Summary 50

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Title: **"Effect of caffeic acid on the color of red wine"**

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The authors investigate the effect of pre-fermentation additions of different doses of caffeic acid on red wine color. The study takes place in the Canary Islands, Spain, using wines made in 1997 with two main local varieties, the deep-colored *Listan negro* and the poorly colored *Negramoll*.

• Anthocyanins play a major role in the color of young red wines, but their reactivity causes them to fade away with aging. One way to stabilize color is by the formation of "polymeric pigments" between anthocyanins and flavanols. Another way is by the reaction of anthocyanins with non-color factors, which not only stabilizes but also enhances color. This effect is called **copigmentation**. The mechanism behind copigmentation seems to be that the non-colored factors involved –called cofactors or copigments- associate predominantly with the colored form of anthocyanins (called flavylium). Because several forms of anthocyanins -purple, colorless, red, yellow- exist in a wine in a delicate equilibrium, the association of the colored form with cofactors shifts this equilibrium towards the formation of more "red forms", thus enhancing color. These color complexes are then stabilized by surrounding sugar molecules.

• In a preliminary study, the authors determined that caffeic acid was a very efficient cofactor. In this study, they added 120, 240, 480, and 960 mg/l of caffeic acid to either Listan negro or Negramoll juices (50 liter fermentations). Wines for each addition level were made in triplicate. Then the wines were analyzed for color by spectrometry (380-780 nm spectra) and high-pressure liquid chromatography (HPLC) to see the effect of the caffeic acid.

• **Absorbance results**. Color as measured at both 520 and 420 nm was increased as a result of adding caffeic acid (12-75% increase for A520, and 7-18% increase for A420). Higher doses caused a greater color increase. The color increase was greater in Negramoll, the cultivar with the least color intensity, than Listan negro. Because the effect around 520 nm (blue) was higher than the effect around 420 nm (red), the hue index (A420/A520) decreased for all wines [*This emphasizes the limited value of such index*]. The increase in absorbance agreed with an increase in perceived visual color. The authors also found that, in their study, the color increase due to caffeic acid was enhanced through time. However, the overall color intensity (A420+A520) decreased for all wines after 8 months, even though the intensity decrease was greater in the Control, without caffeic acid, than in the treatments.

• **HPLC results**. At the highest dose of caffeic acid, the authors were able to detect two new chromatographic pigments (one showing in Negramoll, and both of them showing in Listan negro). A closer look at these peaks revealed that they had maximum absorbances in the same zones as anthocyanins (520 nm) and caffeic acid (320 nm). This confirmed the nature of the peaks as anthocyanin-caffeic acid complexes.

• **Potential mechanism**. Using water/alcohol solutions, rather than the more complex actual wine, a previous author found the increase in the maximum absorption to be a linear function of the amount of copigment added (with the slope of that line reflecting the amount of molecules of pigment that react with

each molecule of anthocyanin). Using actual wine, the current authors found a similar relationship. Because the linear function they found had a slope equal to one, they were able to propose that the initial reaction of caffeic acid with anthocyanins happens in a ratio of 1:1. Of course, as the wine aged, this relationship stopped being linear, probably reflecting the formation of further complexes.

In conclusion, the authors demonstrated that caffeic acid had a color-enhancing, color-stabilizing, oxidation-resisting effect on two red varieties when added to the musts. This means that the non-pigmented content of the grapes may be just as important to wine color extraction and stability as the extraction techniques we use in the cellar. Our next focus obviously is *how do we increase non-color cofactors in the vineyard?!*

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