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Tannin Management in the Vineyard

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What is Tannin?

Tannins are bitter and astringent compounds found in most plants. They are polyphenolic compounds that fall into two classes, hydrolysable and condensed tannins. There are many hundreds of individual compounds, but they have a few things in common. All tannins bind protein, which is the basis of their role in tanning hides for leather and gave rise to their name. This property of binding tannin also gives rise to the term astringent from the Latin, *ad astringere*, "to bind".

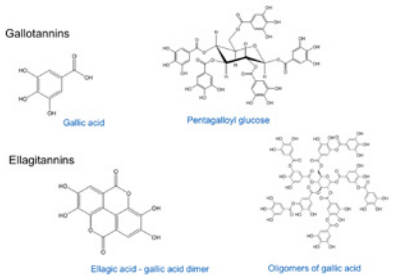
There are TWO classes of tannins, HYDROLYSABLE and CONDENSED tannins

Roles of tannin

Tannins play two defensive roles, one against micro-organisms and the other against herbivores. In both cases tannins bind to protein making it unavailable as a nitrogen source. Tannins also bind to cell walls and other phenolics to create a defensive barrier when cell integrity is lost. Being bitter and astringent, their taste is a deterrent to herbivores.

Hydrolysable tannins

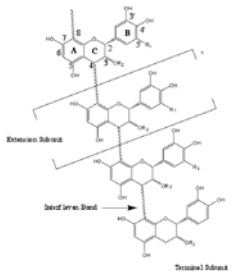
Hydrolysable tannins include gallotannins and ellagitannins. Gallotannins are made up of gallic acid bound to a sugar molecule, eg. pentagalloyl-glucose. Ellagitannins are complexes of gallic acid. Hydrolysable tannins are primarily derived from wood.



Hydrolysable tannins, showing gallic acid, and pentagalloyl glucose (gallotannin) as well as ellagic acid and an ellagitannin polymeric complex.

Condensed tannin

Condensed tannins, also know as proanthocyanidins, are polymeric compounds made up of flavan-3-ols subunits. The flavan-3-ols are based on a three ring structure, the flavan skeleton, and have an alcohol (OH) at position 3 on ring C of the flavan. Further modifications of the flavan give rise to different flavan-3-ols.
In grapes, these include catechin, epicatechin, epicatechin-gallate and epigallocatechin. The biosynthesis of flavan-3-ols is well-studied, but the mechanism of polymer formation is unknown. The flavan-3-ols can be combined in different sequences and proportions in tannin polymers.



A generic condensed tannin structure showing terminal an extension subunits and the ring labelling and carbon numbering convention for the flavan skeleton (uppermost extension subunit).

Condensed tannin polymers have a terminal subunit and a series of extension subunits linked by an inter-flavan bond; a bond between flavan units. The terminal and extension subunits can be any of the flavan-3-ols, but in most studies of grapes, catechin is the common terminal subunit and epicatechin is the most common extension subunit. Published research suggests that grape polymers range in length up to 30-40 subunits.

In grapes, condensed tannins are present in high concentration in the seeds and skins, but are also present in the leaves and stems. Tannins are also present in wood, but become bound to cell walls and other phenolics during lignification and are not easily extracted.

Measurement of tannin

There are many methods for measuring tannin. Most methods measure phenolics, rather than tannins. The most common phenolic measure is absorbance at 280nm (A280). As a surrogate for tannins it is quite poor because it assumes that most of what was measured by A280 is tannin, which is often not the case. All of the small non-tannin phenolic compounds absorb at 280nm, as do anthocyanins, flavonols, proteins, and RNA & DNA. A number of methods are based on stains that react with tannins with varying degrees of specificity. These include: DMACA (dimethylcinnamaldehyde), vanillin-HCL, Folin-Denis, Folin-Ciocalteau and iron chloride ($FeCl_3$). Some of these methods are quite reproducible, but they assume non-tannin material is either insignificant or at least constant; there isn't good evidence for either assumption.

The French method and the Butanol-HCL method rely on boiling the tannin in acid to produce the coloured anthocyanidin precursors to anthocyanins. This approach is useful because it will release bound tannin. However, the lack of standards make it difficult to quantify.

Protein precipitation assays are well established and robust. Some, like haeme analysis that required blood have lost favour, but gelatine and albumin assays are still common. Currently in grape and wine research, precipitation with BSA (bovine serum albumin) is widely used in research and numerous wineries in the USA. Because tannin will also bind polysaccharides, precipitation with methylcellulose has also been quite extensively used by the Australian Wine Research Institute (AWRI).

Thin-layer chromatography (TLC) has also been used, but requires tannin extraction and purification prior to separation. The method is good for monomers, although these are in low abundance relative to polymers, which appear as a blob or smear depending on solvent choice. The separated spots require visualisation by a reagent such as DAMCA or $FeCl_3$ and can be difficult to quantify.

High-performance liquid chromatography (HPLC) is widely used as a research tool and some wineries also employ this technology. The equipment is costly and requires a skilled technician to operate and in-depth knowledge of tannins to interpret the data. HPLC methods are either based on separating monomers from the tannin "blob" similar to TLC or rely on depolymerising the tannin with acid and separating the individual subunits. There are also some HPLC methods that separate polymers based on size.

More recently, rapid analytical methods that don't require chemical analysis have been developed, but are not yet ready for

widespread adoption. For industry, the BSA and methylcellulose precipitation methods are most accessible.

Tannins in wine

Tannins contribute to the mouthfeel and colour stability of wine. The colour of grapes and of young red wine is due to the presence of anthocyanins. Anthocyanins are structurally similar to tannins, but are relatively unstable. In wine they breakdown or interact with other elements to form new compounds, some of which are coloured.

The most accessible method for Australian wineries to measure tannin is the methylcellulose method offered by AWRI.

The largest single class of the coloured compounds in older wines are the pigmented polymers. Polymeric pigments are tannin polymers with anthocyanins attached. There is limited information on the structure of pigmented polymers or their formation in wine. Pigmented polymers are only formed during winemaking not in the grape.

Because tannins are bitter and astringent they contribute to the mouthfeel of the wine. Tannins precipitate the salivary proteins that lubricate the mouth and make it easy to chew and swallow food. This causes the drying and puckering sensation associated with drinking red wine. In addition to protein, tannins also interact with polysaccharides in saliva as well as cells in the oral cavity. These interactions are impacted upon by the alcohol concentration and viscosity of the wine and can be masked by residual sugar and other flavour and aroma characters, such as fruit intensity. The perception of mouthfeel is also affected by tannin concentration, polymer length and polymer composition. We know that epicatechin-gallate is perceived as coarser than epicatechin, while epigallocatechin is perceived as smoother, however the sensory character of oligomers and polymers and combinations of tannins and other elements such as polysaccharides is unknown.

Tannins added to wine

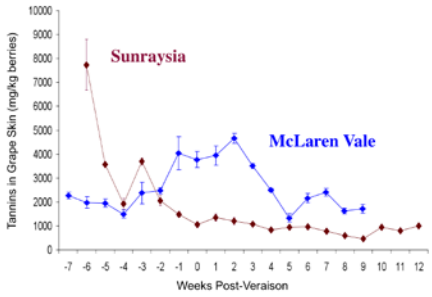
While winemakers recognise there are tannins in grapes, often the level of extracted from the grapes is not sufficient for a particular wine style, so it is common practice to add tannin to wine during fermentation or aging. The main reasons for adding tannin are to stabilise colour or to alter mouthfeel.

The tannins that are added to the wine can come from oak barrels, chips and staves (hydrolysable tannin), or can come from the addition of oenotannins. The range of tannin additives includes both hydrolysable and condensed tannins separately and in combination. The condensed tannin products are often extracted from pomace after winemaking or made from unfermented seeds and skins. Condensed tannins are also extracted from a range of non-grape sources. Hydrolysable tannins are all sourced from non-grape species. The composition of many products is poorly defined and varies from batch-to-batch. To date there is no evidence of hydrolysable tannins forming stable complexes with anthocyanins nor has the sensory character of hydrolysable tannins in wine been rigorously examined.

How and when are tannins made?

Tannin synthesis occurs by the same biosynthetic pathway that makes anthocyanins, flavonols (eg. quercetin) and phenolic acids (eg, caffeic acid, resveratrol). This pathway also makes the precursors for lignin (wood) production.

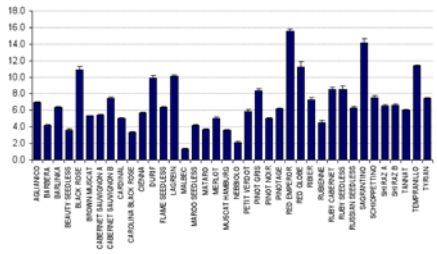
During berry development the main phase of tannin accumulation is between flowering and veraison. Studies conducted in McLaren Vale (South Australia) on Shiraz skin showed a high level of tannin at flowering followed by a period of tannin synthesis towards veraison, after which tannin levels declined towards harvest. From season to season there was variation in the timing of the peak in tannin accumulation, but generally this was accepted as the typical pattern of tannin accumulation in grape skin. However, samples collected from the Sunraysia region of northwest Victoria showed a very different pattern with the peak in tannin occurring at fruit-set and declining from then on. This pattern was seen in Shiraz, Cabernet Sauvignon and Chardonnay skin over several seasons and indicates a strong regional effect on tannin accumulation in grapes.



The pattern of tannin accumulation in skin of Shiraz berries from two regions, Sunraysia (NW Victoria) and McLaren Vale (South Australia) during berry development.

Variation between varieties

Anecdotal evidence indicates significant differences in tannin levels between grape varieties, with some varieties considered high in tannins, eg. Tannat and Nebbiolo, and others low in tannin, eg. Merlot and Barbera.

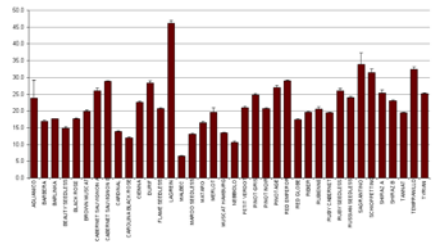


Variation in total skin tannins (mg/g FWT skin) between varieties.

A study of grape skin tannins from 36 cultivars showed that many of the cultivars with high tannin levels were not those traditionally considered high in tannin, but were actually table grapes. The tannin concentration in Nebbiolo skin was one of the lowest and Tannat was similar to other winegrape varieties like Shiraz and Cabernet Sauvignon.

Polymer length

The average length of tannin polymers has also been examined in these 36 varieties. The longest polymers were observed in Lagrein with 46 subunits and Malbec had the shortest polymers at



Variation in average skin tannin polymer length (subunits) between varieties.

around 7 subunits. Cabernet Sauvignon, Shiraz, Pinot Noir and Tannat were mostly in the range of 20-25 subunits in length.

Other research that has tried to separate individual tannin polymers indicates that the longest polymers in Cabernet Sauvignon may be more than 80 subunits in length, while the longest polymers in the skin of Flame Seedless table grapes were over 100 subunits in length.

Tannin polymer composition

The composition of individual tannin subunits in both the seeds and skins of grapes has been studied quite extensively. While all grapes contain tannins in differing amounts, the actual composition of the polymers is not constant. Terminal subunits are generally catechin, with lesser amounts of epicatechin and epicatechin-gallate. In some varieties like Black Rose, catechin accounts for 80% of terminal subunits, while in Rubienne grape skin epicatechin is the main terminal subunit. In some varieties, epicatechin-gallate is not detected as a terminal subunit.

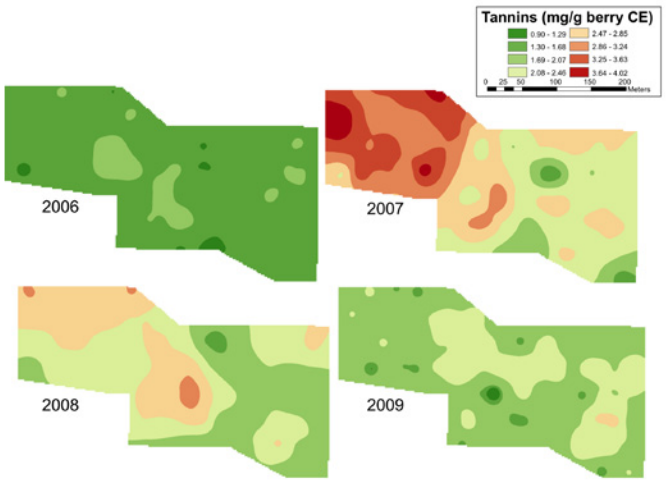
Extension subunits in grape polymers can include all four flavan-3-ols, with epicatechin the most common followed by epigallocatechin, although there are recent reports of epigallocatechin being the most common extension subunit in some varieties. Catechin and epicatechin-gallate extension subunits are reported at low concentrations or absent.

Many table grape varieties have higher skin tannin concentrations than winegrapes

Given that tannin concentration, polymer length and polymer composition impact on mouthfeel, it is important to examine the factors that influence tannin content and composition in grapes.

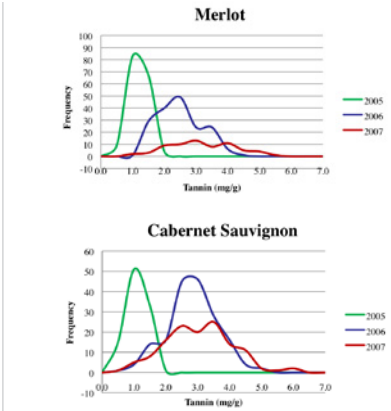
Site and season effects on tannin

Differences in grape and wine quality and composition between growing regions are well documented, although this hasn't often extended to berry composition. In European viticulture, regional variation is attributed to terroir, i.e. the effect of geography. While terroir is a handy phrase, from a research and management perspective it's necessary to break it down into individual components of soil type, temperature, rainfall, sunshine, terrain, prevailing wind and so on. While variation is well recognised at a regional level, significant variation also occurs at a much smaller scale.



Variation in total tannin in whole berries (mg/g FWT berry) at harvest across 100 GPS tagged vines in a vineyard in Sunraysia for four seasons.

Within vineyard variability has always been an issue in vineyards. Managing that variation requires an understanding of what causes variation. A study of tannins in a vineyard in Sunraysia showed considerable variation across the block in some seasons, but also indicated areas that were regularly lower or higher in tannin. This indicates an underlying source of variation that is independent of the year-to-year variation in the weather.



Distribution of total tannins (mg/g FWT berry) in whole berries for Merlot and Cabernet Sauvignon from California over three seasons.

Season is probably the biggest driver of variability in plant production as the spatial maps from the Sunraysia vineyard demonstrate. If we look at data from a broader area, for example central California for three seasons, the range of values is greater as we would expect, but not a lot more than we observe within a single vineyard from one year to the next or even within some years.

Looking at seasonal variation is really only looking at changes in the weather. Some of those changes are happening right now and some happened last year while the vines were laying down the buds for this season. This makes managing the changes harder than it might be for wheat for example, and of course there's not much we can do about the weather. However, there may be things we can do about managing the impacts that seasonal variation has on grapes.

Bunch exposure

Bunch exposure and canopy management have long been touted as tools for managing colour in winegrapes. In some varieties, this has proven effective while in others there was no response. Similar results were observed for tannin in the skin of 27 varieties that were shaded from fruit-set to harvest. In varieties, like Shiraz and Tannat, there was no difference with shading. In some cultivars tannin levels were lower in shaded fruit, eg. Cardinal, Flame Seedless and Brown Muscat; however in other varieties the shaded fruit was higher in tannin, eg. Black Rose, Merlot, Russian Seedless and Tyrian grapes. This suggests there are responsive and unresponsive grape varieties, or that another driver independent of either light or temperature is affecting tannin levels in these fruit.

Rootstocks

The study of the rootstock effects on winegrape tannins has only recently attracted research attention. Data shows that Shiraz on Paulsen and Freedom rootstocks had lower tannin than on Dogridge or Ruggeri. Also, Merlot and Pinot Noir grafted to 120A Mgt were consistently high in tannin than on Riparia Gloire. However, from these few studies there is not

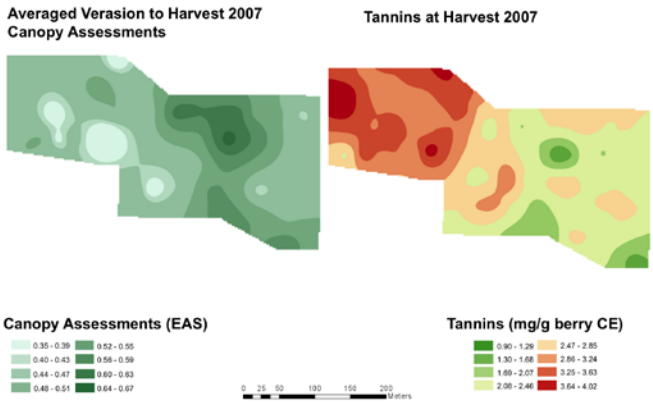
enough data to make an unequivocal statement on their effect on tannins in the fruit.

Irrigation

A number of studies have looked at the effect of irrigation on tannin levels in grapes and wine. All of these have concluded that irrigation, or deficit irrigation, has no effect on grape tannin levels other than reducing berry size and thereby concentrates tannins; similar to the effect on anthocyanins.

Vine vigour

A comprehensive examination of Shiraz vineyards across Australia studied berry composition and many other viticultural parameters. The data has shown a relationship between tannin concentration in the fruit and vine vigour. High vigour vines tended to have lower tannin levels while low vigour vines had higher tannins. The consistency of these data and their similarity to work on Pinot Noir in the USA suggests that vine vigour is the major driver of tannin variability.



Correlation between total tannins in whole berries (mg/g FWT berry) at harvest and veraison canopy assessments as an indicator of vine vigour.

Tannin extractability

Managing grape tannin in the vineyard may not translate directly into wine tannin. Discrepancies between grape tannin and wine tannin levels results from interactions between tannins and grape cell wall material.

In the seed the tannins are located in the seeds coat and this layer of cells dies as the seed matures. When this happens, tannins bind to other phenolics and cell wall proteins and polysaccharides. In grape skin, tannins have long been hypothesized to be located in vacuoles in a similar manner to anthocyanins. However, there is a body of evidence that shows tannins bound to the cell walls. It appears that this happens during berry development as part of the grapes defence mechanism against mirco-organisms and indicates that in the living cell, tannins are closely associated with the cell wall.

Recent studies have isolated cell wall material and measured tannin showing that as much as 50% of tannin could be located in the cell wall. The presence of tannin in the cell wall could also account for the apparent decrease in tannins during berry development. That tannins are associated with the cell wall suggests that rather then being released into the wine during winemaking, cell walls bind additional tannins, which accounts for the discrepancy between grape and wine tannin levels.

Preliminary studies have quantified the tannin binding capacity of cell walls and indicate that the tannin binding capacity of cell walls is not constant. Just how much this varies and what causes this variation is not yet known.

What we do know

- Tannins are bitter and astringent.
- All grapes contain tannin in the seeds and the skin.
- Some grapes contain tannin in the flesh.
- There is variation between varieties.
- There is variation between sites and seasons and even within sites for a single variety.
- Many viticultural practices have small, inconsistent or poorly understood impacts on tannin.
- One of the major drivers of variability is vine vigour.
- Exogenous tannin can be added during winemaking; much of this is hydrolysable and non-grape tannin.
- Tannin extraction is influenced by winemaking.
- Extractability is affected by cell wall interactions.

What we don't know

- The ideal grape tannin content & composition for any given wine.
- The relationship between tannin chemistry and sensory characters.
- How to achieve specific chemistries in the vineyard or winery.
- What the action of many tannin additives is.
- How to manage interactions between tannins and cell walls during winemaking.

Further reading

Downey, et al. (2006) Cultural Practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. Am. J. Enol. Vitic. 57:257-268.

Hanlin, et al. (2010) Review: Condensed tannin and grape cell wall interactions and their impact on tannin extractability into wine. Aust. J. Grape Wine Res. 16:173-188.

Harbertson & Spayd. (2006). Measuring phenolics in the winery. Am. J. Enol. Vitic. 57:280-288.

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