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Review

Mechanisms of oxidative browning of wine

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Abstract

Oxidative browning is a long-standing problem in winemaking and sulphur dioxide is the generally used antioxidant to control wine browning. However, concerns over its ability to induce severe allergic reactions have created a great need for its reduction or replacement in recent years. Furthermore, ascorbic acid seems unsuccessful as an alternative antioxidant in winemaking. So a comprehensive understanding of the chemistry of wine browning is essential to study new antioxidant(s) for the protection of wine from browning. The review systematically outlines the mechanisms of wine browning including enzymic and non-enzymic browning happening in winemaking and discusses the central roles performed by iron and copper in the chemistry of wine oxidation. Iron and copper might play a central role in initiating wine non-enzymic browning, and this has important implications in studying antioxidant substitute for sulphur dioxide in wine.

Keywords: Wine; Sulphur dioxide; Browning; Copper; Iron; Oxygen

Contents

Abbreviations: CHD, coronary heart disease; ROS, reactive oxygen species; SO_2, sulphur dioxide; WHO, World Health Organization; O.I.V., International Organization of Vine and Wine; PPO, polyphenoloxidase; GRP, 2-*S*-glutathionylcaftaric acid; POD, peroxidase; CTAQ, caffeoyltartaric acid *o*-quinones; GSH, glutathione; HMF, 5-(hydroxymethyl)furfural; E_H, oxidation–reduction potential; ΔrGm, free energy change.

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1. Introduction

Considerable amount of data obtained *in vitro* and *in vivo* experiments have shown that phenolic compounds in wine have great antioxidant activities, such as scavenging of reactive oxygen species (ROS), relaxing blood vessels, anticarcinogen, anti-inflammation, and antivirus, among others (Fernández-Pachón, Villaño, García-Parrilla, & Troncoso, 2004; Giugliano, 2000; Lodovici et al., 2001; Makris, Kallithraka, & Kefalas, 2006). However, many constituents of wine, including phenolic compounds, certain metals, tyrosine and aldehydes, are susceptible to oxidation during the winemaking process and lead to browning. Browning is an oxidative process involving sugars, lipids, amino acids or phenols in food. It is one of the main problems encountered during the vinification of wine as it on one hand, adversely affects the sensory properties of wine (loss of colour, flavour and aroma, and increase of astringency) (Escudero, Asensio, Cacho, & Ferreira, 2002; Ferreira, Escudreo, Fernández, & Cacho, 1997; Schneider, 2001; Silva Ferreira, Guedes de Pinho, Rodrigues, & Hogg, 2002) and on the other, results in the loss of nutritional value of wine (Bonilla, Mayen, Merida, & Medina, 2001; Sioumis, Kallithraka, Tsoutsouras, Makris, & Kefalas, 2005).

Sulphur dioxide, often abbreviated to sulphite or SO_2, is the most important and widely used chemical to prevent wine from browning. Besides antioxidant activities, SO_2 also has antimicrobial properties and other important functions, which has been successfully used for years. However, the addition of SO_2 to wines raises health-related objections due to serious allergic reactions incurred by sulphite-sensitive individuals, and concerns over sulphites have resulted in regulatory restrictions set by World Health Organization (WHO) and International Organization of Vine and Wine (O.I.V.) (Li, Wang, Yuan, & Wang, 2005; Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006; Warner, Diachenko, & Bailey, 2000; Yang & Purchase, 1985; Zhou, Zhang, & Xin, 2004). Furthermore, winemakers are also concerned about SO_2 addition to juice and wine for quality reasons: its excessive use can drastically compromise the quality of wine and excessive quantities of SO_2 can actually give the wine unpleasing flavours and aromas or may favour the wine to turn cloudy during its keeping (Li et al., 2005). Therefore, a growing awareness concerning the use of SO_2 in wine has created the need for its reduction or replacement in recent years (Comuzzo & Tat, 2003; Divol, Strehaiano, & Lonvaud-Funel, 2005; Ribéreau-Gayon, Dubourdieu, et al., 2006; Threlfall & Morris, 2002). To date, a single replacement product performing the same roles without the disadvantages of SO_2 is unsuccessful, though a lot of work has been done (Barbe, Revel, Joyeux, Lonvaud-Funel, & Bertrand, 2000; Comuzzo & Tat, 2003; Main, 1992; Ribéreau-Gayon, Dubourdieu, et al., 2006). However, it is perfectly operable to study partial replacement that has antioxidant properties as or better than SO_2 does in winemaking.

Ascorbic acid (vitamin C) and its optical isomer, erythorbic acid, probably the alternative to sulphites, have been widely used as antioxidants in winemaking, especially white wine production, primarily because of their chemical properties (including reducing character and oxygen-scavenging capacity) and their approval for wine use (Bradshaw, Prenzler, & Scollary, 2001; Marks, 1990). However, more and more extensive studies have shown that ascorbic acid may have a pro-oxidant role rather than antioxidant depending on its levels in wine under some conditions (Bradshaw, Cheynier, Scollary, & Prenzler, 2003; Bradshaw et al., 2001; Main, 1992; Scollary, 2002). Many recent research results in model systems and white wines indicate that if ascorbic acid is used in combination with SO_2 then it can cause accelerated consumption of SO_2 and accelerate the production of yellow pigments (browning) (Bradshaw, Scollary, & Prenzler, 2004; Oliveira, Silva Ferreira, Guedes de Pinho, & Hogg, 2002). Presently, the use of ascorbic acid is not widespread in winemaking and not authorized in France (Ribéreau-Gayon, Dubourdieu, et al., 2006).

Therefore, it is essential to find an appropriate antioxidant(s) other than SO_2, and then a comprehensive understanding of the mechanisms of wine oxidative browning should be considered. However, the mechanistic chemistry of the process in its entirety is not outlined, though some advanced research on wine oxidative browning has been achieved in recent years (Danilewicz, 2003; Waterhouse & Laurie, 2006). In this paper, an overview of wine oxidative browning including enzymic and non-enzymic browning is offered, and this is important to fully understand the chemistry of wine oxidation, thus to study alternative antioxidant for wine protection and eventually produce wine with high quality, stability and safety.

2. Phenolic compounds

Some metal ions, SO_2, organic acids, ethanol, and phenolic compounds present in grape must and wine, are susceptible to oxidation, and among them phenolic compounds are considered the major substances to cause browning (Junquera, Gonzalez-San Jose, & Diez, 1992; Macheix, Sapis, & Fleuriet, 1991; Toit, Marais, Pretorius, & Toit, 2006; Vivas, Vivas de Gaulejac, & Nonier, 2001). However, the oxidation of phenols is very complicated, largely depending on phenolic compositions and their levels, and that *o*-diphenols are the most oxidizable ones (Boulton, Singleton, Bisson, & Kunkee, 2001; Sioumis et al., 2005; Toit et al., 2006; Zhai, Du, Guan, Qiao, & Pan, 2001). In short, phenols' regenerative capacities in coupled oxidation with other compounds and their non-oxidative browning reactions constitute the major reason for browning (Boulton et al., 2001). SO_2, ascorbic acid and some transition metals are important factors affecting the process (Sioumis et al., 2005).

Singleton (1987) summarized the oxidation process in wine and considered that phenols' regeneration was involved in the coupled oxidation process and the system worked as a "buffer" in oxidation because the regenerated phenols were more readily oxidized than their corresponding original ones, which prevent the complete oxidation of the initial phenols. Cheynier, Fulcrand, Guyot, Oszmianski, and Moutounet (1995) examined the mechanisms of major reactions responsible for grape must browning and consequences of such reactions for wine in model systems. They revealed that browning reactions in wines appeared related to flavanol contents; autoxidation of flavanols (e.g. catalyzed by Fe^{2+}) was one reaction responsible for wine discolouration, but spontaneous acid-catalyzed hydrolysis and polymerization reactions of flavan-3-ol oligomers (proanthocyanidins) might lead to larger, less soluble coloured compounds. Fernández-Zurbano et al. (1995) and Fernández-Zurbano, Ferreira, Escudero, and Cacho (1998) studied the changes in phenolic compounds during the oxidation of eight white wines to investigate the roles of hydroxycinnamic acids and flavanols in the oxidation and browning of white wines, suggesting that the content of hydroxycinnamic acids and esters decreased significantly during the process but was seemingly unrelated to the extent of wine browning; however, the flavanol content of the wines was found to be correlated with the degree of browning at the end of the oxidation process. Sioumis, Kallithraka, Makris, and Kefalas (2006) studied the browning capacity of white wines, in which browning was approached from a kinetic point of view and efforts were focused on the investigation of plausible correlations with major redox-active polyphenols, including substances with an *o*-diphenol feature, such as gallic acid, caftaric acid, 2-*S*-glutathionylcaftaric acid (known as Grape Reaction Product or GRP), caffeic acid, catechin, and epicatechin. Regression analysis between k values and concentration of individual phenols provided strong evidence that epicatechin was the principal browning agent ($r^2 = 0.8033$, $p < 0.01$).

3. Mechanisms of wine oxidative browning

As described above, phenols especially *o*-diphenols are responsible for the oxidative browning of wine. The browning of wine can be classified into enzymic browning and non-enzymic browning in terms of the initiative mechanism: the former almost entirely occurs in grape must; the latter can happen both in grape must and wine, while as a result of fermentation and some operations it prevails in wine (Es-Safi, Cheynier, & Moutounet, 2003b; Main, 1992; Spagna, Barbagallo, & Pifferi, 2000; Sullivan, 2002).

3.1. Enzymic browning

The enzymic oxidation of phenols, particularly in the presence of atmospheric oxygen and polyphenoloxidase (PPO), takes place in the early stages of processing and is well known to be a cause of browning in foodstuffs (Wang, 1990). In the intact cells of fresh fruit or vegetable tissues, phenols located predominantly in the vacuole and oxidoreductases located in cytoplasm cannot meet due to different cell membrane systems, whereas enzymic browning will arise once the cells are bruised or wounded in air (Wang, 1990). The main oxidoreductases responsible for browning during grape processing are PPO and peroxidase (POD) (Li et al., 2005). PPO is a copper-containing enzyme and able to oxidize substrates characteristic for tyrosinase and laccase. Tyrosinase, also called catecholase (E.C. 1.10.3.1), is naturally produced in grape berry and can catalyze the oxidation of monophenols and *o*-diphenols (Singleton, 1987). However, laccase (E.C. 1.10.3.2) is produced by molds and able to oxidize lots of substrates, especially 1,2- and 1,4-dihydroxyphenene (Li, 2000; Main, 1992; Toit et al., 2006). POD (E.C. 1.11.1.7) is a Fe-containing enzyme and its activity depends on the available hydrogen peroxide (H_2O_2) in medium. However, the browning caused by POD seems insignificant in fruits with few exceptions, such as *litchi* and pineapple (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999), although some researcher found that it did enhance the degradation of phenols when coexisting with PPO (Robards et al., 1999).

In grape must, enzymic browning is largely correlated with the content of hydroxylcinnamates such as caffeoyltartaric acid (caftaric acid) and *p*-coumaroyltartaric acid (coutaric acid), and is promoted by flavanols (Cheynier, Basire, & Rigaud, 1989; Cheynier, Rigaud, Souquet, Duprat, & Moutounet, 1990; Oszmianski, Cheynier, & Moutounet, 1996; Rigaud, Cheynier, Souquet, & Moutounet, 1991). Caftaric acid or *p*-coumaric acid is oxidized by PPO to produce caffeoyltartaric acid *o*-quinones (CTAQ), which are powerful oxidants and able to oxidize other compounds in wine to cause great changes in wine tone and colour intensity depending on the phenols and the reactive situations (Cheynier et al., 1995; Robards et al., 1999). Meanwhile, the *o*-quinones produced in enzymic oxidation will go on with further reactions according to their redox properties and electronic affinities: (1) Firstly, the hydroxylation of monophenols into *o*-diphenols and diphenols into *o*-quinones occurs with rapid coupled oxidations of the quinones with another phenol molecule leading to the formation of dimers or regeneration of the original phenols, which is catalyzed by acid (Fig. 1) (Wang, 1990). (2) The produced quinones can also polymerize and condensate with many other compounds (including phenolic and non-phenolic species), and finally forms brown pigments, which is accelerated by higher pH (Fig. 1) (Robards et al., 1999; Wang, 1990). Being oxidants, *o*-quinones can almost oxidize any substrate with lower potentials such as other phenols, ascorbic acid and SO_2, and such quinones are reduced to their initial phenols in the process; as electrophiles, they can react with amino derivatives and water; on the other hand, *o*-quinones can react with appropriate hydroquinones, probably undergoing condensation and polymerization reactions slowly, and leads to browning pigments (Fig. 1) (Robards et al., 1999).

Glutathione (GSH), however, will affect the process of must oxidation through reacting with CTAQ to produce

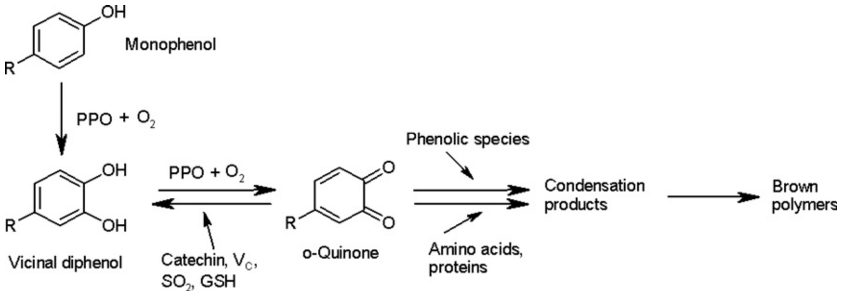


Fig. 1. Enzymic browning process in grape must.

2-*S*-gluthathionyl caftaric acids (GRP). GRP cannot be oxidized by catecholase and it will limit the oxidative browning to some extent, which implies that enough GSH in grape must can trap the quinones produced during enzymic oxidation, and decrease the amount of browning pigments (Rigaud et al., 1991; Singleton & Cilliers, 1995; Singleton, Salgues, Zaya, & Trousdale, 1985; Toit et al., 2006). On the other hand, the interaction between catecholase and SO_2 may prevent the production of GRP, which will maintain much more free caftaric acid and *p*-coutaric acid with high browning potential. Moreover, GRP is ready to oxidation by laccase or CTAQ to brown (Main, 1992). Copper also reacts with GSH and competes with CTAQ, and to some extent, decreases the effective content of GSH and enhances the browning of grape must (Cheynier et al., 1989; Rigaud et al., 1991). In general, not all the enzymic oxidation occurring in white grape must is bad to the final product. For example, white must hyperoxygenation decreases the browning potential of wine in two ways: the disappearance of tyrosinase and the depletion of oxidizable phenolic substrates during the oxidation reactions (Ribéreau-Gayon, Dubourdieu, et al., 2006); the formation of GRP that is resistant to further browning (Main, 1992). This results in wine with low polyphenols content and high GRP content and these wines are more stable than those wines made from non-oxidized juice, in which high polyphenols are maintained with a high browning potential. However, during red wine processing the impact of endogen enzymic oxidation is limited, but on the other hand chemical oxidation (non-enzymic oxidation) strongly affects the wine maturation process (Cheynier, Fulcrand, & Moutounet, 2000).

It is accepted that catecholase is so sensitive to SO_2 that a small amount will inactivate it, while laccase is more active, readily soluble and resistant to SO_2, and it may be present in the final wine (Boulton et al., 2001; Li, 2000; Ribéreau-Gayon, Dubourdieu, et al., 2006; Zoecklein, 2000). However, the concentration and activity of PPO are gradually lowered during oxidation (Li, 2000), and with the development of fermentation and such operations as fining, SO_2 treatment and ethanol production, no PPO exists in wine (Cheynier et al., 1989; Ribéreau-Gayon, Dubourdieu, et al., 2006; Spagna et al., 2000).

Presently, the study on enzymic browning is so profound that the control managements carried out in winemaking have been able to completely remove the oxidative spoilage and much more attention is paid to non-enzymic browning happening in wines, particularly white wines (Labrouche, Clark, Prenzler, & Scollary, 2005).

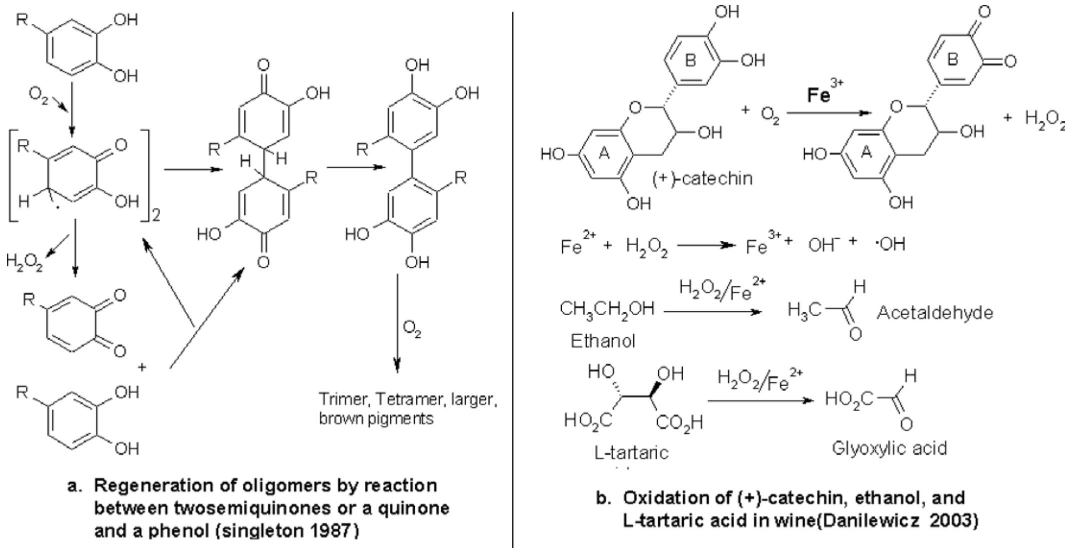
3.2. Non-enzymic browning

Non-enzymic oxidation, also called chemical oxidation, prevails in wine with the characteristics of regeneration and autocatalysis, and it may also occur through the direct reaction with light (Main, 1992). *o*-Diphenols, mainly including caffeic acid and its esters, catechin, epicatechin, anthocyanins and their derivatives, and gallic acid, are considered to be the most susceptible to oxidation in non-enzymic browning process, and the levels of flavan-3-ols are most significantly correlated to the browning degree of most white wines (Fernández-Zurbano et al., 1995, 1998; Lopez-Toledano, Mayen, Merida, & Medina, 2002; Oszmianski et al., 1996; Saucier & Waterhouse, 1999).

In brief, non-enzymic browning in wine can arise through several pathways related to phenols, and one of them is the oxidation of phenols and subsequent polymerization of the oxidized products, the first process catalyzed by copper and iron. Other routes involve polymerization reactions between phenols and other compounds present in wine, including condensation with acetaldehyde or glyoxylic acid (derived from the oxidation of tartaric acid). Some researchers state that the condensation with acetaldehyde is a sole reaction, generally present in sherry wines, for the total concentration of acetaldehyde produced by flor yeasts may reach 300 mg/L during biological aging (Merida et al., 2005; Palma & Garcia-Barroso, 2002). However, the oxidation of ethanol catalyzed by transition metals or through coupled oxidation of phenols may also produce some acetaldehyde in wine (Sullivan, 2002; Wildenradt & Singleton, 1974).

3.2.1. Oxidation of wine phenols

During the process of non-enzymic oxidation of wine, *o*-diphenols are oxidized to *o*-quinones and semi-quinone free radicals may be produced, while oxygen is reduced to H_2O_2 (Danilewicz, 2003; Sullivan, 2002; Waterhouse & Laurie, 2006; Wildenradt & Singleton, 1974), as exemplified in Fig. 2. The quinones formed during the process as the primary products are unstable and may undergo fur-



a. Regeneration of oligomers by reaction between twosemiquinones or a quinone and a phenol (singleton 1987)

b. Oxidation of (+)-catechin, ethanol, and L-tartaric acid in wine(Danilewicz 2003)

Fig. 2. Non-enzymic oxidation reactions in wine.

ther reactions. These reactions, which may cause pigment formation, are similar to those taking place in enzymic browning despite the quinones from enzymic or non-enzymic oxidation (Robards et al., 1999; Singleton, 1987), for instance, condensation reactions to form coloured products with high molecular weight, or reduction reactions to generate original phenols through trapping hydrogen atoms from other compounds (Fig. 2a).

Quinones can spontaneously combine with nucleophilic compounds (including some phenols, sulphydryl compounds and amines) due to their high electronic affinity, and in the process the produced dimers or polymers may rearrange their structure through an enol-like conversion reaction to form new diphenols (Zhai et al., 2001). For example, a quinone–phenol dimer can be converted into a new diphenol dimer (Fig. 2a). Moreover, the produced dimers or polymers in coupled oxidation have lower redox potentials than their initial phenols and are much easier to oxidation (Boulton et al., 2001; Li et al., 2005). These regenerated *o*-diphenols will renewably be oxidized, ultimately accelerating the polymerization reactions of phenols (Boulton et al., 2001; Zhai et al., 2001). Meanwhile, more H_2O_2 is produced and more ethanol is oxidized to acetaldehyde in the presence of transition metals, and some phenols that are not easy to be oxidized originally, seem susceptible to oxidation through the structure rearrangement above (Boulton et al., 2001; Zhai et al., 2001). However, Oszmianski et al. (1996) considered that catechinic acid rearrangement could be impeded by some additives, such as silica, boric acid, aluminum trichloride, and zinc (II) acetate, and could not occur at pH values below 8. The non-enzymic oxidation of phenols occurs very fast under alkaline conditions but also takes place slowly in acidic media like wine at common pressure and temperature, especially if metal ions are present (Boulton et al., 2001; Oszmianski et al., 1996; Tulyathan, 1985).

As indicated above, H_2O_2 may be formed during the oxidation of wine phenols, which has been widely accepted. Some authors also state that the main antioxidant function of SO_2 in wine is to react with H_2O_2, thus limiting the oxidation of ethanol and other saturated hydroxy compounds (Boulton et al., 2001; Danilewicz, 2007). This should be taken into account, because H_2O_2 in association with ferrous ion (Fe^{2+}) tends to generate reactive oxygen species such as hydroxyl radical ($^{\bullet}OH$), which is known as the Fenton reaction (Choe & Min, 2005; Waterhouse & Laurie, 2006; Zhao, 1999). The process of the Fenton reaction is shown in Fig. 3. Presently, although the Fenton reaction is well studied in beer, its importance in wine is considered only in recent years (Laurie & Waterhouse, 2006; Waterhouse & Laurie, 2006).

Hydroxyl radical, a reduced product of oxygen with short existing life of about 10^{-6} s in water, is presently the most powerful oxidant in reactive oxygen species and capable of rapidly oxidizing most organic substances (Choe & Min, 2005; Zhao, 1999). Some authors suggest that $^{\bullet}OH$ is able to oxidize almost any component found in wine, nearly in proportion to their concentrations (Laurie &

Reaction A: Fenton reaction

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \cdot OH$$
$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + 2H^+ + O_2^{\cdot -}$$
$$\text{Net: } 2H_2O_2 \longrightarrow H_2O + 2H^+ + O_2^{\cdot -} + \cdot OH$$

Reaction B: Haber-Weiss reaction

$$Fe^{3+} + O_2^{\cdot -} \longrightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + \cdot OH$$
$$\text{Net: } O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + OH^- + \cdot OH$$

Fig. 3. Fenton reaction.

Waterhouse, 2006). However, ˙OH is non-selective and only reacts with adjacent molecules on account of its smaller action radius (Waterhouse & Laurie, 2006; Zhao, 1999). Thus, ethanol and tartaric acid being the prime substrates due to their relatively large concentrations in wine would likely be oxidized by ˙OH to form acetaldehyde and glyoxylic acid, respectively, and other abundant components of wine such as glycerol, sugars and organic acids probably undergo the same kind of oxidation reactions to yield corresponding products (Waterhouse & Laurie, 2006). These expected oxidation products, mainly aldehydes and ketones, are good nucleophiles, which may be important to colour development and other changes to tannin structure (Waterhouse & Laurie, 2006).

3.2.2. Browning pathways involving acetaldehyde and glyoxylic acid

3.2.2.1. Acetaldehyde. Presently, the chromatic changes during wine browning are well documented, though the aromatic deterioration seems to occur prior to the colour change (Escudero et al., 2002; Silva Ferreira et al., 2002). At the same time, flavour degradation during wine browning has received little attention, and less on the relationship between the changes of flavour and colour in wine (Ferreira et al., 1997; Silva Ferreira, Oliveira, Hogg, & Guedes de Pinho, 2003). Traditionally, acetaldehyde is considered to possess an offensive odour and taste, which brings bitterness and oxidized flavour to wine, and if its level exceeds 50 mg/L in a table wine, it means that the wine has been oxidized (Zhai et al., 2001). However, acetaldehyde appears to be the typical substances of flavour like ripen nuts in some dry sherry wines subjected to biological or oxidative aging (Ferreira et al., 1997). Timberlake and Bridle (1976) first proposed one of the mechanisms that acetaldehyde could contribute to the formation of dimer and trimer between flavanols (tannins), and later it was confirmed by other researchers (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999; Fulcrand, Doco, Es-Safi, Cheynier, & Moutounet, 1996; Saucier, Guerra, Pianet, Laguerre, & Glories, 1997). The outcome of this increases the colour of the yellow spectral region as it does the condensation degree (Lopez-Toledano, Villano-Valencia, Mayen, Merida, & Medina, 2004). As mentioned above, the acetaldehyde present in wine is derived from the yeast metabolism during fermentation or eventual biological aging and from the oxidation of ethanol catalyzed by transition metals or through coupled oxidation of phenols. The first route in which acetaldehyde is produced mostly takes place in certain wines such as sherry wines, and the second route is the most important in most wines. Wildenradt and Singleton (1974) showed that the coupled oxidation of ethanol to acetaldehyde at an appreciable rate during phenols oxidation process (Fig. 2b).

The reaction between acetaldehyde and phenols is believed to start with the protonation of acetaldehyde to a carbocation under acidic conditions, followed by the nucleophillic addition of the flavanol (via the position C-6 or C-8 of the A-ring) to the carbocation (Fig. 4). After losing a water molecule, the ethanol adduct forms a new carbocation intermediate that is attacked by a second nucleophilic flavanol unit, probably at its position C-8 to yield an ethyl-linked flavanol dimer (Fig. 4). The reaction begins again from the newly formed dimers, leading to polymers in the end. With acetaldehyde, the ethyl linkages generated in the polycondensated flavanol polymers are not stable and cleave into vinylflavanol oligomers (Fig. 4(1)) (Fulcrand, Dueñas, Salas, & Cheynier, 2006). However, xanthylium pigments have not been detected when acetaldehyde is reacted with flavanols, but the orange colouration of the initial solution is characteristic of the xanthylium chromophore (Es-Safi, Cheynier, & Moutounet, 2002; Fulcrand et al., 2006).

As shown in model systems and red wines, direct condensation would be achieved between anthocyanins and tannins or catechins to form anthocyanin–tannin and tannin–anthocyanin adducts, but the reaction is very slow, and the eventual products are yellow xanthylium salts, which always changes the colour of red wine into orange (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Dueñas, Fulcrand, & Cheynier, 2006; Monagas, Bartolomé, & Gómez-Cordovés, 2005; Remy, Fulcrand, Labarbe, Cheynier, & Moutounet, 2000; Timberlake & Bridle, 1976). Compared to direct condensation between anthocyanins and tannins or catechins, rapid polymerization between them mediated by acetaldehyde occurs with increased wine colour intensity and stability, but further polymerization with flavanols gives rise to instability, precipitation and decreased colour (Es-Safi et al., 2002, 2003b; Liu & Pilone, 2000). In addition, acetaldehyde might form new types of pigments such as Vitisin B and other proanthocyanidins that are more stable to SO_2 bleaching and the pH effect than free anthocyanins in model systems, which may be important in stabilizing wine colour (Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Morata, Calderón, González, Gómez-Cordovés, & Suárez, 2007).

3.2.2.2. Glyoxylic acid. In 1996, Oszmianski et al. found that in a model wine, the oxidation products of the iron-induced oxidation of (+)-catechin, were colourless or yellow, and the yellow compounds with maximum absorbance region at 440–460 nm. Fulcrand, Cheynier, Oszmianski, and Moutounet (1997) subsequently analyzed and verified the structural characteristics of these major colourless and yellow compounds, and proposed that a second browning pathway might exist: when iron was present, the oxidation did not happen on catechin directly, but on tartaric acid to give glyoxylic acid; once glyoxylic acid was formed, it would react with two flavanol units to form a colourless dimer linked through carboxy-methine bridge, the reaction mechanism resembling that of acetaldehyde-induced condensation (Fig. 4), and the colourless dimer would form yellow pigments by a dehydration followed by an oxidation process (Fig. 4(2)). Later, more studies confirmed the new browning pathway (Es-Safi, Le Guernevé, Cheynier,

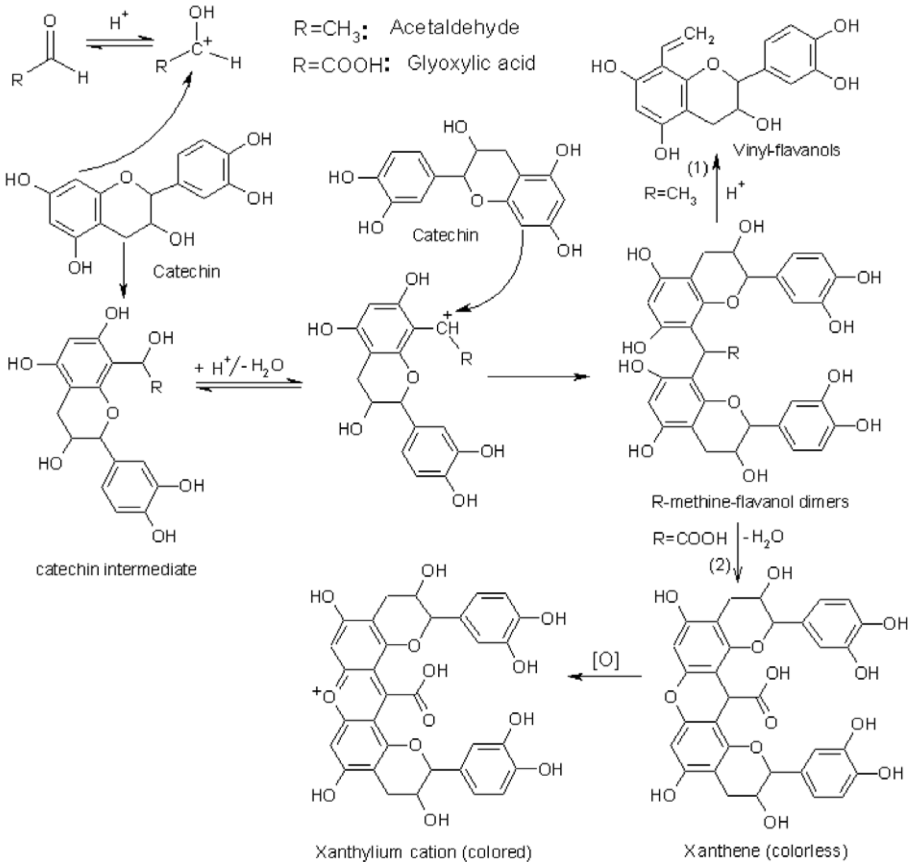


Fig. 4. Mechanisms of acetaldehyde-mediated and glyoxylic acid-mediated polymerization of flavanols (Fulcrand et al., 2006).

& Moutounet, 2000; Es-Safi, Le Guernevé, Fulcrand, Cheynier, & Moutounet, 1999). When copper existed, the same phenomena were found in model wines (Clark, Prenzler, & Scollary, 2003; Es-Safi, Cheynier, & Moutounet, 2003a). Considering the contents of iron and copper and tartaric acid in wine, the browning pathway may be an important one during wine aging (Fulcrand et al., 1997), but until now, the specific roles of metal ions in the formation of xanthylium cations are not clear. Clark et al. (2003) investigated the effect of copper on the reaction between (+)-catechin and glyoxylic acid in a white model wine. They proposed that copper increased the total levels of carboxymethine-linked catechin dimer and xanthylium cation pigment, which accelerated the bridging of two catechin units by glyoxylic acid, but no enhancement of copper was observed when acetaldehyde replaced glyoxylic acid in the reaction, which was probably due to the acid groups of glyoxylic acid. A current study in model wine suggests that (−)-epicatechin is slightly more reactive with glyoxylic acid than (+)-catechin, however, the xanthylium cations generated from (−)-epicatechin absorb twice as strong at 440 nm than those derived from (+)-catechin (Labrouche et al., 2005).

Besides glyoxylic acid and acetaldehyde, furfural and 5-(hydroxymethyl)furfural (HMF), which are sugar dehydration products formed during the processing and storage of grape-derived food, can also react with flavanols to form xanthylium pigments or black precipitates, and the reaction seems faster with furfural than with HMF (Es-Safi, Cheynier, & Moutounet, 2000). However, in the presence of anthocyanins, like in red wines, these aldehydes (acetaldehyde, glyoxylic acid, furfural and HMF) interact with anthocyanins to give more stable coloured compounds, which have been well documented in model solution and wines (Es-Safi et al., 2003b). But during wine storage and aging, different from the reactions induced by acetaldehyde, in which the involvement of flavanols seems to largely contribute to the stability of wine colour, the reactions caused by glyoxylic acid, furfural and HMF, are predominantly responsible for giving rise to colourless and yellowish compounds that are more important in browning and astringency decrease than in colour stability (Es-Safi et al., 2002).

3.2.3. Other possible non-enzymic browning mechanisms

In addition to the major non-enzymic browning involved phenols in wine, the following chemical mechanisms may also occur in winemaking. The main non-enzymic oxidation reactions occurring during grape juice heating or storage are caramelization and Maillard reaction, temperature affecting them most (Bozkurt, Göğüş, & Eren, 1999; Buglione & Lozano, 2002). Caramelization can occur with carbohydrates but requires higher temperatures than products would typically be subjected to during distribution and storage (Steele, 2004). In wine, caramelization occurs during production of baked sherry and during excessive pasteurization of sweet wines; similarly, browning overtones derived from caramelization may be present in red and white wines vinified from raisined berries (Zoecklein, Fugelsang, Gump, & Nury, 1995). Maillard reaction, a reaction involving condensation of reducing sugars with amino acids and proteins, which was discussed earlier, occurs in foods during processing and cooking, even during storage. To the best of our knowledge, although the Maillard reaction has been found in beer and other foods, there is little supportive evidence for its occurrence in wine browning (Main, 1992; Zoecklein et al., 1995). However, because wine contains the necessary Maillard reaction substrates such as amino acids, proteins, and reducing sugars, the possibility of its occurrence should not be overlooked (Zoecklein et al., 1995). Furthermore, certain conditions may promote Maillard reaction when wines are made using technologies such as thermovinification (heating the grapes) and high-temperature bottling (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Many recent studies indicate that a great number of volatile compounds responsible for typical aroma or aging aromas of some sweet natural wines seem to be linked with chemical like Maillard reactions between sugars and amino acids (Cutzach, Chatonnet, & Dubourdieu, 1998; Cutzach, Chatonnet, Henry, & Dubourdieu, 1999; Marchand, De Revel, & Alain Bertrand, 2002). On the other hand, the unsaturated lipids or complex lipids naturally present in wine contain unsaturated fatty acids (Lauretta, 1993), which are susceptible to oxidation by molecular oxygen. Vivas, Bourgeois, Glories, and Vitry (1995) reported that autoxidation of these unsaturated fatty acids in wine might lead to the formation of peroxides which were transformed into compounds causing off-flavours.

As addressed above, oxidative browning phenomena occurring in winemaking can be classified into enzymic browning and non-enzymic browning, and the most difference between them lies in the rate of quinone production or the reaction rate of browning taking place. Enzymic browning is much quicker, in which PPO is the initiator of enzymic oxidation reactions (Fernández-Zurbano et al., 1995; Robards et al., 1999; Toit et al., 2006), and the following polymerization reactions caused by quinones lead to browning pigments with higher molecular weight, although molar absorbance relies on the types of products (Fernández-Zurbano et al., 1995; Fulcrand et al., 1997). However, during the process of non-enzymic browning, the reactions of phenols oxidation to quinones are very slow due to the low pH value of wines, always depending on the presence of iron and copper, although they are quick in alkaline medium (Benítez, Castro, Antonio Sanchez, & Barroso, 2002; Lasanta, Caro, & Perez, 2005; Oszmianski et al., 1996). An early study showed that, when iron and copper were possibly removed from a wine with the use of potassium ferricyanide, the wine almost did not absorb oxygen, or did extremely slowly; if renewing copper and iron in the treatment wine, the specific consumption of oxygen was markedly increased (Zhu, Li, & Guo, 1983). Numerous recent literature studies have confirmed the conclusion that some transition metals especially copper and iron catalyzing the oxidation is of great importance during wine non-enzymic browning process (Clark & Scollary, 2002; Danilewicz, 2003; Es-Safi et al., 2003a; Lopez-Toledano et al., 2002; Oszmianski et al., 1996; Palacios Macias, Caro Pina, & Perez Rodriguez, 2001); without copper and iron, molecular oxygen is not active enough to directly oxidize the phenols in wine (Danilewicz, 2003; Peynaud, 1992). The "autoxidation" of wine seems to be the catalyzed result by transition metals.

4. Central roles of metals in non-enzymic oxidation of wine

4.1. Activation of oxygen in wine

A redox reaction is a process involving electron transfer from reducing species to oxidizing species, and whether the redox reaction can happen, or carry out completely, both depend on the difficulty of the electron transfer or on the potentials (E_H) of them. E_H is considered as an important criterion to evaluate a redox reaction occurring, reaction succession and performance level (Pi, 1987). Traditionally, standard potentials are described as potentials when pH value is 0 and solute molarity equals to 1, which are generally denoted by E_0. However, in order to compensate for effects from the ionic strength and side reactions on the E_H forms, researches propose to adopt conditional potentials (or formal potentials) to replace standard potentials in calculating the actual potentials (Pi, 1987). According to Nernst's equation, the potential depends not only on the proportion of oxidizing species to reducing species in a solution, but also on the pH, as well as the influence from temperature and ionic concentration (Li et al., 2005). In wine, there are lots of oxidation–reduction electron pairs, such as quinones–phenols, O_2–H_2O_2, O_2–H_2O, sulphate–sulphite, acetaldehyde–ethanol, acetate–acetaldehyde, pyruvate–lactate, fumarate–succinate, oxalacetate–malate, Cu^{2+}–Cu^{+}, and Fe^{3+}–Fe^{2+}, and pH may affect the E_H values of some pairs and change their stability (del Álamo, Nevares, & Cárcel, 2006).

The process of catechol oxidized to quinone, along with that of oxygen reduced to H_2O_2 and other reactive oxygen species, is shown in Fig. 5. At 298 K, pH 0 and pH 3.5,

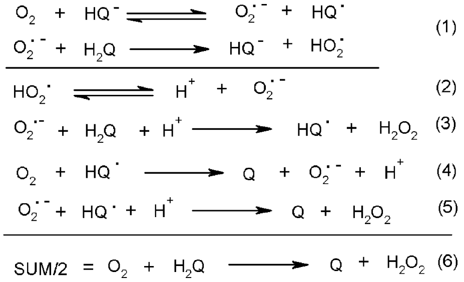


Fig. 5. Autoxidation of wine's catechols (H_2Q) (Main, 1992).

their corresponding reduction potentials are given in Fig. 6. At pH 3.5, the two electron potential of quinone–catechol is 0.58 V, O_2–H_2O_2 is 0.57 V, combining the two semi-reaction equations, as we can see, $\Delta E_{3.5} = -0.01$ V. According to free energy equation $\Delta rGm = -nF\Delta E$, the reaction free energy changes are: $\Delta rGm = 1.93\ \text{kJ mol}^{-1} > 0$, which means the reaction cannot occur in thermodynamics. The study on oxidation of caffeic acid and their derivatives also give the same results (Hapiot et al., 1996). This indicates that H_2O_2 cannot oxidize catechol directly under the above conditions, while it can be implicated indirectly because it is the precursor for the generation of $^{\bullet}OH$, which may be a strong initiator of wine oxidation. Danilewicz (2003) thought that the reason for it was due to the difficulty of the first reaction taking place (Fig. 5, reaction 1) in thermodynamics, owning to $\Delta E_{3.5} = -0.94$ V, $\Delta rGm = 90.70\ \text{kJ mol}^{-1} > 0$. Numerous literature reports also indicate that phenols in wine cannot react with oxygen directly, and the presence of iron and copper is necessary (Danilewicz, 2003; Fulcrand et al., 1997; Peynaud, 1992; Waterhouse & Laurie, 2006).

That direct reaction between oxygen and phenols cannot occur or extremely slow in acidic medium, is mostly due to its difficulty in thermodynamics, whereas some transition metals, particularly iron and copper, can change the reaction pathways, decrease the free energy needed by reaction, and increase the reaction rate (Dong, Li, & Lu, 2001), and they may play a great role in overcoming the initiation of the first reaction (Fig. 5, reaction 1) (Danilewicz, 2003).

4.2. Iron and copper ions in wine

In wine, transition metal ions such as iron and copper are widely present, whose contamination is dominantly related to soil and winemaking operation equipments (Lasanta et al., 2005; Pyrzyńska, 2004). With the growing use of stainless steel units, the levels of transition metals in wine are greatly decreased, and typical wines contain average iron levels at around 0–5 mg/L, copper at about 0.1–0.3 mg/L (Li et al., 2005). However, they may cause oxidative spoilage at the low concentration (Green, Clark, & Scollary, 1997; Lasanta et al., 2005; Robards et al., 1999). Besides their concentrations, the present forms and specification of copper and iron in wine are most probable factors leading to wine browning (Scollary, 2002). Ferric ion (Fe^{3+}) present in wine is not in free form but almost in complex form with other compounds, and ferrous ion (Fe^{2+}) can also form complex in wine, but the complex is easier to ionization (Zhu et al., 1983). That most iron is present in complex form in wine is important in understanding the chemistry of wine browning.

Complexation reduces the system pH because of releasing hydrogen ion (H^+), and with increased pH, it is promoted and the reduction potential of Fe^{3+}–Fe^{2+} couple is decreased (Yang, Wang, & Chen, 2003). Although the solubility of the complex compound of polyphenols associated with metal ions decreases with the substitution degree and may form precipitation, the initial pH to precipitate varies with metal ions (Yang et al., 2003). Under basic conditions, on the one hand, phenols are unstable and more readily to be oxidized to quinones with the loss of hydroxybenzene ligand, and on the other, iron ions largely form ferric hydroxide to precipitate through hydrolysis reactions in which its coordination linkage is broken (Yang et al., 2003).

In acidic aqueous solutions, although most Fe^{3+} and Fe^{2+} are present in hexaaquo complex form (i.e. $[Fe(H_2O)_6]^{3+}$ and $[Fe(H_2O)_6]^{2+}$, respectively), $[Fe(H_2O)_6]^{2+}$ only reacts with oxygen at a low speed due to the impossibility in thermodynamics (Danilewicz, 2003). However, water molecules are easy to be replaced by other stronger ligands such as some phenols bearing catechol, galloyl or 3-hydroxyl-4-carbonyl groups (such as chlorogenic acid, caffeic acid, gallate and protocatechuic acid), which can bind iron, the amounts and location of the hydroxyl groups on them also affecting the complexation (Andjelkovic et al., 2006; Khokhar & Apenten, 2003; Moran, Klucas, Grayer, Abian, & Becana, 1997; Yang et al., 2003). The ligand amounts of polyphenol–iron complex depend on pH: displacing one molecular water to give single ligand complex (FeL^+) with their two *ortho*-hydroxyl groups entering the coordination sphere in mono dissociation or double dissociation form; with the increase of pH, two and three water molecules can be substituted to produce FeL^- and FeL^{3-}, respectively, the pH range leading to the structural difference related to polyphenolic structure (Yang et al., 2003).

As some phenols chelate with Fe^{3+}, oxidation–reduction reactions also occur: phenols are oxidized to corresponding quinones, and Fe^{3+} is reduced to Fe^{2+} (Fig. 2). The lower the pH values of the media, the stronger the reducing power of the phenols (Danilewicz, 2003; Moran et al., 1997; Yang et al., 2003). Thus, complexation reduces the potential of Fe^{3+}–Fe^{2+}, to some extent, stabilizes the oxidation state of

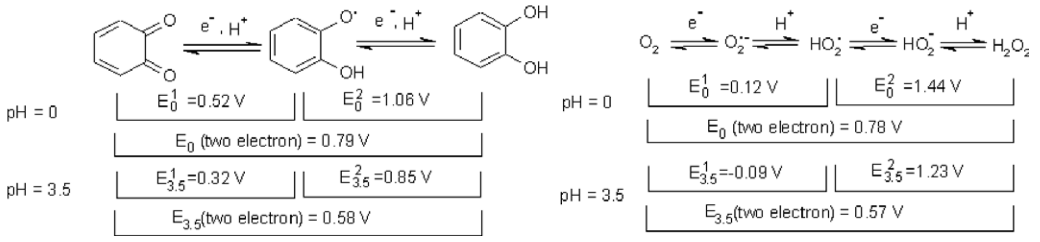


Fig. 6. First, second, and two-electron reduction potentials of the 1,2-benzoquinone/catechol system and the oxygen/hydrogen peroxide system (Danilewicz, 2003).

Fe^{3+} and increases the reducing power of Fe^{2+}, which indicates that the organic ligands present in wine ensure the fast oxidation of Fe^{2+}. As a result, in the presence of oxygen, oxygen is reduced to H_2O_2, and Fe^{2+} is reoxidized back to Fe^{3+}, which enhances the oxidation–reduction cycles of Fe^{3+}–Fe^{2+} or the mobility of iron in oxidation process (Danilewicz, 2003; Roginsky, Barsukova, Bruchelt, & Stegmann, 1997; Wensheng & Millero, 1996).

In addition, when iron ion as well as its complex compounds is present with H_2O_2 in a solution, the Fenton reaction takes place and produces powerful oxidant $^{\cdot}OH$. In acidic medium, the production of $^{\cdot}OH$ becomes easy, but due to the limitation of reactive conditions, iron (IV) may be formed in neutral medium (Danilewicz, 2003). Different concentrations of iron ion and H_2O_2 will produce different radicals; when chelating agents exist, the reactions above will change: some agents accelerate the Fenton reaction, but other ones may do the opposite (Zhao, 1999). On the other hand, copper can also react with H_2O_2 to produce $^{\cdot}OH$, which seems much more stronger than iron in the reaction; besides the production of $^{\cdot}OH$, some other oxidants may be involved (Hawkins & Davies, 1997). Trace copper would promote the catalyzing capacity of iron in wine (Peynaud, 1992; Zhu et al., 1983).

5. Conclusions

The advanced research on wine oxidative browning reveals that phenols' (especially vicinal diphenols) regenerative capacity in coupled oxidation with other compounds and their non-oxidative browning reactions constitute the major reason for wine browning. Enzymic browning almost entirely occurs in grape must, which has been thorough studied and can be controlled at present; non-enzymic browning prevails in fermented wine, essentially depending on the presence of iron and copper. It indicates that iron and copper may play a central role in the initiation of wine non-enzymic browning, and as possible, a better control of wine browning mainly depends on these metal ions. Although the critical hypothesis is not confirmed and has to be made sure by further research, it indeed has many important potential enological implications, especially to study antioxidant substitute for SO_2 in wine, which is very important to protect wine from browning and to decrease SO_2 content in final product. In addition, $^{\cdot}OH$ and other reactive oxygen species may be fundamental in fully understanding the oxidative chemistry of wine phenolics, which is in great need of further research and more debates on this subject.

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