Summary 36

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Title: **"Effect of maturity and vine water status on grape skin and wine flavonoids"**

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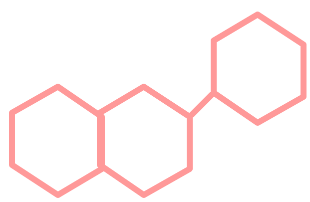
The authors study the effect of both water status and berry maturation on the category of phenolic compounds called the flavonoids.

• Flavonoids are important because they are one of the major components extracted from grape skins during winemaking, playing a role in a red wine's body, astringency, bitterness and color. Let's not let the different types of flavonoids, and their seemingly identical names, intimidate us. They may very well have similar names because their structures are relatively similar. Flavonoids all have in common a characteristic 3-ring structure. Depending on the oxidation state and chemical substitutions on these basic rings, the different types of flavonoids arise.



Flavonoid 3-ring structure

• There are 4 main types of flavonoids: 1) **anthocyanins** (intact aromatic rings) are responsible for the color of red wine, 2) **flavonols** (keto and alcohol substitutions in the ring) are particularly effective as cofactors in copigmentation, 3) **flavanol monomers** (mostly saturated ring with an alcohol substitution) contribute to the bitterness of seeds, and finally, 4) **proanthocyanidins** (formed by association of flavanol monomers) are responsible for the bitter and astringent properties of red wine. Flavanol monomers are also called **catechins**. Flavanol chains the size of proanthocyanidins and larger are also called **condensed tannins**. The two first categories -anthocyanins and flavonols- are found only in the skins; flavanol monomers are found mainly in the seeds; and proanthocyanidins are found in both skins and seeds.

• To study the effect of irrigation on flavonoids, the authors applied the following irrigation treatments: **standard irrigation** (4 liter/hr for 8 hours weekly), **double irrigation** (8 liter/hour for 8 hours weekly), and **minimal irrigation** (4 liter/hr for 8 hours only when water potential fell below -16 bars). The trial took place in Oakville, Napa Valley, and was a randomized block design with 5 replications of 18 Cabernet Sauvignon vines in each replication.

• To study the effects of maturation on flavonoids, berry samples (300 berries) were collected throughout the season starting at veraison. After extracting the flavonoids from the skins with acetone, the flavonoids were analyzed by reverse-phase HPLC (high pressure liquid chromatography). The authors compared the retention time between each peak and known standards to identify the different compounds.

• **Effect of water status on berry size**. Before moving on, the authors made sure that the different irrigation treatments resulted in varying leaf water potentials. These were -1.0 to -1.3 MPa for standard irrigation, -0.7 to -1.0 MPa for double irrigation, and -1.3 to -1.6 for minimal irrigation. The author's 3 main findings were: 1) berry weight at harvest was reduced by 15% in the minimal irrigation, 2) the skin amount was significantly less is minimally irrigated berries, and 3) sugar accumulation was accelerated by water deficit.

• **Effect of water status on flavonoids.** Before seeing the impact of irrigation on the different categories of flavonoids, let's emphasize again the difference between seeing the results expressed as **content** *versus* as **concentration.** When we talk about "content, we are referring to how much of something is there. Period. When we talk about "concentration", we are referring how much is there <u>per</u> unit of something (per volume, per weight, per size). As a result, **content is insensitive to berry size; concentration is not**. 1) **Anthocyanin** concentration was twice as high in the minimal irrigation treatment as in the other treatments. While this is significant on a weight basis, there was no difference on a per-berry basis. 2) Concentration of **flavonols** was not affected by water status. 3) Concentration of **flavanol monomers** (catechins) was significantly greater in the minimal irrigation treatment. Still, both flavonols and flavanols combined are a small percentage compared to anthocyanins. So the authors believe that the potential for water deficit to alter copigmentation via skin flavonols is limited. 4) **Proanthocyanins** were significantly increased by minimal irrigation.

• **Effect of maturity on flavonoids.** 1) **Anthocyanin** content in all irrigation treatments increased rapidly from veraison until September. As stated before, there were no differences in anthocyanins content with water status. 2) **Flavonols** followed a pattern similar to anthocyanins. Even though water stress caused a decrease in flavonols early on, after September the content became similar for all irrigation treatments. 3) The small **flavanol** amounts present in the skins (catechin) decreased rapidly from veraison on. The flavanols present in the seeds (catechin, epicatechin, epicatechin gallate) also decreased from veraison on, but, at their peak, they were 50 times higher than skin flavonols. In other words, flavanol monomers in red wines are essentially of seed origin.

4) The authors analyzed **proanthocyanidins** in two forms: intact (with normal phase HPLC), and digested (with reseverse phase HPLC after acid degradation). **Intact proanthocyanindins** provide information on amounts that are extractable into the wine. **Digested proanthocyanidins** provide information on subunit composition, as well as a sense of degree of polymerization. Intact proanthocyanidins increased 4-fold in all irrigation treatments throughout fruit ripening. But at harvest proanthocyanidins were highest in the minimal irrigation. Digested proanthocyanidins also increased after veraison, but much less. So why this discrepancy? If proanthocyanidins increased with one analysis method, they should have increased with the other. What the authors noticed is that, along with the increase in 280 nm during ripening, there was also an increase in 520 nm that was due to "high molecular weight material". They concluded that anthocyanins were being incorporated into proanthocyanidins during ripening. This was increasing the tannins that, in turn, were causing the increase in both A280 and A520.

• **Flavonoids in skins versus flavonoids in wine.** To what extent do differences in fruit composition carry over to wine? To find out, grapes from the different irrigation treatments were combined (24^{o}B) and small scale wines were made (20 liter). In general, the concentration of each type of flavonoid in the wines as a function of water status was similar to those levels in grapes. But **wine flavonols were particularly sensitive to water status**. The authors attribute this to the complex associations of flavonols with anthocyanins (copigmentation): wines with a higher anthocyanin concentration (minimal irrigation) would be able to support higher flavonol concentrations.

Implications? The predominant factor affecting flavonoids in red wines seems to be the pronounced effect of irrigation on berry size. So how much of the changes resulting from water deficit are due to phenolic metabolism and how much to berry size? Dr. Matthews and his team find in a later paper *(Summary 2 of this series)* that water deficit grapes have more anthocyanins and skin tannin, and that this is true no matter how you look at it -content or concentration.

	Effect of water deficit on concentration	**Effect of maturation on concentration**
Anthocyanins	↑↑ (- per berry)	Rapid ↑ from veraison
Flavonols	-	Rapid ↑ around veraison, then ↓
Flavanol monomers	↑↑	Rapid ↓ from veraison (both in skins and seeds)
Proanthocyanidins	↑↑	↑↑ throughout ripening (Intact) ↑ from veraison (Digested)

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