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Title: "Incorporation of malvidin-3-glucoside into high molecular weight polyphenols during fermentation and wine aging"

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What do you think would happen if we threw a radioactive anthocyanin molecule into a wine fermentation and tracked it? That is what the authors do in this paper.

• The mechanism by which anthocyanins react with the flavan-3-ol units of tannins is not completely understood. Suffice it to say that this condensation can happen either directly, or through the intervention of acetaldehyde, which acts as a bridge. Even though this is an oversimplification, in general, the amount of anthocyanins tends to decrease towards the end of the fermentation, and the amount of anthocyanins adducts (adducts refering to combinations of anthocyanins with a variety of large tannin molecules) tend to increase. At the same time that the incorporation of anthocyanins into tannins takes place during aging, wine color changes from red-purple to red-brown.

• To gain information about the fate of anthocyanins during fermentation, the authors radioactively-labeled the most abundant anthocyanin species in wine: *malvidin-3-glucoside* - which we will now call "labeled malvidin". Then, using a powerful analytical method able to distinguish monomers (or anthocyanins), oligomers (dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers), and polymers (or tannins), they were able to trace where within these groups the radioactivity (^{3}H-malvidin-3-glucoside) "ended up". To learn how the wine was aging, they extended the experiment to 8 months after bottling.

• The authors set up 6 "very small" Cabernet Sauvignon fermentations (340 g of berries each!) After inoculation, labeled malvidin-3-glucoside was added to 3 of them, the remaining serving as Controls. After fermentation, anthocyanin monomers were separated from the rest of the molecules (this was done through isoamylic alcohol extraction, which yields an alcoholic phase (containing the monomers) and an aqueous phase (containing mostly larger molecules, harder to extract). The change with time in the partitioning of radioactivity (due to the labeled malvidin) between monomers and polymers was then quantified.

• **Fate of anthocyanins during fermentation**. Anthocyanins reached a peak on Day 3 and then decreased. One day after the addition of the labeled anthocyanins, only approximately 50% of the radioactivity was recovered in the wine solution. The authors interpreted this as a **massive adsorption of about half of the original anthocyanin to the grape solids**. After that, both the radioactivity and the total amount of anthocyanins continued to decrease. By the end of the experiment (10 months of aging), *anthocyanins had decreased substantially, but the radioactivity did not change*. This was proof that all the radioactivity in the monomer fraction (anthocyanins) was shifting, or being incorporated, into the larger molecule fraction (tannin adducts), and none of the anthocyanins was chemically disintegrating.

• **Effect of different factors during aging**. The authors next studied how different factors affected the incorporation of anthocyanins into tannins during aging, by setting up the following treatments (in duplicate):
- *Control*: wine adjusted to pH 3.6 and stored in the dark at 20°C.
- *Temperature*: as above but stored at either 5°C or 35°C.
- *pH*: as control but wine was adjusted to either pH 4.1 or pH 3.1. - *Seed extract*: as control but a seed extract (60% of which was tannin) was added to the wine before storage (1 g/l).
- *Copigmentation*: as control but a cofactor (chlorogenic acid, 10nM) was added to the wine before storage.

• And here is how these factors affected the distribution of radioactivity:
- *Temperature*: as temperature increased, less radioactivity was present as anthocyanins (monomers), and more appeared as oligomers and polymers. This suggested that **there was more formation of polymeric pigment taking place at the higher temperature**. The authors did not find an important effect of any of the remaining factors (pH, tannin addition, cofactor addition, 3 additional months of aging) on the amount of radioactivity incorporated into tannin adducts, when compared to the Control.

• Even though the influence of *oxygen* was not included as one of the factors studied, the authors noticed that the radioactivity for all of the treatments was much lower in the oxygen-exposed bottles than in the corresponding un-opened bottles (opened only the day of the analysis). Thus, it would seem that the wines that were sheltered from oxygen exposure presented higher levels of both anthocyanins and tannin adducts. This led the authors to conclude that oxygen may have a dramatic effect on the retention of anthocyanin molecules into polymeric pigments, and should be an important factor to pay attention to in future experiments.

In conclusion, most of the loss of malvidin-3-glucoside during fermentation is likely due to substantial adsorption to solids. During aging, most of the anthocyanins were incorporated into larger molecules. Aging variables like *pH*, *tannin levels*, and *copigmentation* factors, had little effect on this incorporation. In contrast, *temperature* had pronounced effects, accelerating the amount of anthocyanins incorporated into tannin adducts. This work is just one more effort towards understanding the complex mechanisms that lead to polymeric pigment formation.

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