

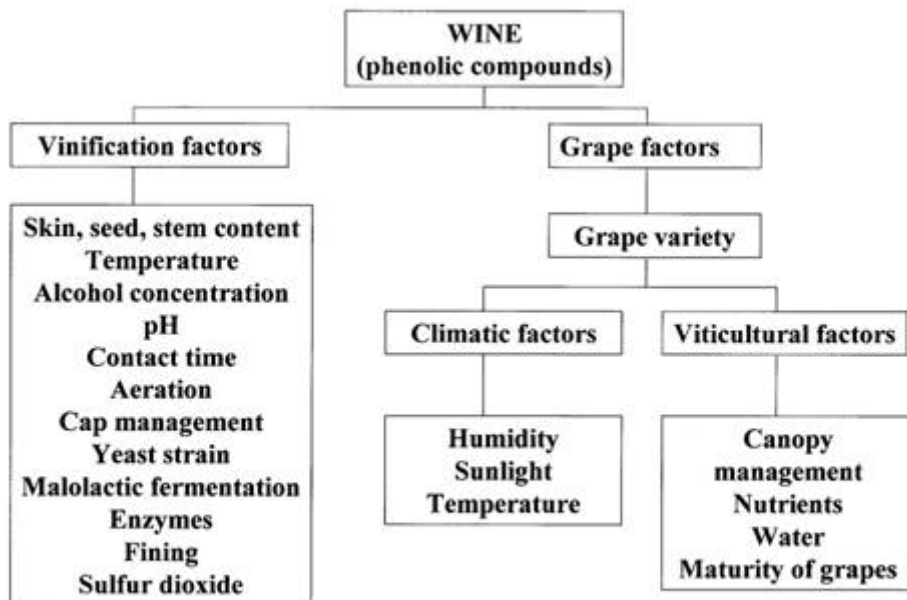
Grape and Wine Color and Tannins

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“The faster the scientific advances, the greater the risk of widening the gap between what we know and what we do.” Emile Peynaud (1984)

The phenolic compounds in wines are the result of a host of factors, including those depicted in Figure 1. The following is adapted, in part, from a presentation titled “Grape and Wine Phenols,” that the author gave in Chile in late 2005. Additional information on the subjects discussed are available at the Enology-Grape Chemistry Group web site at www.vtwines.info. Click *Enology Notes*.

Figure 1



Source: in part, Stockley and Hoj (2005)

Approximately one-third of the carbon produced by grapevines is used to produce phenolic substances. Therefore, phenols are an important constituent of both vines and wines. Grape and wine color receives attention due to the impact on sensory perception, because color absorption spectra are easy to measure, and because of certain assumptions. As Boulton (2005) suggested, these beliefs drive our practices, but are they correct?

- **Grape and wine color strongly correlate to aroma/flavor.** These are not always strongly correlated.
- **Anthocyanin concentration strongly correlates to aroma/flavor.** The biochemical pathways for the production of anthocyanins and aroma/flavor compounds are different and operate independently. Why should they relate? Indeed, the biochemical pathways for anthocyanins and tannins are also different.
- **Color is a good indicator of yield.** The correlation between color and yield depends on what component(s) of yield are investigated.
- **Desirable grape color assures desirable red wine color.** While correlated, these two are different, as outlined below under Factors Impacting Red Wine Color.

Tannins. The term *tannin* defines a very heterogeneous group of phenolic compounds that are identified, based on certain properties:

- astringency
- bitterness
- reaction with ferric chloride
- the ability to bind with proteins, e.g., tannin leather - hence the term *tannins*

It was their characteristic interaction with proteins that traditionally differentiated tannins from other phenols. However, not all phenols that bind with proteins elicit an astringent response, and tannins are not the only compounds in wines that cause astringency.

Phenols, including tannins, have the ability to polymerize, or associate, with themselves and other compounds, including anthocyanin pigments. As polymerization occurs, the molecule becomes larger. The number of subunits bound together is referred to as the DP number, or degree of polymerization. The DP number increases with wine age, a reason, in part, why wines become more supple or less astringent, and go from bright red to tawny in color.

So-called tannin “quality” refers to:

- degree of polymerization
- the association of tannins with other molecules
- stereospecific nature of the tannin molecule, which can make it harsh and hard, or supple

Grape tannins derived from the skin, seeds, and stems differ in their length, overall subunit concentration (DP number), and sensory properties. They differ in the following:

- astringency
- stereospecific nature
- size
- number of hydroxyl groups

In general, seed and stem tannins are harsh and have a relatively short DP length, the average being about 15. Skin tannins, on the other hand, have a DP number that averages about 40.

Polymeric Pigments. In grapes and wines, anthocyanin pigments can be either free monomers, that is, unbound, or associated with other phenols to form polymers. In the lab, we can easily characterize two sizes of polymers: small polymeric pigments (SPP), which are five subunits or smaller, and large polymeric pigments (LPP), which are more than five subunits. Polymerization can impact the sensory quality of the pigments by increasing color stability, and by changing the spectral color from bright red to brick-red. Additionally, the LPPs are loosely associated with softer, less harsh astringency.

A limited degree of polymerization occurs in the fruit during maturation. In wines, tannin polymerization continues until an anthocyanin molecule binds the terminal end, then polymerization is believed to stop. Therefore, the ratio of anthocyanins to tannins is important in impacting polymerization. The ratio of free anthocyanins to tannin is important, due to the following:

- tannin and anthocyanin concentrations affect concentrations of LPP
- low concentrations of tannin cannot form polymers
- whichever is limiting will determine amount of polymeric pigment
- impacts color, color stability, and mouthfeel

Wine quality may be dependent, in part, on the ratio of anthocyanins to tannin, a reason why some add enological tannins. The anthocyanin-to-tannin ratio of red wines for optimum color stability is usually about 1:4. An example of Bordeaux red wine ratios is as follows (source, Scott Labs):

- Red wines: Anthocyanin 0.5 to 1 g/L, Tannin 1.5 to 5 g/L
- Rosé wines: Anthocyanin 0.2 to 0.5 g/L, Tannin 0.25 to 1 g/L

What Determines Red Wine Color? Color is an important wine attribute, because humans are visually oriented. As such, wine color can certainly bias evaluations. A classic example of color bias is to change the color of a white wine, such as Chardonnay, to red. In blind evaluations, the color-adjusted wine frequently receives a different sensory rating for attributes such as fullness, body, and complexity.

As such, richly-colored wines are assumed to have high volume or body, and softer tannins. Conversely, a wine with less color is automatically assumed to have 'green' or 'harsh' tannins. Spectral color in wine is a function of these three elements:

- anthocyanin concentration
- concentration of cofactors, or certain non-colored compounds, which bind with anthocyanins
- polymeric pigments

Hyperchromicity, also known as copigmentation, is an interesting phenomenon that allows more visible red color than would be expected due to the anthocyanin concentration alone. Cofactors are non-colored compounds that have the ability to bind with anthocyanins, creating more color than the unbound pigment, hence the term *hyperchromicity*.

The concentration and type of cofactors vary greatly from variety to variety, and season to season, but include some non-flavonoid phenols, flavonols, and the amino acid arginine. It is not likely that enological tannins contain compounds that act as cofactors.

We need to learn more about the impact of seasonal and processing variations on cofactors. Some are easily oxidized, which can affect red wine color intensity dramatically. Previously, we assumed that fining agents bound with anthocyanin molecules directly, thus reducing red color. It appears that their action may also be removing cofactors and other macromolecules, or impacting cofactor binding with anthocyanins.

Because red color is a function of three elements (anthocyanin concentration, cofactor concentration, and polymeric pigments), it is possible to have the following (Boulton, 2005):

- Change in grape anthocyanin concentration = Change in wine color
- Change in grape anthocyanin \neq Change in wine color
- No change in grape anthocyanin = Change in wine color

The above highlights several points:

- Variation in cofactors and polymeric pigment concentration may be more important to spectral color, than simply anthocyanin concentration.
- Grape pricing based on anthocyanin concentration alone may not be desirable.
- Harvesting based on anthocyanin concentration will not necessarily assure desirable red wine color.

Phenolic Compounds and Red Wine Processing. The concentration of phenols in red wines is dependent on the grape and vinification factors. Generally, no more than 50% of the total phenols present in the skins, seeds, and flesh of grapes can be extracted during conventional winemaking. The extraction rate of phenols during processing is primarily a function of the following:

- maturity of the phenolic elements in the skins, seeds, pulp, and stems (including cap stems)
- temperature
- contact time
- alcohol content at dejuicing

The main effects from winemaking techniques are galloylation, the association with seed tannins. Increased galloylation can increase astringency.

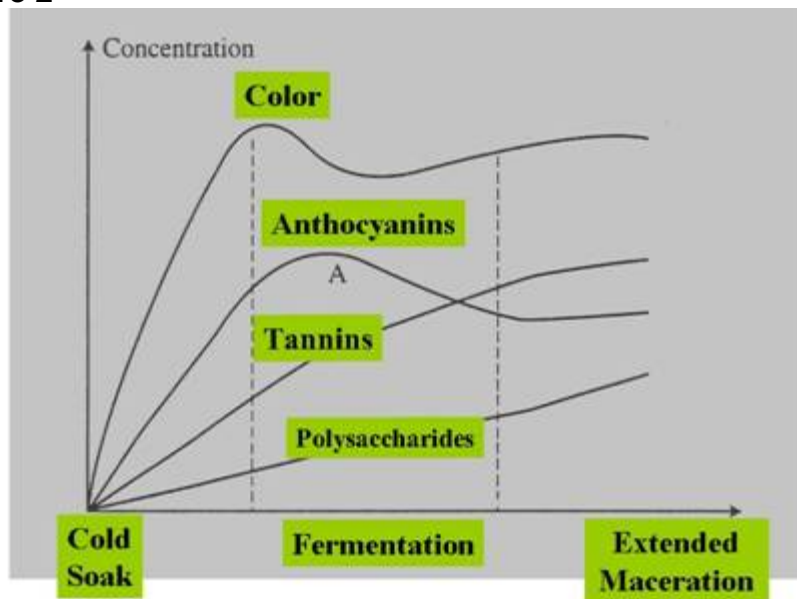
We know that prefermentation sorting is important with regard to phenolic composition of wines, as is gentle fruit handling. Gentle handling is reported to be one of the benefits of gravity flow processing, as a result of minimizing the extraction of phenols, by limiting the non-soluble solids extraction.

An area traditionally overlooked is post-destemming sorting to remove cap stems or jacks. Stem tannins are chemically different (different DP number) from skin tannins, and impart a different sensory profile. Stem tannins, including cap stem tannins, are more astringent and harsher than skin tannins. Jack stems in the fermentor can be a problem, if the concentration is high and the stems are green or not lignified, resulting in increased tannin intensity and astringency.

Various cap management protocols, such as punch down, pump over, etc., have an impact on the following:

- non-soluble solids concentration
- rate of phenolic extraction
- DP number (degree of polymerization)

Figure 2



Source: Glories (1993)

Figure 2 shows hypothetical curves, illustrating the relative changes in spectral color, anthocyanins, total tannins, and polysaccharides, from cold soak, through

extended or postfermentation maceration. Extraction of individual classes of phenolic compounds differs during processing.

Cold Soak. An important initial processing question is the desirability of cold soak, or pre-fermentation maceration. The rationale behind cold soak is that aqueous extraction in a non-alcoholic matrix improves wine color and color stability, and may increase aromatic intensity. Is that true? Sometimes, but not always. Monomeric anthocyanins are extracted during cold soaking, which may result in increased finished red wine color (Figure 2).

Tannin extraction during cold soak is limited by solubility. As can be noted from Figure 2, tannins are extracted at a slightly lower rate than anthocyanins, but the extraction continues throughout the maceration period. Seed tannin extraction requires ethanol. Therefore, tannins extracted during cold soak are from the skins and stems, not the seeds. Important cold soak variables include the following:

- varietal
- season
- time
- temperature

Varieties like Pinot noir may actually have less red wine color following cold soak. Highly-colored varieties, like Cabernet Sauvignon, frequently demonstrate an increase in color absorbance at 520 nm as a result of cold soak, but this increase may not persist through wine aging. The impact of cold soak on various varieties needs further review. Large variations, mainly in cofactor concentration, may be responsible for both varietal and seasonal variations on the impact of cold soak.

Most winemakers practicing cold soak use a temperature low enough to limit the growth of unwanted microorganisms, such as *Kloeckera*. As such, a temperature below 10°C is considered desirable.

The duration of cold soak varies considerably. During a trip to Chile in 2004, I visited with winemakers who suggested the desirability of a 20-day cold soak. A student in the Enology-Grape Chemistry Group examined the impact of cold soak time on Cabernet Sauvignon wine chemistry at seven months post-fermentation (Figure 3 and 4). NS indicates not significant, * indicates significance at 0.05.

Figure 3

Effect of 4-Day vs. 20-Day Cold Soak at 7°C of Prefermentation Must on Cabernet Sauvignon Wine Chemistry

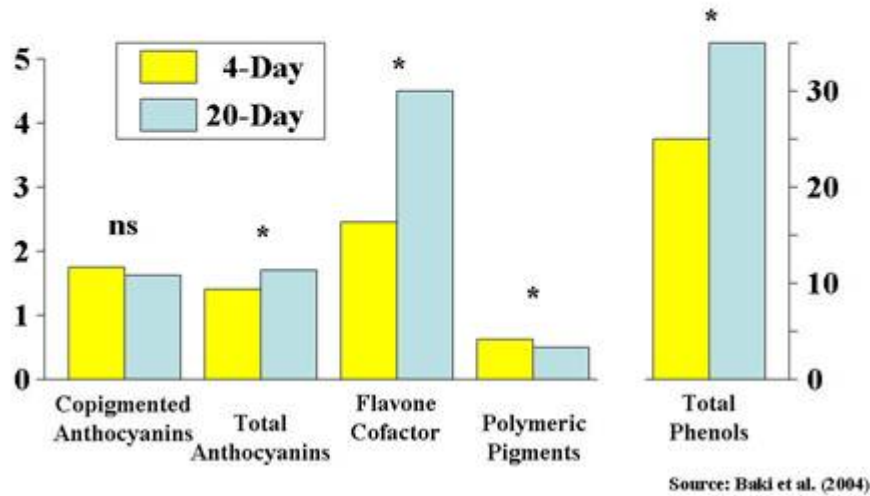
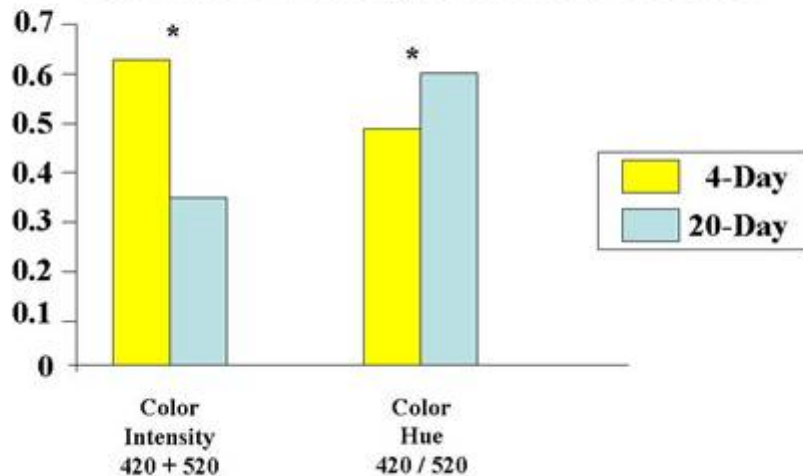


Figure 4

Effect of 4-Day vs. 20-Day Cold Soak at 7°C of Prefermentation Must on Cabernet Sauvignon Wine Colors



Source: Baki et al. (2004)

Red-colored pigments have a maximum absorption at 520 nm. Brown pigments have a maximum absorption at 420 nm. Color intensity (520 nm + 420 nm) actually decreased with increased cold soaking time. Color hue, defined as the ratio of these two wavelengths (absorbance at 420 nm/absorbance at 520 nm), went up, likely due to the increased relative concentration of tannins, which contribute to brown color.

Practical effects of cold soak are a function of time and cultivar. There may be little benefit from more than three days soaking. The impact of cold soak on wine aroma has not been fully resolved.

Cap Management. How the cap is managed impacts the rate of extraction of phenolic compounds. Mechanical punch down, pump over and, in particular, rotation methods, can significantly increase the rate of extraction and the extent of phenolic polymerization.

For the first five to seven days of fermentation, extraction comes predominantly from the skins and stems (Figure 2). Thereafter, the seed coat cuticle is dissolved by the ethanol, and extraction from seeds predominates. From this point on, increases in tannin concentration are mainly from the seeds. This highlights the importance of seed tannin maturity. Free anthocyanins begin to decline when alcohol concentration reaches a certain level. It is at this point that anthocyanins begin to be incorporated into tannins to form polymers. Generally, the DP of the phenols increases with fermentation.

Polysaccharides are mainly extracted from the grape at the beginning of the vatting period (Figure 2). High polysaccharide-producing yeasts have become important winemaking tools. Such yeasts can modify the wine palate profile, notably by lowering the perception of the phenolic and acidic elements.

Color rises during cold soak, as the development of copigmentation and polymerization continues. Color intensity will reach a maximum, and then decline (Figure 2). This represents extraction from the fruit and binding of anthocyanin with tannins, to form small and large polymeric pigments that are red-brown.

Maceration Variables. Important maceration variables include:

- duration - impacts the percentage of phenols from the seeds
- temperature - impacts the rate of extraction from the skins, stems, and seeds

For example, increasing the temperature from 15°C to 35°C increases the rate of extraction, and may increase the concentration of total phenolic compounds by as much as 200%.

Sulfur Dioxide. What is the effect of sulfur dioxide on phenolic extraction and color formation? Sulfur dioxide mainly binds to sugars, but can bind phenols at the carbon-4 position of the flavonoid phenol. As such, too much sulfur dioxide can have a negative impact on phenolic polymerization. Sulfur dioxide generally has little impact on the extraction of phenols from the fruit. Sulfur dioxide may be of benefit in helping to minimize the extent of oxidative degradation of color cofactors.

Maceration Time. What is the impact of maceration time on phenols? Longer maceration during fermentation can increase both the anthocyanin and the tannin content in the wine. As such, there is a big difference between 4-5 days and 10-days fermentation, in the percentage of polymeric pigments after the wine has aged for one year. The greater concentration of polymeric pigments occurs with the longer fermentation.

Extended, or postfermentation, maceration does not increase the anthocyanin content, but does increase the concentration of tannins (Figure 2). The increase in tannins is from the skins, and notably from the seeds. This is usually accompanied by increased phenolic polymerization rates, and may result in wines that have a softer mouthfeel.

What is the optimum time period for extended maceration? The answer relates to vineyard uniformity, fruit chemistry, and stylistic goals. During extended maceration, both extraction from the skin and seeds, and polymerization, are occurring. Some choose to monitor these changes by sensorially comparing a top sample with a bottom sample. Bottom samples, due to the proximity of the seed load, will have relatively high seed tannin concentrations. There are usually limited changes within the first 6-10 days postfermentation. Wines with this extent of maceration are often hard and harsh. Afterward, polymerization, and the possible association of phenols with other compounds, aids in suppleness.

Pectic Enzymes. The effect of pectic and maceration enzymes depends on a number of factors, including cultivar, maturity, the specific enzyme used, and the alcohol at dejuicing. It also depends on when you dejuice, as to how significant enzymes may be.

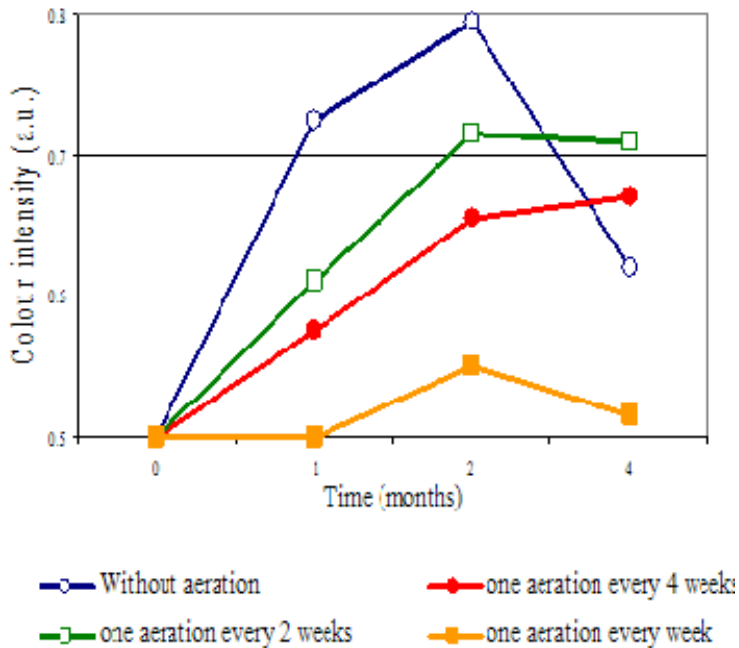
Generally, pectic enzymes impact tannin extraction more than anthocyanin extraction, due to the fact that tannins are not as soluble. Therefore, the use of pectinolytic enzymes can change the ratio of anthocyanins to tannins. Pectinolytic enzymes may be an important wine processing tool, particularly in the case of short vatting, and can impact the following:

- increased extraction of color, notably if you are short vatting
- increased extraction of tannins
- increased rate of polymerization

There are a number of enzyme preparations available for different winemaking functions.

Oxygen and Red Wine Color. Several years ago, an interesting red wine color phenomenon was noted, which is illustrated in Figure 5 (Ribereau-Gayon and Glories, 1983).

Figure 5



Wines made without any air or oxygen exposure developed more red color initially, but soon the color declined. In other words, the red color was not stable. Conversely, red wines produced with some air exposure developed more color and color stability, while too much exposure demonstrated loss of color.

Clearly, oxygen was playing a strong role in red wine color formation and stability. What is the role of oxygen in color, and how much oxygen is needed for optimizing color?

Oxygen helps to form cross-links between anthocyanins and tannins, thus helping to stabilize color. How much oxygen is desirable depends on the percentage of anthocyanins in the monomeric (non-polymerized) form, among other factors. In solution, the unbound anthocyanin molecule is positively charged, which enables it to absorb light, and thus have color. Anthocyanins have a carbohydrate (sugar), usually glucose, attached (esterified) at the 3-position. The presence of this sugar helps the anthocyanin maintain solubility and stability. If the sugar is hydrolyzed or lost, the solubility and stability decrease. What is equally important for red color is the association of this molecule with tannins and other phenols. There are several mechanisms (oxidative and non-oxidative) by which pigment polymers are formed.

One example illustrates the role of tannin depolymerization. If a tannin of DP-10 size is hydrolyzed under acidic conditions in wine, it can break up into shorter lengths, such as two DP-5s. In such a case, the hydrolysis would produce one electron-neutral and one positively-charged tannin. Depending on the concentrations of available tannins and anthocyanins, the positively-charged tannin will react with either another tannin or an anthocyanin molecule. This explains why the ratio of tannin to anthocyanin in the must is important, regarding color and color stability. If it reacts with another tannin, a longer non-colored polymer will be formed. However, the process is different if it reacts with an anthocyanin. If the anthocyanin reacts with a tannin, this new larger anthocyanin-tannin polymer acts as an electron sink and stabilizes color.

A unique aspect of this binding is that the anthocyanin molecule is no longer available to react, and the anthocyanin acts as a terminus for any further reaction at that end of the polymer.

In addition to the direct reaction described above, anthocyanins and tannins can be linked by aldehydes. This is the basis for microoxygenation's impact on color and stability. Aldehydes are able to act as cross-linkers, binding anthocyanins and tannins. Many aldehydes can be derived from toasted oak. Aldehydes are also produced as a result of ethyl alcohol oxidation, which occurs during wine microoxygenation. Thus, microoxygenation (including splash racking) of very young wines (when there is a high percentage of monomeric or unbound anthocyanins) can aid in color and color stability, as a result of oxidative formation of anthocyanin-tannin pigment polymers. There is a structural difference in tannins cross-linked with aldehydes, versus reductively-formed tannin pigment polymers which may impact stability.