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Title: **"Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching"**

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In a previous article, the authors developed a tannin assay for grapes based on protein precipitation. In this article they combine this assay and another pigment-detecting assay (Somers and Evans, 1977) to develop a third assay that has the advantage of being able to measure different types of phenolic compounds. This new assay is then used to characterize how these different phenolic fractions change during grape ripening. And here is what they find.

- Because phenols are so complex, it is not unusual to base their classification on the method used to separate them. Bisulfite is known to bleach monomeric pigments (also called anthocyanins), but is unable to bleach larger polymeric pigments. Therefore, treating compounds with bisulfite allows separation between: **1) monomeric pigments (free anthocyanins)** and **2) polymeric pigments**. Because polymeric pigments are made up of tannins bound to anthocyanins, they should precipitate to some extent when treated with protein. Therefore, treating these compounds with protein further classifies them into two new fractions: **3) large polymeric pigment (LPP)**, or those pigments that are precipitated with protein, and **4) small polymeric pigment (SPP)**, or those pigments that are not precipitated with protein. Finally, when the protein precipitation assay is used without bisulfite bleaching, the result is a mix of colored and non-colored species, called **5) total tannin**.

- Here is some information to help us become more familiar with all these phenolic names. We know that it is both anthocyanins and polymeric pigments that are responsible for color in red wine. During winemaking and aging, anthocyanins from the skins are thought to react with tannins from the skins and seeds to give rise to polymeric pigments, the stable color compounds in wine. This reaction can happen either directly, or indirectly through cross-linking of individual units - flavanols and anthocyanins - with acetaldehyde. As wine ages, the tannins continue to polymerize, and large polymeric pigments are formed at the expense of the small polymeric pigments. But how do all of these fractions actually change during ripening and winemaking?

- When several wines of the same vintage, and of the same or different varieties, were studied, the authors found that **the ratios of LPP and SPP were strikingly different among red wines, even from the same variety**.

• When the authors looked at pigments in the skins of Cabernet sauvignon and Syrah, they found very little LPP, compared to SPP, and found a large amount of anthocyanin. This anthocyanin content increased as ripening progressed and, interestingly, continued to increase through harvest in Cabernet, whereas it reached a maximum and then declined around harvest in Syrah.

• How do levels of LPP and SPP in the fruit compare to levels in the wine? From this research project, it is clear that grapes contain very little LPP, whereas the corresponding wines have large amounts of LPP. Thus, **most of the finished wine color due to LPP is formed during fermentation**. In contrast, **the color due to SPP is mostly contributed by the berry**, since the levels in the berry are nearly the same as in the finished wine. As for **anthocyanins**, the levels **tend to be higher in the grape than in the corresponding wine**. This was particularly true in Cabernet sauvignon, less so in Pinot noir, and finally, Syrah presented almost the same anthocyanin levels in both fruit and wine.

• How does knowing the levels of LPP/SPP help the winemaker? A very direct application that the authors discuss is the ability to predict a wine's response to a particular fining treatment. For example, commercial fining agents such as gelatin or casein tend to remove only the LPP fraction, leaving the SPP fraction unaffected. Therefore, a wine high in LPP/SPP would be expected to respond more to the fining than one low in LPP/SPP, which might not justify the same treatment. The assay will also be useful to obtain tannin values that could be used as a reference year after year to trigger best winemaking practices, such as press time, blending decisions or aging potential.

• The implementation of this assay requires some familiarity with lab techniques, inexpensive reagents, and only two important pieces of equipment - a spectrophotometer and a bench top centrifuge. If you would like to try it, an easy-to-follow assay recipe can be found at the UCDavis Viticulture & Enology department website:
http://wineserver.ucdavis.edu/people/Faculty/adams/tannin/index.htm

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