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Understanding phenolic compounds in red winemaking

Contents

Terminology

Abiotic stresses: negative impact of non-living factors on the grapevine

Acylation: the process of adding an acyl group to a compound (e.g. incorporation of acetic acid or p-coumaric acid into an anthocyanin structure)

Astringency: dry tactile sensation due to the formation of precipitable aggregates between tannin and saliva proteins

Bitterness: gustatory taste recognised by the receptor cells of the taste buds

Bleaching: anthocyanin decolouration through SO_2 incorporation into the anthocyanin molecule

Branching: incorporation of secondary chains to the main tannin chain leading to more compact and symmetrical conformations

Cold soaking (cold maceration): a period of skin contact at a cool / cold temperature occurring before fermentation

Condensations: reactions between phenolic compounds through chemical bonding

Copigment: non-coloured phenolic compounds that reinforce the pigmented structure

Copigmentation: anthocyanin associations with copigments leading to a pigmented polymer

Degree of polymerisation: average molecular size of a group of compounds (generally applied to tannins)

Acetaldehyde (ethanal) linkages: anthocyanin-tannin molecules linked through acetaldehyde (derived from the oxidation of ethanol)

Extractability: release of phenolic substances from the solid parts of grape berries

Galloylated tannins: gallic acid incorporated into the tannin structure

Interactions: weak associations among compounds (or between a phenolic structure and wine components)

Maceration: skin contact period during alcoholic fermentation

Polymeric pigment: coloured pigment composed of an anthocyanin and other grape and wine components (including other phenolic compounds)

Polymerisation: reactions among phenolic compounds leading to larger molecules

Proanthocyanidins (or tannins): polymers composed of several units of flavanols

Pyranoanthocyanin: pigments composed of an anthocyanin containing a pyran ring (e.g. pyruvic acid)

Ripening: biochemical changes occurring in the grape berry during the vine growth cycle

Self-associations: reactions involving the combination of a compound with itself

Spectroscopy: use of light absorption to quantify analytes (e.g. phenolic compounds)

Veraison: anthocyanin accumulation in the skins of red berries

1

Introduction

Phenolics are probably the most sought after compounds in red wines. Despite representing less than 1% of the grape berry composition the role they play in the sensorial properties of wine is widely acknowledged. This is because two of the most important red wine attributes are largely due to its phenolic content as well as its phenolic composition. It follows that phenolic compounds are responsible for red wine's colour. The simplest forms of anthocyanins and their combinations with other grape and wine components, including phenolic compounds, are responsible for red wine colour intensity and hue. On the other hand, tannin compounds define to a large extent the mouthfeel properties of red wines. Tannins are also responsible for wine bitterness, astringency and flavour. This is not only due to the tannin compounds themselves, but also because of their self-combinations, interactions and reactions with other phenolic substances. The reaction of tannins with anthocyanins is one of the major combinations occurring in wine and plays a very important role in colour, mouthfeel and flavour properties.

The presence of phenolic compounds in the final wine is dependent on several factors. A large number of challenges and conditions occur during the journey phenolic compounds have to undertake. This journey starts with their biosynthesis in the grape vine. Next up is the grape ripening process, followed by winemaking and aging and finally the wine is consumed, which marks the end of this journey. The accumulation of phenolic substances during grape ripening and their extractability at harvest time are mainly dependant on the vineyard characteristics and winegrowing conditions of a specific year. During fermentation the winemaker's strategy and winemaking conditions will lead to a young wine with a particular phenolic content and composition. The phenolic compounds released from grapes during the winemaking process will undergo further transformation during barrel and bottle aging. The finished wine's phenolic composition will thus be a collection of phenolic substances formed during ripening in the vineyard, extracted during fermentation and matured during aging.

During fermentation the winemaker's strategy and winemaking conditions will lead to a young wine with a particular phenolic content and composition.

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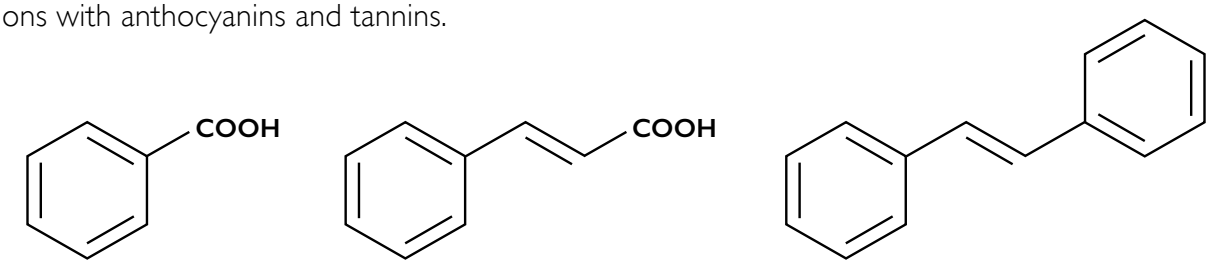
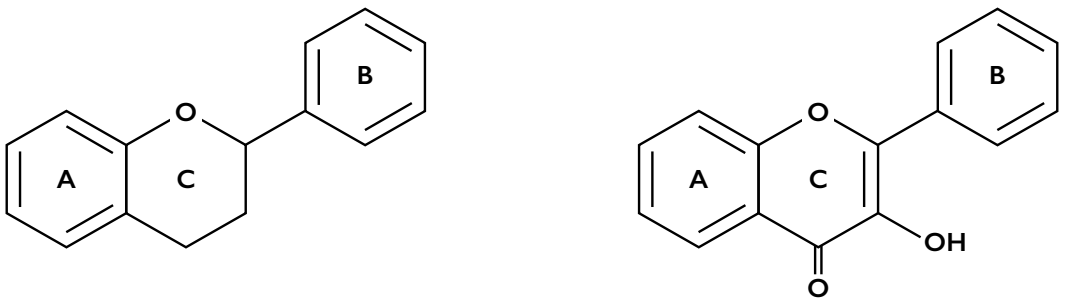
Phenolic compounds

Red wine phenolic compounds are divided in two main families – the non-flavonoids and the flavonoids. Non-flavonoids include hydroxycinnamic and hydroxybenzoic acids, and stilbenes. They are simple phenolic structures, generally containing only one phenolic ring (Figure 1). On the other hand, the flavonoids correspond with a more complex group of compounds, with a common three-ring C6-C3-C6 structure (Figure 2). Anthocyanins, flavonols and flavanols (tannin monomers) are the main phenolics included within the flavonoid group. Anthocyanins and flavanols (tannins) are found at higher levels in red wine and are therefore mainly responsible for colour and mouth feel properties. However, phenolic acids, stilbenes and flavonols, found at much lower levels, also play a significant role in the sensorial properties of red wines – on their own or through their involvement in numerous reactions and interactions with anthocyanins and tannins.

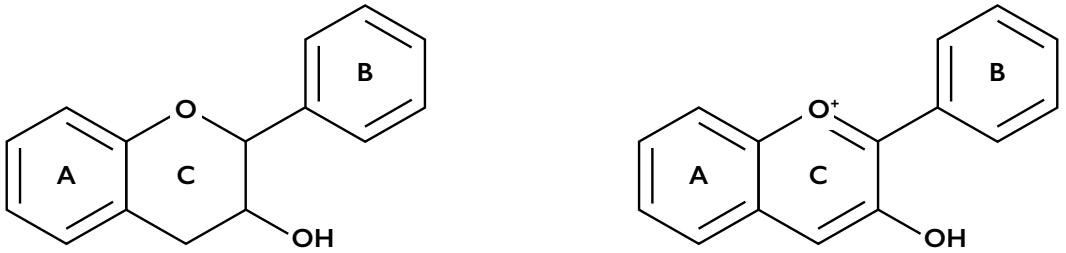


Benzoic acid | Cinnamic acid | Stilbene

Figure 1. Non-flavonoids



Flavanol skeleton | Flavonol skeleton



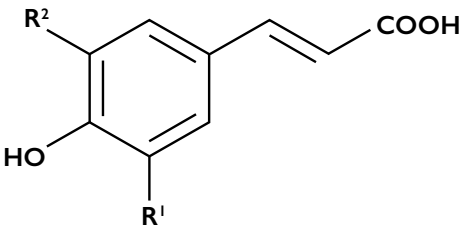
Flavan-3-ol | Anthocyanidin

Figure 2. Flavonoids

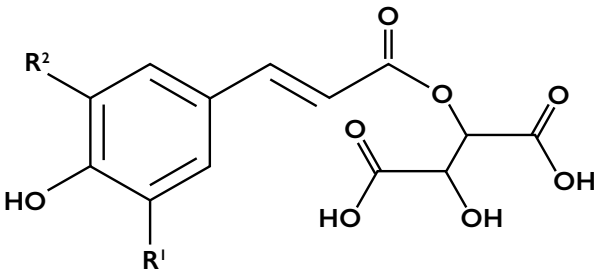
a. Non-flavonoids

i. Hydroxycinnamic acids

Hydroxycinnamic acids are mostly located in the flesh of the grape berries and are one of the major phenolic compounds found in white wine (Figure 3). They are mostly esterified with tartaric acid in wine. Hydroxycinnamic acids play a major role in the colour of white wines due to their highly oxidative nature, being partially responsible for the browning phenomena. In red wine these compounds are found in combination with anthocyanins to form acylated pigments with mainly *p*-coumaric and caffeic acids. Hydroxycinnamic acids can also be precursors of volatile phenols, which are responsible for the medicinal / barnyard aroma, through the activity of microorganisms such as *Brettanomyces* (a yeast) and certain bacteria.



R^1=R^2=H: *trans*-*p*-coumatic acid
R^1=OH; R^2=H: *trans*-caffeic acid
R^1=OCH_3; R^2=H: *trans*-ferulic acid



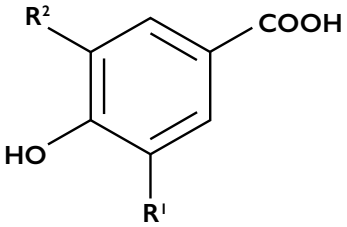
R^1=R^2=H: *trans*-*p*-coutaric acid
R^1=OH; R^2=H: *trans*-caftaric acid
R^1=OCH_3; R^2=H: *trans*-fertaric acid

Figure 3. Hydroxycinnamic acids

> Hydroxycinnamic acids can also be precursors of volatile phenols, which are responsible for the medicinal / barnyard aroma, through the activity of microorganisms such as *Brettanomyces* (a yeast) and certain bacteria.

ii. Hydoxybenzoic acids

Hydroxybenzoic acids are mainly found in the seeds and skins of grape berries. They occur mostly as glucoside combinations or in the form of esters (Figure 4). Hydroxybenzoic acids are found in red wines as free forms due to acid hydrolysis reactions.

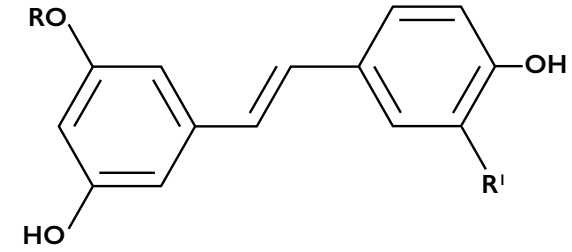


R^1=R^2=OH: gallic acid
R^1=OH; R^2=H: protocatechuic acid
R^1=OCH_3; R^2=H: vanillic acid
R^1=R^2=OCH_3; syringic acid

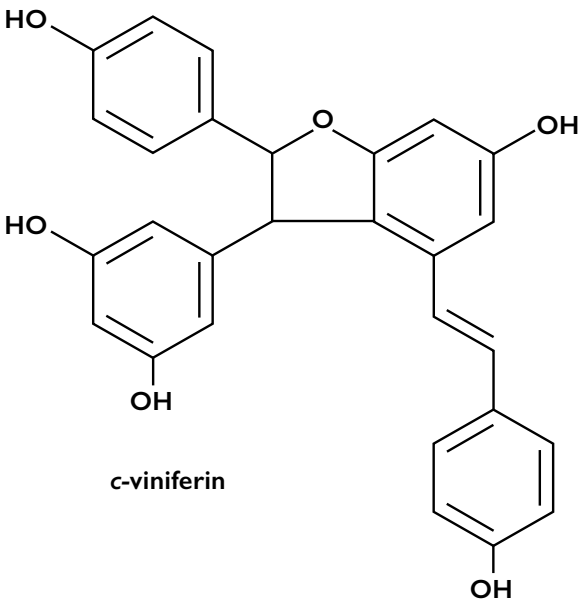
Figure 4. Hydroxybenzoic acids

iii. Stilbenes

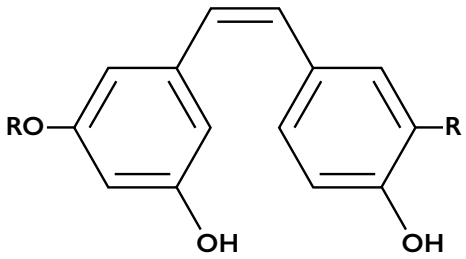
Stilbenes accumulate in grape vines due to fungal infection and abiotic stresses. Stilbenes are located mainly in the skins and seeds of berries. The most renowned stilbene is resveratrol, to which important health properties have been attributed (Figure 5).



R=H, R¹=H: *trans*-resveratrol
R=H, R¹=OH: *trans*-picetannol
R=Glc, R¹=H: *trans*-piceid
R=Glc, R¹=OH: *trans*-astringin



***c*-viniferin**



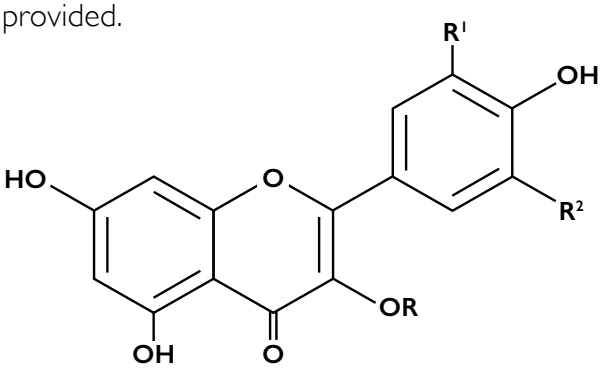
R=H, R¹=H: *cis*-resveratrol
R=H, R¹=OH: *cis*-picetannol
R=Glc, R¹=H: *cis*-piceid
R=Glc, R¹=OH: *cis*-astringin

Figure 5. Stilbenes

b. Flavonoids

i. Flavonols

Flavonols are yellow pigments that accumulate in the skins of both red and white grapes due to abiotic stresses (such as UV light exposure), thus acting as a natural sunscreen (Figure 6). Due to its location in grape berries these phenolic compounds are found at higher levels in red wines. Flavonols' role in red winemaking is mainly due to its potential interactions with anthocyanins to create co-pigmented molecules. The copigmentation phenomenon plays a crucial role in the colour of young red wines and is believed to also contribute to the longevity of red wine colour, due to the early anthocyanin protection provided.

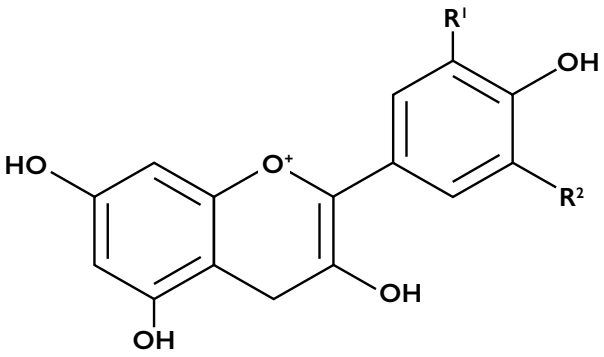


R¹=OH, R²=H, R=H: quercetin
R=Glc: quercetin 3-glucoside
R=GlcA: quercetin 3-glucuronide
R¹,R²=OH, R=H: myricetin
R¹,R²=H, R=H: kampferol
R¹=OCH_3, R²=H, R=H: isorhamnetin
R¹=OCH_3, R²=OH, R=H: laricitrin
R¹,R²=OCH_3, R=H: syringetin

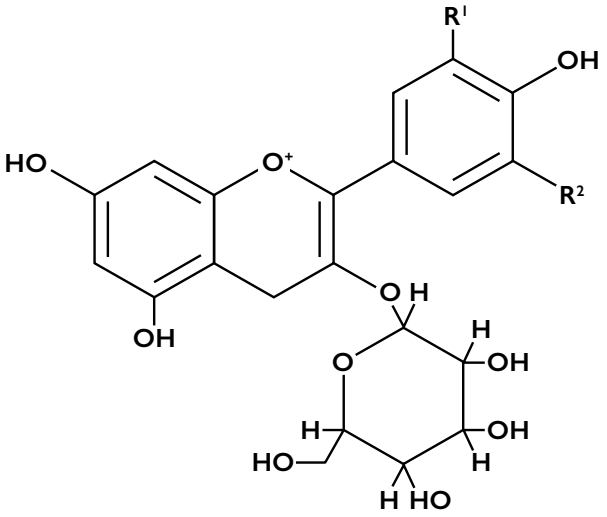
Figure 6. Flavonols

ii. Anthocyanins

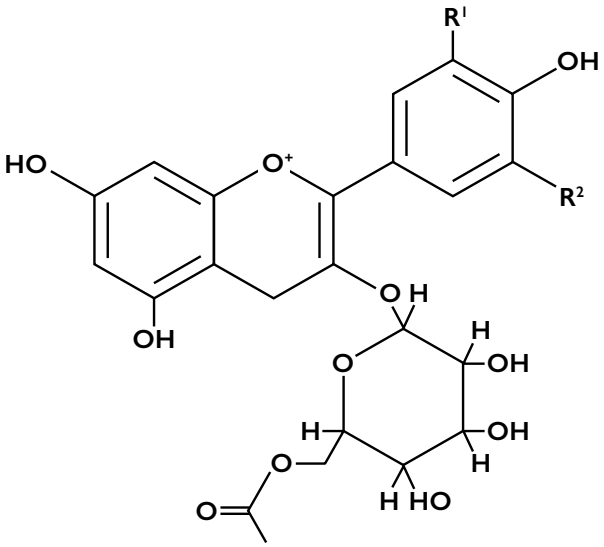
Anthocyanins are the compounds responsible for the red colour of wine (Figure 7). They are located in the skins of red cultivars and also in the flesh of a few teinturier varieties. Anthocyanins are part of a wide range of interactions and reactions with a variety of grape components, including phenolic compounds. Anthocyanins in their different forms can display from bluish hues in young wines to yellowish/brown hues in aged red wines. The colour of anthocyanins is also dependent on the medium's conditions, with pH and SO_2 playing major roles. Lower pH values (higher acidity) increase the colour intensity and the red tonality of red wines. In addition, at wine pH (typically 3 to 4) only a small fraction of the anthocyanins in young wines are in the red coloured form. On the other hand, the role of SO_2 is due to its ability to combine and bleach the anthocyanin in what is known as the bleaching effect of SO_2. Anthocyanins are thus involved in a number of reactions including: acylation, self-associations, inter-molecular copigmentation interactions and intra-molecular copigmentation reactions, pyranoanthocyanin formation and also through combinations with tannins.



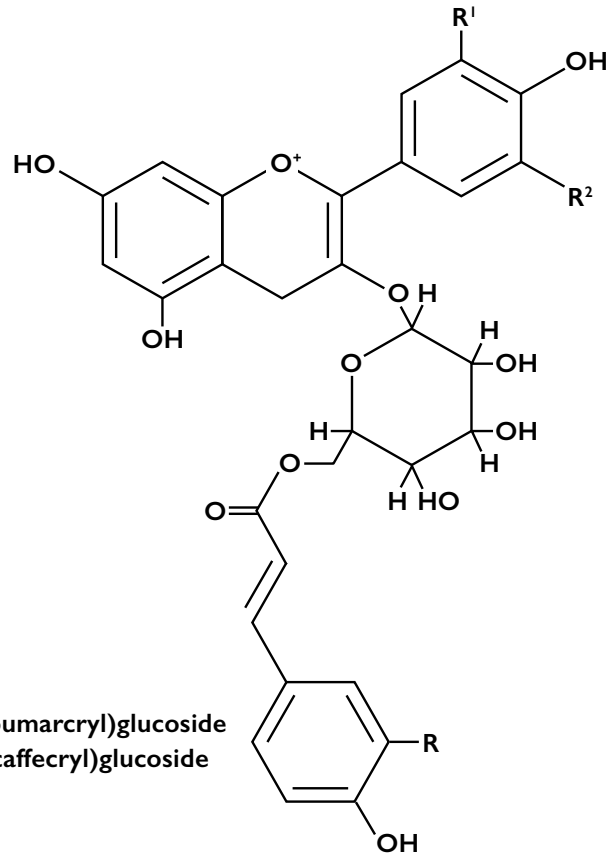
R^1=OH, R^2=H: cyanidin
R^1=OCH_3, R^2=H: peonidin
R^1=R^2=OH: delphinidin
R^1=OCH_3, R^2=OH: petunidin
R^1,R^2=OCH_3: malvidin



Anthocyanidin 3-glucoside



Anthocyanidin 3-(6-acetyl)glucoside

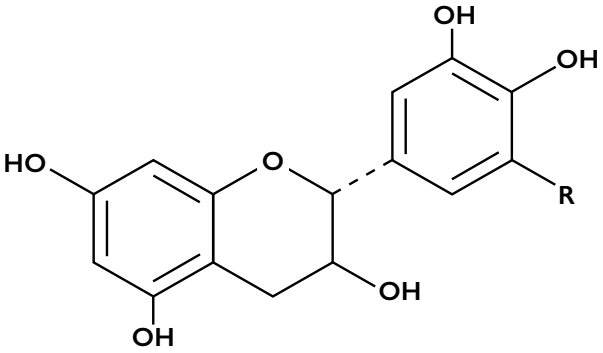


R=H: anthocyanidin 3-(6-*p*-coumarcryl)glucoside
R=OH: anthocyanidin 3-(6-caffecryl)glucoside

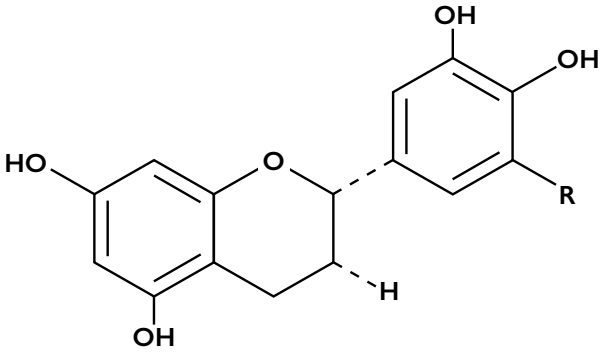
Figure 7. Anthocyanins

iii. Flavanols (tannins)

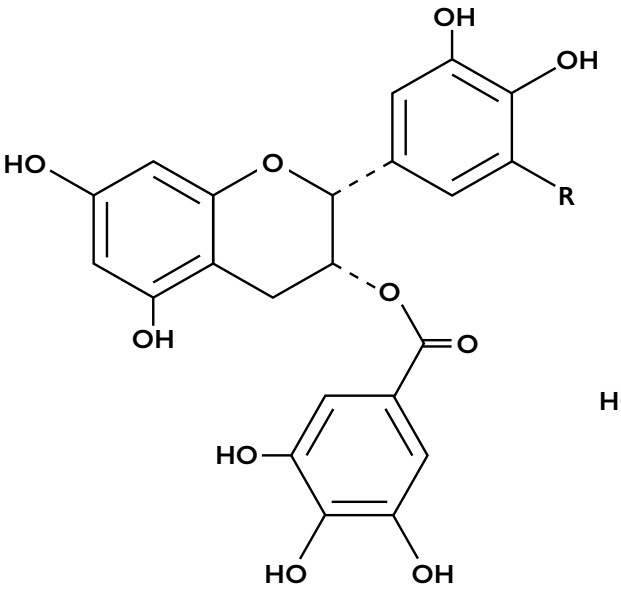
Flavanols are the monomeric forms of proanthocyanidins or tannins (Figure 8). Flavanols and tannins are located in the skins and seeds of grape berries and are responsible for the bitterness and astringency of red wines. They are also the major phenolic compounds found in red wines. During winemaking and aging, flavanols and tannins undergo polymerisation reactions to form larger molecules. On the other hand, monomeric and low polymerised flavanol forms are mostly bitter, but they become more astringent as the size of the molecule (number of sub-units) increases. Large tannin molecules are structured into a spiral form that places the reactive groups of the molecule towards the exterior. These reactive sites thus become more available to interact with salivary proteins, creating an insoluble macromolecular complex that precipitates from solution and produces the drying and puckering sensation characteristic of astringency.



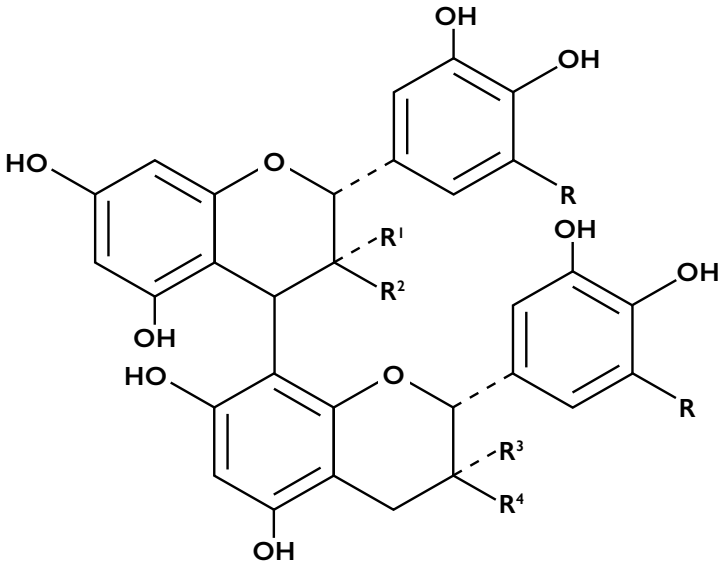
R=H: (+)-catechin
R=OH: (+)-gallocatechin



R=H: (+)-epicatechin
R=OH: (+)-epigallocatechin



R=H: (–)-epicatechin 3-*o*-gallate

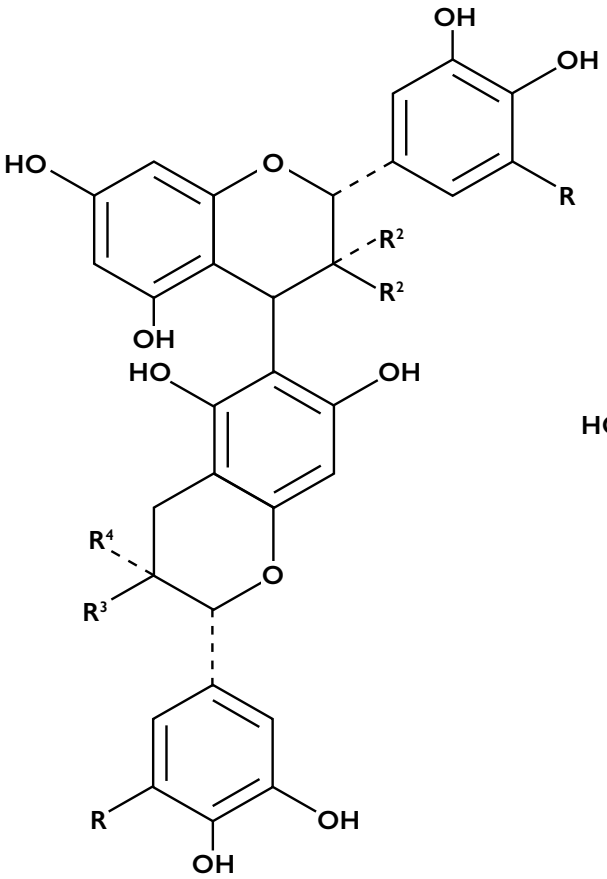


C4 – C8 proanthocyanidin dimer

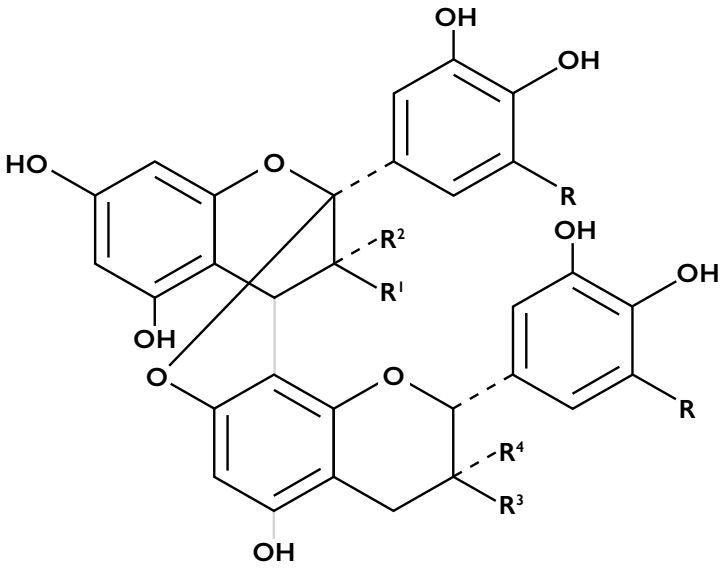
R=H; R^2=R^3=H; R^1=R^4=OH: procyanidin B_1
R=H; R^2=R^4=H; R^1=R^3=OH: procyanidin B_2
R=H; R^1=R^3=H; R^2=R^4=OH: procyanidin B_3
R=H; R^1=R^4=H; R^2=R^3=OH: procyanidin B_4

Figure 8. Flavanols and tannins

Flavanols are the monomeric forms of proanthocyanidins or tannins



C4 – C6 proanthocyanidin dimer



A-type proanthocyanidin dimer

R=H; R^1=R^3=H; R^2=R^4=OH: procyanidin A_2

Figure 8. Flavanols and tannins (continued)

3

Biosynthesis, accumulation and extractability of phenolic compounds during ripening

Phenolic compounds (with the exclusion of anthocyanins) start accumulating in the grape berry from berry set (Figure 9 shows where the various phenolic compounds are located in the grape berry). Anthocyanins on the other hand, only start to accumulate in the berry from veraison onwards. The concentration of anthocyanins increases up to a point where degradation of anthocyanin compounds may occur (in overripe grapes). During this period anthocyanins start reacting with other grape compounds, including phenolic compounds, giving rise to acylated anthocyanins, and to a limited extent, polymeric pigments.

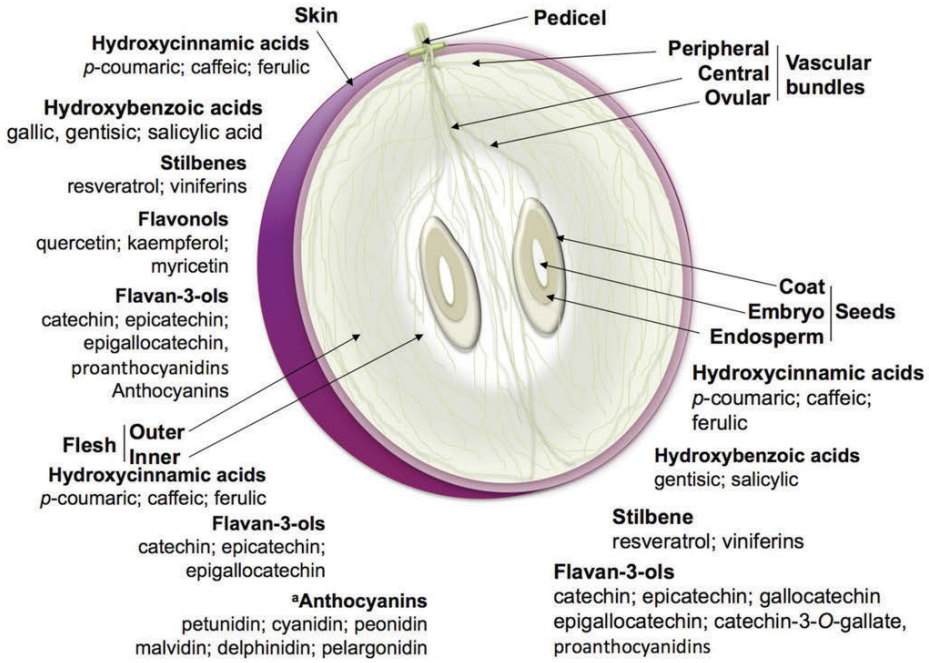


Figure 9. Location of phenolic compounds in the grape berry.

Flavanols and their polymeric forms, proanthocyanidins, start accumulating in the berry from blooming, reaching a maximum concentration at veraison. A subsequent decrease is observed during the last stages of the ripening period. Due to the nature of these compounds and their role in the perception of mouth feel, it is also important to evaluate the polymerisation extent of the tannin compounds. Seed tannins are generally considered more bitter and astringent than skin tannins. This is explained by the molecular size and composition of the different tannins found in both berry tissues. Seed tannins are characterised by

smaller molecules (lower degree of polymerisation) and also by a higher presence of tannins incorporating galloyl (gallic acid) units. Galloylated tannins are more astringent than non-galloylated tannins. On the other hand, skin tannins are bigger in size and less galloyl units are present. The ability of tannins to elicit astringency is proportional to their molecular size (the higher the tannin's size, the more astringent activity the tannins will have). Based on this skin tannins should therefore be more astringent than seed tannins, however the greater galloyl presence in seed tannins seems to play a major role. During ripening the degree of polymerisation of skin tannins seems to increase, while for seed tannins only minor changes have been reported. As skin tannins polymerise during ripening they are gradually deactivated seeing that they have an improved ability to combine with grape components, thus losing their aggressiveness and astringency.

Table 1. Location and accumulation of phenolic compounds in the grape berry.

Compound	Level of synthesis[a]			Location	Berry phenological scale[b]			
	Skin	Flesh	Seed		Blooming	Green stage	Veraison	Ripening
Nonflavonoids								
Hydroxycinnamic acids	++	+++	++	Hypodermal cells and placental cells of the pulp; primaily in the vacuoles of mesocarp cells.	+++	+++	+	+
Hydroxybenzoic acids	+	-	++					
Stilbenes	+++	+	++	Berry skin and seeds.	-	+	++	+++
Flavonoids								
Flavonols	++	-	-	Dermal cell vacuoles of the skin tissue and call wall of skin and seeds.	++	+	+++	++
Flavan-3-ols	++	+	+++	Specific vacuoles of hypodermal skin cells and seeds coat soft parenquima.	+	++	+++	++
Anthocyanins	+++	-[x]	-	Cell layers below the epidermis; storage confined to the vacuoles and cytoplasmic vesicles named anthocyanoplasts.	-	-	+	+++

[a,b]Very abundant compound (+++) to absent (-); [x]Teinturiers contain anthocyanins also in mesocarp cells.

The increased phenolic extractability in riper grapes is not exclusively dependant on increased phenolic levels during ripening. Additional factors may explain the higher phenolic content normally found in wines made with riper grapes. First of all, cell wall status seems to play a crucial role in phenolic extractability. The degradation of grape cell wall structures during ripening favours the release of phenolic compounds into the wine. Despite seed tannins being more easily released from unripe seeds, the higher alcohol content in wines made with riper grapes positively influences extraction of seed tannins during winemaking. Single or a combination of the aforementioned factors might explain the generally higher phenolic content observed in wines made with riper grapes.

4

Extraction of phenolic compounds during winemaking

Phenolic compounds are released into the must as soon as the solid parts of berries (skins and seeds) come into contact with juice during crushing. The period of skin contact during fermentation is known as maceration. During the initial stages of fermentation extraction takes place under different conditions than those found at the end of the fermentation. The first stages of maceration are characterised by the extraction of water soluble phenolic compounds. The released phenolic compounds are mainly those located in the skins of the grape berries and correspond mainly to anthocyanins, flavonols, flavanols and skin tannins. Therefore, if pressing is done before the end of the fermentation the resulting wine will have very particular sensorial properties. Influenced by the different nature of skin tannins the wines will be perceived softer, with a more pleasant and rounded mouth feel perception. The incorporation of seed tannins into the phenolic content will also change the sensorial properties of wine, albeit in a different manner. In this case the mouth feel perception will shift towards a more intense astringency that will need a longer aging period to achieve roundness. These scenarios are mainly influenced by the alcohol produced by yeast during fermentation which intensifies the extraction of seed tannins towards the end of the fermentation.

The first stages of maceration are characterised by the extraction of water soluble phenolic compounds.

5

Winemaking practices affecting phenolic extraction and stability

A number of winemaking practices can be applied during the winemaking process in order to achieve a more or less intense phenolic extraction from the berry tissue. Some of these techniques, such as cold maceration or cold soaking, can be initiated before the start of the fermentation. Another example is post-alcoholic or extended maceration, which can be performed after the conclusion of fermentation. The usage of enzymes and commercial tannins are also common in winemaking.

a. Cold maceration (cold soaking)

The cold maceration technique is based on an extended contact period after the grapes are crushed and before the start of the alcoholic fermentation. This is achieved by keeping the grapes at lower temperatures (4 to 10°C) to avoid the start of the fermentation. The extraction thus takes place in an aqueous medium which strongly influences the type of compounds released. The duration of successful cold maceration should be 4 to 10 days. This technique can have an influence on the anthocyanin fraction, however some other low molecular weight phenolics, including small tannins, are also released during the process. If successful, cold maceration will yield wines with an improved phenolic content and composition. The combination of cold maceration treatments with enzymes or increased SO_2 additions may increase the efficacy of this technique. Cold maceration can also make use of solid CO_2 (dry ice). The cold generated by the change of the state of dry ice from solid to gas without going through the liquid phase produces an intense decrease in temperature. This can lead to the freezing of parts of the solid tissues of grape berries. The increase in size of the water content inside cells then causes degradation or breaking of the cell wall, which enhances the release of intercellular substances such as phenolic compounds. The high cost of this product and safety issues at industrial scale are the main constraints of this technique.

b. Tannin addition

The addition of oenological tannins during winemaking is gaining acceptance among winemakers worldwide. Oenological tannins can be added to the wine at different stages of the winemaking process and also with different purposes. Different tannins can also be added depending on the origin of these tannins. The majority of oenological tannins are wood derived tannins (hydrolysable tannins) or combinations of wood and grape tannins (condensed tannins). Oenological tannin products derived mainly from grape seeds are also available. Oenological tannins are added very early during fermentation, not simply to increase the final tannin content of wine, but to interact with grape proteins. These initial interactions will precipitate from solution allowing the grape native tannins to remain in the wine as the proteins that might precipitate them are no longer available.

The second purpose of tannin additions is done with the objective of colour stabilisation or, in other words, to promote the formation of long lasting tannin-anthocyanin reactions. Finally, oenological tannins can be added later during the winemaking process to improve body, structure and the mouth feel properties of wine.

c. Enzyme addition

Commercial enzyme addition improves the release of phenolic compounds from the cells of berries. The efficacy of the enzymes in enhancing the release of phenolic compounds is affected by a number of factors such as grape characteristics (level of ripeness at harvest), the specific enzyme cocktail mix, enzyme dosage, must temperature, length of maceration, etc. Enzymes facilitate an increased extraction of the anthocyanin and tannin fractions, with the latter usually the more profound.

d. Punch downs and pump overs

The CO_2 produced by yeast during fermentation is responsible for cap formation. Punch downs and pump overs are done with two main purposes. The first one is to wet the cap, thus avoiding wine spoilage by acetic acid bacteria. The second purpose is to maximise the contact period between the solid parts of berries and the must/wine during the maceration. During a pump over wine is pumped from the lower part of the tank and sprayed onto the cap formed above the wine. Pump overs can be done with or without aeration. Aeration complements yeast development during the early stages of fermentation by supplying oxygen. Aeration also promotes early colour stabilisation, favouring anthocyanin-tannin reactions through acetaldehyde linkages. A current trend is the increase of pump overs per day (while decreasing the amount of wine being pumped over) to ensure that an intense extraction is achieved. The main drawback of a pump over is the possible formation of channels within the cap area which leads to poor cap homogenisation.

Punch downs, on the other hand, consist of breaking up the structure of the cap by literally pushing down and thus homogenising the solid parts of the berries with the liquid part of the fermentation. Increased extraction should theoretically be achieved as a more intense mechanical action involving the skins and seeds is performed. The frequency of pump overs varies between the standard three punch downs per day to six to eight punch downs per day if an intense extraction is desired. Punch downs can however only be performed in relatively small tanks seeing that this technique is impractical for higher tanks.

e. Fermentation temperature

Temperature conditions during fermentation will have a major influence on the extent and composition of phenolic compounds extracted from the solid parts of berries. Depending on the desired intensity of phenolic extraction a lower (approximately 20°C) or higher (25 to 28°C) temperature will be established. Higher temperatures will influence cell wall integrity with subsequent enhanced phenolic extraction, whereas lower temperatures will limit the extraction phenomena. Higher temperatures will also accelerate the chemical reactions among phenolic compounds with an increased presence of polymeric pigments and larger tannin molecules. Lower temperatures will be suitable for younger/fruitier wine styles with decreased phenolic content, but increased aromatic profile. Also note that fermentation temperatures higher than 28 to 30°C could be detrimental to wine quality, leading to yeast stress as well as alcohol and aroma losses.

f. Extended maceration

The pressing operation in red wines takes place once all fermentable sugars are consumed. However, the maceration process can be extended beyond fermentation in what is known as extended maceration. The main purpose of extended maceration, commonly lasting one to three weeks, is to increase the phenolic content of wine, resulting in an enhanced tannin fraction. Anthocyanin content, on the contrary, shows a decrease mainly due to degradation phenomena and re-adsorption on skins and lees cell walls. Note that an increase in polymeric pigment content is observed. Tannin extraction will thus take place under high alcohol conditions with an increase in the concentration of seed tannins being released. This comes with the associated risk of over extraction of seed tannins which will be difficult to manage during the aging process. Grape characteristics at harvest and more specifically the skin and especially seed phenolic maturity will thus be key factors to consider.

Another important observation when applying this technique is the enhanced release of mannoproteins and polysaccharides from grape cell wall components. These substances have a high affinity for tannin compounds and the resulting wine will thus be perceived as softer, but with and improved roundness and volume.

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g. Micro-oxygenation

The addition of controlled amounts of oxygen at levels that can promote phenolic structural modifications is the main purpose of micro-oxygenation applications. A number of reactions among phenolic compounds make use of the oxidation of mainly ethanol as reaction facilitator. Micro-oxygenation is commonly applied between alcoholic and malolactic fermentation, or once malolactic fermentation is completed. During this process small oxygen bubbles are released at the bottom of the tank. If the height of the tank is appropriate, the released oxygen bubbles will dissolve in the wine thus becoming available for the oxidation of the ethanol, which will later on be involved in the reaction between phenolic substances.

Two main effects are expected after a micro-oxygenation treatment is completed. First of all, increased anthocyanin-tannin combinations will be present thanks to the ability of the oxidised ethanol to serve as reaction facilitator in what is known as acetaldehyde bridges. Secondly, the participation of tannin compounds in this reaction gives rise to the decreased ability of these tannin molecules to elicit astringency as they become partially deactivated. In other words, their tannin activity is compromised due to the presence of the anthocyanin partner in the molecule.

Tannin extraction will thus take place under high alcohol conditions with an increase in the concentration of seed tannins being released.

6

Interactions and reactions between phenolic compounds

a. Copigmentation interactions

Copigmentation interactions take place between anthocyanins and non-coloured copigments, including other phenolic compounds. The molecules are formed through weak hydrophobic forces, creating sandwich-like structures where the sugar moieties of the anthocyanins are placed towards the external part of the molecule. The resulting copigmented molecule is in this way protected against water decolouration. Copigmented molecules are to a large extent responsible for the colour of young red wines. As the wine ages, these copigmentation reactions become less important due to their low stability. The main outcome of copigmentation interactions is a hyperchromic effect (higher red colour intensity) in the anthocyanin's red coloration in conjunction with a bathochromic shift towards bluish hues. Higher copigmentation in young wines might lead to enhanced anthocyanin concentrations in older wines due to the protection of the anthocyanins under the copigmented structure. Finally, flavonols are the phenolic compounds with the highest copigmentation ability suggesting that wines rich in flavonols and anthocyanins will retain enhanced colour properties over time.

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b. Pyranoanthocyanins

Pyranoanthocyanins are formed by an anthocyanin and other molecules, including phenolic compounds and yeast derivates such as acetaldehyde or pyruvic acid. The flavilium form of the anthocyanin reacts with a double bond compound and the pyran ring is incorporated into the anthocyanin structure. These molecules are characterised by a hypsochromic shift (red colouration shifting towards orange hues) in the absorption maxima. The formation of these compounds occurs during the first months of winemaking. After a stable period of two to three years a second increase is observed due to the reaction of anthocyanins with hydroxycinnamic acids. The positioning of the pyran ring at position four of the anthocyanin provides increased stability to the pigment against water and SO_2 decolouration. These reactions are also more stable against pH changes and have a significant impact on the long term colour stability of red wines.

c. Anthocyanin-tannin reactions

Anthocyanins and tannins can react directly or by making use of oxidised ethanol in what is known as acetaldehyde linkages. In direct reactions both tannins (T) and anthocyanins (A) can be positively or negatively charged. In the first scenario (A+-T-), the formed pigments are colourless, but become red in the presence of oxygen. If the opposite scenario (A--T+) is evident, the resulting molecule is initially colourless, but is rapidly rehydrated into a stable red-orange pigment. The second reaction is completely independent of oxygen presence. On the other hand, the indirect reaction involves the presence of acetaldehyde acting as bonding agent between the tannins and the anthocyanins, leading to a pigment which is mauve in colour. The main outcome of this reaction is increased stability of the anthocyanins as they become protected by the new structure. Moreover, the interaction between these compounds also compromises the ability of the tannin counterpart to elicit astringency. The incorporation of tannin into the pigmented structure partially deactivates the reactive groups of the tannin molecule, which makes them less available to interact with the salivary proteins, precipitate them and thereby causing astringency.

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d. Tannin polymerisation

Tannins are able to polymerise with themselves creating longer organised structures of varying sizes and conformations. The flavanol monomers polymerise, giving initially oligomer structures and later polymers with differing abilities to elicit astringency. The levels of these long chain polymers are influenced by the precipitation of compounds that become too bulky, hydrophobic or insoluble. The size, composition and conformation of these compounds define their ability to elicit astringency. These polymerisation reactions are completely independent of oxygen and are promoted by higher temperatures. The role of these molecules are related to the tannin activity of the corresponding structure that will provide a more or less intense astringent sensation.

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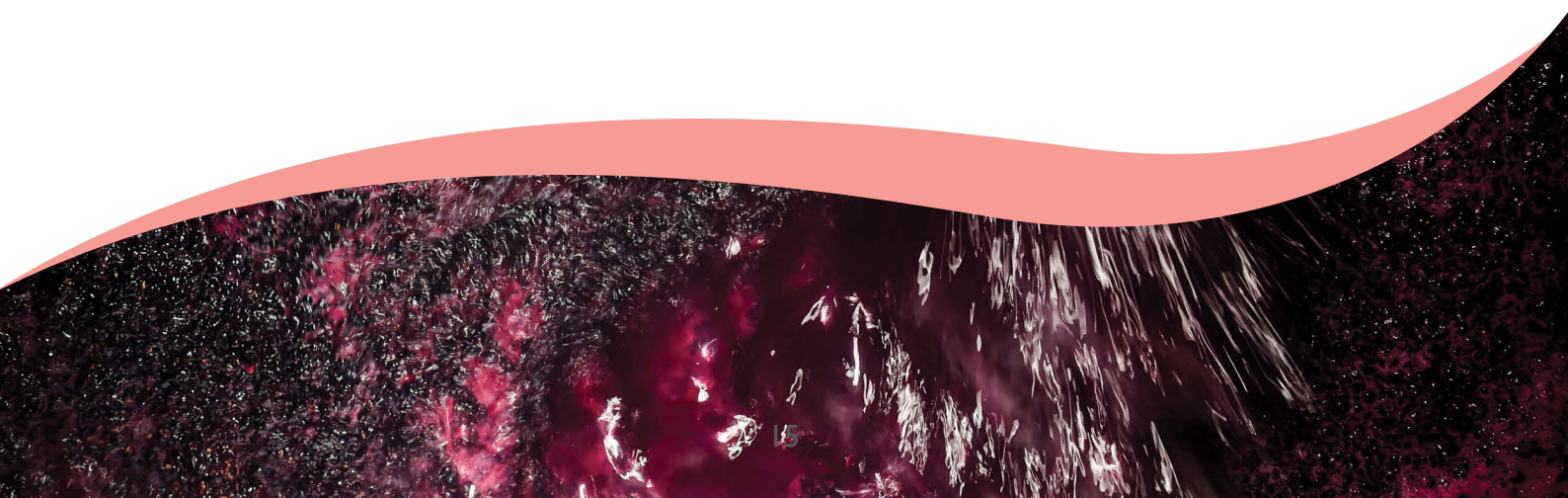
e. Tannin condensation with polysaccharides and proteins

Tannins can also be involved in condensation reactions and interactions with other wine components such as proteins and polysaccharides. These combinations occur through weak hydrophobic forces or hydrogen bonds and are dependent on the type of polymer. As tannins polymerise they are organised into longer chains with spiral structures placing their reactive sites towards the external part of the molecule, thus becoming available to interact and react with proteins or polysaccharides. Tannin-protein interactions lead to decreased astringency as the tannin molecule is deactivated by precipitation. On the other hand, tannin-polysaccharide structures remain in solution, thereby improving the mouth feel properties of wine in terms of enhanced volume and roundness



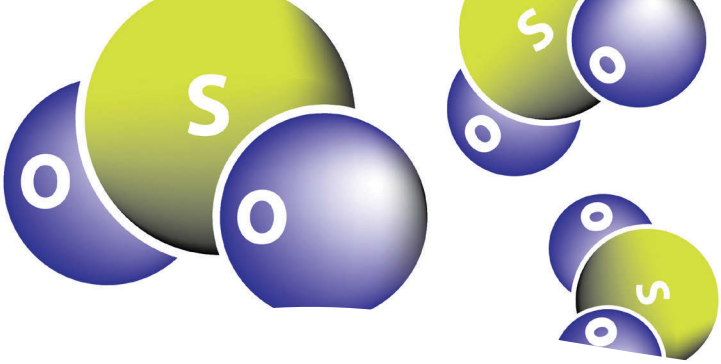
7

Evolution of phenolic compounds during wine aging

Copigmentation interactions are to a large extent responsible for the colour of young red wines. These molecules, which are thought to be involved in the very first step towards the stabilisation of red wine colour, have limited stability and the colour due to copigmented anthocyanin decreases to a large extent during the first months of aging. In addition, monomeric anthocyanins are highly reactive substances and their levels continuously decrease during aging due to various phenomena such as degradation, oxidation and precipitation. Monomeric anthocyanins however take part in polymerisation reactions to form polymeric structures known as polymeric pigments. The concentration of polymeric pigments starts increasing from the very beginning of fermentation. The presence of these pigmented molecules represents a bigger part of the wine colour as the wine ages. These molecules, including tannin-anthocyanin and pyranoanthocyanin pigments, are responsible for the colour of aged wines. The polymerisation and stabilisation of anthocyanins are thus important phenomenons that should be handled through the application of wine-making practices aimed at protecting the individual anthocyanins from degradation reactions. During wine aging the colour of wine becomes less intense (due to anthocyanins degradation), but more stable over time and with increased orange tonalities (due to tannin-anthocyanin and pyranoanthocyanin pigments).

The level of flavanols and tannins during wine aging initially remains relatively stable, with only a moderate decrease seen over the years. A number of phenomena related to flavanols and tannins and their ability to elicit bitterness and astringency occur in wine during the aging period. Flavanols (monomers and small molecules) are bitter compounds that become astringent as new, larger compounds are formed through polymerisation reactions. Larger tannin compounds become more astringent as the size of the molecule increases, with no size limit indicated. Based on this, the average tannin's molecular size will increase during aging, but at the same time the wine becomes softer or less astringent, which seems to be a contradicting result. This can be explained by a number of parallel phenomena.

First of all, the interactions of tannins with polysaccharides and proteins, as well as phenolic components (anthocyanins) might compromise the astringent activity of the tannin structure. Secondly, larger tannin molecules might become too bulky, with branching occurring along the main chain. This leads to more symmetrical and compact conformations that compromises the ability of tannins to combine with salivary proteins. Thirdly, it is also possible for the tannin polymers to reach insoluble molecular sizes, thus leading to precipitation from the wine matrix. Finally, cleavage reactions might also take place, which transforms highly polymerised tannin to smaller, less astringent molecules. A combination of these phenomena probably explains why, despite tannins continuously polymerising during wine aging, the astringency perception is decreased during this process.



SO_2

SULFUR DIOXIDE

Analysis of phenolic compounds in grapes and wine

a. Free anthocyanins (monomeric pigments)

The quantification of monomeric (free) anthocyanins is achieved by making use of the chemical properties of these compounds. Two principles are mainly applied to quantify monomeric anthocyanin levels. The ability of SO_2 to combine with and bleach monomeric anthocyanins is used to quantify the concentration of these compounds. In this case a control sample is compared with a sample where the anthocyanin monomers have been bleached. The difference will provide the monomeric anthocyanin concentration of the particular grapes or wine.

Furthermore, the effect of low pH in the colouration of monomeric anthocyanins, with more anthocyanins in the red flavilium form at lower pH values, is also used to measure the levels of these compounds. The sample is diluted with hydrochloric acid, thereby decreasing the pH and moving the anthocyanins into the red flavilium form. Both approaches quantify these compounds by measuring the absorption at 520 nm (red colour in the visible region of the electromagnetic spectrum).

b. Bound anthocyanins (polymeric pigments)

The bleaching ability of SO_2 is used again to quantify the levels of pigmented compounds found in wines. Simple (monomeric) anthocyanins are vulnerable to SO_2 combination due to the availability of position four in the phenolic ring of the anthocyanin molecule. SO_2 can thus combine with and bleach the anthocyanin, i.e. the molecule loses the red colouration and becomes colourless. On the other hand, polymeric pigments are structures with increased protection against SO_2 decolouration. The reactive site in the anthocyanin molecule is used by other substances to create the pigment. The remaining red colour after SO_2 addition will thus correspond to polymeric pigments that are also quantified by measuring the absorption in the red colour region (520 nm).

The ability of SO_2 to combine with and bleach monomeric anthocyanins is used to quantify the concentration of these compounds.

c. Colour

Colour measurements in grapes and wines are mainly carried out by recording the absorption intensities at three key wavelengths corresponding to the main colourations found in wines, i.e. yellow (ABS 420nm), red (ABS 520 nm) and blue (ABS 620 nm). The addition of these three absorptions provides what is known as the colour intensity or the colour density of the wine. On the other hand, an alternative method provides a more detailed characterisation of wine colour. This colour determination simulates the way the human eye perceives the colour through a few colour coordinates (CIElab colour coordinates).

In brief, the visible (coloured) region is measured and the coordinates a* (red/green colour component, a*>0 red; a*<0 green), b* (blue/yellow colour component, b*>0 yellow; b*<0 blue), L (clarity or luminosity, L*=0 black; L*=100 colourless) and its derived magnitudes chromaticity (C*) and tone (H*) are calculated. Moreover, it has been also established that a colour difference (ΔE) between two samples of 2.7 units indicates differences that can be perceived by the human eye between two wines evaluated in standard tasting glasses

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d. Tannins

Tannin compounds can also be quantified using different principles. The ability of tannins to be hydrolysed in an acid medium at high temperatures (acid hydrolysis) was widely used. However, this method contains a number of limitations as it does not provide tannin structural information (potentially related with the astringency intensity) and often overestimates the total content with increasing levels over time that do not correspond to an increase of tannin content. More recent methods make use of the ability of tannins to precipitate with proteins or polysaccharides. These precipitation based methods make use of the same principle occurring in the oral cavity when tannin compounds interact with salivary proteins. These interactions give rise to macromolecular complexes that become insoluble, therefore precipitating from solution and leading to a more or less intense astringency perception. Theoretically these approaches will also provide an indication of the ability of grape and wine tannins to elicit astringency, i.e. they provide an indication of the astringency intensity of wines.

These methods are known as the BSA (bovine serum albumin) tannin assay and the MCP (methylcellulose precipitable) tannin assay. The MCP tannin assay relies on tannin polymer interaction to create a complex that is precipitated from solution by centrifugation. A control sample is compared against a treated sample with the tannins precipitated and measured at 280nm within the ultraviolet (UV) region that corresponds to the absorption maxima of tannins. The total tannin content is calculated with a calibration curve using epicatechin and expressed in mg/L.

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e. Total phenols

The quantification of total phenol levels is achieved by measuring the absorption intensity at 280nm (the main absorption region of all phenolic compounds) after the grape extract or wine is diluted to avoid saturation of the UV detector at this wavelength. The results are generally presented as index values and less often as mg/L of a standard calibration. An alternative method known as the Folin-Ciocalteau method is also commonly employed to quantify the total phenol content in the wines. This more complex procedure provides an estimation of the tannin content through the generation of a blue coloured complex achieved after a redox reaction with acids in an alkaline medium. The results are in this case generally provided as mg/L. In addition, studies have reported strong correlation among methods making the results obtained comparable.

f. Anthocyanin extractability and percentage of seed tannin content

When grapes are analysed for phenolic content it is also important to include other parameters in order to better understand the phenolic status of a specific batch of grapes. During maceration phenolic compounds are released into the must/wine. The extent of this diffusion is correlated not only with the phenolic levels present in the grape at harvest, but also with the ability of those phenolics to be extracted. The anthocyanin extractability test thus provides an indication of the ease with which the anthocyanins can be extracted. Due to the shared location in the berry tissue, anthocyanin extractability can also be used as an indication of the extractability of skin tannins. Theoretically, riper grapes should have more extractable phenolic compounds, however this statement should be considered with caution due to the numerous other factors that influence the extractability phenomena. On the other hand, it is also interesting to evaluate the percentage of seed tannin content as seed tannins are more bitter and astringent than skin tannins, with the subsequent implication on wine mouth feel properties. The estimation is obtained by using anthocyanins to determine the extent of skin tannins and comparing this against the total phenol content of the sample. The remaining tannins thus correspond to seed tannins and a percentage is then calculated. Higher percentages of seed tannin content indicate that an increased concentration of these tannins, with their intrinsic properties, would be present in the final wine

g. Ultraviolet-Visible (UV-Vis) spectroscopy applications for phenolic content quantification

The ability of phenolic compounds to absorb UV light in combination with the coloured nature of some phenolic compounds (absorption in the visible region) makes UV-Visible spectroscopy suitable for the quantification of phenolic compounds. Quantification is in this case possible through the correlation of the spectral properties of grapes and wine with the phenolic content obtained by the conventional reference analytical methods for phenolic analysis. The spectral information and the measured levels of phenolic compounds of a large number of samples are then correlated through regression techniques and after the appropriate process of model calibration and validation accurate algorithms are obtained. The algorithms only need the spectral properties of the samples (or the key information contained within the UV-Vis spectrum) to calculate the levels of the phenolic compounds of interest. The measurement of phenolic content using spectroscopy calibrations thus becomes an exercise of measuring the spectral properties of samples and thereby avoiding the laborious reference method protocols. A large number of information is thus obtained from a single spectral measurement (multi-parametric approach). Furthermore, the use of spectroscopy calibrations simplifies the analytical procedure and significantly reduces the analytical costs in terms of reagents, facilities, equipment and personnel.

Polymeric pigments are structures with increased protection against SO_2 decolouration.

9

Conclusion: Using phenolic analysis in your winemaking decisions

The concept of phenolic maturity involves the measurement of phenolic content during grape ripening. The monitoring of phenolic accumulation is complemented by extractability measurements (mainly in terms of anthocyanin extractability). Skin extractability measurements may apply to other phenolic compounds contained in the skins of the berries due to similar location in the vacuoles of cell walls, i.e. it also provides an indication of skin tannin extractability. Information on the proportion of seed vs. skin tannin can be also obtained before deciding on the optimum harvest date. This information can be used to optimise winemaking strategy by considering practices such as the addition of enzymes, cold maceration (with or without dry ice), varying SO_2 doses, oenological tannin addition and the frequency and duration of pump overs and punch downs.

The extraction of phenolic compounds during the skin contact phase of fermentation is influenced by many factors. Monitoring phenolic extraction during this process potentially yields optimisation of the final phenolic content. The efficacy of a cold maceration, commercial enzyme or extended maceration applications can easily be assessed. Decisions on the winemaking conditions could also be made based on real life information and then adapting the winemaking strategy during the course of the fermentation. This includes optimisation of fermentation temperature, punch down and pump over frequency and duration, as well as tannin addition, to name but a few. Finally, the decision when to press could be better timed based on the early achievement of targeted phenolic content or wine style, with large implications in terms of space availability and wine quality. The phenolic information could also indicate the need for micro-oxygenation between alcoholic and malolactic fermentation.

The phenolic content of wine continuously changes during aging. Degradation of phenolic compounds and reactions and interactions involving phenolic compounds are taking place constantly. Phenolic information coupled with tasting evaluation is used to decide the extent of the barrel and bottle aging periods. Increased polymeric pigment formation and a relatively stable phenol content thus provides a good indication of a wine's suitability for longer aging. On the other hand, the loss of phenols and wine colour without observing an increased polymeric presence could indicate that the aging period is reaching its conclusion. Additionally, phenolic content can also be used for the grading and benchmarking of grapes and wine in order to meet market specifications.

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