



RESEARCH FOCUS

Comparing Red Wine Color in *V. vinifera* and Hybrid Cultivars

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Bench-top anthocyanin kinetic experiments help researchers describe color formation in red hybrid wines.

Photo by Claire Burtch

Red hybrid grapes have a broader and more varied collection of phenolic compounds than their *Vitis vinifera* counterparts, and consequently show greater diversity in wine color, structure, and mouthfeel. Hybrid red wine quality, however, is often measured through comparison to more familiar *V. vinifera* varietal wines, so obvious color differences may detract from perceived quality. Winemakers complain that hybrid red wines vary from *V. vinifera* in color density, hue, and development during aging, but the reasons for these differences have not been extensively studied. To determine the source of the differences in hybrid and *V. vinifera* wine color, we charted the reaction between tannins and anthocyanin mono- and diglucosides in a benchtop model. Anthocyanins were used alone and in combination to elucidate the changes in color and formation kinetics in various situations.

So little work has been performed on hybrid cultivars that the winemaking variables impacting wine color are largely unknown. Hybrids contain high levels of tannin-binding protein that limit the efficacy of color-enhancing practices used in *V. vinifera* production, as endogenous or added tannins are removed from solution before they can form stable complexes with anthocya-

KEY CONCEPTS

- The color of red wine comes from pigments called anthocyanins.
- Wines produced from *V. vinifera* have anthocyanin-3-monoglucosides, which polymerize with other wine compounds to form stable color.
- Wine produced from interspecific hybrids usually contain high concentrations of anthocyanin-3,5-diglucosides.
- Anthocyanin-3,5-diglucosides don't form polymeric pigment as quickly as monoglucosides.
- Hybrid cultivars have less extractable tannins.
- Interspecific hybrid wines will have low concentrations of stable color, polymeric pigment, due to high anthocyanin-3,5-diglucoside concentration and low tannin concentration.

nins. For this reason, understanding the rate and types of reactions that occur with hybrid grape anthocyanins is the first step in developing hybrid-specific processing protocols that would allow winemakers to modify wine color, and to ensure stable color over time.

In this work, we found substantial differences in the rate at which the anthocyanins most common in *V. vinifera* and hybrid grapes reacted with tannins and with each other. Further, the visible color produced by different anthocyanin combinations varied a great deal. While this work goes only a little way in explaining the differences found in the two wine types, it clearly demonstrates that anthocyanin type and binding capacity are key factors, and warrant further study.

Background. Red wine color has captivated scientists for decades, and as a result there are hundreds, if not thousands, of studies on the subject. However, a very large majority of these studies are based on wines produced from *Vitis vinifera* wine grapes. This is understandable, considering the much more recent introduction of interspecific hybrid grapes to the wine scene.

In *V. vinifera* wines, a transition of wine color from purple-red to brick red during aging has been documented time and again. The same color transition, however, has not been described in most hybrid wines. Interspecific hybrid grapes have important chemical differences when compared to *V. vinifera* grapes, which raises questions concerning the color of red wines produced from these grapes. To better understand hybrid wine color, we will start with what is known about *V. vinifera* wine color and combine this with what is known about the chemical composition of interspecific hybrid wines.

Vinifera wine color. Red wine is colored by phenolic pigments called anthocyanins. The base of an anthocyanin, called an anthocyanidin, is an aromatic ring [A] bound to an oxygen-containing ring [C], which is bound to a second aromatic ring [B] (see Figure 1) (De Freitas & Mateus 2006). When a sugar is attached, the anthocyanidin is called an anthocyanin. In *V. vinifera* grapes and wines, there are five main anthocyanins: the 3-glucosides of cyanidin, delphinidin, malvidin, peonidin, and petunidin, with malvidin-3-glucoside being the most common (Mazza & Francis 1995).

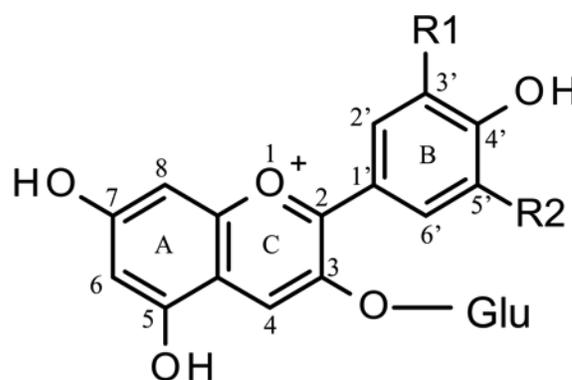
Anthocyanins have the B-ring, and different substitutions (hydrogen (-H), hydroxyl (-OH), or methoxy (-OCH₃)) lead to different colors and levels of reactivity (Figure 1). Hydroxyl groups promote blueness, while methoxy groups promote redness (He et al. 2012). As the number of hydroxyl groups on the B-ring increases, the color of the molecule changes from red to violet (De Freitas & Mateus 2006). For example, delphinidin (with hydroxyl groups on R1 and R2) appears purple in color, while pelargonidin (which lacks hydroxyl groups) appears orange. Anthocyanin color is dependent on anthocyanin concentration and the pH of the solution. Figure 2 shows the color of the five primary wine anthocyanins at 0.1 mM in model wine (pH=3.6).

Color stability. Anthocyanins are very unstable. If they become hydrated with water or bleached by sulfites, they change into a colorless compound. Once released from grape skins, they begin to react with other compounds to stabilize in a colored form. In young red wines, this process is due to copigmentation. Copigmentation occurs when anthocyanins form molecular stacks with other small compounds (copigments) in juice and wine. The copigment protects the anthocyanin structure from hydration, and associated loss of color. In fact, copigmentation is responsible for up to 50% of the color in young red wines (Boulton 2001).

As anthocyanins are extracted during fermentation, some portion of them will bind into their copigmentation forms,

depending on the concentration of copigmentation cofactors. Copigmentation shifts the equilibrium between anthocyanins in the skin and anthocyanins in the wine, and allows more anthocyanins to be extracted from the skins. Therefore, grapes that have high concentrations of copigmentation cofactors extracted during fermentation will also have greater concentrations of extracted anthocyanins and a greater copigmentation effect (Boulton 2001).

As ethanol increases during fermentation, copigmentation complexes are disrupted and anthocyanins are free to form more permanent (covalent) bonds with other chemicals, such as flavanol monomers (ex. catechin), dimers, trimers, and polymers (condensed tannins), other phenolic compounds, acids (ex. pyruvic acid), and aldehydes (ex. acetaldehyde). These newly formed pigments have dif-



Name	R1	R2
Cyanidin-3-glucoside	OH	H
Delphinidin-3-glucoside	OH	OH
Malvidin-3-glucoside	OCH ₃	OCH ₃
Peonidin-3-glucoside	OCH ₃	H
Petunidin-3-glucoside	OCH ₃	OH
Pelargonidin-3-glucoside	H	H

Figure 1: Monoglucoside form of the primary wine anthocyanins.

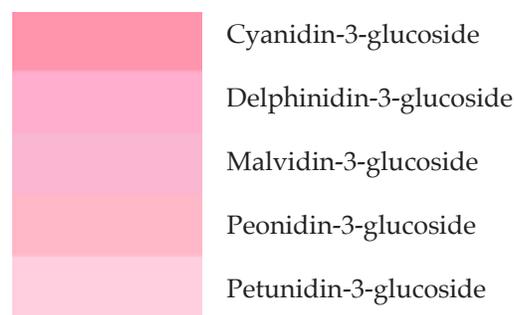


Figure 2: Color of the monoglucosidic form of the primary wine anthocyanins at 0.1 mM in model wine.

ferent colors than their anthocyanin parents. For example, pyranoanthocyanins are a group of orange pigments that form when certain small molecules, such as pyruvic acid, react with an anthocyanin, forming an additional ring (Alcalde-Eon et al. 2006). The new ring makes the pyranoanthocyanin very stable because it protects the anthocyanin from hydration and sulfite bleaching.

The orange pyranoanthocyanins can further react with compounds such as flavanols to form blue compounds (De Freitas & Mateus 2006). Anthocyanins can also react with other anthocyanins to form red or colorless compounds depending on which conformation each of the anthocyanins is in (Vidal et al. 2004). If this reaction is mediated by acetaldehyde, the resulting compound might also be blue or purple (Atanasova et al. 2002).

One of the most significant reactions is between anthocyanins and flavanols. These can be monomers (catechin and epicatechin) monomers or polymers formed from long chains of condensed tannins. When anthocyanins react with flavanols in the presence of acetaldehyde, ethyl-linked purple pigments form.

Ethyl-linked anthocyanins are 50% or more colored at wine pH (Cheynier et al. 2006). However, the ethyl bridge in these compounds is highly unstable, so they typically break down and form more stable pyranoanthocyanin derivatives, which produce an orange color (Cheynier et al. 2006). When anthocyanins react with flavanols directly (no acetaldehyde), the resulting compound can be red or colorless depending on the conformation of the anthocyanin.

Reaction products of anthocyanins and condensed tannins form another class of stable color referred to as polymeric pigment. These anthocyanin—tannin reaction products

are at least partially responsible for the stable color in red wine, as they are more pH stable than anthocyanins alone. Like pyranoanthocyanins, anthocyanin—tannin products have red-orange color, though other colors are possible.

To summarize *V. vinifera* wine color, copigmentation is the driving force of the purplish-red color of young red wines. As ethanol concentration increases, copigmentation links disassociate, and reactions between anthocyanins and other wine compounds take place. These reactions tend to move the wine towards a brick-red color produced by pyranoanthocyanins and polymeric pigment. The pyranoanthocyanins, pyranoanthocyanin-derivative pigments, anthocyanin-anthocyanin pigments, anthocyanin-flavanol pigments, and anthocyanin-tannin pigments described above may all form in *V. vinifera* wines. The exact likelihood of the formation of each of these compounds, however, is not well understood.

Interspecific hybrid wine color. Fruit composition differs from interspecific hybrid grapes. *V. vinifera* grapes leads to differences in sensory characteristics, including color in the finished wines. Anecdotal evidence suggests that non-*vinifera* and hybrid wines do not undergo the same color evolution from purple-red to brick-red (Manns et al. 2013) as wines made from *V. vinifera* (Alcalde-Eon et al. 2006). This lack of color evolution has multiple contributing factors, including unique anthocyanin types and concentrations and lower concentrations of condensed tannins in many hybrids (Manns et al. 2013; Springer & Sacks 2014).

Hybrid wines can have very high concentrations of total anthocyanins. A recent study profiling anthocyanins in 17 hybrid grape samples found concentrations between 500 and 6600 mg/L juice (Figure 3). These anthocyanins are different than those found in *V. vinifera* wines in two main

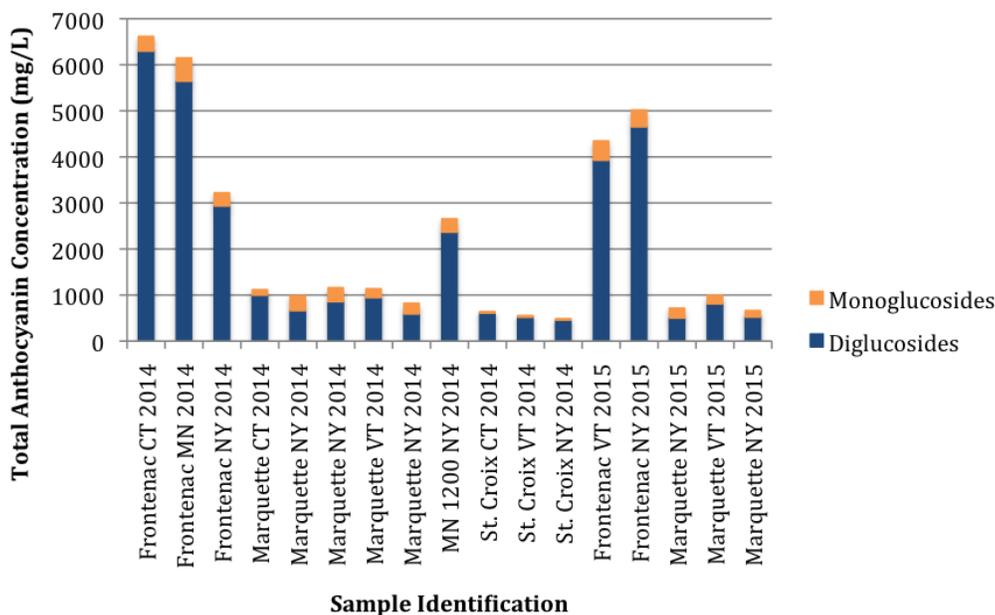


Figure 3: Total anthocyanin concentration (mg/L) in juice of hybrid grapes measured by High Performance Liquid Chromatography (HPLC).*

*CT=Connecticut; MN=Minnesota; NY=New York; VT=Vermont

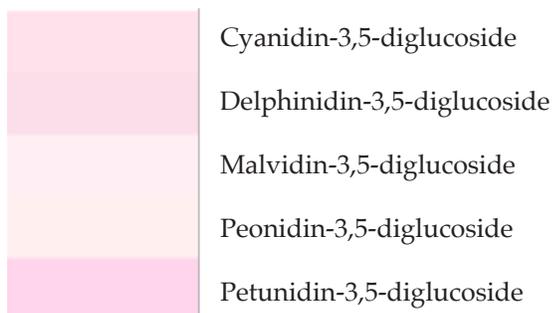


Figure 4: Color of the diglucosidic form of five common wine anthocyanins at 0.1 mM in model wine.

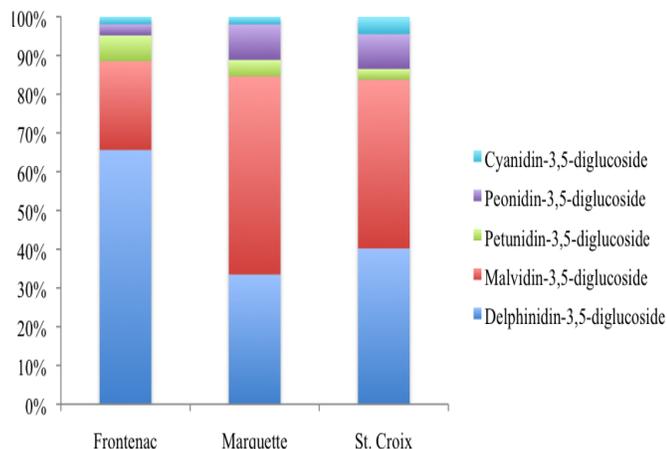


Figure 5: Average proportions of five types of anthocyanin diglucosides in juices from sample grapes.*

*Frontenac (n=5), Marquette (n=8), and St. Croix (n=3)

ways. First, hybrid wines have the potential to contain high concentrations of anthocyanin-3,5-diglucosides; up to 100% of the anthocyanins can be in this form (Van Buren et al. 1970). In the hybrid samples mentioned above, the maximum diglucoside proportion was 95% in a Frontenac sample (Figure 3). Anthocyanin diglucosides have two attached glucose units, compared to the anthocyanin-3-monoglucosides (with one glucose unit) found in *V. vinifera* wines. The diglucosides have different colors than the monoglucosides, though color is still dependent on concentration and pH (Figure 4).

Secondly, hybrid wines tend to have higher concentrations of blue and purple anthocyanins, including delphinidin, petunidin, and malvidin, than *V. vinifera* wines (Manns et al. 2013). Young hybrid wines tend to be more blue and purple than young *V. vinifera* wines. Figure 5 shows a breakdown in average concentration of anthocyanin diglucoside types in Frontenac, Marquette, and St. Croix. Note the high proportions of delphinidin-3,5-diglucoside.

Diglucosides and color. The anthocyanin diglucosides have a significant impact on wine color. Unlike monoglucosides, diglucosides are unable to form the orange-red pyranoanthocyanins and their derivatives. Moreover, diglucosides are less reactive than their monoglucoside

counterparts. Furthermore, a recent study showed that monoglucosides react 7.5 times faster than diglucosides (Figure 6). This means that anthocyanin monoglucosides form polymeric pigments, stable color, much more quickly. When the mono- and diglucosidic forms of the same anthocyanidin base (ex. malvidin-3-glucoside and malvidin-3,5-diglucoside) were placed in a model wine solution together, the rates of the monoglucosides decreased (Figure 7), but the diglucoside rates remained unchanged. Wines containing anthocyanin diglucosides are likely to form less polymeric pigment than *V. vinifera* wines, even if the hybrid wines contain monoglucosides.

In addition to the unique anthocyanin profiles, hybrids contain lower levels of condensed tannins than *V. vinifera* wines (Springer & Sacks 2014). This means that polymers of anthocyanins and tannins are less likely to form, and color is less likely to be stabilized. One proposed reason for low tannin levels is that tannins are bound to protein in the cell walls of hybrid grapes (Springer & Sacks 2014). Therefore, tannins cannot be extracted. In the samples studied, we found lower protein concentrations than those previously reported for hybrids (Table 1) (Springer et al. 2016), and tannin concentrations were similar to those reported in the literature and lower than those found in *V. vinifera* grapes (Springer & Sacks 2014).

Table 1. Tannin and protein concentration of hybrid grapes. Results are expressed as mean (n=2 for tannins; n=3 for protein) with standard deviation in parentheses.

Variety	Vintage	Location	Tannin (mg/L CE)	Protein (mg/L)
Frontenac	2014	New Haven, CT	28.5 (3.4)	83.2 (18.6)
Frontenac	2014	Excelsior, MN	39.7 (0.4)	73.3 (1.4)
Frontenac	2014	Willsboro, NY	8.1 (5.1)	74.0 (4.5)
La Crescent	2014	New Haven, CT	15.0 (6.1)	116.7 (27.2)
Marquette	2014	New Haven, CT	62.4 (0.3)	42.7 (1.4)
Marquette	2014	Trumansburg, NY	15.7 (6.7)	77.6 (5.4)
Marquette	2014	Geneva, NY	22.8 (5.9)	37.4 (5.6)
Marquette	2014	Burlington, VT	30.1 (2.9)	37.7 (1.5)
Marquette	2014	Willsboro, NY	15.7 (3.1)	46.5 (3.5)
MN 1200	2014	Willsboro, NY	24.7 (4.1)	118.8 (9.7)
St. Croix	2014	New Haven, CT	86.2 (5.8)	61.8 (2.9)
St. Croix	2014	Burlington, VT	28.6 (0.9)	36.6 (6.3)
St. Croix	2014	Willsboro, NY	37.6 (0.4)	87.0 (16.5)
Frontenac	2015	Burlington, VT	37.3 (11.3)	78.5 (7.3)
Frontenac	2015	Willsboro, NY	85.5 (31.3)	57.9 (3.0)
Marquette	2015	Trumansburg, NY	35.0 (1.1)	59.6 (1.0)
Marquette	2015	Burlington, VT	91.3 (62.1)	52.1 (2.2)
Marquette	2015	Willsboro, NY	30.7 (8.9)	49.3 (1.0)

MONOGLUCOSIDES VS. DIGLUCOSIDES

Anthocyanin diglucosides have a significant impact on wine color. A recent study showed that monoglucosides react 7.5 times faster than diglucosides (Figure 6). In other words, anthocyanin monoglucosides form polymeric pigments (stable wine color) much quicker. Mono- and diglucosidic forms of the same anthocyanidin base had decreased monoglucoside rates (Figure 7), but diglucoside rates remained unchanged. As a result, wines containing anthocyanin diglucosides are likely to form less polymeric pigment than *V. vinifera* wines, even if the hybrid wines contain monoglucosides.

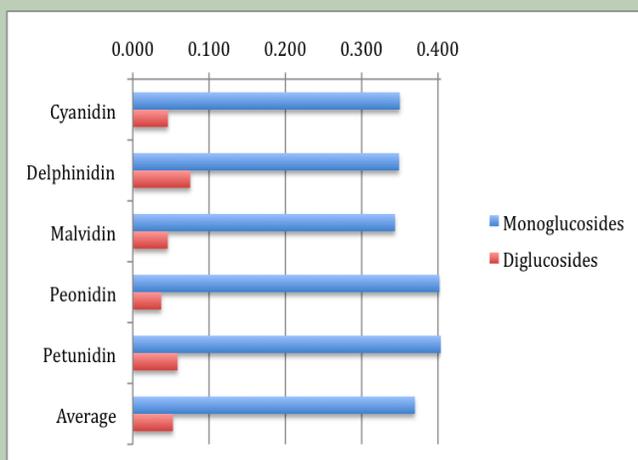


Figure 6. First-order rate constants (k) of the decrease in monomeric anthocyanin concentration in single (mono- or diglucoside) anthocyanidin reactions.

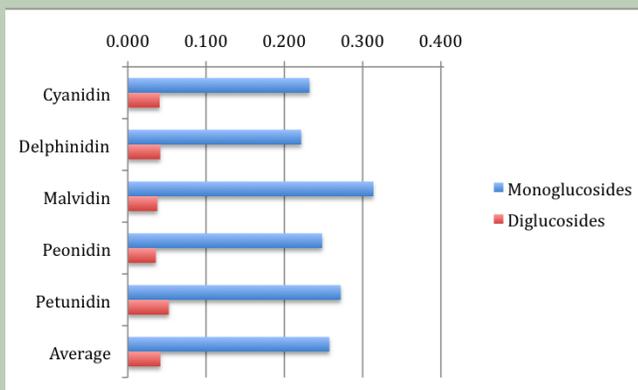


Figure 7. First-order reaction rate constants (k) of monomeric anthocyanin decrease in coupled (mono- and diglucoside) anthocyanin reactions.

To summarize, interspecific red wine color will not behave in the same way as *V. vinifera* wines. While copigmentation will be responsible for color stabilization in young hybrid wines, copigmentation complexes will disassociate throughout fermentation and polymeric pigment formation will not proceed as rapidly due to high concentrations of anthocyanin diglucosides and low condensed tannin concentration. Consequently, hybrid wines will exhibit less stable and less predictable color. It is important to continue the study of hybrid red wine color to better understand the pigments that are breaking down and being formed over time.

Implementations for winemaking. In hybrid winemaking, high color intensity should be expected in young wines due to high anthocyanin concentrations. Winemakers might also expect more of a blue tint than is found in *V. vinifera* wines, though the intensity of this effect will be cultivar dependent. For hybrids high in anthocyanin diglucosides, a transition in color to brick-red is unlikely to occur, as there are simply fewer anthocyanins capable of forming the pigments that contribute to the brick-red color. If this type of color is desirable, winemakers should consider choosing a cultivar that has a low concentration of diglucosides. Finally, hybrid wine color will be less stable over time because less polymeric pigment will form. The diglucosides are slow to react, and there is less tannin to help stabilize the anthocyanins. As we learn more about hybrid wine color and the components responsible for it, we can begin to understand how to manipulate it to produce desired wine colors.

Further research. Interesting future studies could include a focus on monitoring both the color parameters and anthocyanin levels of hybrid red wines of different cultivars over time. Better understanding the pigments that are forming and breaking down in hybrid wines and the associated color transitions will be helpful in influencing wine color. Other studies could focus on describing why anthocyanin diglucosides are slow to produce polymeric pigment and why they slow down monoglucosides. By learning more about the chemical nature of hybrid wine color, we can begin to work towards more stable and predictable color in these wines.

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