Tannins: from reactions to complex supramolecular structures

Hélène Fulcrand¹, Cécile Morel-Salmi, Carine Mané, Céline Poncet-Legrand, Aude Vernhet and Véronique Cheynier

INRA, UMR Sciences pour l’œnologie, 34060 Montpellier cedex, France
¹Corresponding author: fulcrand@ensam.inra.fr

Introduction

Oenology research aims, ultimately, to master wine quality. It is a multidisciplinary approach that makes use of the empirical knowledge and experience of winemakers and oenologists. With phenolics, which are essential for wine quality, several skills contribute to understanding their influence on the colour and taste properties of wines. Firstly, oenologists and winemakers, who are able to evaluate ‘good’ and ‘bad’ tannins by sensory tasting; then organic chemists, who try to characterise the chemical structures of the phenolics and their reactions in the course of winemaking and ageing (identification of reaction processes and the factors favouring or limiting their occurrence); finally, physical-chemists, who study the three dimensional (3D) structures of these components and how it is influenced by their interactions with other wine macroconstituents (such as proteins, polysaccharides). All of these fields have their own language and the difficulty is to link them together. For a chemist, what structure lies behind a ‘good’ or ‘bad’ tannin? For a physical-chemist, are there specific interactions between wine constituents that make them ‘bad’ or ‘good’, or ‘soft’ or ‘hard’? Answering these questions is the challenge to overcome for mastering wine quality. This article reviews our current knowledge on wine phenolics and their properties.

Grape composition: anthocyanins and tannins

In wine, the anthocyanins and tannins are the major grape polyphenol constituents. As anthocyanins are red pigments, they are specific to the red varieties and are usually localised in the berry skins. The anthocyanin composition primarily depends on the cultivar. For instance, Pinot Noir grapes have a very simple anthocyanin profile with only five anthocyanidin 3-glucosides whereas most other varieties also contain the corresponding acylated derivatives.

Flavanols are encountered in the grape berry as monomers, oligomers and polymers. They are mainly localised in the solid parts of the clusters, the seeds, the skins and stems. The polymeric forms are commonly called tannins because of their strong propensity to interact with and precipitate proteins. The tannins are made up of flavanol units. Epicatechin is the isomer that appears mainly in the extension and upper units, while catechin is the isomer that is mostly encountered in the terminal units. Additionally, specific substitutions of the flavanol and the polymer chain length (degree of polymerisation ‘DP’, i.e. number of units in the polymer) are characteristic of the tannin source. Thus, seed tannins contain some flavanols that are galloylated on the hydroxyl at C-3 of the flavanol unit (corresponding to epicatechin gallate units); and they have degrees of polymerisation ranging from 1 to ~20 (Prieur et al. 1994). However, skin tannins have a third hydroxyl group on the B ring of some flavanol units (corresponding to epigallocatechin units) and are much larger than seed tannins, up to 80 units (Souquet et al. 1996) but ~30 units on average. Therefore, tannins show a great diversity of structures and molecular weights. Furthermore, the heterogeneity of this polyphenolic fraction increases exponentially since the sequence of the constitutive units is randomly determined within the polymer chain so that the number of isomers increases as the chain length increases.

Anthocyanin and tannin reactivity and reactions

From a chemistry point of view, anthocyanins and tannins belong to the flavonoid class. The common skeleton of these compounds is made up of two phenolic nuclei referred to as the A and B rings (Figure 1a), connected through a central oxygen-containing ring referred to as the heterocyclic ring or the C ring. The number of double bonds (unsaturation) in the C ring is variable and it distinguishes some of the different sub-groups of flavonoids (Figure 1b). Thus, no double bond in ring C corresponds to the flavanol series, one double bond corresponds to the flavonol series and two double bonds give the anthocyanins. As the number of double bonds increases in the central ring, the electron delocalisation in the molecule is extended from one to three phenolic nuclei, which is reflected by the colour variation within the flavonoid class: colourless, yellow and red for flavanols, flavonols and anthocyanins, respectively. Besides the colour, the electron delocalisation is responsible for the chemical reactivity of the molecules. The reactivity depends on the nature, number and position of the substituents located on the molecules, as the substituents induce partial or full positive or negative charges on the molecule. Positive charges indicate electron deficient sites, and the functional group or the molecule containing such a charge is designated as an electrophile. Conversely, negative charges correspond to electron rich sites, called nucleophilic sites, and the molecule containing such a site is said to be a nucleophile.

With these flavonoids, the A and B rings have different hydroxylation patterns, and this difference leads to different reactivity. The adjacent position of two hydroxyl groups in the B ring is called an ortho-substitution pattern, and the main reaction associated with it is oxidation. The position of the hydroxyl groups...
on the A ring is called a meta-substitution pattern, and the major reaction associated with it is electrophilic aromatic substitution. In other words, the hydrogen attached to the carbon in between the two carbons bearing the hydroxyl groups becomes replaced by an electrophile (an electron deficient species) because of the negatively charged character of that carbon. The most famous reaction in oenology illustrating this A ring reactivity is the polycondensation reaction mediated by acetalddehyde (Timberlake and Bridle 1976), in which the aldehyde is the electrophile.

Lastly, ring C, the central, heterocyclic ring of the flavonoids can also be reactive provided that it is positively charged. For tannins, this happens under acidic conditions. Such conditions promote the cleavage of bonds that connect the constituent flavanol units within the polymeric chains of the tannins, the interflavanyl linkage. The flavanol units of grape tannins are linked through the C4 of the upper unit and either C8 or C6 of the lower unit (Figure 2a). The breakage of these bonds releases the extending flavanol units and the uppermost flavanol unit as carbocations (positively charged molecules at C4), while the lowermost flavanol unit is released without any change. This acid catalysed reaction, leading to a complete depolymerisation under drastic conditions (heat and strong acid), is classically used for analysing tannins. In this process, the carbocations released by the bond cleavage can be further oxidised to red anthocyanidins (Bate-Smith 1954), hence the designation of tannins as proanthocyanidins. Alternatively, when analysing tannins, the carbocations may be trapped by nucleophilic reagents such as toluene-t-thiol or phloroglucinol and the corresponding reactions are then referred to as thiolysis (Thompson et al. 1972; Rigaud et al. 1991; Prieur et al. 1994) or phloroglucinolysis (Matsuo et al. 1984, Peyrot Des Gachons and Kennedy 2003), respectively. Even in a medium as acidic as wine, tannins are prone to such cleavage reactions (Haslam 1980, Vidal et al. 2002a). In fact, although tannins are usually nucleophiles through their A ring reactivity, the carbocations released from them through bond cleavage are electrophiles (Figure 1a) and can be involved in direct reactions with anthocyanins, leading to flavanol-anthocyanin (F-A) products with a C4-C8 linkage, in which the flavanol unit is in the upper position (Salas et al. 2003).

Although anthocyanins are usually represented by their red form, the flavylium cation, they exist at wine pH mainly in their uncharged, colourless form, the hydrated form (Figure 2b). This occurs because the positive charge of the flavylium cation makes that form electrophilic in character and particularly reactive towards nucleophilic attack of water. However, as the positive charge of the flavylium cation form is not exclusively localised on C4 of the central heterocyclic ring but is distributed by resonance onto other positions, including C2, the red flavylium cation form is a less reactive species than the carbocation released from tannins by interflavanyl linkage cleavage.

The electrophilic character of the flavylium cation form of anthocyanins makes that form reactive towards nucleophilic attack by the A ring of flavanol units in tannins; this produces C4-C8 linked anthocyanin-flavanol (A-F) products (with the anthocyanin unit in the upper position). However, this reaction becomes important only if the bond cleavage of tannins, which gives a more reactive carbocation for nucleophilic attack by a flavanol unit, is no longer effective; that is, when the acidity is too weak (pH >3.8) (Salas et al. 2003). Given the small proportion of anthocyanins in the flavylum cation form at this pH value (~6%), the direct reaction of anthocyanin and flavanol units to yield anthocyanin-flavanol (A-F) products is not a major reaction in wine. Consequently, anthocyanins are expected to react mainly as nucleophiles (through the A ring of their uncharged hydrated form) rather than electrophiles (through the positive charge of the flavylium cation form) and give predominantly flavanol-anthocyanin (F-A) products. However, the presence of both F-A and A-F type polymers has been detected by mass spectrometry in a wine fraction (Hayasaka and Kennedy 2003).

To summarise, all the rings of the flavonoid framework are reactive and can undergo specific types of reaction. The B ring, with an ortho-dihydroxy substitution pattern, is prone to oxidation reactions. The A ring has a meta-hydroxy substitution pattern that promotes polycondensation reactions through A rings and aldehydes. The central heterocyclic ring, when it is positively charged, is involved in direct reactions with the A ring of the other flavonoids.

Wine composition: structures and reactions

Due to the high reactivity of flavonoid compounds, a tremendous number of new molecules are formed in the course of winemaking and, later on, during wine storage and ageing. Reactions between anthocyanins and tannins have been thoroughly studied in the last decade and the main types of product have been characterised. Oxidation, polycondensation and direct reaction have been shown to generate specific types of linkages that are, for most of them, resistant to thiolysis cleavage.

Many of these reactions have been demonstrated in model solutions using monomeric and, to a lesser extent, dimeric flavanols. However, the molecules identified in such cases do not reflect actual wine composition because the most abundant polyphenol fraction, the grape flavanols, are mainly higher oligomers and polymers (Czochanska et al. 1980, Souquet et al. 1996). While monomers and oligomers can be analysed by HPLC, the heterogeneity of tannins, which increases with their chain length, leads to poor resolution of components in all separation techniques. Only thiolysis, by yielding resistant fragments and derivatives of specific wine reaction markers gives access to qualitative information on the 'history of wine', but there is still a huge lack of quantitative data. For this reason, quantitative analysis of wine tannins, to determine the constituents formed during the winemaking process (the derived tannins), is the principal challenge to be faced over the next few years. The different products and pigments identified in wines and model solutions will now be reviewed on the basis of the linkage type that connects their flavonoid units (Figure 3).
Products arising from oxidation reactions: $\varphi_B$-$O$-$\varphi_A$ and $\varphi_B$-$\varphi_A$ linkages

The first product obtained by oxidation of an ortho-dihydroxy benzene (commonly called an ortho-diphenol), is an ortho-quinone which is very reactive, particularly towards other polyphenols. The ortho-quinone can oxidise molecules with lower redox potentials. It is also a powerful electrophile, and it can react with a nucleophile; this can include its non-oxidised ortho-diphenol form or another phenol. The resulting product is a dimer containing at least one ortho-diphenol function. The dimer, then, is susceptible to further oxidation and can be converted into a trimer. Therefore, oxidation can be a polymerisation process.

The formation of the quinone can occur by both biochemical and chemical processes. In the biochemical process, an enzyme acts as a catalyst and initiates the first step of oxidation. The chain reactions that follow the formation of the ortho-quinone are driven by the reactivity of the quinone itself. This biochemical process takes place in the early stages of winemaking, in the presence of oxygen, with grape polyphenoloxidase (PPO) as the enzyme. The ortho-diphenol substrates for PPO-induced oxidation are the major grape phenolic acids, namely cinnamic and cinnaric acids (Gutala et al. 1987). As the ethanol content increases during fermentation, enzymatic systems become less effective, and chemical oxidation gradually becomes the predominant process.

Chemical oxidation is primarily governed by the redox potential of polyphenols and it can affect a wider range of molecules than enzymatic oxidation. Therefore, the polyphenol substrates of enzymatic reactions are not necessarily the most reactive molecules towards chemical oxidation. Thus, the capacity of white wine to brown was shown to be directly related to its flavanol content (Simpson 1982, Cheynier et al. 1989). Additionally, oxidation is not restricted to phenolic constituents; it can affect wine matrix constituents, such as ethanol and potassium bitartrate, to produce aldehydes that are reactive towards flavonoids (Fulcrand et al. 1997). Furthermore, it seems that catalytic species, like radicals and/or metals (Fe, Cu), induce chemical oxidation reactions in the presence of oxygen and polyphenols. In fact, reaction between polyphenols and oxygen, also called auto-oxidation, is not straightforward and requires that either oxygen or the polyphenol be activated (by radicals and/or metals). The resulting chain reaction can be summarised by the following equation:

$$O_2 + \text{ortho-diphenol} \rightarrow H_2O_2 + \text{orthoquinone}$$

The hydrogen peroxide ($H_2O_2$) generated from phenol auto-oxidation can further oxidise ethanol to acetaldehyde (Wildenradt and Singleton 1974) only in the presence of Fe$^{2+}$ (Inui et al. 2004). Oxidation of bitartrate in the presence of ferrous ions (Fe$^{2+}$) leads to the formation of various acids and aldehydes (Genevoix and Ribéreau-Gayon 1947, Mourgues and Deibner 1967), among which, glyoxylic acid was shown to be the precursor of yellow pigments through its polycondensation with flavonoids (Fulcrand et al. 1997). Polycondensation reactions with aldehydes will be further discussed in the next section.

In summary, grape phenolic acids are the major substrates of enzymatic oxidation, while flavanols are rather prone to auto-oxidation. The compound called ‘grape reaction product’ (GRP) that results from the addition of glutathione to cattaric acid orthoquinone is a marker of enzymatic oxidation (Singleton et al. 1985, Cheynier et al. 1986). Oxidation products of flavanols exhibit specific linkages in their structures (Guyot et al. 1996b). The interflavanyl linkage of the dimers obtained from catechin oxidation at pH 3 is always located between the B ring of the upper unit and the A ring of the lower unit. It is also either a biphenyl ether linkage ($\varphi_B$-$O$-$\varphi_A$, where $\varphi_A$ and $\varphi_B$ represent the flavonoid ring A and B, respectively, and O is the linking oxygen atom), resulting from a radical coupling, or a biphenyl linkage ($\varphi_B$-$\varphi_A$) from nucleophilic addition of the catechin A ring to the ortho-quinone (Figure 4). It is worth noting that all the interflavanyl linkages yielded by oxidation are totally resistant to acid cleavage and, hence, are much stronger than the interflavanyl linkages of genuine tannins.

Further oxidation of the direct biphenyl-linked dimer ($\varphi_B$-$\varphi_A$) leads to yellow pigments by an intramolecular rearrangement of the oxidised dimer. This is probably a competitive pathway to further oxidation-induced polymerisation. Furthermore, flavanol auto-oxidation can be modulated by the presence of phenolic acids; it was recently shown (Fulcrand 2005) that addition of caffeic acid to a catechin solution actually enhances the oxidation-induced orange-

![Figure 3](image-url)  
**Figure 3** Overview of the different types of linkages and pigments yielded by flavonoid reactions: oxidation, polycondensation and direct reaction.

![Figure 4](image-url)  
**Figure 4** Structures characterised in catechin oxidation performed at pH3 and pH6.
brown hue of the solution, whereas addition of protocatechuic acid (3,4-dihydroxybenzoic acid) slows down the formation of yellow pigments by limiting the formation of their precursor, the biphenoxy dimer. Instead, a colourless product was formed, corresponding to the addition of a catechin molecule to the ortho-quinone of protocatechuic acid. This latter study emphasizes the complexity of wine oxidation processes, and shows how it can be very dependent upon wine composition, particularly the different classes of polyphenols and their relative proportion.

**Products arising from polycondensation reactions:** $\Phi_A \cdot C \cdot \Phi_A$

As explained above, polycondensation reactions involve the A ring of flavonoids and an aldehyde which, under the acidic conditions, becomes electrophilic through protonation (Figure 5). After substitution of the hydrogen on the A ring by the protonated aldehyde (Figure 5, Step 1), the reaction continues since a new carbocation can be formed by loss of a water molecule from the substitution product (Figure 5, Step 2). In fact, this transitory carbocation competes favourably with the initial protonated aldehyde as an electrophile for reaction with another flavonoid molecule, leading then to a dimer (Figure 5, Step 3) which continues to polymerise (Figure 5, Step 4). Both the flavanols and the hydrated forms of anthocyanins have been demonstrated to follow this pathway, yielding different kinds of polymers depending upon whether they react separately or in mixtures: polyflavanols (Fulcrand et al. 1996b), polyanthocyanins (Atanasova et al. 2002a, b), and copolymeres of anthocyanins and flavanols (Es-Safi et al. 1996, Es-Safi et al. 1999a).

The polycondensation products identified in wines have usually been dimeric pigments resulting from acetaldehyde polycondensation between a flavanol monomer and an anthocyanin, although the reaction has been demonstrated with other aldehydes (Es-Safi 2004), including glyoxylic acid, the oxidation product of tartaric acid (Fulcrand et al. 1997). The main reason for acetaldehyde-derived products being predominant is the relatively high natural abundance of acetaldehyde in wines in comparison to the other aldehydes. A further reason is that some rearrangements occur with acetaldehyde-polycondensation because the interflavanyl linkages of polymers depending upon whether they react separately or in mixtures: polyflavanols (Fulcrand et al. 1996b), polyanthocyanins (Atanasova et al. 2002a, b), and copolymeres of anthocyanins and flavanols (Es-Safi et al. 1996, Es-Safi et al. 1999a).

The pyranoanthocyanins are stable orange pigments found in aged wines. Some of them, also known as vitisins (Bakker and Timberlake 1996a), are derived polyethyl-flavanols. After cleavage of tannins occurs over a large range of wine pH (3.0–3.6) (Haslam 1980, Vidal et al. 2002a) but is no longer observed at pH 3.8 (Salas et al. 2003).

A complementary study has shown that two kinds of A-F polymer pigments can be obtained, according to the flavanol chain length. The reaction leads to a xanthylium pigment when the flavanol is a monomer, but the reaction gives the expected A-F pigment when the flavanol is a higher DP (a dimer or trimer) (Malien-Aubert et al. 2002, Dueñas-Paton et al. 2005). Furthermore, along with A-F polymers, an anthocyanin dimer (A-AO)H was also detected. This confirms, first, that anthocyanins can react both as a nucleophile and an electrophile and, secondly, that they can polymerise. In fact, the formation of anthocyanin polymers from different reactions (polycondensation and direct reactions) is currently the most relevant finding regarding red wine phenolic composition.

**Products arising from direct reactions:** $\Phi_C \cdot \Phi_A$

Direct reactions occur between the positively charged central ring C of a flavonoid unit and ring A of a second flavonoid unit. As discussed before, the most expected reaction, between the flavylum cation of an anthocyanin and a flavanol unit yielding an A-F product, is not the major reaction. More important appears to be the acid-catalysed cleavage of the interflavanyl bond of tannins (Salas et al. 2003). Consequently, flavanyl carbocations $F^+$, released from the acidic bond cleavage of tannins, can react with the hydrated form of anthocyanins to give F-A products (flavanol as the upper unit). Results from related studies have indicated that acid-catalysed cleavage of tannins occurs over a large range of wine pH (3.0–3.6) (Haslam 1980, Vidal et al. 2002a) but is no longer observed at pH 3.8 (Salas et al. 2003).

Among the polymers and copolymers produced by anthocyanin reactions, those having the anthocyanin moiety as the terminal unit (an F-A type) are not coloured at wine pH while polyethyl-anthocyanins or polyethyl-flavanyl-anthocyanins remain coloured. Therefore, a wine’s colour will depend on what reactions prevail all along the winemaking process.
From a chemical point of view, the rate of a reaction depends on the reactant concentrations as well as on environmental factors such as pH, temperature, presence of oxygen. In the context of oenology, the reactant concentration ‘polyphenol content’ is better expressed by the tannin to anthocyanin ratio (T/A). It depends primarily on the grape variety, on the maturity at harvest, and then on extraction conditions that can be greatly modulated by winemaking technologies. The proportion of anthocyanins and tannins in grapes, expressed as the T/A (tannin/anthocyanin) ratio, is very different from one cultivar to another, with both anthocyanin and tannin concentrations varying. These differing proportions are also found in the corresponding wines (Figure 6). Monitoring the phenolic composition of the fermenting red grape must shows that diffusion of anthocyanins and skin proanthocyanidins is usually faster than extraction of seed proanthocyanidins, which require higher levels of alcohol and temperature (Cheynier et al. 1997b). Winemaking experiments have shown that pre-fermentation maceration at low temperature leads to a higher concentration of anthocyanins and proanthocyanidins from skins, whereas extending the maceration time after the end of alcoholic fermentation results in higher levels of proanthocyanidins from seeds and a lower anthocyanin content (Figure 6). In subsequent work, a flash release treatment was tried. The process quickly heats the grapes to high temperature (>80°C), then a strong vacuum is applied to them, resulting in rapid vaporisation and cooling. The cellular structure of the grape is degraded, improving phenolic extraction (Moutoutnet and Escudier 2000). The flash release treatment was shown to extract all phenolic compounds faster, giving an enriched must and juice with a significant increase in concentrations of flavanols mostly from skins (monomers and polymers).

This information shows that the largest difference in anthocyanin and tannin concentrations results from the grape variety. Subsequently, winemaking techniques can modulate their levels in wine, especially the tannin content, and influence their extraction kinetics, modifying the competition between anthocyanins and tannins in reactions. Moreover, the use of high temperature, in winemaking processes like flash release, probably promotes specific reactions.

**Wine composition: structures and properties**

**Pigment and pigmented tannin properties: structure and colour relationships**

When dissolved in water, the red form of an anthocyanin, the flavlylium cation, undergoes proton transfer (Figure 7a) and hydration (Figure 7b) reactions generating, respectively, the blue quinonoidal base and the colourless hemiketal (also known as a carbinol). These reactions are reversible, and the balance of the equilibrium between the flavlylium cation and the other forms is characterised by the relevant equilibrium constant, namely the acidity (or proton transfer) constant (pK_a) and the hydration constant (pK_h). These have values that are specific to the anthocyanin structure. These constants indicate that when pH = pK_h, the same quantity of anthocyanin is in the flavlylium cation form and in the hydrated (colourless hemiketal) form, and when pH = pK_a, the same quantity of anthocyanin is in the flavlylium cation form and the quinonoidal base form. The proportion of the various forms at equilibrium is thus determined by the pH, the acidity constant (pK_a) and the hydration constant (pK_h). Flavlylium ions are predominant only in very acidic media (pH < 2). At usual wine pH values, genuine grape anthocyanins occur mostly as the colourless hydrated hemiketal form, since the value of their hydration constant (pK_h = 2.6) is lower than the wine pH value. In addition, sulphite ions add to the anthocyanin flavlylium cation in the same way as water to form colourless products, through a reaction known as sulphite bleaching (Figure 7c), which is also reversible. As a result, retention of colour in red wines is dependent upon two colour stabilising processes: conversion of grape anthocyanins to more stable pigments, and processes referred to as copigmentation.

Reactions that result in C-4 substitution on the parent anthocyanin usually produce derived pigments that exhibit greater colour stability than the parent anthocyanin. The presence of a substituent on the C-ring at this position impedes the nuclophilic addition of water (Brouillard 1982) or sulphites (Timberlake and Bridle 1967). This is observed particularly with pyranoanthocyanins, which remain coloured over a wide pH range and in the presence of sulphites (Sarni-Manchado et al. 1996, Bakker and Timberlake 1997). The colour of pyranoanthocyanins is usually orange (λ_max 480-510 nm) (Sarni-Manchado et al. 1996, BenAbdeljalil et al. 2000) although flavanylvinyl-pyranoanthocyanins, called porstins (since

![Figure 6. Impact of winemaking processes on tannin/anthocyanin (T/A) ratio; G = grenache; +FR = flash release](image)

![Figure 7. a) hydration equilibrium (AH+: flavlylium cation; AOH: hydrated form) and related hydration constant b) proton transfer equilibrium (AH+: flavlylium cation; A: neutral quinoidal base) and related acidity constant c) sulphite discolouration equilibrium (HSO3-: hydrogen sulphite, AHSO3-: anthocyanin sulphite adduct)](image)
they were first isolated from Port wines) are blue (λmax 575 nm) (Mateus et al. 2003).

On the other hand, direct reactions and polycondensation reactions yield various pigmentated tannins in which the anthocyanin moiety is not substituted at C4 (except for structures of the A-F type) and thus, in principle, is not chemically protected from water addition. The different kinds of polymers show a variety of colours: direct tannin-anthocyanin adducts are red, like their anthocyanin precursors (λmax 515-526 nm), while ethyl-linked species are more purple (λmax 528-40 nm) (Timberlake and Bridle 1976, Atanasova et al. 2002a, b, Salas et al. 2005). Moreover, these new structures are susceptible to colour changes, induced by pH variation, in differing ways.

Flavanol-anthocyanin dimers (F-A type structures, of type ϕ_A-C-ϕ_A), like anthocyanins themselves, are red only in very acidic medium and are almost colourless in the usual wine pH range, indicating that hydration of the flavylum cation takes place (Salas et al. 2004). No such bleaching is observed with flavanol-ethyl-anthocyanin pigments (ethyl bridged structures, of type ϕ_A-C-ϕ_A), they turn blue as the pH increases. Spectrophotometric studies have shown that the hydration constant for catechin-malvidin-3-glucoside (a dimeric structure of the F-A or ϕ_A-C-ϕ_A type) is 2.6, like that of malvidin-3-glucoside; so no formation of quinonoidal base was observed for this pigment in the pH range 1-4. In contrast, the hydration constant for catechin-ethyl-malvidin-3-glucoside (a dimeric ethyl bridged structure, ϕ_A-C-ϕ_A of a flavanol and anthocyanin) was found to be 4.17, much higher than the usual wine pH value, explaining why the ethyl-linked pigments remain coloured in wine. Moreover, the proton transfer constant of catechin-ethyl-malvidin-3-glucoside was found to be 3.44, indicating that about half of this pigment is present as the blue quinonoidal base in wine. For malvidin-3-glucoside-ethyl-malvidin-3-glucoside (a dimeric ethyl bridged structure, ϕ_A-C-ϕ_A of two anthocyanins), the constants calculated (1.81 and 4.63, respectively, for the first and second hydration constants) indicated that only one form, in which one of the anthocyanin moieties is in the red flavylum form and the other is hydrated, is present in significant quantities at wine pH (Atanosova et al. 2002b).

The colour-stabilising mechanism of these ethyl-linked pigments seems to be linked to their propensity for self-aggregation, as suggested earlier by Escribano-Bailon et al. (Escribano-Bailon et al. 2001). This assumption is further supported by copigmentation studies presented below that support colour-stabilisation through interaction phenomena, referred to as copigmentation. Interactions of anthocyanins with colourless polyphenols (copigments) is an important process for colour stabilisation in flowers, fruits and wine. Anthocyanin copigmentation gives more intense and more stable colours than those expressed by the free anthocyanins (Brouillard et al. 1989; Mazza and Brouillard 1990; Goto and Kondo 1991). The hyperchromic effect (an increase in colour intensity) typical of copigmentation results from a displacement of the hydration equilibrium of the anthocyanin (Figure 7a) toward the flavylum cation form due to its selective interaction with the copigment. This occurs because the planarity and polarisability of the chromophores of the coloured forms of anthocyanins (the red flavylum cation and the blue quinonoidal form), tend to stack with themselves (self-association), or with their own aromatic acyl residues (intramolecular copigmentation), or with other phenols (inter-molecular copigmentation). These mechanisms have been thoroughly studied (Brouillard and Dangles 1993). Copigmentation is particularly important in the wine pH range, where hydrated forms normally predominate. It is reflected by a colour enhancement and a slight shift towards a more purple colour (a bathochromic effect). Studies using chlorogenic acid (an esterified caffeic acid) as the copigment have shown that the various pigmentated tannins behave differently with respect to copigmentation. Thus, an F-A type product (catechin-malvidin-3-glucoside, a φC-φA type dimer) showed good interaction with the copigment, since a 42% colour increase was observed when using a copigment to pigment ratio of 100:1. Under the same conditions, the visible spectrum of ethyl-linked pigments (polycondensation products, of φ A-C-φ A type) showed a weak bathochromic shift (a shift of a spectral band to longer wavelengths, in this case of ~10 nm) that points to an interaction taking place. Moreover, no hyperchromic effect (increase in the intensity of a spectral band) was observed, in agreement with the fact that hydration is negligible for these pigments, so only very low concentrations of colourless forms are available for conversion into the flavylum cation upon interaction with the copigment. Self-aggregation is also responsible, to a lesser extent, for the resistance of ethyl-linked pigments to sulphite bleaching. The lower efficiency of self-aggregation towards sulphite discoulouration than towards hydration may reflect the greater accessibility of the flavylum C4 centre (compared with C2) within the aggregates.

In summary, reactions of anthocyanins with tannins produce a variety of structures, some coloured, some not. Among these reactions, the conversion of genuine anthocyanins into pyrananthocyanins reinforces the orange component of wine colour (Figure 8). Some chemical processes, like direct reactions to yield flavanol-anthocyanin (F-A) products (structures of type ϕC-ϕA) have no effect on colour, since the anthocyanin component of the corresponding derived tannin has the same hydration constant as the original anthocyanin. However, some reaction processes, although they don't directly produce newly coloured molecules, contribute to wine colour by the physico-chemical behaviour of their products; the conversion of anthocyanins into polyethyl-flavanol/anthocyanin copolymers (structures of type ϕA-C-ϕA type) much enhances the wine colour by self-aggregation, preserving more than 80% of the anthocyanin residues that are incorporated into the polymers in coloured forms. Nevertheless, the latter pigments are not as stable as the genuine anthocyanins and thus gradually converted into flavanyl pyrananthocyanins. These successive chemical modifications (from ethyl-linked structure to pyrananthocyanin structure) fit properly the colour variation observed as the wine ages, changing from purple hue, characteristic of young red wines, to tawny nuance of older wines.

Additionally, it is also worth emphasising that pigments resistant to sulphite bleaching are not necessarily polymeric, for example the pyrananthocyanins. Moreover, that some polymeric pigments are not resistant, such as flavanol-anthocyanin (F-A) products.
Structure / taste relationships

Bitterness and astringency are two essential descriptors of wine sensory perception that involve phenolics. The former is a taste mediated by sensory receptors, while the latter is a tactile sensation caused by a loss of mouth lubrication following salivary protein precipitation. As a consequence, bitterness is restricted to rather small molecules with particular structural features enabling them to enter the receptor and activate the signal transduction process, whereas astringency is linked to the number of protein interaction sites in the molecule and thus specifically caused by tannins.

Within the flavanol series, bitterness decreases from monomer to trimer while astringency increases (Noble 1998), as expected from these mechanisms. More generally, astringency increases with tannin concentration, molecular size, and degree of galloylation (Vidal et al. 2003) like their ability to complex with peptides and proteins (Sarni-Manchado et al. 1999a; Sarni-Manchado et al. 1999b; Sarni-Manchado and Cheynier 2002). However, conformational flexibility and water solubility of polyphenols can modulate their associations with proteins and other molecules, as demonstrated in protein tannin interaction studies (Vidal et al. 2003).

Contrary to popular opinion, there is no simple relationship between tannin solubility and either tannin molecular size or the mean degree of polymerisation (mDP) of the tannin fraction (Poncé-Legrand et al. 2003). The propensity of tannins to aggregate and precipitate from solution is directly related to tannin solubility. The propensity to aggregate increases with mDP, but it reaches a maximum for quite low molecular weights (mDP5 for grape seed tannins, mDP10 for apple tannins) before decreasing with higher molecular weights. Beyond the DP at which aggregation decreases, two assumptions can be made: either the polymer adopts a conformation that increases its solubility, or the aggregates formed have different structures. Two additional parameters related to the wine matrix have also been shown to play a crucial role in self-aggregation of tannins: the ionic strength and the ethanol content. Increasing the ionic strength results in a significant loss of tannin solubility, and the aggregates that stay in solution are bigger and much more polydisperse. Increasing the ethanol content of the medium produces the reverse effect: tannin solubility is increased, and colloidal aggregates are smaller and less polydisperse. An increase in either ionic strength or acidity also increases the perception of astringency (Noble 1998) but ethanol does not affect it. However, aggregation and astringency are not directly linked. While the tendency of tannin to aggregate reaches a maximum at a certain molecular size, this is different to the trend of astringency, which keeps increasing with increasing tannin molecular size. As well, ethanol decreases tannin aggregation (increases tannin solubility) but does not change astringency. In fact, colloid formation is relevant to a very tiny part (<5%) of the overall tannins in solution that occur as isolated molecules largely prevalent and available for protein binding.

In wine-like solutions that contain tannin, wine polysaccharides affect both tannin aggregation and tannin astringency. The polysaccharides do not prevent initial tannin aggregation but they do modify the evolution of particle size. However, the effect depends on the chemical nature of the wine polysaccharide. For instance, aggregation is strongly inhibited by mannanproteins, whereas the dimeric form of rhamnogalacturonan II (RGII), a pectic polysaccharide, promotes the enhancement of particle size, probably because of co-aggregation between this polysaccharide and the tannin particles (Riou et al. 2002). However, to sensory tasting, RGII decreased the astringency of a wine-like solution with tannins, whereas the mannanproteins had no effect (Vidal et al. 2002b). The difference in astringency between the two polysaccharides suggests that competition with salivary protein binding is affected by the particle size of the tannin-polysaccharide complexes.

Although changes in wine taste, with time, have long been ascribed to tannin reactions, studies on the taste properties of derived tannins are still scarce. Oxidation products of catechin interact with proteins in the same way as native tannins, as demonstrated by their inhibitory effect on β-glucosidase activity (Guyot et al. 1996a). Therefore, flavanol oxidation products are likely to contribute an astringency like their native forms. Polyethyl-catechin oligomers were shown to be as astringent as, and more bitter than, native tannins of close molecular size (Vidal et al. 2004a). Consequently, derived tannins that are based on flavanol units only are likely to taste as astringent as native tannins. On the other hand, grape anthocyanins and polymeric pigments fractions isolated from wine or pomace were perceived to be neither bitter nor astringent when tasted in 5% ethanol (Vidal et al. 2004a). Another sensory tasting of wine extracts has shown astringency to be correlated with the concentration of proanthocyanidin units in the polymeric fraction (Brossaud et al. 2001). These results taken together suggest that incorporation of anthocyanin units in the core of derived tannins may lead to a decrease in astringency in the proportion to the flavanol units replaced by anthocyanin units.

Conclusions

Development of analytical techniques has now allowed the characterisation of some derived tannin structures of low molecular weight (dimers) and has demonstrated the mechanisms of their formation. However, these derived tannins are present in very low amounts in wines and do not reflect the actual wine tannin composition. Instead, they should be regarded as qualitative markers of the various reaction processes that can occur and the new linkage types that can be formed. No quantitative information on the composition of wine tannins (such as the relative proportions of native and derived tannins) is available to date. This highlights the crucial lack of quantitative methods for tannin analysis. Consequently, the trend of anthocyanin and tannin conversion is only speculative and lacks precision. The process can form small pigments such as the pyranoanthocyanins and the xanthylum pigments, but there is also a slow and gradual polymerisation, as most of the linkages created in derived tannins appear resistant to acid-catalysed cleavage. Therefore, it is very difficult to predict the average tannin molecular size as a wine ages. Furthermore, wine quality may be affected not only by the size but also by the diversity of structure within the derived tannins. The primary factor influencing this may be the tannin to anthocyanin ratio from the grape, and its further modulation by winemaking processes.

Colour and taste studies on derived tannins have only just been initiated, so the influence of tannin structure on wine quality is only just being revealed. For instance, while colour stabilisation can be attributed to some structural changes (for instance, conversion into pyranoanthocyanins), it is also an outcome of the propensity of derived structures to stack with themselves or with other molecules. The stability of derived tannin colour towards pH changes and to sulphite bleaching, thus, seems not to be related to the molecular weight of the derived tannins. Astringency is related to the tannin content and increases with their molecular weight, without size limit. Moreover, incorporation of anthocyanin units in the core of tannins likely leads to a decrease in astringency. Other wine components, such as polysaccharides and proteins, also change the astringency presumably through competition with salivary proteins in the formation of tannin complexes.
Acknowledgments
Part of this study was carried out with the financial support from the Commission of the European Communities, specific RTD program ‘Quality of Life and Management of Living Resources’, proposal number QLRT-2002-02364. It does not reflect the Commission’s views and in no way anticipates the Commission’s future policy in this area. A part of this work received the financial support of the Interprofessional Committee of Champagne Wine (CIVC)-Moët and Chandon.

References