Summary 51

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Title: **"Effect of copigments and grape cultivar on the color of red wines fermented after addition of copigments"**

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[Reviewer's note: Throughout this article, the authors use the word "copigment" to refer to a "cofactor". Even though we believe "cofactor" would have been a better choice, both are fine provided you are aware that a copigment is not a pigment, but rather a colorless molecule.]

In the previous summary, we saw how the addition of a copigmentation cofactor (caffeic acid) to certain Spanish red varieties increased wine color. These authors go two steps further: they study the effect of adding different types of cofactors, and they compare two major varieties, Tempranillo *versus* Cabernet Sauvignon. This is a dense paper that covers a lot of ground.

• To start on a good foot, let's make sure we feel comfortable with the terms. *Copigmentation* is the loose association between anthocyanins and colorless phenolic compounds - called"cofactors" or "copigments"- that causes an enhancement of wine color. *Cofactors* –or copigments- are the colorless phenolic compounds responsible for enhancing wine color. This color enhancement is normally due to two effects: 1) the equilibrium of the anthocyanin is shifted towards the flavylium (red) form (this is called *hyperchromic effect*), and 2) the wine also tends to gain more blue hue (this is called *bathochromic effect*). As research has shown, **levels of copigments in red wines are at least as important as levels of anthocyanins in determining the color of red wines.** This is particularly important in young wines, where copigmentation can account for as much as 30-50% of total red color.

• Which are the best copigments? As we will see, copigments can comprise a number of phenolic categories. Many studies have focused on the role of 1) *flavanols*, either as monomers (catechin and epicatechin), oligomers (dimeric and trimeric proanthocyanidins), or polymers (tannins). Recent research has assigned more importance to cofactors of 2) *hydroxynnamic acid* nature. One such example is caffeic acid (see *Summary 42*). According to the authors, the use of 3) *flavonols* as cofactors has not previously been investigated.

• So the goal of this study was to evaluate the possible color improvement of red wines by the addition of 3 naturally occurring copigments: a flavonol (*rutin*, which is similar to *quercetin* but more soluble), and two hydroxynnamic acids (*caffeic acid* and *coumaric acid*). As varieties, they chose a widely-grown Spanish variety, Tempranillo, and a variety grown world-wide, Cabernet Sauvignon. They conducted each small-scale fermentation (5 kg of fruit) in triplicate.

• To evaluate the effect of these copigments on the color of the wines from each of the two varieties, the authors measured color in a variety of ways. They measured *chromatic characteristics* (by classic absorbances and by CIELAB coordinates). They also measured the contribution to color of *copigmentation* and of *polymeric pigments* (Boulton method). Finally, they measure the detailed composition of the different pigments and the different colorless copigments (HPLC).

• 1) **Effect of copigments on color at the end of fermentation**. The addition of the flavonol *rutin* had a significant effect on color absorbance (A520) at the reference pH (3.6). The magnitude of this effect (*hyperchromic effect*) was 9% for Tempranillo, and 35% for Cabernet. The authors expected this result, since flavonols have shown the most powerful effect as copigments in model systems. Addition of *rutin* also gave the darkest wines (lowest values of *L**), the most saturated red colors (highest values of *c**), with the most purple hues (very low *h**). In this study, rutin-added wines always had the highest total anthocyanin content and the highest percentage of copigmentation, compared to the rest of the wines. These results suggest that **rutin favored the extraction of grape anthocyanins during winemaking through the formation of stable copigment complexes**.

• In contrast, the authors found an unexpected result with the addition of hydroxycinnamic acids (caffeic and coumaric acids): they found these had the opposite effect on color. The addition of caffeic acid, in particular, resulted in a decrease of color, or *hypochromic effect*. The authors noted that these results contradict previous results found with the Spanish varieties *Listan Negro* and *Negramoll* (*Summary 42*), for which caffeic acid increased color. The addition of hydroxycinnamic acids always resulted in lower content of total anthocyanins, and lower percent copigmentation than the Control. In accordance with these observations, wines with hydroxycinnamic acid added were always the lightest in color, with less saturated values and the weakest purple tonalities.

• 2) **Effect of aging**. The positive effects on color of the addition of rutin were maintained over the aging time (9 months). Even though A520 at reference pH decreased for all wines during the aging time, this decrease was smaller for rutin-added wines. As for the wines that had received caffeic acid, the trend was reversed, particularly in Tempranillo. Despite some initial effect, the addition of cofactors to the Tempranillo wines had no significant effect after 9 months of aging. Cabernet Sauvignon, however, still benefited after 9 months from the addition of rutin.
• As expected, total anthocyanin concentration decreased with time for all wines. But rutin-added wines still displayed the highest total anthocyanin levels, whereas wines that had hydroxycinnamic acid added had the lowest anthocyanin levels. This decrease of monomeric anthocyanins during aging is mainly due to the formation of polymeric pigments, as confirmed by the increase in percent polymerization. As a consequence of the decrease in monomeric anthocyanins available, the percent copigmentation also decreases until it becomes zero. Addition of rutin resulted in wines with the highest copigmented anthocyanins over time, whereas addition of hydroxycinnamic acids resulted in the lowest percent copigmentation. Eventually, percent copigmentation in all the wines decreased due to an observed precipitation of colored matter.

• The evolution of the CIELAB coordinates confirmed the previous trends. All the wines turned lighter during aging (increasing L* values), and the resulting colors were less saturated (decreasing c*). As copigmentation decreased, so did the blue hues (increasing h* values, due to less bathochromic effect). The decreased copigmentation, coupled to the increased polymerization with time, led to wines which were finally described as "purely red", and "without any purple". All of these changes occurred more slowly in the rutin-added wines. The changes were also slower in Cabernet Sauvignon than in Tempranillo.

• 3) **Effect of grape variety**. Cabernet Sauvignon was more sensitive to the prefermentation addition of copigments than Tempranillo. Copigmentation depends on the concentration at equilibrium of both components forming the complex, anthocyanins and copigments. The authors discuss how the higher ratio of flavonols/anthocyanins of Cabernet at the end of fermentation might have to do with these results. Each variety also has a very specific anthocyanin profile. For example, Tempranillo has less acylated anthocyanins and more "coumaroylated" anthocyanins, which the authors believe might have neutralized the action of the added copigments.

In summary, the **nature** of the copigment added before fermentation has an important effect on copigmentation (color enhancement). Indeed, *rutin* addition enhanced copigmentation and anthocyanin extraction, improving red color. In contrast, *hydroxynnamic acids*, especially *caffeic acid*, had the opposite effect. In addition to the type of cofactor, this study also emphasized the importance of the **variety** we are working with. To this respect, Cabernet Sauvignon was more responsive than Tempranillo to rutin additions. Two rigorously conducted previous studies showed that caffeic acid improved color in Listan Negro and Negramoll, but it did not have a positive effect in Tempranillo. Finally, this same treatment decreased color in Cabernet Sauvignon. Whenever the copigment had a positive effect on color, this effect persistent after 9 months, meaning the compounds formed were stable.

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