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

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

54equilibrium: deprotonation to form the violet quinonoidal base and hydration at the C-2 position
55yielding the non-coloured hemiketal form. These forms are also in equilibrium with the *cis-* and
56*trans*-chalcone forms which present a yellow colour (Pina, 1998). With a pH rise to values up to
576, the quinonoidal base can be ionized to form the respective blue anionic quinoidal form
58[(Brouillard and Lang, 1990; Santos et al., 1993) (**Figure 1**). According to these equilibria, it
59would be expected that at wine pH (3.2-3.8), anthocyanins would be present in a great extent in
60their non-coloured hemiketal form. Nevertheless, there are some mechanisms to stabilize the
61coloured forms of anthocyanins. These include self-association (Gonzalez-Manzano et al., 2008;
62Mistry et al., 1991), intra and intermolecular copigmentation (Mistry et al., 1991; Yoshida et al.,
632009) and metal complexation mechanisms. Copigmentation helps to stabilize the flavylium
64form and protects it from the nucleophilic attack by water, thereby preventing the formation of
65colourless forms (Goto and Kondo, 1991; Yoshida et al., 2009, Dangles et al., 1994). However,
66some years ago, Asenstorfer and his co-workers using paper electrophoresis postulated that at
67wine pH, malvidin-3-glucoside is present as the uncharged quinonoidal base as major coloured
68component of the equilibria (Asenstorfer et al., 2003).

69.
70During wine ageing, anthocyanins participate in several reactions (i.e. reduction, oxidation, and
71polymerization) involving other wine molecules, leading to the formation of more stable
72anthocyanin-derived compounds (Oliveira et al., 2009; Oliveira et al., 2014).

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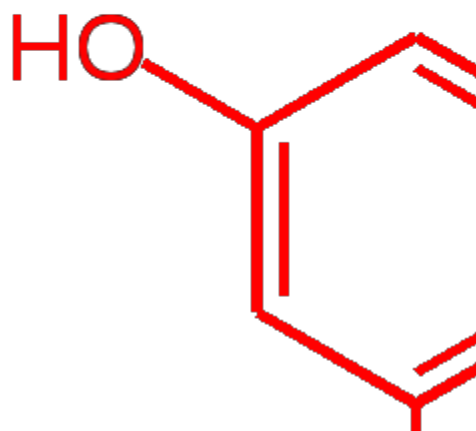
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Bearing all this, this paper will focus on reviewing the current knowledge of red wine colour, especially concerning colour stabilization mechanisms and colour evolution during wine ageing and maturation.

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 [30]. 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 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.

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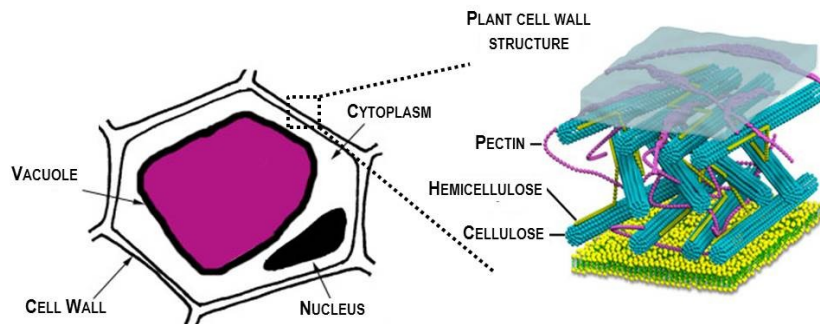
Figure 2 – Schematic representation of the grape cell structure evidencing the presence of the vacuoles where anthocyanins are located and schematic representation of the primary cell wall evidencing the most important polysaccharides.

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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, 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-

170acylated anthocyanins were better extracted than the acylated ones. On the other hand, total
171insoluble CW material has shown to exhibit the biggest opposition to anthocyanin extraction,
172slightly higher for non-acylated anthocyanins, while the highest amounts of cellulose,
173rhamnogalacturonans II and polyphenols were positively correlated with anthocyanin
174extraction. Xyloglucans, homogalacturonans and rhamnogalacturonans I evidenced opposite
175behaviour to anthocyanin extraction (Hernández-Hierro et al., 2014).
176The positive relationship between polyphenol content in CW and anthocyanin extraction
177probably suggests the existence of copigmentation processes referenced before between these
178components while the extraction is taking place, so they could be able to assist the extraction
179process (Boulton, 2001).
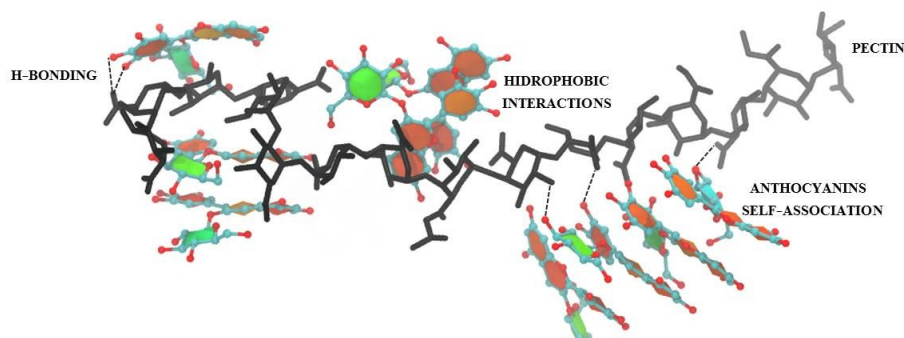
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181Figure 3. Schematic representation of some interactions established
182between anthocyanins and a low methoxylated pectin model involving
183hydrophobic interaction and H bonding. Pectin molecule is depicted with
184sticks and coloured in dark gray **(Fernandez *et al.*, 2014).**
185
186Gonçalves *et al*. reported a higher retention capacity of acylated anthocyanins by CW polymeric
187material, compared to the non-acylated anthocyanins. The acylation of the glucose residue was
188shown to enhance anthocyanin hydrophobicity, suggesting that the association of polymeric
189material with anthocyanins could be mainly due to hydrophobic interactions. In addition, CH-π
190interaction can occur between the aromatic rings on anthocyanins and carbohydrates (Gonçalves
191at al., 2012). Aggregation of anthocyanin pigments to yeast CW during fermentation has also
192been explained to occur as a result of hydrophobic interactions between sugars and phenols,
193although this adsorption has been found to depend also on the degree of methoxylation and
194acylation of anthocyanins (Morata et al., 2003). Previous reports also showed that the physical
195properties of grape skins, like skin hardness, thickness, number of cell layers and CW thickness,
196and the grape variety are also linked to anthocyanin extractability (Río Segade et al., 2011 ; Río
197Segade et al., 2008).
198

199 2. Copigmentation

200As mentioned before, wine anthocyanins are present in different forms, depending essentially on
201wine pH, thus affecting the hue and intensity of wine colour. Red wine is a very complex matrix
202that contains several polyphenolic compounds that help to stabilize the flavylium form of
203anthocyanins and thereby the colour of red wine. However, the occurrence of copigmentation in
204young red wine is easily demonstrated by successive dilution at constant pH: the dilution breaks
205the pigment-pigment and pigment-copigment complexes and no constant spectral behaviour is
206observed, as it would be expected if no interactions were involved (Boulton, 2001; Trouillas et
207al., 2016).
208Copigmentation can be defined as several physical-chemical mechanisms that result in the
209formation of non-covalent molecular complexes and in subsequent changes in the solution
210optical properties. It generally consists in van der Waals interactions (vertical π-π stacking)
211between the planar polarizable nuclei of two anthocyanin molecules or an anthocyanin and a
212colourless copigment (**Figure 4**) (Trouillas et al., 2016 ; Brouillard et al., 1989).

213

214 *2.1. Intermolecular and intramolecular copigmentation*

215 Intermolecular copigmentation occurs when the interaction involves an anthocyanin and a 216 colourless copigment and intramolecular copigmentation occurs when the acylated residues of 217 the anthocyanin act as copigment (**Figure 4**). These complexes adopt a sandwich-like structure 218 that stabilizes the flavylium cation chromophore and partially protects it from nucleophilic 219 attack by water, thus preventing colour loss (Goto, 1987; Santos-Buelga and De Freitas, 2009).

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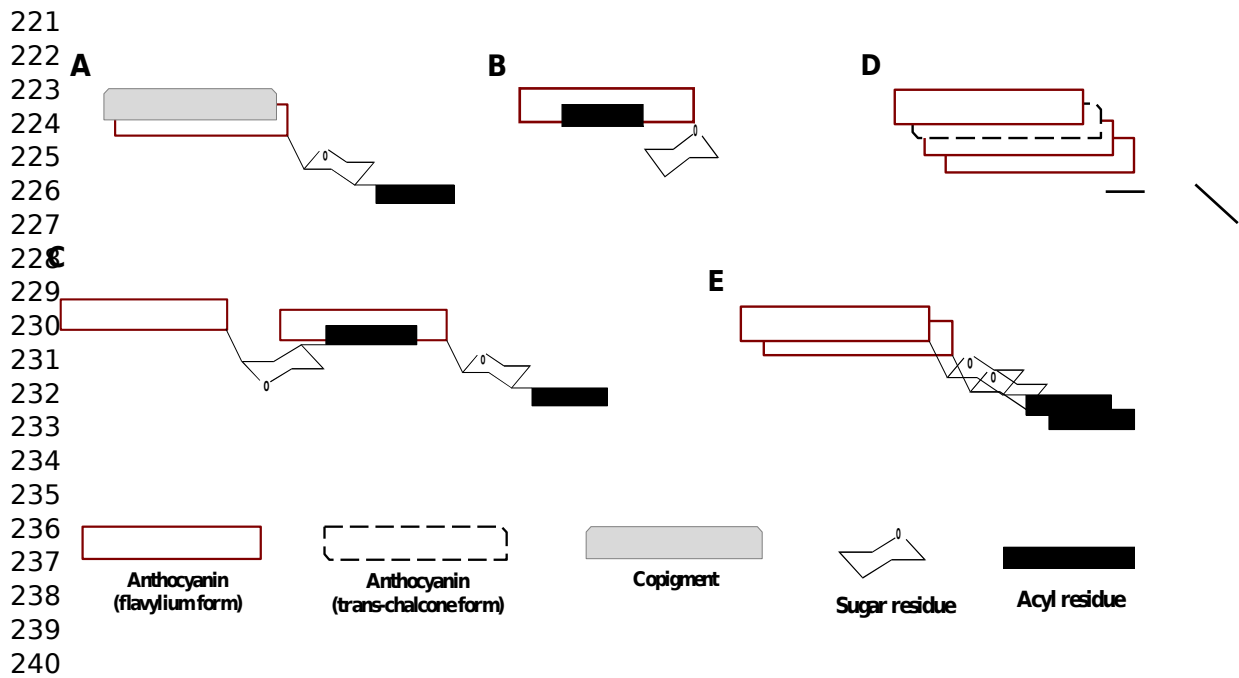
Figure 4. Copigmentation non-covalent molecular complexes resulting from π-π stacking interactions. A : Intermolecular copigmentation between an anthocyanin and a colourless copigment; B : Intramolecular copigmentation between an anthocyanin and its acyl residue; C : Intermolecular copigmentation between 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.

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

275and Dubois, 1977; Brouillard and Delaporte, 1977; Brouillard et al., 1978).]. However, acylated
276anthocyanins are usually more stable than non-acylated ones. In fact, acylated anthocyanins
277extracted from flowers (which present much more complex anthocyanin structures than grapes)
278display deep stable colours at neutral pH in the absence of any copigment (Goto and Kondo,
2791991, Saito et al., 1972; Tatsuzawa et al., 2012). This can be explained by intramolecular
280copigmentation where the chromophoric part of the anthocyanin and the acyl residue exist in a
281folded conformation. The formation of such intramolecular copigmentation complexes also
282protects the chromophore against hydration in an even more efficient way than that conferred by
283intermolecular copigmentation, because one side of the anthocyanin chromophore is protected.
284Copigmentation usually produces an increase in absorbance (hyperchromic effect) and a
285positive shift of the visible absorption maximum (bathochromic effect). The hyperchromic
286effect occurs due to the formation of the flavylium cation-copigment (or acyl residue) complex.
287The bathochromic effect occurs due to the affinity of the copigment for the quinoidal forms of
288the anthocyanin. Copigmentation is an exothermic process, and it increases with copigment
289concentration (in intermolecular copigmentation) and with sugar residue di-acylation (in
290intramolecular copigmentation), reflecting the preferential behaviour and shifting the
291equilibrium towards dehydration (Dangles et al., 1993; Teixeira et al., 2013; Trouillas et al.,
2922016).
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294*2.2. Self-association of anthocyanins*
295Self-association is considered to be a special type of copigmentation since it also induces optical
296changes in the involved pigments. It occurs when the anthocyanin undergoes a vertical stacking
297of the aromatic rings, again preventing the nucleophilic attack by water and the loss of colour
298caused by the formation of the hemiketal and chalcone forms (**Figure 4**). It can be easily
299observed by UV-Vis absorption spectroscopy in relatively concentrated solutions of any
300anthocyanin. This phenomenon was first suggested by Asen *et al.* in 1972 and ten years later it
301was demonstrated by Hoshino *et al.* by NMR and circular dichroism (CD) measurements
302(Boulton, 2001; Asen, 1972; Hoshino, 1992). NMR analysis allows the observation of the
303chemical shifts of the aromatic protons moving upfield with an increase of the anthocyanin
304concentration, suggesting the occurrence of hydrophobic interactions. The proof that
305anthocyanins can be organized into larger molecular structures can be achieved by the
306determination of the diffusion coefficient, where it should decrease with increasing
307concentrations of anthocyanins (Fernandes et al., 2015).
308Houbiers *et al.* reported that for malvidin-3-*O*-glucoside self-association can involve both the
309flavylium cation and the *trans*-chalcone (**Figure 4**) (Houbiers et al., 1998). They observed that
310the corresponding chemical shifts vary with different concentration values of each form at
311different pH values. In fact, and opposite to what is observed for hemiketal forms and *cis*-
312chalcone, flavylium cation and *trans*-chalcone chemical shifts move to higher frequencies upon
313increasing temperature. This contradictory behaviour between the two chalcone forms may be
314explained by the more planar structure of the *trans*-chalcone form. Comparing this effect at pH
3150.6 and 3.5 the results on temperature dependence are more expressive at pH 3.5 where both
316forms co-exist. However, it can still be questioned if, in the case of acylated anthocyanins, self-
317association cannot be mistaken with intramolecular copigmentation. This can probably be
318answered with ^{1}H-NMR measurements at different temperatures. When studying the self-
319association between flavylium cations and *trans*-chalcones, Houbiers *et al*. also performed [1]H-
320NMR measurements at different temperatures (Houbiers et al., 1998). They observed that the
321chemical shifts of flavylium cations and *trans*-chalcones moved to higher frequencies upon
322increasing the temperature while the chemical shifts of hemiketals and *cis*-chalcones remained
323identical. Knowing that by increasing the temperature the formed aggregates can split and that
324copigmentation is an exothermic process, these observations support the idea that flavylium
325cations and *trans*-chalcones prefer self-association to copigmentation (Houbiers et al., 1998). To
326these observations it can also be added the fact that the equilibrium constant of malvidin-3-*O*-
327(6-coumaroylglucoside) is almost two times higher than that for malvidin-3-*O*-glucoside,
328suggesting a higher affinity to aggregation (Fernandes, 2015).

329As already mentioned, Hoshino *et al*. have performed a series of studies demonstrating the self-
330association of anthocyanins. CD experiments allowed to conclude that anthocyanidins can stack
331both in a right- or left-handed axis, while quinoidal bases of cyanidin and pelargonidin form
332right-handed adducts and peonidin, delphinidin and malvidin form left-handed adducts
333(Hoshino, 1981 : Hoshino, 1992). However, all the flavylium cations lead to left-handed adducts
334due to an asymmetry imposed by oligomeric species, as shown by Rodger and Nordén (1997).
335Later, Gavara *et al*. demonstrated that the flavylium cations of anthocyanidin 3-glucosides can
336self-associate in a monomer-dimer aggregation type and further aggregation is prevented by
337electrostatic repulsion between the positively charged molecules (Gavara et al., 2013). However,
338and knowing that *trans*-chalcones may also be involved in self-association mechanisms, some
339authors reported that self-association models should not always be thought of as simple dimeric
340models, but rather as higher order mixed aggregates with intramolecular copigmentation as well
341as for acylated anthocyanins (Houbiers et al., 1998 ; Leydet et al., 2012).
342The substitution pattern of the anthocyanin B ring has little influence on its thermodynamic and
343kinetic properties but it is extremely important for aggregation processes. Leydet *et al*.
344demonstrated that methoxyl and hydroxyl substituents favour the aggregation of flavylium
345cations probably due to hydrogen bonding (Leydet et al., 2012).
346There are not many studies on self-association in wines due to the complexity of the subject. In
3472009, González-Manzano *et al*. reported that self-association in wine-like solution models
348accounts for 8 to 60% of absorbance increase (González-Manzano et al., 2009).

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351These non-covalent interactions can be the first step in the formation of covalent bonds between
352two molecules, resulting into new anthocyanin-derived pigments as described below. In fact,
353during storage or ageing, copigmentation decreases and the concentration of polymerized
354structures increases, with consequent changes in wine colour (Liao et al., 1992, Brouillard and
355Dangles, 1994 ; Gutiérrez, 2005). One of these new types of structures, pyranoanthocyanins (see
356next section), may also undergo copigmentation phenomena. Pyranoanthocyanin-flavanol
357pigments generally do not undergo hydration processes and display 30 to 50% stronger
358absorption values at wine pH (around 3.6) than at pH 1.0 (Oliveira et al., 2009; He et al., 2012;
359Escribano-Bailon and Santos-Buelga, 2012). For instance, a copigmentation assay with
360malvidin-3-*O*-glucoside-vinylguaiacol pyranoanthocyanin as pigment and catechin as
361copigment resulted in a larger increase of the visible absorption than in the case of malvidin-3-
362*O*-glucoside (Quijada-Morín et al., 2010). In another study, a dimeric-type malvidin-3-*O*-
363glucoside-(O)-catechin adduct was tested and showed to be a far more effective copigment than
364other flavan-3-ols (Teixeira et al., 2013). This shows the potential of anthocyanin-derived
365pigments in wine copigmentation and therefore in red wine perception of colour.
366
367*Colour evolution during wine ageing and maturation*
368During ageing and maturation, the colour of wines changes from red/violet to red/orange due to
369several chemical reactions (oxidation, reduction and polymerization) where anthocyanins
370participate, thus being the precursors of new compounds. In the first stages of wine evolution,
371anthocyanins can react with flavan-3-ols (catechin monomers and condensed tannins) directly or
372indirectly via aldehydes, yielding new purple pigments (anthocyanin-alkyl-catechin) that further
373lead to the formation of pyranoanthocyanin-catechin compounds displaying an orange colour
374(Pissarra et al., 2003; Liao et al., 1992; Rivas-Gonzalo et al., 1995; Francia-Aricha et al. 1998)
375(**Figure 5**). Acetaldehyde is particularly important for anthocyanin chemical transformation
376reactions. This aldehyde is present in high amount in wine spirits (40-260 mg/L) and is also
377formed during fermentation and wine ageing from ethanol oxidation (Pissarra et al., 2005).

379
380Figure 5 – Anthocyanin-derived pigments identified in red Port wines.
381
382Several pyranoanthocyanin pigments resulting from the reaction of anthocyanins with small
383molecules arising from grapes or from yeast metabolism have been identified in red wines over
384the last years, like acetaldehyde (Bakker and Timberlake, 1997), pyruvic acid (Fulcrand et al.,
3851996), acetoacetic acid (He et al., 2006), diacetyl (Gomez-Alonso et al., 2012),
386hydroxycinnamic acids (Schwarz et al., 2003; Rentzsch et al., 2007) and *p*-vinyl-phenols
387(Hayasaka and Asenstorfer, 2002; Schwarz et al., 2003) (**Figure 5**).
388Pyruvic acid derivatives of anthocyanins (A-type vitisins) are the pyranoanthocyanins detected
389in higher concentration in Port wines (Mateus and de Freitas, 2001). The characteristic chemical
390and physical properties of Port wine could be at the origin of higher levels of yeast metabolites
391likely to yield anthocyanin derivatives. Indeed, when wine spirit is added to stop fermentation,

392 pyruvic acid concentration is expected to be higher than when the fermentation is allowed to go
393 to dryness. In fact, pyruvic acid released by yeast at the beginning of the fermentation is further
394 used in yeast metabolism. Its levels increase right after wine fortification, and the initial
395 formation of anthocyanin-pyruvic acid adducts is concurrent with the degradation of
396 anthocyanin monoglucosides. A-type vitisins were found to be very resistant to wine ageing
397 when compared to the original grape anthocyanins (Oliveira et al., 2013, Mateus and de Freitas,
398 2001; Asenstorfer and Jones, 2007). Yellowish α-pyranone-anthocyanins were also described to
399 be present in aged red wines derived from the direct oxidation of A-type vitisins (He et al.,
400 2010).

401 A few years ago, a new class of pyranoanthocyanin compounds (vinylpyranoanthocyanins)
402 named portisins, presenting an unusual bluish colour at acidic pH values, was identified in a
403 young Port wine (Mateus et al., 2003) (**Figure 5**). The studies performed in model solutions
404 have demonstrated that these compounds can be formed in wines from the reaction between A-
405 type vitisins and flavanols in the presence of acetaldehyde (Mateus et al., 2004). Similar
406 compounds were identified in Port wines resulting from the reaction of A-type vitisins with
407 hydroxycinnamic acids such as *p*-coumaric, caffeic, ferulic and sinapic acids (Oliveira et al.,
408 2007). More recently, a turquoise blue pyranoanthocyanin dimer pigment was found to occur in
409 a 9-year-old Port wine and in the respective lees derived from the reaction of the A-type vitisin
410 with a methylpyranoanthocyanin (Oliveira et al., 2010).
411 The occurrence of these novel pigments in aged wines points to a second-generation pathway
412 where the main precursors are no longer the native anthocyanins but secondary anthocyanin
413 products such as A-type vitisins involved in the formation of new pigments in subsequent stages
414 of wine ageing that may contribute directly or indirectly to its colour evolution.
415 Moreover, studies performed in aqueous solutions using UV-Visible spectroscopy and NMR
416 techniques revealed that pyranoanthocyanins present a higher colour stability when compared to
417 their anthocyanin precursors due to the absence of hydration reactions (Oliveira et al., 2009;
418 Oliveira et al. 2013; Oliveira et al., 2014).
419 On the other hand, polymeric pigments have been described to play an important role in the
420 long-term colour stability of aged red wines (Boulton, 2001). However, there is still a lot to
421 know about their identity and chemical pathways in wines. The first evidence of the presence of
422 oligomeric anthocyanins (dimeric and trimeric) was described by Vidal and co-workers in grape
423 skins using mass spectrometry techniques (Vidal et al., 2004). Later, the dimeric compounds
424 were also reported to occur in red wines by Salas *et al.* (2005) and Alcalde-Eon *et al.* (2007)
425 using the same technique. A few years later, Oliveira *et al*. demonstrated the presence of an A-
426 type malvidin-3-glucoside trimer in a young Port wine using LC-MS and NMR methodologies
427 (Oliveira et al., 2013) (**Figure 6**). The origin of these compounds in red wine could result from
428 their extraction from grape skin during the winemaking process, as the presence of these
429 trimeric pigments was detected in a grape skin extract by LC/DAD-MS spectrometry (Oliveira
430 et al., 2013). Moreover, this pigment revealed to be much less prone to hydration reactions than
431 the anthocyanin monomers, with hydration accounting for less than 10% of the overall
432 reactivity (Oliveira et al., 2014). A dimeric acetaldehyde malvidin-3-glucoside condensation
433 pigment was also found to occur in red wine (Atanasova, 2002).
434 All these new families of anthocyanin-derived pigments make an important contribution to
435 colour hue and stability in wine and, in some cases, they could constitute a quality factor.

436
Figure 6 – Structure of A-type malvidin-3-glucoside trimer detected in a young wine and in grape skins.

439

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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