-berry basis (Table 1). In previous studies (albeit with a different methodology) slightly less severe water deficits imposed on Cabernet franc increased the concentration of anthocyanins in skin tissue (mg/cm<sup>2</sup>) by about 25% [20], which, when expressed on a berry volume basis, is 42% greater than fruit from well-watered vines. In a study conducted on Shiraz vines [7], an increase in the concentration of anthocyanins on a weight basis, yet lower values on a per berry

**Table 2** Effect of irrigation treatments on berries harvested at commercial maturity, with flavonoids expressed on a weight basis.

Parameter	Treatment <sup>a</sup>		
	DI	SI	MI
Anthocyanins, mg/g	0.534 a <sup>b</sup>	0.635 a	0.822 b
Flavonols, mg/g	0.087	0.091	0.105
Catechin, mg/g	2.74 ab	2.02 a	2.81 b
Proanthocyanidins			
Intact			
280, mg/g	0.88 a	0.84 a	1.22 b
Degraded			
Terminal units, nmol/g	32 a	38 ab	43 b
Extension units, nmol/g	721 a	896 b	1173 c
eGC, nmol/g	343 a	429 b	558 c

<sup>a</sup>DI = double irrigation, SI = standard irrigation, MI = minimal irrigation (MI).

<sup>b</sup>Values with different letters are significantly different at  $p = 0.05$ .

basis, was observed, although under somewhat higher water deficit regimes.

This would appear to call into question the physiological significance of indirect evidence of water deficit stimulation of anthocyanin biosynthesis [4,20]. The lack of an increase in anthocyanin content on a per-berry basis may be related to the isolation of the berry from the low water potentials that develop in stem xylem during water deficits. Greenspan et al. [9] showed that although the xylem tension at the insertion of the rachis to the stem responded to water deficits similar to leaves on the same vine, the sensitivity of berry water content to low leaf water status was greatly diminished after veraison. Thus, although there is evidence of abscisic acid accumulation in water-stressed berries [Matthews and Wang, unpublished], the postveraison berry may not experience the water deficits indicated by the leaf water status.

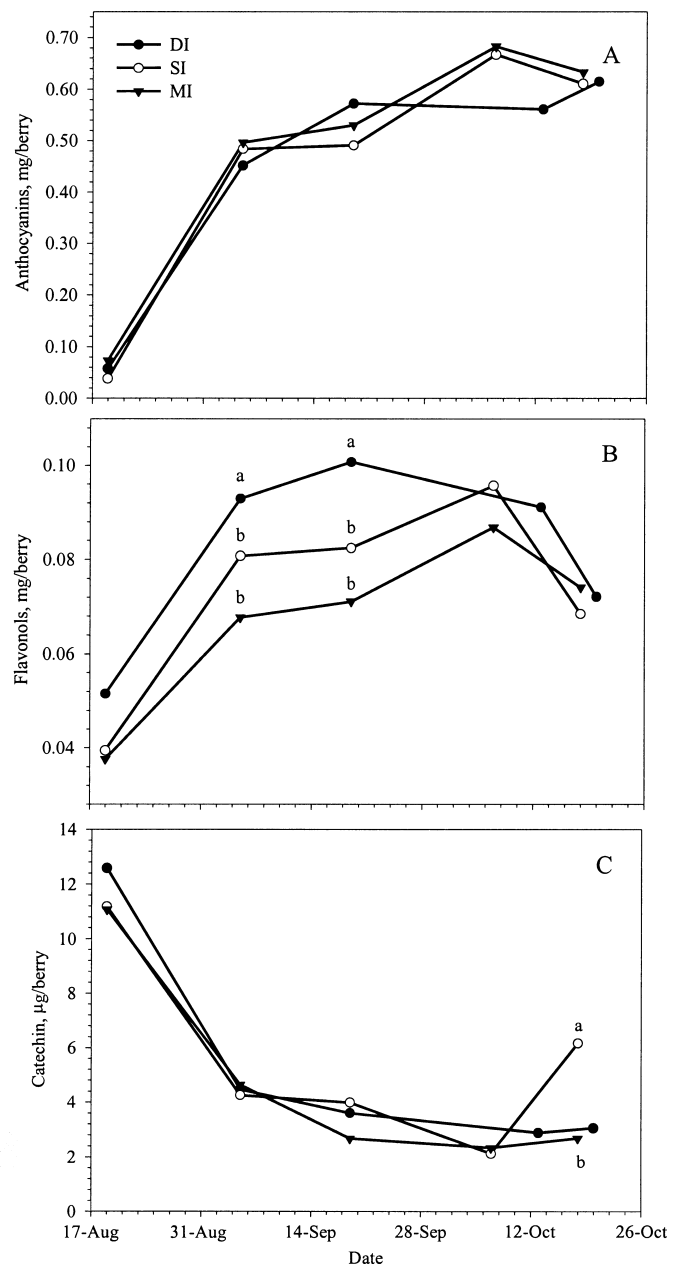
Concentration of catechin was significantly greater in MI than SI fruit, but intermediate in DI fruit (Table 2). Although both catechin and flavonols can participate in copigmentation with anthocyanin (albeit weakly in the case of catechin) [24], the concentration of catechin and flavonols was only 1 to 2% and 15%, respectively, of anthocyanins (Table 2, converted to approximate molar equivalents). The lack of significant differences in skin flavonol in the presence of large differences in vine water status suggests that the potential for water deficits to alter copigmentation via skin flavonol is limited. Nevertheless, the ratio of flavonol:anthocyanin did increase with vine water status, from 0.13 for MI to 0.16 for DI (Table 2).

Total and pigmented proanthocyanidins were significantly increased by MI compared to SI and DI (Table 2). Concentration of intact proanthocyanidins ( $A_{280}$  assay) was increased about 40% in MI fruit compared to DI (Table 2). Concentration of pigmented proanthocyanidin ( $A_{520}$  assay) was increased over 45% in MI compared to DI fruit.

Irrigation treatment differences in concentrations of flavonoids raise two obvious questions. First, to what extent were the differences in concentration due to changes in phenolic metabolism and changes in the fruit size (that is, solvent), since growth is clearly inhibited by water deficits and the response to water deficits was increased concentrations? Second, to what extent are such differences in fruit composition carried over into wines? To address the first question, flavonoid composition was evaluated during fruit ripening, expressed here as the total amount of flavonoid on a per-berry basis and referred to hereafter as content.

**Developmental analysis of flavonoid content: Anthocyanins.** Anthocyanin content in all treatments increased rapidly from veraison until early September and considerably more slowly thereafter (Figure 3A). Although a more continuous increase has been observed, as noted above, the method of analyzing total absorbance at 520 nm of total skin extracts could not distinguish between anthocyanins and pigmented tannins. The proportion of methoxylated anthocyanins was greater in all postveraison samples than at veraison (data not shown).

As noted above, anthocyanin analyses have generally reported anthocyanin concentration expressed on a per skin area



**Figure 3** Anthocyanin (A), flavonol (B), and catechin (C) amounts during fruit ripening for treatments with double irrigation (DI), standard irrigation (SI), and minimal irrigation (MI). Values with different letters indicate significance at  $p = 0.05$ .

or per unit berry fresh weight. In this study, an increase in concentration was observed (above), but there were no significant differences in the anthocyanin content at any sample date.

**Flavonols and flavan-3-ol monomers.** The flavonols increased in a pattern similar to anthocyanins, rapidly from veraison to early September and then more slowly before attaining a maximum approximately two weeks before harvest (Figure 3B). Water stress significantly decreased flavonol content until late September, but thereafter the flavonol contents decreased and became similar for all three treatments.

Very low amounts of the flavan-3-ol monomers were present in the skin extracts. The only flavan-3-ol monomer present at a

significant concentration was (+)-catechin (C), which decreased rapidly from veraison until reaching rather stable content of about 3  $\mu\text{g}/\text{berry}$  in early September (Figure 3C).

Substantial amounts of flavan-3-ol monomers are also found in the seed. In addition to C, seed extracts also contain (-)-epicatechin (eC) and (-)-epicatechin-3-*O*-gallate (eCG) [14,34]. The flavan-3-ol monomer amounts in skin tissue were much lower throughout ripening than those found in seeds isolated from the same fruit [14]. At veraison and for seed extracts, C, eC, and eCG were 350, 145, and 73  $\mu\text{g}/\text{berry}$ , respectively,  $\sim 50$  times the amount present in skin extracts. By harvest, seed flavan-3-ol monomers had dropped to 35, 33, and 2  $\mu\text{g}/\text{berry}$  for C, eC, and eCG, respectively,  $\sim 14$  times the level found in skins. The large differences between flavan-3-ol monomers found in seed and skin suggest that the flavan-3-ol monomers in red wine are essentially of seed origin.

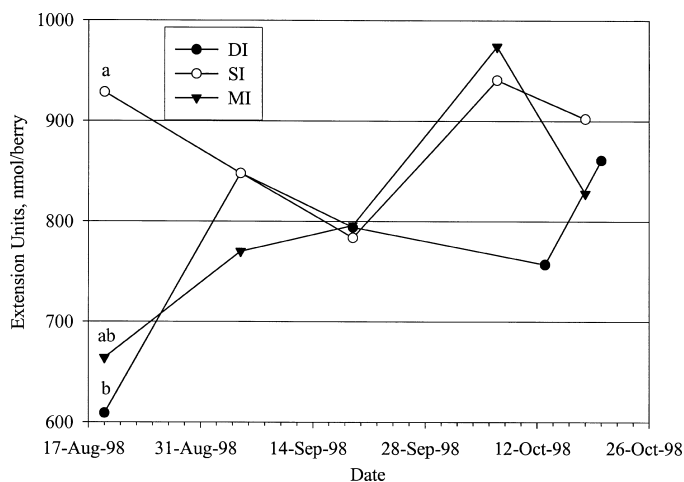
**Proanthocyanidins and pigmented tannin.** The measurement of intact proanthocyanidin content as measured chromatographically at 280 nm increased approximately 4-fold in all treatments throughout fruit ripening (Figure 4). There was no consistent difference among treatments. However, the mean proanthocyanidin content at harvest was greatest in MI, least in DI, and intermediate in SI, perhaps because after mid-September the rate of proanthocyanidin increase decreased in DI and increased in the water-stress treatments.

In sharp contrast, the amount of proanthocyanidin observed by thiolysis generally increased after veraison, but only by about 40% (Figure 5). This large discrepancy in accumulation suggests that the material detected in the intact analysis by light absorption at 280 nm is not only proanthocyanidin. In support of this, the intact skin proanthocyanidin analysis showed a large accumulation of 520 nm absorbing high molecular weight material that was associated with the increases observed at 280 nm (Figure 6). On the basis of the associated increases in apparent tannin when monitored at 280 and 520 nm, a reasonable conclusion is that anthocyanins are being incorporated into proanthocyanidins during fruit ripening. Consistent with this conclusion, the  $\sim 800$  nmol/berry of extension units observed

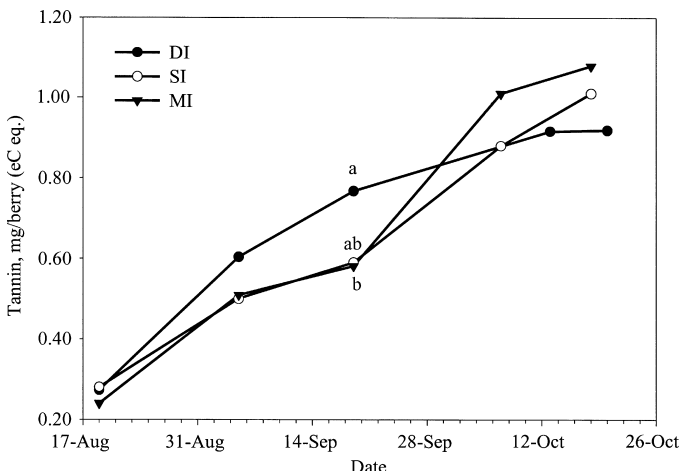
by thiolysis during fruit ripening (Figure 5) is equivalent to 0.23 mg/berry in epicatechin equivalents, the amount of proanthocyanidin present in the skins at veraison (Figure 4), suggesting that skin “tannin” accumulation after veraison is due to the incorporation of anthocyanin subunits.

The composition of extension units changed significantly during ripening (Figure 7). The contribution of eCG was constant, but the fraction of the total in (-)-epigallocatechin (eGC) increased until early October, with a corresponding decrease in eC proportion. The increase in eGC subunits—subunits that are more susceptible to oxidation [33]—suggests that the proanthocyanidins do not become oxidized during fruit ripening.

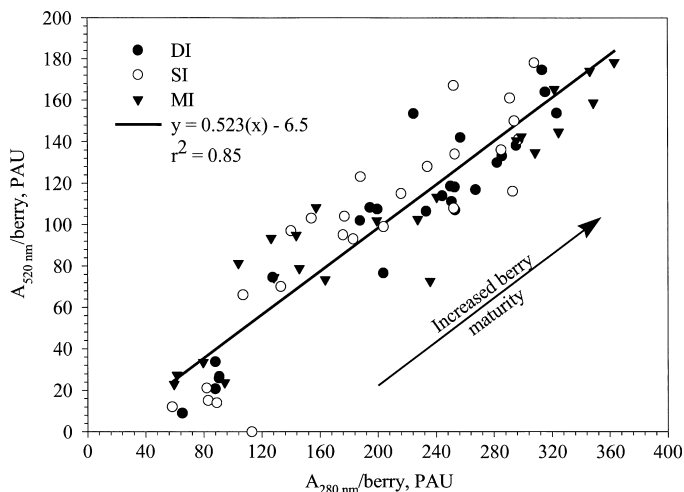
From thiolysis, catechin was the only terminal subunit of proanthocyanidin detected in skin tissue of Cabernet Sauvignon. Other flavan-3-ol terminal subunits, if present, comprised a small proportion of the total pool. This is consistent with the similar analysis of Merlot skin tissue by Souquet et al. [32].



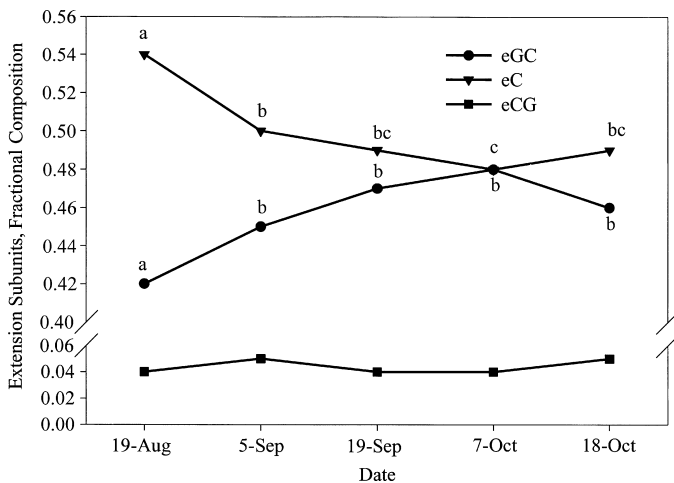
**Figure 5** Quantity of extractable proanthocyanidin extension subunits from skin during fruit ripening, for treatments with double irrigation (DI), standard irrigation (SI), and minimal irrigation (MI). Values with different letters indicate significance at  $p = 0.05$ .



**Figure 4** Quantity of extractable high molecular weight phenolics during fruit ripening.



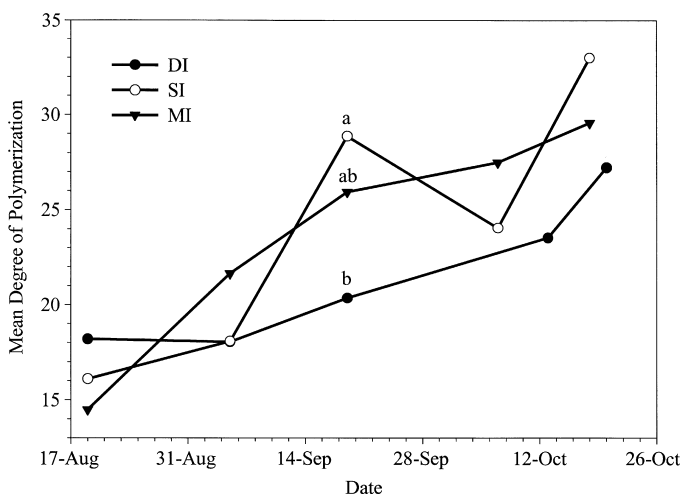
**Figure 6** Relationship between absorption at 520 nm and 280 nm of high molecular weight phenolic material.



**Figure 7** Change in the relative proportion of proanthocyanidin extension subunits (eGC = (-)-epigallocatechin, eC = (-)-epicatechin, eCG = (-)-epicatechin-3-O-gallate) during fruit ripening for the SI treatment. Values with different letters indicate a significant change across maturity for individual extension units at  $p = 0.05$ .

The extension subunits observed here included eC, eGC, and small amounts of eCG and C. Only 2% of the total extension unit pool was C in Merlot [32], and it was not quantified in this study.

The mean degree of polymerization (mDP) of skin proanthocyanidin increased fairly steadily during fruit ripening in all treatments from approximately 15 to 30 (Figure 8). The mDP was generally lowest for DI fruit, although there was only one sample date where the difference was significant at  $p = 0.05$ . At commercial harvest, the mDP of skin material was greater than that reported for Merlot [32] and much greater than the mDP of seed material from these same berries [14]. However, the discrepancy between the amount of extension subunits and the intact “tannin” suggests that these estimates of mDP may not be accurate.



**Figure 8** Mean degree of polymerization during fruit ripening for treatments with double irrigation (DI), standard irrigation (SI), and minimal irrigation (MI). Values with different letters indicate significance at  $p = 0.05$ .

**Table 3** Flavonoids in wines made from grapes harvested at commercial maturity.

Parameter	Treatment <sup>a</sup>		
	DI	SI	MI
Anthocyanins, mg/L	320	335	402
Flavonols, mg/L	23	26	36
Proanthocyanidins			
eGC, $\mu\text{mol/L}$	116	130	136

<sup>a</sup>DI = double irrigation, SI = standard irrigation, MI = minimal irrigation (MI).

**Comparison of skin and wine flavonoids.** When flavonoid concentrations were determined in wines (Table 3), the concentrations as a function of the vine water status were similar to those observed in the fruit expressed on a weight (that is, concentration) basis (Table 2). The MI wine contained 120 and 126% of the anthocyanin concentration in SI and DI wines, respectively. For flavonols, the MI wine contained 138 and 156% of the concentration in SI and DI wines, respectively. For proanthocyanidins, monitoring eGC provided information on grape skin proanthocyanidins without interferences due to grape seed proanthocyanidins [14]. The MI wine contained 105 and 117% of the eGC concentration of SI and DI wines, respectively.

Treatment effects on the concentrations of anthocyanin and proanthocyanidin in wines were about 50% of those in berries. Diminished differences may be related to the solvent system used for extraction. The acetone used in the extraction of skins is an effective hydrogen bond disrupter; therefore, anthocyanin and proanthocyanidin adsorption onto skin tissue (via hydrogen bonding) is reduced. In wine production, however, these compounds would likely adsorb onto skin tissue. The effect would be most pronounced in MI because of the higher proportion of skin tissue in the fermenting must. The differences between these wine flavonoids and those found in grapes was greatest for MI, followed by SI and DI in that order.

Amounts of wine flavonols differed among treatments by about twice as much as in the fruit. Flavonols are poorly soluble in a wine solvent system; however, complex formation with anthocyanins (copigmentation) would likely increase flavonol solubility. Wines with a higher anthocyanin concentration, such as the MI wine, would be able to support a higher flavonol concentration.

## Conclusions

Modified irrigation appeared to affect only flavonol synthesis moderately, but the pronounced effect of irrigation on berry size suggests it is the predominant factor affecting flavonoids in red wine. The analytical methodology revealed a new dynamic in anthocyanin concentration, showing little change late in ripening, but a steady increase in pigmented tannin. The latter effect is most likely the factor that drove the observation of increasing “anthocyanin” levels in previous studies.

Preveraison vine water deficits may have more pronounced effects, however [8]. Water deficits applied during the first

month after anthesis reduce berry size at harvest [11], may affect the biosynthesis of flavonoids differently than postveraison deficits, and may be beneficial in other ways, including vigor control and increased bud fruitfulness.

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