Development of Anthocyanins and Tannins in Pinot noir Grapes and their Relative Importance in Wine

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INTRODUCTION

The objectives of this research project are to understand tannin and anthocyanin changes during berry development in Pinot noir fruit and wines. Specifically, the objectives of this research project are:

- Determine the effect of berry development on the composition of tannins in cv. Pinot noir berries
- Determine the effect of berry development on the composition of anthocyanins in cv. Pinot noir berries
- Isolate and purify sufficient quantities of tannin and anthocyanin for sensory and stability studies
- Determine the sensory properties of wines manipulated with anthocyanins and tannins representing extremes in composition based on grape and wine data.

These goals are designed to understand how tannin and anthocyanin composition in grapes affects the color stability and texture of wine, and to begin to understand the extent to which vineyard and winery practices could be used to influence their composition.

Color stability and textural quality are two important aspects of red wine appreciation. Anthocyanins and tannins are responsible for these attributes, and based on broad anecdotal evidence, grape maturity can have a dramatic effect on the quality of these components in wine (Heald and Heald, 1993,1994; Long, 1997). For cool climate Pinot noir production, where obtaining optimum fruit ripeness can be challenging, it is particularly important that an understanding of anthocyanin and tannin development is as complete as possible.

The development of tannins in grapes have typically been limited to low molecular weight tannins, which make up a small portion of the tannins present in wine, or have assumed that the tannins behave similarly. Recent studies on tannin development in cvs. Cabernet Sauvignon (Kennedy et al., 2000b) and Shiraz (Kennedy et al., 2000c, 2001) indicate that tannins change during development.

Research on anthocyanins is more extensive. Beginning at véraison, anthocyanins accumulate in the grape berry, and are correlated with increased sugar accumulation (Pirie and Mullins, 1980). Many factors affect their accumulation in the berry, including temperature (Kliewer and Torres, 1972; Hale and Buttrose, 1974), light (Dokoozlian and Kliewer, 1996; Keller and Hrazdina, 1998), vine water status (Bravdo and Hepner, 1986;

Matthews and Anderson, 1988), and cultural practice (Kliewer and Schultz, 1973; Reynolds et al., 1996; Yokotsuka et al., 1999). Sensory studies indicate that red wine color is generally positively associated with overall red wine quality (Somers and Evans, 1974; Jackson et al., 1978).

Tannins and anthocyanins are predicted to interact with each other to improve color stability; and therefore, their proportionality becomes an important aspect wine production (Bissel et al., 1989; Singleton and Trousdale, 1992). This interaction has been associated with general reactivity of tannins under wine pH conditions (Haslam, 1980; Hemingway and McGraw, 1983). Based on this interaction, it is conceivable that the mouth feel of wines could be affected as well.

Sensory studies indicate that small molecular weight tannins are dominated by bitterness, and secondarily by astringency. As the molecular weight of the tannin increases, astringency becomes the dominant perception (Robichaud and Noble, 1990). Based on these findings and on tannin development in grapes, it is predicted that wines made from grapes that are increasingly ripe will become increasingly astringent and less bitter. Based upon research in Ciders, these differences are associated with hard and soft tannin descriptors.

Studies specific to cv. Pinot noir indicate that it may be dangerous to draw conclusions about phenolic behavior in grapes and wines based on data gathered from other varieties. Pinot noir for example generally has higher amounts and different proportions of low molecular weight seed tannins than other varieties (Thorngate and Singleton, 1994; Bourzeix et al., 1986). In addition, Pinot noir lacks acylated anthocyanins. For these reasons and because of its importance to the Oregon wine industry, it is important to study the development of these compounds in Pinot noir.

The development of analytical techniques (Kennedy and Waterhouse, 2000; Kennedy and Jones, 2001, Kennedy et al., 2001) has enabled the characterization of tannins much more thoroughly and more routinely than only a decade before. With these analytical techniques and the ability to purify large quantities of tannins and anthocyanins, this research will provide a thorough understanding of how tannin composition (i.e.: amount, mean degree of polymerization, subunit composition, chemical change), and anthocyanin composition change during fruit ripening. This research will also provide an understanding of how different proportions of anthocyanin and tannin affect color stability and mouth feel of red wine.

MATERIALS AND METHODS

This study was conducted on grapes grown at the Oregon State University experimental vineyard located in Alpine (Woodhall vineyard, maturity/C block, Pommard clone), planted in 1984. Two rows containing 55 vines each were selected for study. Vineyard operations were in keeping with commercially accepted practice. Beginning approximately 1 week prior to véraison, 6 cluster samples (5 replicates) were collected for analysis. Clusters were kept at –20 °C prior to analysis. On September 27th, when sugar concentrations had reached approximately 24 °Brix, the fruit from alternating vines was harvested for experimental wine production. On October 4th, the remaining fruit was collected for wine production.

Sample Analysis
For sample analysis berries were removed from the cluster within each replicate while still frozen. Berries were randomized and from this, a 200-berry subset was used for extraction and phenolic analysis. The remaining berries were thawed and the must was analyzed for titratable acidity, pH, and sugar.

To the 200-berry subset, seeds and skin were separated from the pulp. Seeds were counted, weighed, freeze-dried and then reweighed. Seeds and skins were then extracted separately in a 2:1 v/v acetone:water extraction solvent as described elsewhere (Kennedy et al., 2000c). After extraction, extracts were filtered through Whatman #1 filters and evaporated under reduced pressure prior to analysis using various chromatography methods (Lamuela-Raventos and Waterhouse 1994, Kennedy et al. 2000c, Kennedy and Jones 2001, Kennedy et al., 2001).

Wine Production
Harvested grapes were crushed , destemmed, divided into two lots, and 50 mg/L SO2 was added to each lot. Musts were inoculated with 1 g/L Lalvin RC 212 Bourgorouge yeast and the fermenting wines were punched down two times/day. Fermentation rates were monitored with a hydrometer, and samples were collected every other day. Fermentation temperatures reached a maximum of ~32 °C for approximately two days. One lot was pressed after 1 week and the second lot was pressed after 2 weeks. New wines were kept at ~15-20 °C during the first 2 months to complete malolactic fermentation and to settle.

RESULTS AND DISCUSSION

Berry Development
Overall berry development is summarized in Figures 1-4.

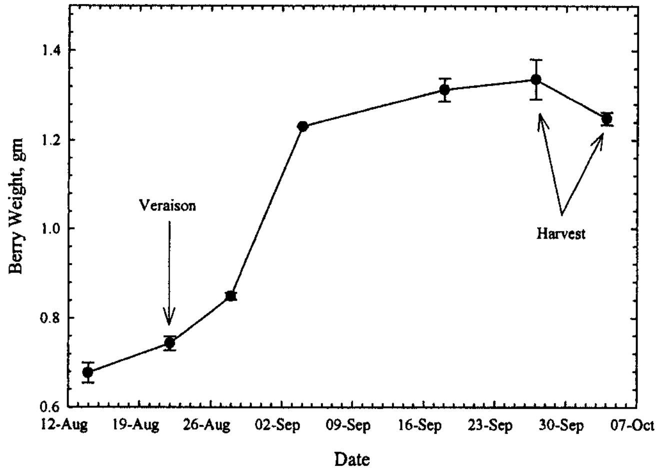

Figure 1. Berry weight change during the 2001-growing season with error bars indicating ± SEM (N=5).

Berry weight after véraison developed in a typical manner, increasing from 0.74 g/berry at véraison and reaching a maximum berry weight on 27 September (1.33 g/berry). Afterwards, berry shrinkage occurred, reaching 1.25 g/berry on 4 October (94% of maximum). The berry shrinkage is consistent with the comparatively rapid sugar increase at the end of berry development (Figure 2).

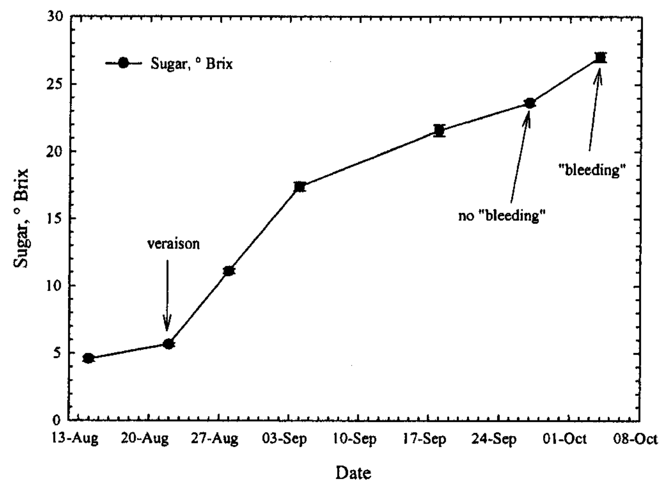

Figure 2. Sugar accumulation during the 2001-growing season with error bars indicating ± SEM (N=5).

The pH and titratable acidity (TA), summarized in Figure 3, followed expected trends, yet the final TA and pH suggest that extensive organic acid metabolism has taken place.

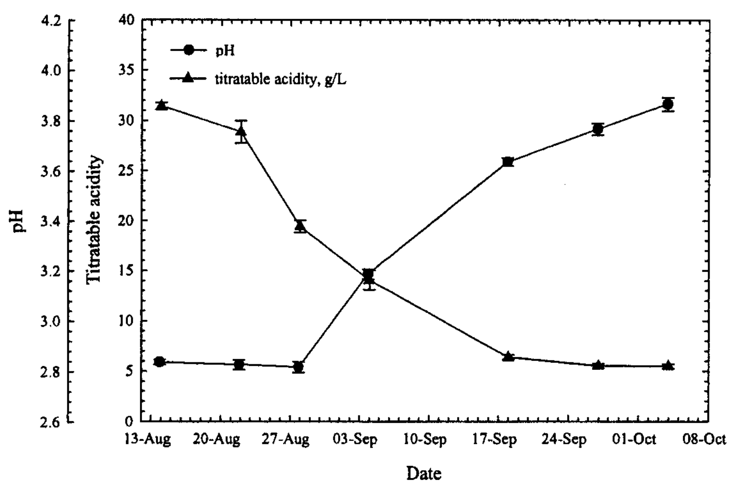

Figure 3. pH and titratable acidity change during the 2001 growing season with error bars indicating ± SEM (N=5).

Seed Development

The seeds developed similarly to those in cv. Shiraz (Kennedy et al., 2000c). Initially, prior to véraison, the seeds were green with a soft seed coat. Beginning at véraison, and continuing through harvest, the seeds lost their green color and became progressively browner with time.

The difference between fresh and dry seed weight provides some indication of dessication status, and can be used as an indicator of seed developmental status. In this study (Figure 4), the difference between fresh and dry seed weight is not stable at harvest suggesting that changes were occurring in the seed at harvest. In comparison with cv. Shiraz (Kennedy et al., 2000c), which is a later ripening variety, some differences in seed development were observed. First, the difference between fresh and dry seed weight for Shiraz was stable 3-4 weeks prior to harvest, considerably different than this study. Second, the seed coat color in Pinot noir at harvest was a much lighter brown than that for Shiraz.

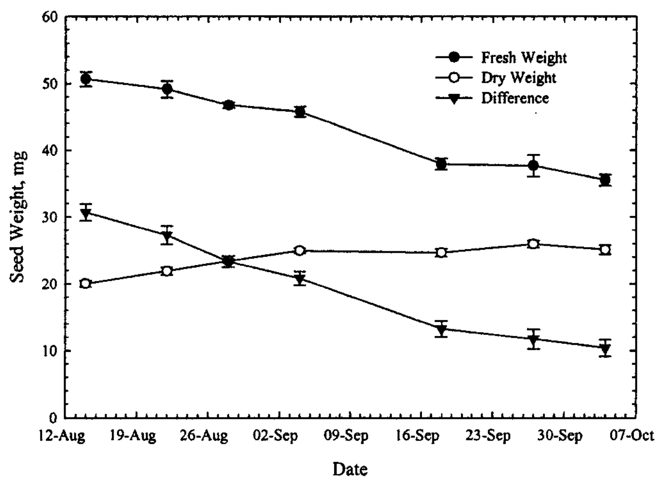

Figure 4. Fresh and dry seed weight indicating desiccation status, with error bars indicating ± SEM (N=5).

Tannin Development

Being composed of flavan-3-ol subunits, the tannins in grapes belong to the proanthocyanidin class of compounds (Figure 5). These tannins are a heterogeneous group of compounds and vary in molecular weight (typically reported from dimers up to over 30 subunits in length), subunit composition (Figure 6), and the way in which they are linked (the interflavonoid bond). In this report, the flavan-3-ol monomers (often refered to as catechins) will be considered as tannins. The most common tannin interflavonoid bond (shown in Figure 5) is susceptible to cleavage under wine pH conditions.

Seed tannins developed in a manner consistent with other varieties, although significant differences were apparent (Figures 7-14). Consistent with previous results, the seed flavan-3-ol monomers in Pinot noir were synthesized in the few weeks preceding véraison whereas the majority of the tannins were already present (Figure 8). From Figures 7 and 9, it is clear that there is a considerable amount of flavan-3-ol monomer produced in Pinot noir. This is in stark contrast to other studied varieties where they make up a much smaller proportion of the total tannin pool (Figures 10 -12). When the development of skin tannin is considered (Figures 13 and 14), it is clear that Pinot noir has an abundance of tannin when compared to Cabernet Sauvignon.

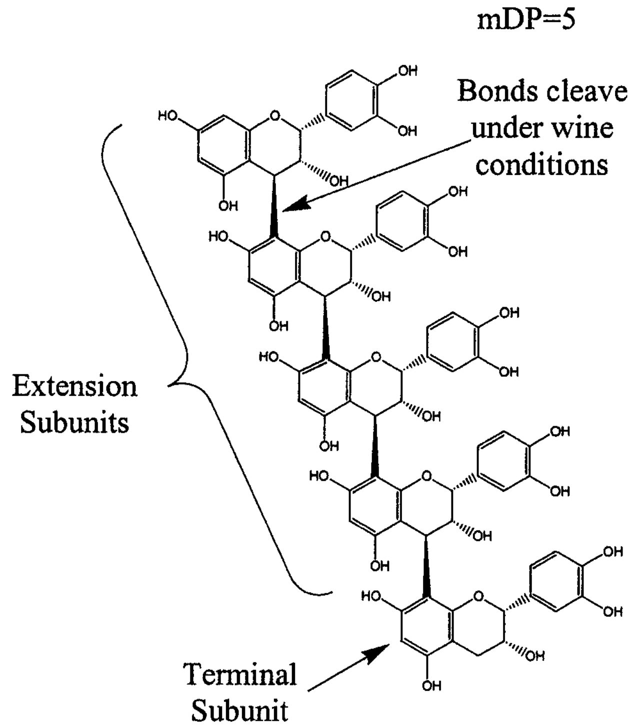

Figure 5. Tannins in grapes belong to the proanthocyanidin class of compounds and are composed of flavan-3-ol subunits.

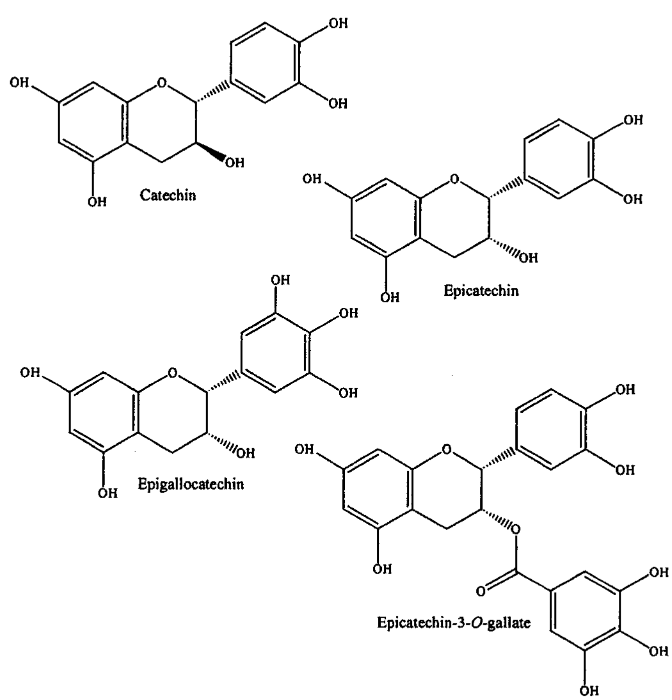

Figure 6. Flavan-3-ol monomers and/or proanthocyanidin subunits found in grapes.

The presence of a large amount of flavan-3-ol monomers may have important implications in winemaking if the following is considered. First, because of their smaller size, low molecular weight tannins are expected to diffuse into wine more readily than larger molecules. Second, because of their much higher concentration, wines made from Pinot noir grapes should have a much higher concentration present than varietals such as Cabernet Sauvignon and Shiraz. Third, because tannins hydrolyze under acidic conditions, it is expected that they will recombine with other molecules, in this case the flavan-3-ol monomers. This will result in a reduction in the size of the tannins. This is significant because smaller tannins are dominated by the perception of bitterness, and have been described as hard in other beverages (Noble, 1990; Peleg et al., 1999; Lea and Arnold, 1978).

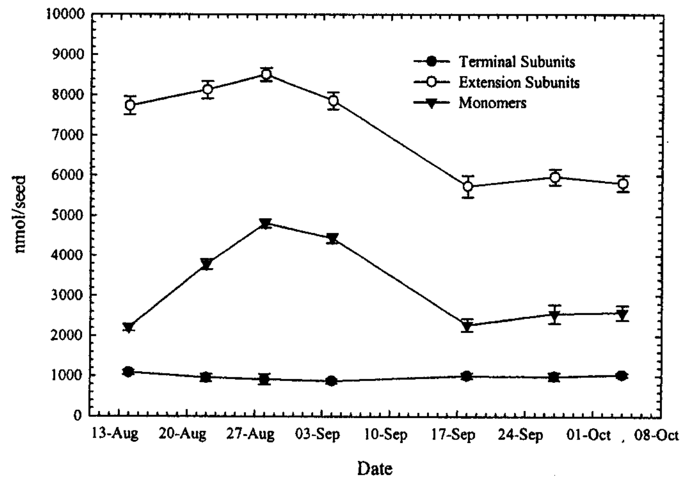

Figure 7. Development of the different tannin flavan-3-ol components in grape seed, with error bars indicating ± SEM (N=5).

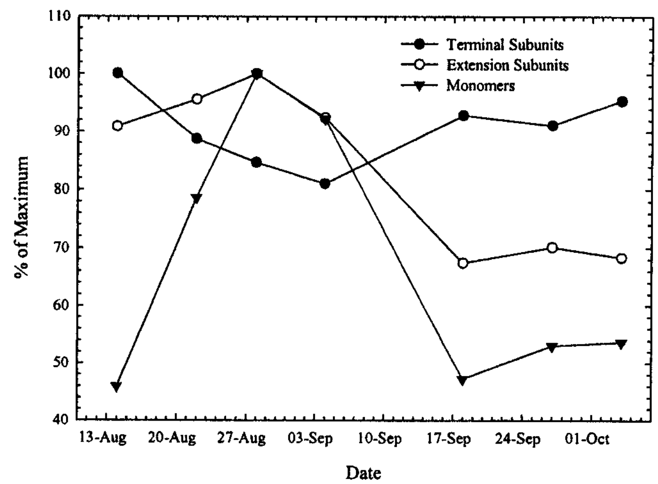

Figure 8. Development of the different "tannin" flavan-3-ol components in grape seed with development expressed as the % of maximum within each component.

When considering overall tannin development, and for all of the varieties studied to date, the single greatest outlier is with Pinot noir grape seed flavan-3-ol monomers, and secondly overall grape seed tannin. With respect to wine style, and excluding flavan-3-ol monomers, Pinot noir has an abundance of tannin, far exceeding (>6x) varieties known for their larger wine structure, yet the average polymer size is consistent with other varieties.

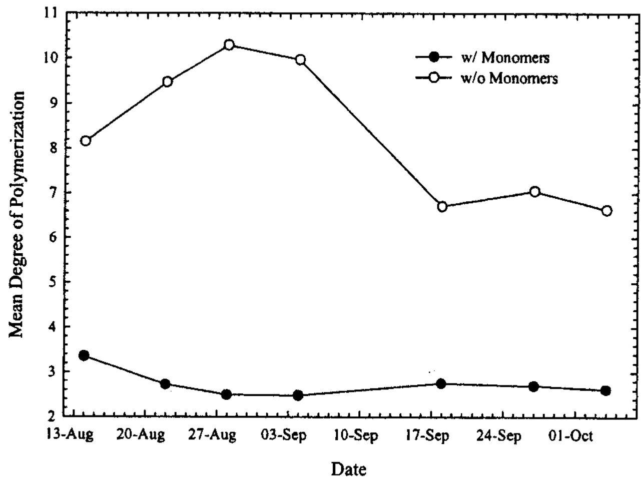

Figure 9. Mean degree of polymerization of grape seed tannins for cv. Pinot noir during berry development with and without the inclusion of flavan-3-ol monomers.

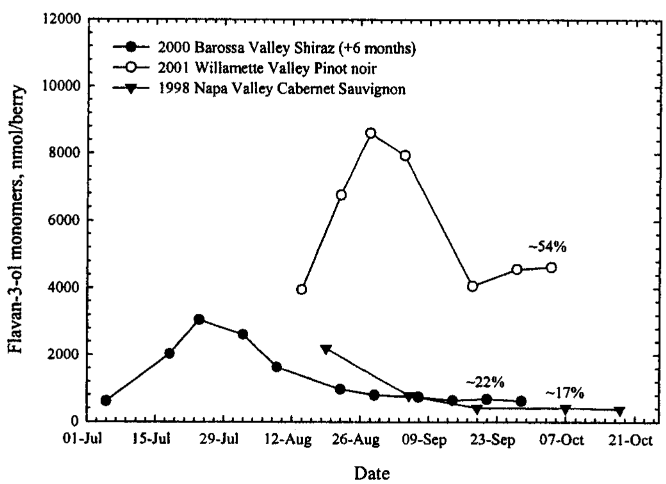

Figure 10. Development of the flavan-3-ol monomers in different grape seed varieties with % of maximum present at harvest indicated.

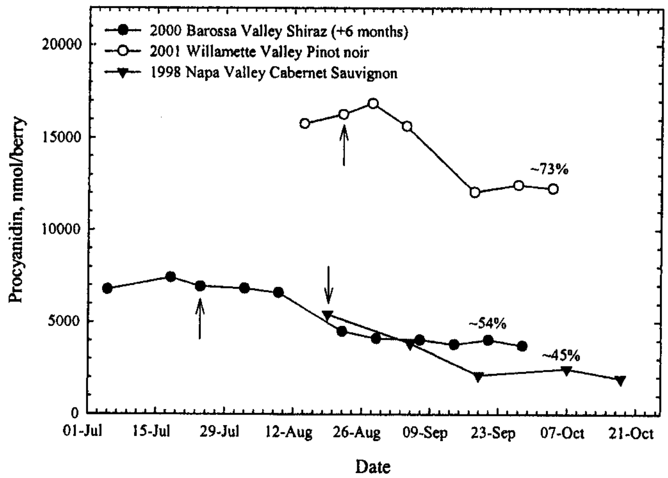

Figure 11. Development of the higher molecular weight tannins (procyanidins) in different grape seed varieties. Arrows indicate véraison within each variety and numbers indicate the % of maximum remaining at harvest.

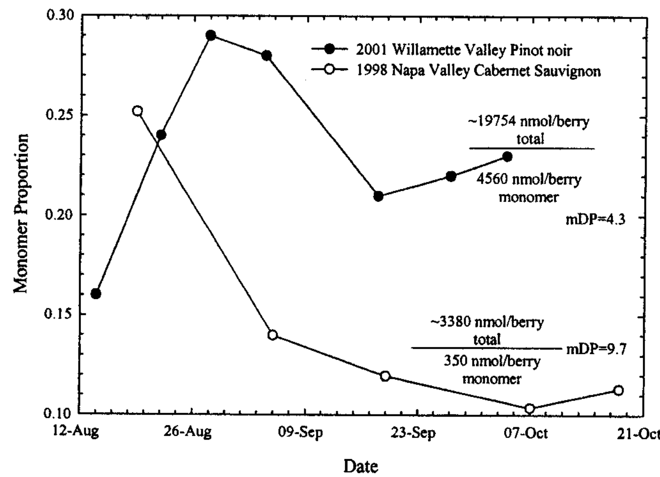

Figure 12. Proportion of the total tannin pool that is flavan-3-ol monomer for cvs. Cabernet Sauvignon and Pinot noir. Numbers indicated are the values at harvest.

Wine Phenolics

Wines were made from grapes harvested at two times, 27 September and 4 October. The results of the phenolic analyses are shown in Figure 15. From these results, it can be concluded that later harvest times favor a reduction in seed tannin extraction after short maceration times. However, longer maceration times and later harvests can also promote seed extraction. This may be due to higher alcohol concentrations resulting from higher berry sugar concentration.

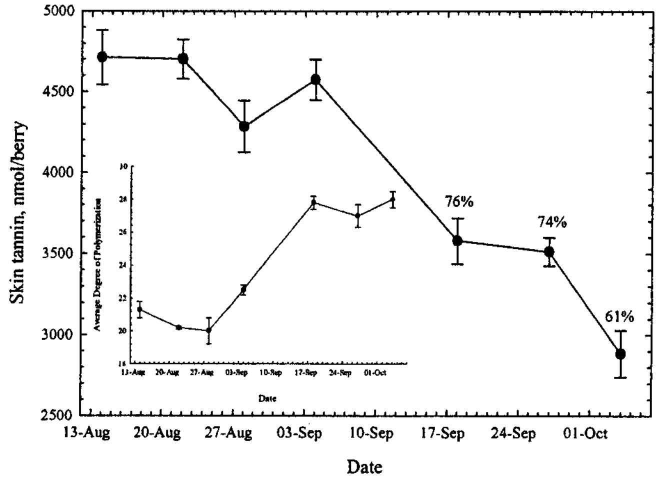

Figure 13. Development of the grape skin tannin and the corresponding mean degree of polymerization (mDP, inset) with error bars indicating ± SEM (N=5). Numbers toward the end of the growing season indicate the % of maximum present.

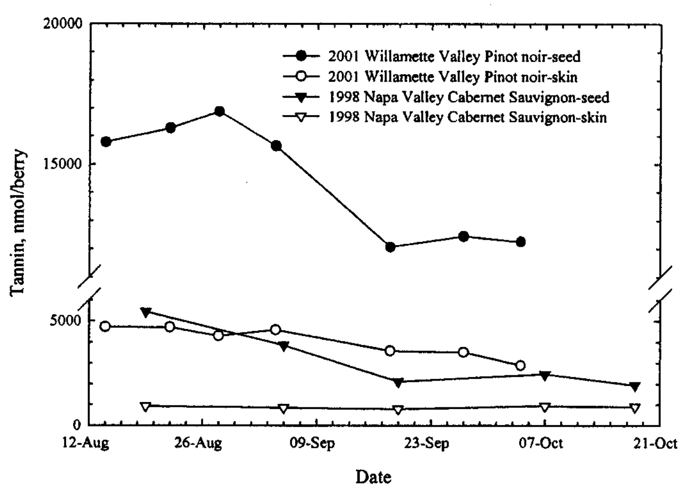

Figure 14. Comparison of Grape seed and skin tannin development (including flavan-3-ol monomers) for cvs. Cabernet Sauvignon and Pinot noir.

One of the reported values in Figure 15 is the proportion of skin tannin present in the wines. Given the different impact that seed and skin tannins could have on overall wine textural quality, it is desirable to determine the proportion of skin and seed tannin extraction into red wine to determine if stylistic differences in texture can be associated with differences in the source of tannin. From this study and others (Kennedy et al. 2000a, 2000b, 2000c, 2001), it is clear that the tannin extension subunit composition differs between seed and skin tannins within the same variety. Moreover, it is apparent that the composition does not vary with berry development. Assuming that fermentations are carried out without cluster stems, this observation makes it possible to determine the tissue source and quantity of tannin extracted into wine based on a comparison of the proportional composition of extension subunits (Tables 1 and 2).

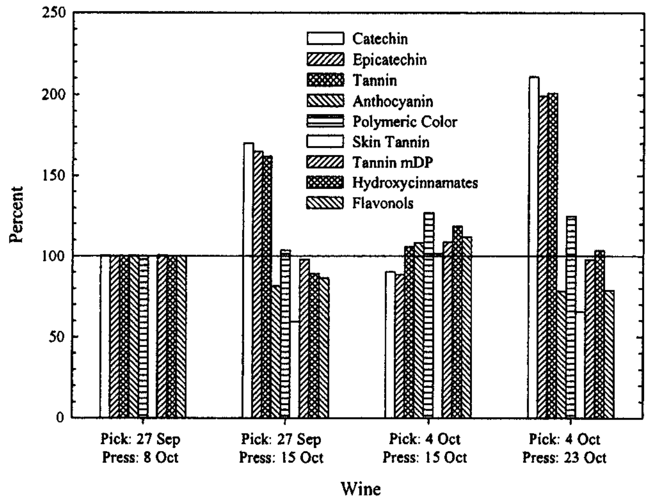

Figure 15. Composition of wine phenolics for the 4 wines prepared in this study, and expressed as relative concentration to the phenolics in the initial wine.

Table 1. Proportional extension subunit composition for cv. Pinot noir grape seed and skin tannins and corresponding wines.

Sample	Epigallocatechin	Catechin	Epicatechin	Epicatechin-3-O-gallate
Skin	0.327	0.029	0.627	0.017
Seed	-	0.093	0.772	0.135
Pick: 27 Sep Press: 8 Oct	0.141	0.124	0.706	0.030
Pick: 27 Sep Press: 15 Oct	0.108	0.120	0.733	0.040
Pick: 4 Oct Press: 15 Oct	0.159	0.101	0.705	0.035
Pick: 4 Oct Press: 23 Oct	0.099	0.127	0.729	0.045

Table 2. Average proportional skin tannin composition in wines.

Sample	% Skin Tannin by Epigallocatechin	% Skin Tannin by Epicatechin
Pick: 27 Sep Press: 8 Oct	43.1	45.5
Pick: 27 Sep Press: 15 Oct	33.0	26.9
Pick: 4 Oct Press: 15 Oct	42.4	46.2
Pick: 4 Oct Press: 23 Oct	26.4	29.7

One of the primary goals of this study is to understand how the relative composition of tannins and anthocyanins affects both color stability and texture in red wine. The application of new analytical tools to monitor tannin composition in red wine makes this determination possible. From Figure 16, three components have been quantified for the red wines produced: flavan-3-ol monomers and terminal subunits, anthocyanins, and extension subunits. One of the hypotheses being tested in this study is that the relative composition of these species will influence both color stability and texture. These proportions are very different in these wines. Also shown in Figure 16 is a targeted composition that is being prepared with tannins that have been isolated and purified.

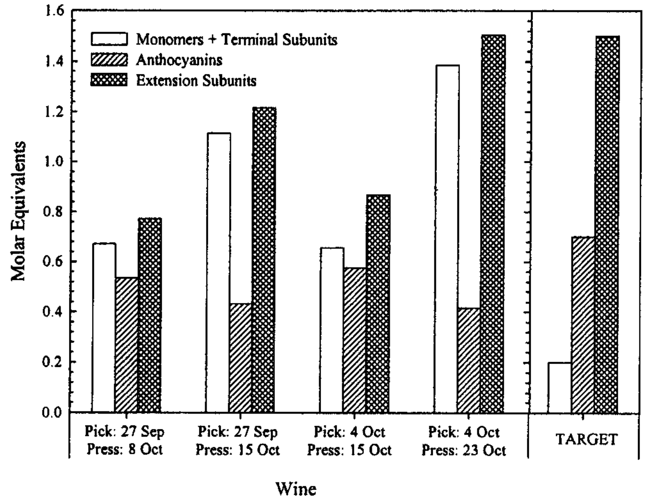

Figure 16. Composition of flavan-3-ol monomers (including terminal subunits), anthocyanins and extension subunits in molar equivalents for the 4 wines prepared in this study. Also included is the targeted wine that will be prepared to understand the effect of tannin composition on color stability.

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