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A review of the current knowledge of red wine colour

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Abstract

Anthocyanins are the main compounds present in young red wines, being responsible for their intense red colour. These pigments are mainly located in grape skins and their extractability during winemaking depends on many factors, such as their concentration in vacuoles and interaction with the cell-wall polysaccharides, affecting their stability and concentration in the must. The red colour of anthocyanins at wine pH is explained by the stabilization of the flavylium cation form which displays a red colour; otherwise at this pH the colourless hemiketal is the dominant form, bleaching the wine. Besides the contribution of free anthocyanins, a phenomenon called copigmentation influences the colour of young red wines. Copigmentation can be defined as a series of stabilization mechanisms involving van der Walls interactions that occur naturally in red wines and that can explain this unanticipated colour behaviour. Copigmentation is also pointed as the first interaction between anthocyanins and other wine components, leading to the formation of new coloured compounds during red wine ageing. Some of these pigments have already been identified and characterized but many are still undiscovered. The detection and structural characterization of new pigments and the knowledge of their chemical formation pathways are crucial to better understand the evolution of the colour of red wine during ageing.

Keywords: anthocyanins, cell-wall components, colour stabilization, copigmentation, anthocyanin-derived pigments, wine ageing

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Introduction

Colour is an important feature of red wine, being the first attribute to be perceived by wine consumers, and is directly associated with its quality. Red wines have a very complex matrix due to the wide variety of compounds extracted from grapes and to the metabolites released by yeast during the fermentation process. In specific cases, such as fortified wines like Port wines, the complexity is even higher owing to the addition of wine spirit to stop the fermentation. Wine spirits possess a great variety of compounds such as higher alcohols, esters, and mainly aldehydes, acetaldehyde, 2-methylpropanal, 2-methylbutanal and benzaldehyde, among others), Pissarra *et al.*, 2005). Moreover, wine ageing in oak barrels, addition of oak chips, or must fermentation in contact with oak staves can also have an impact on the chemical transformation of wine components. During these processes, different compounds can be extracted from oak, such as ellagitannins, phenolic acids, and furanic and phenolic aldehydes, Gonzalez-Centeno *et al.*, 2016; Garcia *et al.*, 2012), that can participate in wine colour stabilization. Among these molecules, aldehydes should have a higher impact on wines due to their increased reactivity towards flavonoids like flavan-3-ols and anthocyanins, Pissarra *et al.*, 2003; Sousa *et al.*, 2012). The latter are the main pigments in many flowers and fruits, e.g. grapes. being responsible for the great diversity of colours, i.e. red, violet and blue. found in nature and the red/violet colour observed in young red wines. In aqueous solution, the colour of anthocyanins is dependent on their structure and is strongly affected by the pH, Brouillard and Lang, 1990). At very acidic pH, pH~1), anthocyanins are present in their flavylium cation form which has a red colour. When the pH is raised to 3-4, the flavylium cation is involved in two parallel reactions in equilibrium: deprotonation to form the violet quinonoidal base and hydration at the C-2 position yielding the non-coloured hemiketal form. These forms are also in equilibrium with the *cis*- and *trans*-chalcone forms which present a yellow colour, Pina, 1998). With a pH rise to values up to 6, the quinonoidal base can be ionized to form the

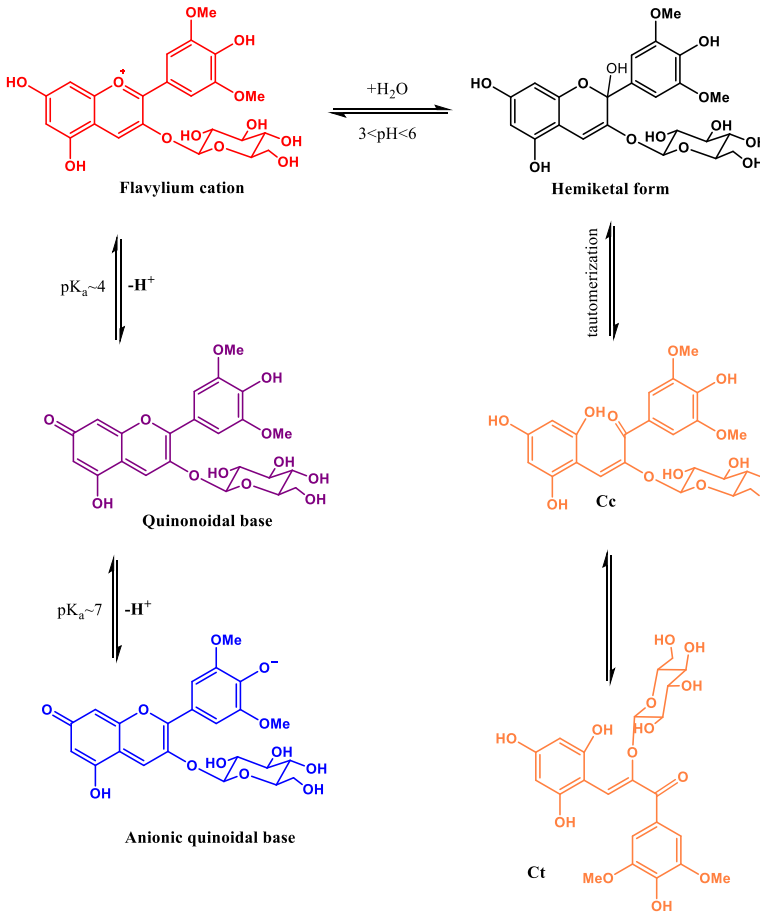


Figure 1 - Anthocyanin equilibria in aqueous solutions at different pH values (adapted from Brouillard and Lang, 1990).

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respective blue anionic quinoidal form, Brouillard and Lang, 1990; Santos *et al.*, 1993), **Figure 1**). According to these equilibria, it would be expected that at wine pH, 3.2-3.8), anthocyanins would be present in a great extent in their non-coloured hemiketal form. Nevertheless, there are some mechanisms to stabilize the coloured forms of anthocyanins. These include self-association, Gonzalez-Manzano *et al.*, 2008; Mistry *et al.*, 1991), intra and intermolecular copigmentation, Mistry *et al.*, 1991; Yoshida *et al.*, 2009. and metal complexation mechanisms. Copigmentation helps to stabilize the flavylium form and protects it from the nucleophilic attack by water, thereby preventing the formation of colourless forms, Goto and Kondo, 1991; Yoshida *et al.*, 2009, Dangles *et al.*, 1994). However, some years ago, Asenstorfer and his co-workers using paper electrophoresis postulated that at wine pH, malvidin-3-glucoside is present as the uncharged quinonoidal base as major coloured component of the equilibria, Asenstorfer *et al.*, 2003).

During wine ageing, anthocyanins participate in several reactions, i.e. reduction, oxidation, and polymerization. involving other wine molecules, leading to the formation of more stable anthocyanin-derived compounds, Oliveira *et al.*, 2009; Oliveira *et al.*, 2014).

Bearing all this, this paper will focus on reviewing the current knowledge of red wine colour, especially concerning colour stabilization mechanisms and colour evolution during wine ageing and maturation.

Colour stabilization

In red wines made from *Vitis vinifera* L. grapes, the monomeric anthocyanins present are 3-*O*-glucosides of delphinidin, cyanidin, in very small amounts), petunidin, peonidin and malvidin. They differ from each other on the hydroxylation and/or methoxylation pattern of ring B, which affects directly the hue and colour stability, Mateus *et al.*, 2001). In red wines, the glucose moiety can also be esterified at the hydroxyl group present at the C-6 position with different acids, namely acetic, *p*-coumaric and caffeic acids, Mateus *et al.*, 2002). Malvidin-3-*O*-glucoside and its derivatives are usually the most abundant anthocyanins in young red wines, Mateus *et al.*, 2001).

1. Interaction with cell-wall components

Due to the strong influence of anthocyanins on red wine quality, many studies have been conducted to determine how anthocyanins are extracted during winemaking. In red grapes, these pigments are mainly located in skins, He *et al.*, 2012). Inside the cells, anthocyanins are found in the vacuoles enclosed by tonoplast and cytoplasmic lipid membranes, which are in turn encapsulated by the skin cell-wall, CW), **Figure 2**), Rodríguez *et al.*, 2004). During winemaking, the rate and the extent of anthocyanin extraction depend not only on their concentration but also on the grape skin CW polysaccharide composition, Busse-Valverde *et al.*, 2012. In fact, during the maceration process, anthocyanins and soluble and insoluble CW polysaccharide fragments resulting from pectolytic enzyme activity come into contact and a substantial proportion of anthocyanins may be adsorbed by the CW in suspension in the must. Such interactions affect the extractability of anthocyanins during fermentative maceration, their solubilization and the final colour of the wines, Padayachee *et al.*, 2012; Bautista-Ortín *et al.*, 2016), **Figure 2**).

Previous works have shown that the anthocyanin content of a given cultivar is not always positively correlated with the anthocyanin concentration in the

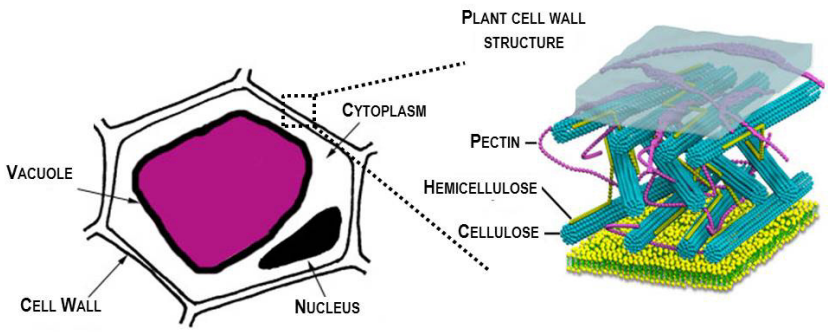


Figure 2 - Schematic representation of the grape cell structure evidencing the presence of the vacuoles where anthocyanins are located and schematic representation of the primary cell wall evidencing the most important polysaccharides.

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resulting wine, Romero-Cascales *et al.*, 2005). This lack of correlation was attributed to the partial retention of these anthocyanins in the skin cells due to the barrier effect of the CW polymers, Ortega-Regules *et al.*, 2006; Rolle *et al.*, 2009; Rolle *et al.*, 2012). The grape CW have a very complex structure, composed essentially of a network of cellulose microfibrils inter-knotted with a hemicellulosic polysaccharide matrix consisting mainly of xyloglucan polymers. Pectic polysaccharides and other components such as enzymes, structural proteins, ions and low molecular weight polyphenols are also embedded within this matrix, Pinelo *et al.*, 2006). Pectic polysaccharides are a group of polysaccharides rich in galacturonic acid and classified in three types of polymers: homogalacturonan polymer and two branched side chain polymers containing a rhamnosyl residue, the so-called rhamnogalacturonans I and II, Vicens *et al.*, 2009 ; Nunan *et al.*, 1997). The grape skin CW are made up of 30% neutral polysaccharides, cellulose, xyloglucan, arabinan, galactan, xylan and mannan), 20% acidic pectic polysaccharides, of which 62% are methyl esterified. and approximately 15% insoluble proanthocyanidins, Lecas et Brillouet, 1994). During fruit ripening, the degradation of CW polysaccharides by pectolytic enzymes and other enzymes able to catalyze the hydrolysis of bonds in plant CW polysaccharides increases the extractability of anthocyanins from grape skins.

Different studies using purified grape CW material or pure cellulose and cellulose-pectin composites as CW models have also demonstrated the high affinity of these CW materials for anthocyanins and highlighted that these interactions may affect anthocyanin extraction, Padayachee *et al.*, 2012; Bindon *et al.*, 2014). Likewise, it was evidenced that some anthocyanin structural features may influence their extractability from grape skins. In particular, a lower extraction yield was reported for coumaroylated anthocyanins compared to the non-acylated ones, Fournand *et al.*, 2006). Furthermore, the results reported by Saura-Calixto *et al.* suggest that wine polymeric polysaccharides interact with two major groups of polyphenolic compounds: free wine polyphenols and wine polyphenols associated to polysaccharides. About 35 to 60% of total wine polyphenols in red wine and about 10% in white wine are associated to polysaccharides, polyphenol-polysaccharide complexes). Moreover, anthocyanins were the major constituents of red wine polysaccharide-polyphenol complexes, while phenolic acids were the major constituents in white wine complexes, Saura-Calixto and Díaz-Rubio, 2007).

The retention of polyphenols in CW polysaccharides depends on the conformational flexibility and on the molecular weight of the phenolic moiety. Also, physical features of CW polysaccharides like porosity and chemical composition can also influence the eventual association between CW polysaccharides and anthocyanins. Different polarities and the hydrophobic nature of these polysaccharides influence the degree of retention or adsorption of anthocyanins, Renard *et al.*, 2001; Le Bourvellec, 2004). Hydrogen bonds and hydrophobic interactions are the two main driving forces proposed to explain the nature of complex formation between anthocyanins and plant CW polysaccharides, Pinelo *et al.,* 2006). It seems likely that the adsorption of anthocyanins involves the establishment of a number of low energy non-covalent interactions derived from a combination of hydrogen bonding between the hydroxyl groups of phenols and the oxygen atoms of the cross-linking ether bonds of sugars present in the CW polysaccharides and hydrophobic interactions between sugar rings and phenols, Fernandes *et al.*, 2014; Phan *et al.*, 2015), **Figure 3**). By Saturation Transfer Difference – Nuclear Molecular Resonance, STD-NMR. and Molecular Dynamics, MD. studies,

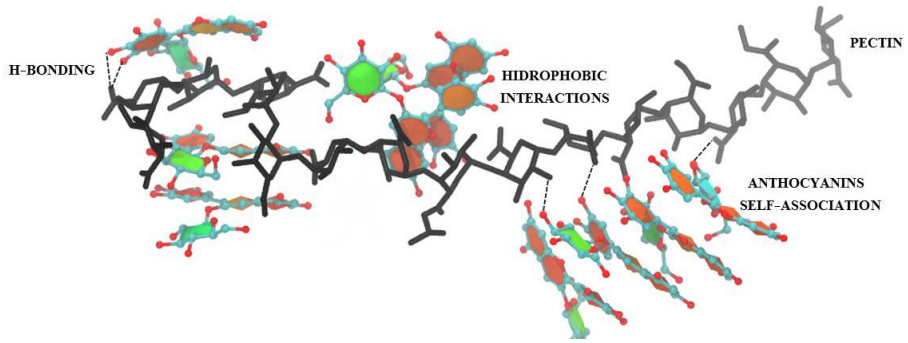


Figure 3 – Schematic representation of some interactions established between anthocyanins and a low methoxylated pectin model involving hydrophobic interaction and H bonding. Pectin molecule is depicted with sticks and coloured in dark gray (Fernandez *et al.*, 2014).

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Fernandes *et al.* described that anthocyanins interact with polysaccharides over a two-stage process – a rapid but limited phase followed by a slower but more extensive binding, which may be due to deposition of anthocyanin molecules onto the occasional sites where primary binding had already occurred, Fernandes *et al.*, 2014). Likewise, some polysaccharides have the ability to form gel-like structures or to develop secondary structures in solution forming hydrophobic pockets able to encapsulate and complex polyphenols, Gonçalves *et al.*, 2012; Le Bourvellec *et al.*, 2005). Differences in polysaccharides based on galactose and arabinose, together with changes in the cellulose content and degree of methylation of pectins, may also affect the extractability rate of anthocyanins from grapes into the must, Ortega-Regules *et al.*, 2006). According to Hernández-Hierro *et al.*, 2012), the ripeness degree and, to a lesser extent, the soluble solids content also influence the amount of anthocyanins released and generally, non-acylated anthocyanins were better extracted than the acylated ones. On the other hand, total insoluble CW material has shown to exhibit the biggest opposition to anthocyanin extraction, slightly higher for non-acylated anthocyanins, while the highest amounts of cellulose, rhamnogalacturonans II and polyphenols were positively correlated with anthocyanin extraction. Xyloglucans, homogalacturonans and rhamnogalacturonans I evidenced opposite behaviour to anthocyanin extraction, Hernández-Hierro *et al.*, 2014).

The positive relationship between polyphenol content in CW and anthocyanin extraction probably suggests the existence of copigmentation processes referenced before between these components while the extraction is taking place, so they could be able to assist the extraction process, Boulton, 2001).

Gonçalves *et al.* reported a higher retention capacity of acylated anthocyanins by CW polymeric material, compared to the non-acylated anthocyanins. The acylation of the glucose residue was shown to enhance anthocyanin hydrophobicity, suggesting that the association of polymeric material with anthocyanins could be mainly due to hydrophobic interactions. In addition, CH-π interaction can occur between the aromatic rings on anthocyanins and carbohydrates, Gonçalves *at al*., 2012). Aggregation of anthocyanin pigments to yeast CW during fermentation has also been explained to occur as a result of hydrophobic interactions between sugars and phenols, although this adsorption has been found to depend also on the degree of methoxylation and acylation of anthocyanins, Morata *et al.*, 2003). Previous reports also showed that the physical properties of grape skins, like skin hardness, thickness, number of cell layers and CW thickness, and the grape variety are also linked to anthocyanin extractability, Río Segade *et al.*, 2011 ; Río Segade *et al.*, 2008).

2. Copigmentation

As mentioned before, wine anthocyanins are present in different forms, depending essentially on wine pH, thus affecting the hue and intensity of wine colour. Red wine is a very complex matrix that contains

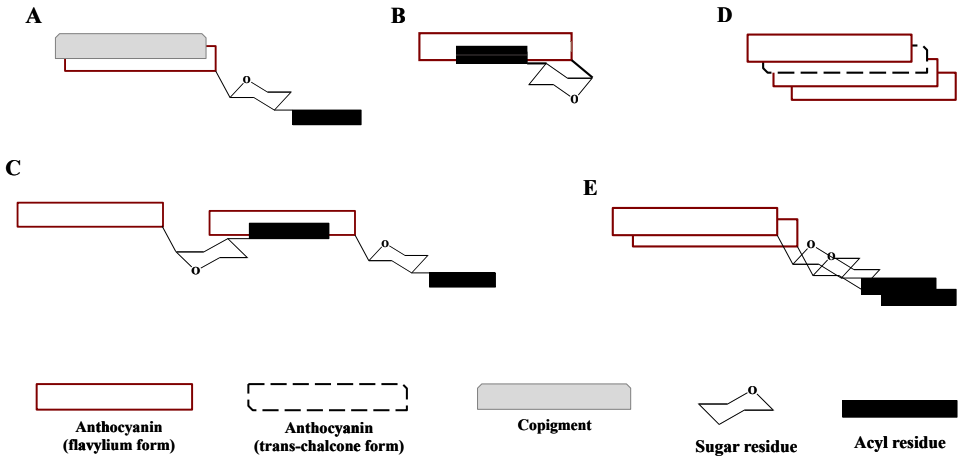


Figure 4 - Copigmentation non-covalent molecular complexes resulting from π -π stacking interactions. A: Intermolecular copigmentation between an anthocyanin and a colourless copigment; B: Intramolecular copigmentation between an anthocyanin and its acyl residue; C: Intermolecular copigmentation between an anthocyanin and an acyl residue from another molecule; D: Self-association between flavylium forms or flavylium and trans-chalcone forms of anthocyanins; E: Self-association between acylated anthocyanins.

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several polyphenolic compounds that help to stabilize the flavylium form of anthocyanins and thereby the colour of red wine. However, the occurrence of copigmentation in young red wine is easily demonstrated by successive dilution at constant pH: the dilution breaks the pigment-pigment and pigment-copigment complexes and no constant spectral behaviour is observed, as it would be expected if no interactions were involved, Boulton, 2001; Trouillas *et al.*, 2016).

Copigmentation can be defined as several physical-chemical mechanisms that result in the formation of non-covalent molecular complexes and in subsequent changes in the solution optical properties. It generally consists in van der Waals interactions, vertical π-π stacking. between the planar polarizable nuclei of two anthocyanin molecules or an anthocyanin and a colourless copigment, **Figure 4**), Trouillas *et al.*, 2016 ; Brouillard *et al.*, 1989).

2.1. Intermolecular and intramolecular copigmentation

Intermolecular copigmentation occurs when the interaction involves an anthocyanin and a colourless copigment and intramolecular copigmentation occurs when the acylated residues of the anthocyanin act as copigment, **Figure 4**). These complexes adopt a sandwich-like structure that stabilizes the flavylium cation chromophore and partially protects it from nucleophilic attack by water, thus preventing colour loss, Goto, 1987; Santos-Buelga and De Freitas, 2009).

Red wine contains several potential copigments extracted from grapes or resulting from chemical reactions during vinification. During early red wine storage, the concentration of anthocyanins decreases exponentially but the same is not observed for copigments, He, 2012; Brouillard *et al.*, 2003). In fact, young red wine generally displays an intense colour and it is reported that copigmentation is responsible for 30 to 50% of total colour intensity, He, 2012 ; Boulton, 2001; Monagas and Bartolomé, 2009). Copigmentation depends on the structure of the anthocyanins and particularly on the structure of the copigments. Comparing the potential of flavonols, like quercetin. and flavan-3-ols to act as copigments, the first group is a better choice since it generally has a planar polyphenolic nucleus capable of forming π-stacking with the anthocyanin planar chromophore, Mistry *et al.*, 1991; Cruz *et al.*, 2010). Such hydrophobic complexation displaces the equilibria towards the formation of more flavylium forms of anthocyanins. Nevertheless, red wine is more concentrated in flavan-3-ols than in flavonols and the contact with anthocyanins is inevitable, Neveu *et al.*, 2010). Several authors performed copigmentation studies in model solutions with several cofactors including, epi)catechin,, epi)gallocatechin, procyanidins, prodelphinidins, myricetin, quercetin and phenolic acids such as caffeic acid and *p*-coumaric acid. Malvidin-3-*O*-glucoside, being the main anthocyanin present in red wines, is usually the chosen pigment for copigmentation studies, Teixeira *et al.*, 2013; Darias-Martin *et al.*, 2001). Among flavan-3-ols,, -)-epicatechin is a better copigment than, +)-catechin due to its C ring conformation, allowing it to be approximately coplanar, Brouillard, 1991; Liao *et al.,* 1992); the presence of a pyrogallol group in the B ring slightly increases the copimentation potential, Teixeira *et al.*, 2013. procyanidin dimers with C-4→C-6 interflavanic linkages seem to be better copigments than their respective C-4→C-8 dimers; and galloylation at C-3 also increases the copigmentation effect, Berké *et al.*, 2005).

In slightly acidic to neutral pure aqueous solutions, common anthocyanins only have around 5% of the colour of total pigment concentration, which results in colourless solutions, Brouillard and Dubois, 1977; Brouillard and Delaporte, 1977; Brouillard *et al.*, 1978). However, acylated anthocyanins are usually more stable than non-acylated ones. In fact, acylated anthocyanins extracted from flowers, which present much more complex anthocyanin structures than grapes. display deep stable colours at neutral pH in the absence of any copigment, Goto and Kondo, 1991, Saito *et al.*, 1972; Tatsuzawa *et al.*, 2012). This can be explained by intramolecular copigmentation where the chromophoric part of the anthocyanin and the acyl residue exist in a folded conformation. The formation of such intramolecular copigmentation complexes also protects the chromophore against hydration in an even more efficient way than that conferred by intermolecular copigmentation, because one side of the anthocyanin chromophore is protected.

Copigmentation usually produces an increase in absorbance, hyperchromic effect. and a positive shift of the visible absorption maximum, bathochromic effect). The hyperchromic effect occurs due to the formation of the flavylium cation-copigment, or acyl residue. complex. The bathochromic effect occurs due to the affinity of the copigment for the quinoidal forms of the anthocyanin. Copigmentation is an exothermic process, and it increases with copigment concentration, in intermolecular copigmentation. and with sugar residue di-acylation, in intramolecular

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copigmentation), reflecting the preferential behaviour and shifting the equilibrium towards dehydration, Dangles *et al.*, 1993; Teixeira *et al.*, 2013; Trouillas *et al.*, 2016).

2.2. Self-association of anthocyanins

Self-association is considered to be a special type of copigmentation since it also induces optical changes in the involved pigments. It occurs when the anthocyanin undergoes a vertical stacking of the aromatic rings, again preventing the nucleophilic attack by water and the loss of colour caused by the formation of the hemiketal and chalcone forms, **Figure 4**). It can be easily observed by UV-Vis absorption spectroscopy in relatively concentrated solutions of any anthocyanin. This phenomenon was first suggested by Asen *et al.* in 1972 and ten years later it was demonstrated by Hoshino *et al.* by NMR and circular dichroism, CD. measurements, Boulton, 2001; Asen, 1972; Hoshino, 1992). NMR analysis allows the observation of the chemical shifts of the aromatic protons moving upfield with an increase of the anthocyanin concentration, suggesting the occurrence of hydrophobic interactions. The proof that anthocyanins can be organized into larger molecular structures can be achieved by the determination of the diffusion coefficient, where it should decrease with increasing concentrations of anthocyanins, Fernandes *et al.*, 2015).

Houbiers *et al.* reported that for malvidin-3-*O*-glucoside self-association can involve both the flavylium cation and the *trans*-chalcone, **Figure 4**), Houbiers *et al.*, 1998). They observed that the corresponding chemical shifts vary with different concentration values of each form at different pH values. In fact, and opposite to what is observed for hemiketal forms and *cis*-chalcone, flavylium cation and *trans*-chalcone chemical shifts move to higher frequencies upon increasing temperature. This contradictory behaviour between the two chalcone forms may be explained by the more planar structure of the *trans*-chalcone form. Comparing this effect at pH 0.6 and 3.5 the results on temperature dependence are more expressive at pH 3.5 where both forms co-exist. However, it can still be questioned if, in the case of acylated anthocyanins, self-association cannot be mistaken with intramolecular copigmentation. This can probably be answered with ^{1}H-NMR measurements at different temperatures. When studying the self-association between flavylium cations and *trans*-chalcones, Houbiers *et al.* also performed ^{1}H-NMR measurements at different temperatures, Houbiers *et al.*, 1998). They observed that the chemical shifts of flavylium cations and *trans*-chalcones moved to higher frequencies upon increasing the temperature while the chemical shifts of hemiketals and *cis*-chalcones remained identical. Knowing that by increasing the temperature the formed aggregates can split and that copigmentation is an exothermic process, these observations support the idea that flavylium cations and *trans*-chalcones prefer self-association to copigmentation, Houbiers *et al.*, 1998). To these observations it can also be added the fact that the equilibrium constant of malvidin-3-*O*-(6-coumaroylglucoside. is almost two times higher than that for malvidin-3-*O*-glucoside, suggesting a higher affinity to aggregation, Fernandes, 2015).

As already mentioned, Hoshino *et al.* have performed a series of studies demonstrating the self-association of anthocyanins. CD experiments allowed to conclude that anthocyanidins can stack both in a right- or left-handed axis, while quinoidal bases of cyanidin and pelargonidin form right-handed adducts and peonidin, delphinidin and malvidin form left-handed adducts, Hoshino, 1981 : Hoshino, 1992). However, all the flavylium cations lead to left-handed adducts due to an asymmetry imposed by oligomeric species, as shown by Rodger and Nordén, 1997). Later, Gavara *et al.* demonstrated that the flavylium cations of anthocyanidin 3-glucosides can self-associate in a monomer-dimer aggregation type and further aggregation is prevented by electrostatic repulsion between the positively charged molecules, Gavara *et al.*, 2013). However, and knowing that *trans*-chalcones may also be involved in self-association mechanisms, some authors reported that self-association models should not always be thought of as simple dimeric models, but rather as higher order mixed aggregates with intramolecular copigmentation as well as for acylated anthocyanins, Houbiers *et al.*, 1998 ; Leydet *et al.*, 2012).

The substitution pattern of the anthocyanin B ring has little influence on its thermodynamic and kinetic properties but it is extremely important for aggregation processes. Leydet *et al.* demonstrated that methoxyl and hydroxyl substituents favour the aggregation of flavylium cations probably due to hydrogen bonding, Leydet *et al.*, 2012).

There are not many studies on self-association in wines due to the complexity of the subject. In 2009, González-Manzano *et al.* reported that self-association in wine-like solution models accounts for 8 to 60% of absorbance increase, González-Manzano *et al.*, 2009).

These non-covalent interactions can be the first step in the formation of covalent bonds between two

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molecules, resulting into new anthocyanin-derived pigments as described below. In fact, during storage or ageing, copigmentation decreases and the concentration of polymerized structures increases, with consequent changes in wine colour, Liao *et al.*, 1992, Brouillard and Dangles, 1994 ; Gutiérrez, 2005). One of these new types of structures, pyranoanthocyanins, see next section), may also undergo copigmentation phenomena. Pyranoanthocyanin-flavanol pigments generally do not undergo hydration processes and display 30 to 50% stronger absorption values at wine pH, around 3.6. than at pH 1.0, Oliveira *et al.*, 2009; He *et al.*, 2012; Escribano-Bailon and Santos-Buelga, 2012). For instance, a copigmentation assay with malvidin-3-*O*-glucoside-vinylguaiacol pyranoanthocyanin as pigment and catechin as copigment resulted in a larger increase of the visible absorption than in the case of malvidin-3-*O*-glucoside(Quijada-Morín *et al.*, 2010). In another study, a dimeric-type malvidin-3-*O*-glucoside-(O)-catechin adduct was tested and showed to be a far more effective copigment than other flavan-3-ols, Teixeira *et al.*, 2013). This shows the potential of anthocyanin-derived pigments in wine

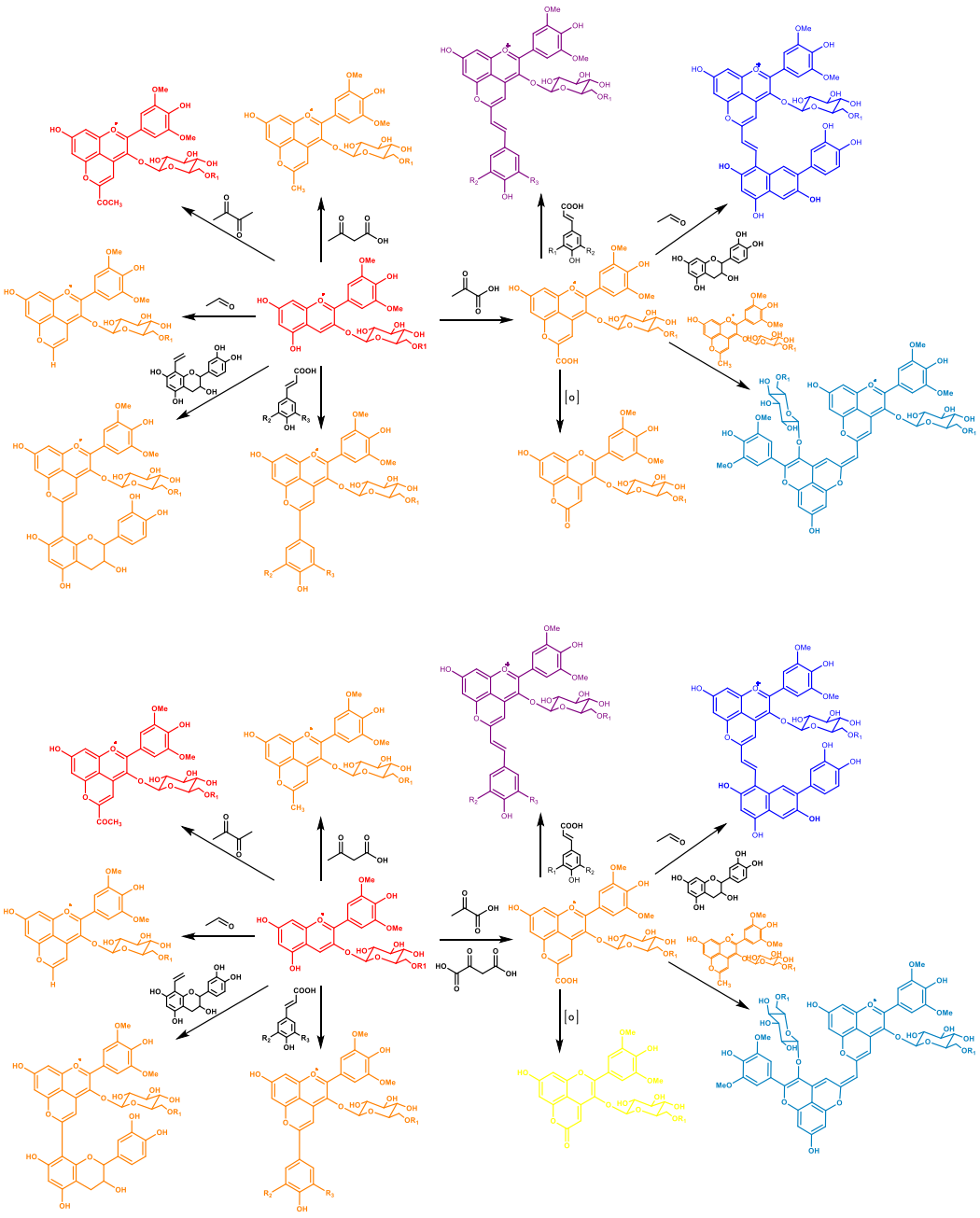


Figure 5 – Anthocyanin-derived pigments identified in red Port wines.

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copigmentation and therefore in red wine perception of colour.

Colour evolution during wine ageing and maturation

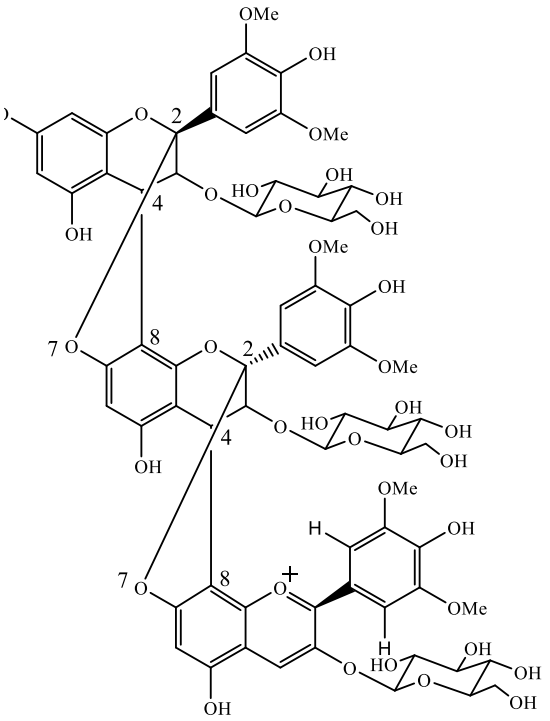
During ageing and maturation, the colour of wines changes from red/violet to red/orange due to several chemical reactions, oxidation, reduction and polymerization. where anthocyanins participate, thus being the precursors of new compounds. In the first stages of wine evolution, anthocyanins can react with flavan-3-ols, catechin monomers and condensed tannins. directly or indirectly via aldehydes, yielding new purple pigments, anthocyanin-alkyl-catechin. that further lead to the formation of pyranoanthocyanin-catechin compounds displaying an orange colour, Pissarra *et al.*, 2003; Liao *et al.*, 1992; Rivas-Gonzalo *et al.*, 1995; Francia-Aricha *et al.* 1998), **Figure 5**). Acetaldehyde is particularly important for anthocyanin chemical transformation reactions. This aldehyde is present in high amount in wine spirits, 40-260 mg/L. and is also formed during fermentation and wine ageing from ethanol oxidation, Pissarra *et al.*, 2005).

Several pyranoanthocyanin pigments resulting from the reaction of anthocyanins with small molecules arising from grapes or from yeast metabolism have been identified in red wines over the last years, like acetaldehyde, Bakker and Timberlake, 1997), pyruvic acid, Fulcrand *et al.*, 1996), acetoacetic acid, He *et al.*, 2006), diacetyl, Gomez-Alonso *et al.*, 2012), hydroxycinnamic acids, Schwarz *et al.*, 2003; Rentzsch *et al.*, 2007. and *p*-vinyl-phenols, Hayasaka and Asenstorfer, 2002; Schwarz *et al.*, 2003. , **Figure 5**).

Pyruvic acid derivatives of anthocyanins, A-type vitisins. are the pyranoanthocyanins detected in higher concentration in Port wines, Mateus and de Freitas, 2001). The characteristic chemical and physical properties of Port wine could be at the origin of higher levels of yeast metabolites likely to yield anthocyanin derivatives. Indeed, when wine spirit is added to stop fermentation, pyruvic acid concentration is expected to be higher than when the fermentation is allowed to go to dryness. In fact, pyruvic acid released by yeast at the beginning of the fermentation is further used in yeast metabolism. Its levels increase right after wine fortification, and the initial formation of anthocyanin-pyruvic acid adducts is concurrent with the degradation of anthocyanin monoglucosides. A-type vitisins were found to be very resistant to wine ageing when compared to the original grape anthocyanins, Oliveira *et al.*, 2013, Mateus and de Freitas, 2001; Asenstorfer and Jones, 2007). Yellowish α-pyranone-anthocyanins were also described to be present in aged red wines derived from the direct oxidation of A-type vitisins, He *et al.*, 2010).

A few years ago, a new class of pyranoanthocyanin compounds, vinylpyranoanthocyanins. named portisins, presenting an unusual bluish colour at acidic pH values, was identified in a young Port wine, Mateus *et al.*, 2003), **Figure 5**). The studies performed in model solutions have demonstrated that these compounds can be formed in wines from the reaction between A-type vitisins and flavanols in the presence of acetaldehyde, Mateus *et al.*, 2004). Similar compounds were identified in Port wines resulting from the reaction of A-type vitisins with hydroxycinnamic acids such as *p*-coumaric, caffeic, ferulic and sinapic acids, Oliveira *et al.*, 2007). More recently, a turquoise blue pyranoanthocyanin dimer pigment was found to occur in a 9-year-old Port wine and in the respective lees derived from the reaction of the A-type vitisin with a methylpyranoanthocyanin, Oliveira *et al.*, 2010).

The occurrence of these novel pigments in aged wines points to a second-generation pathway where the main precursors are no longer the native



Flavan, A-type
(C4-C8; C7-O-C2)

Figure 6 – Structure of A-type malvidin-3-glucoside trimer detected in a young wine and in grape skins.

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anthocyanins but secondary anthocyanin products such as A-type vitisins involved in the formation of new pigments in subsequent stages of wine ageing that may contribute directly or indirectly to its colour evolution.

Moreover, studies performed in aqueous solutions using UV-Visible spectroscopy and NMR techniques revealed that pyranoanthocyanins present a higher colour stability when compared to their anthocyanin precursors due to the absence of hydration reactions, Oliveira *et al.*, 2009; Oliveira *et al.* 2013; Oliveira *et al.*, 2014).

On the other hand, polymeric pigments have been described to play an important role in the long-term colour stability of aged red wines, Boulton, 2001). However, there is still a lot to know about their identity and chemical pathways in wines. The first evidence of the presence of oligomeric anthocyanins, dimeric and trimeric. was described by Vidal and co-workers in grape skins using mass spectrometry techniques, Vidal *et al.*, 2004). Later, the dimeric compounds were also reported to occur in red wines by Salas *et al.*, 2005. and Alcalde-Eon *et al.*, 2007. using the same technique. A few years later, Oliveira *et al*. demonstrated the presence of an A-type malvidin-3-glucoside trimer in a young Port wine using LC-MS and NMR methodologies, Oliveira *et al.*, 2013), **Figure 6**). The origin of these compounds in red wine could result from their extraction from grape skin during the winemaking process, as the presence of these trimeric pigments was detected in a grape skin extract by LC/DAD-MS spectrometry, Oliveira *et al.*, 2013). Moreover, this pigment revealed to be much less prone to hydration reactions than the anthocyanin monomers, with hydration accounting for less than 10% of the overall reactivity, Oliveira *et al.*, 2014). A dimeric acetaldehyde malvidin-3-glucoside condensation pigment was also found to occur in red wine, Atanasova, 2002).

All these new families of anthocyanin-derived pigments make an important contribution to colour hue and stability in wine and, in some cases, they could constitute a quality factor.

Conclusions

Red wine is undoubtedly a complex matrix, and our understanding of all the molecules and events responsible for wine colour is far from being complete. The main limitations are related to the capability of the techniques used, the detection limits, and the increasing complexity of the structures, among others. Many physical and chemical phenomena have been described herein where anthocyanins were reported to interact/react with each other and many other simpler or more complex phenolic and non-phenolic compounds. All of these phenomena interfere or have influence on red wine colour.

The main characteristic of all copigmentation processes is the capacity to hinder the hydration reaction for all the mechanisms involved in vertical π-stacking of the chromophore, preventing nucleophilic attack by water and colour loss. Of all the copigmentation phenomena, intermolecular copigmentation is the most important in red wines due to the variety and concentration of possible copigments that can be found in solution. Moreover, there are evidences of the occurrence of copigmentation between anthocyanins and grape CW components while the extraction is taking place. The only requirement is the ability to adopt a planar configuration that allows the approach and association with the anthocyanins.

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