Oxygen in Must and Wine: A review

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Submitted for publication: April 2006
Accepted for publication: May 2006
Key words: Oxygen, oxidation, wine, phenolic molecules

Oxygen can play an important role during the winemaking process. It can influence the composition and quality of the must and wine. Phenolic compounds are the main substrates for oxidation in must and wine. Oxygen addition leads to colour changes and the polymerisation of phenolic molecules in wine. Oxygen can, however, also influence the flavour and microbial composition of wine drastically, with certain off-flavours being formed and spoilage micro-organisms able to grow at too high oxygen additions to wine. A state-of-the-art, up-to-date review on the effects of oxygen in must and wine has, however, not been published recently. This review focuses on the effects of oxygen in must, during alcoholic fermentation, extended lees contact and during ageing of white and red wines. The effects it has on acetic acid bacteria and *Brettanomyces* are also discussed, as well as micro-oxygenation, a relative new technique used in wine production.

The atmosphere consists of approximately 21% oxygen (O_2). It plays a crucial role in many metabolic and chemical reactions on earth, thus it is of little surprise that it plays a very important role in the winemaking process. Wine can never be completely protected from it. The general use of sulphur dioxide as an anti-oxidant dates back to the early 18th century and the protection of wine from unwanted oxidative spoilage has been recognised (Ribéreau-Gayon *et al*., 2000a). Oxygen can influence the composition and quality of wine drastically, either positively or negatively, and this will be the focus of this review. This review will also focus on the basic steps involved in oxidation, substrates for oxidation in wine and the evolution of wine constituents during the wine production process when in contact with different concentrations of O_2.

Basic reactions of oxygen in wine

Oxidation is the process where electron transfer takes place between reductive and oxidative partners. In wine, O_2 is predominantly responsible for this, with it being reduced to certain intermediates and eventually to hydrogen peroxide and then water. Molecular O_2 exists as a diradical and is thus in a triplet ground state. This limits the reactivity of O_2 and it cannot form bonds by accepting electron pairs. However, the addition of a single electron, originating from reduced transitional metal ions, can overcome this limitation. This leads to an unpaired electron in the resulting negatively-charged superoxide radical, with a second electron transfer resulting in a peroxide anion (Miller *et al*., 1990; Danilewicz, 2003). This phenomenon results in O_2 being involved in various reactions in wine.

Substrates for oxidation in wine

Phenolic molecules originating from grapes can basically be divided into the non-flavonoids and the flavonoids. The non-flavonoids, which are hydroxybenzoic and hydroxycinnamic derivatives, originate from the grape juice, and are normally the principal phenolic molecules in white wines at concentrations ranging from 50-250 mg/L, depending on the cultivar, winemaking techniques, etc. Examples of non-flavonoids are the tartaric esters of caffeic acid, p-coutaric acid and furanic acids. These molecules have been shown to be the main phenolic molecules in white wine that did not receive prolonged periods of skin contact, because they occur at higher concentrations in the grape juice (Margalit, 1997; Monagas *et al*., 2005).

The second main group of grape-derived phenolics is the flavonoids. This group of molecules basically consists of two phenolic rings attached to a pyran ring. The flavanoids have a more complex structure than the non-flavonoids. In a young wine they are normally in a more unpolymerised state, but as wine matures they undergo different polymerisation reactions in which O_2 plays an important role. The most important flavonoids in wine are the anthocyanins, flavanols and flavonols. Anthocyanins occur mainly in the skins of red grape cultivars and are responsible for the colour of red wine. In young red wines their concentrations can range from 250 mg/L to more than 1000 mg/L. Different types occur in wine, depending on the -OH and -OCH_3 constitution of the B-ring of the molecule, and are esterified with glucose at the C3 position of the molecule. This leads to the occurrence in wine of cyanidin, peonidin, delphinidin, petunidin and malvidin-3-monoglucoside, which can also be acylated with a cinnamic acid derivate (Ribéreau-Gayon *et al*., 2000b; Monagas *et al*., 2005). Anthocyanins are amphotheric and pH influences their structure in wine. The positively-charged flavylium ion is mainly responsible for the red colour in a young red wine. It is in equilibrium with the chalcone (colourless to yellow), quinodal base (violet), carbinol pseudo-base (colourless) and bisulphate addition product (colourless) (Hrazdina & Franzese, 1974).

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The other important group of flavonoids in grapes and wine are the flavanols. These consist of flavan-3-ols (catechins) and flavan-3,4-diols. Different -H and -OH group substitutions on the C and B rings lead to different stereo-isomers being found, viz. (+)-gallocatehin, (-)-epigallocatechin, (+)-catechin and (-)-epicatechin, with the latter two occurring in concentrations of up to 200 mg/L in red wine. These molecules can associate through C4/C6 and C4/C8 bonds to form dimers, trimers and oligomers, and thus form procyanidins. Dimeric procyanidins can be divided into types A and B. Type A has interflavan C4/C6 and C4/C8 bonds, with ether bonds between the C5 or C7 carbon units of the terminal unit and the C2 carbon of the upper unit. Type B dimeric procyanidins are characterised by C4/C6 and C4/C8 interflavan bonds. Trimeric procyanidins are divided into Types C and D. Type C has two type B interflavan bonds, and type D has a type A and a type B bond. These molecules can polymerise further to form so-called grape tannins or condensed tannins, which can be classified according to the mean degree of polymerisation (mDP). These molecules are considered oligomers when the mDP is five to ten, and polymers when the mDP is greater than ten. The mDP for stems and pips is about 10, but about 30 for skins, indicating that the flavanoid molecules of skins are more polymerised than those of the pips and stems (Ribéreau-Gayon *et al*., 2000b; Herderich & Smith, 2005). Flavan-3,4-diols can also polymerise in a similar fashion (Monagas *et al*., 2005). These condensed tannins normally exist at 1-3 g/L in red wine and their concentration depends on the cultivar and wine-making techniques, such as skin maceration time, ageing procedures, etc. Other flavonoids that also exist in grapes and wine at lower concentrations are flavonols, such as kaempherol, quercitin and myricetin, which normally occur in white wine at 1-3 mg/L and in red wine at about 100 mg/L, as well as flavanonols (mainly taxifolin) (Ribéreau-Gayon *et al*., 2000b).

Phenolics also originate from the oak when wine comes in contact with it, mainly during ageing. This is the other main source of phenolics. These are mainly oak or hydrolysable tannins that contain a polyhydric alcohol of which the hydroxyl groups have been esterified with gallic acid or hexahydroxydiphenic acid. Hydrolysable tannins can easily be hydrolysed by acid, base, or enzymatically, to form gallic or ellagic acid. Ellagitannins can constitute up to 10% of the dry weight of the heartwood of oak. The most common ellagitannins are castalagin (isolated at up to 21 mg/L from oak-aged wine) and vescalagin (up to 7 mg/L). Additional ellagitannins identified in oak are roburins A-E and grandinin (Puech *et al*., 1999). These tannins normally exist in much lower concentrations in wine compared to their concentrations in oak, but this could be due to their involvement in oxidation processes during the ageing of wine that contribute to their breakdown (Vivas & Glories, 1996a).

The other main substrates for oxidation in wine are ascorbic acid, ethanol and tartaric acid. Ascorbic acid occurs naturally in grapes and it can also be added to wine. Tartaric acid normally occurs at 1-6 g/L in grapes and wine and ethanol normally at 9-15% v/v (Boulton *et al*., 1996).

The oxidation process

It is clear that phenolic molecules are quantitatively and qualitatively important constituents of wine, especially red wines. During oxidation molecular O_2 is reduced in a stepwise manner to $2H_2O_2$, which requires the addition of four electrons. This can be illustrated as follows:

$$O_2 + e^-, H^+ \rightarrow HO_2^{\cdot} + e^-, H^+ \rightarrow H_2O_2 + e^-, H^+ \rightarrow {}^{\cdot}OH\ (+H_2O) + e^-, H^+ \rightarrow (2)H_2O.$$

This leads to the formation of free superoxide (O_2^{-}) and peroxide (O_2^{2-}) radicals. These radicals can be directly reduced by phenolic molecules, and are better oxidants than O_2 (Singleton, 1987; Danilewicz, 2003). Wine phenols, however, exist in either the phenol or phenolate anion forms due to the acidic nature. Electron transfer takes place from the phenolate, leaving a free radical of semiquinone, which is further oxidised to the corresponding quinone. The quinone can thus be formed either from phenolate by molecular O_2 or ionic free O_2 (the intermediate between molecular O_2 and H_2O_2), or from the phenol. The semiquinone can partake in further radical reactions, due to the resonance stabilisation of the delocalised electrons in the ortho and para positions of the aromatic ring (Singleton, 1987; Margalit, 1997).

The constitution of the phenolic molecule will also determine its reduction potential. The phenoxyl radical will more commonly reside on the B ring of catechin than the A ring. The reduction power of a phenolic molecule is determined mainly by the ring constituents, with a lower reduction potential leading to greater reducing power of the reduced component. Electron donating groups (-OMe, -Me, vicinal -OH groups) lower reduction potential, but electron-withdrawing groups (-CO_2Et, -COMe) have the opposite effect. Methyl gallate is thus a weaker reducing agent than -(-)epi-gallocatechin due to its electron-withdrawing carboxylic-ester group. pH also influences this, due to the protonated carboxylic group being electron withdrawing at wine pH levels. This effect is negated at pH 7, where deprotonation takes place and where the reduction potential of methyl gallate becomes comparable to that of -(-)epi-gallocatechin. Malvidin-3-monoglucoside, with two -OMe groups on the B ring, exists mainly in equilibrium in wine: equilibrium exists between the positively-charged flavylium ion and the carbinol pseudobase, which does not have a charge on the C ring. In the carbinol pseudobase the electron-withdrawing -OMe groups on the B ring should make it a strong reducing agent, but an increase in the reduction potential in the flavylium ion is observed. This is due to the positive charge on the C ring of the flavylium ion, which acts as an electron withdrawing system, and which reduces the sensitivity of anthocyanins in the red form to oxidative degradation (Cheminat & Brouillard, 1986; Danilewicz, 2003). The number of O_2 atoms consumed per mole of phenol in white wine is about 5.5 times that in red wine. This is mainly due to malvidine derivatives which occur in high concentrations in red wine and which are not directly oxidisable with O_2 (Boulton *et al*., 1996). Quinones, being electrophiles, can also readily react with nucleophilic centres such as phenols, phloroglucinol, SO_3^{2-}, RSH groups, etc. In the same manner, two semiquinone free radicals can also bind by sharing the unpaired electrons in a shared-pair covalent bond. This process, called regenerative polymerisation, leads to the generation of a reoxidisable hydroquinone. This has a lower reduction potential than its original constituents and increases the O_2 capacity of wine (Singleton, 1987). It is thus no surprise that phenolics act as the principal oxidation substrate for O_2 in wine, with especially the vicinal-1,2-dihydroxyphenyl units readily reacting with O_2. These are found in abundance in hydrolysable and condensed tannins, for example. The total phenolic content of a wine can thus be an indication of its ability to consume O_2 (Boulton *et al*., 1996).

Ascorbic acid, which occurs naturally in grapes or is added during the wine production process, can also act as a substrate for oxidation in wine. In the process it reduces quinones back to the corresponding phenols (Peng *et al*., 1998; Bradshaw *et al*., 2001). It also undergoes two-electron oxidation. The ascorbate radical exists at wine pH mostly in the anion form. The latter loses a second electron to the quinone, and dehydroascorbic acid is formed. The oxidation rate decreases at lower pH levels, becoming very low below pH 2 (Danilewicz, 2003). Ethanol can also be oxidised in wine by the resulting H_2O_2 to form acetaldehyde. This can take place in the presence of SO_2 because ethanol occurs at relatively high concentrations in wine (Boulton *et al*., 1996). Acetaldehyde plays an important role in the polymerisation of different phenolic molecules during the ageing of wine (Dallas *et al*., 1996).

Iron, occurring normally in concentrations of a few mg/L in wine, plays an important role in these oxidation reactions. Fe^{3+} is reduced to Fe^{2+} by phenols during oxidation, but oxidised back to Fe^{3+} in the presence of O_2, until all the phenolic substrates have been consumed (Powell and Taylor, 1982). The addition of ferrous sulphate increases the oxidation rate of (+)-catechin, as found by Oszmianski *et al*. (1996). It is thought that Fe^{3+} acts as a catalyst to overcome the high activation energy in the initial thermodynamically unfavourable electron reduction step of the oxidation process (Miller *et al*., 1990). Fe^{3+} also catalyses the oxidation of ascorbic acid; two moles of Fe^{2+} are produced from one mole of ascorbic acid (Hsieh & Hsieh, 1997). It has also been observed that Fe^{3+} plays an important role in the oxidation of tartaric acid in wine. The overall oxidative process and the role of Fe^{3+}/Fe^{2+} here can be seen in Fig. 1. Fe^{3+} ions are thus required for the oxidation of the phenolic molecule and Fe^{2+} is required for the reduction of H_2O_2, which leads to the oxidation of ethanol to acetaldehyde. Cupric ions can catalyse the aerial oxidation of Fe^{2+}, with the resulting cuprous ions being re-oxidised by O_2. The main anti-oxidative activity of sulphur dioxide in wine is due to the bisulphite ion, which reacts with H_2O_2 to produce sulphuric acid, thereby limiting further oxidation of phenolic molecules or ethanol (Danilewicz, 2003). The use of sulphur dioxide in conjunction with ascorbic acid has been recommended in order to react with the H_2O_2 generated by the oxidation of ascorbic acid (Peng *et al*., 1998).

Factors affecting oxygen pick-up and consumption in wine

Winemaking operations

When wine is saturated with O_2 it contains about 6-8 mg/L O_2 at cellar temperatures. During the normal wine production process wine comes into contact with air, which can result in different O_2 concentrations dissolving in the wine. Must can be almost saturated with O_2 during the crushing and pressing of fresh grapes (Schneider, 1998). How much O_2 dissolves into the wine during fermentation when a pumping over is applied is debatable, because the evaporating CO_2 probably sparges O_2 out of the wine (Boulton *et al*., 1996). Subsequent winemaking operations add more O_2 to the wine. These operations include: pumping (about 2 mg/L), transfer from tank to tank (up to 6 mg/L), filtration (4-7 mg/L), racking (3-5 mg/L), centrifugation (up to 8 mg/L), bottling (0.5-3 mg/L) and barrel ageing (20-45 mg/L/year). During barrel ageing the humidity of the wood and the thickness and the grain of the staves all play a role. Lower humidity, tight grain and thinner staves all allow more O_2 to permeate into the wine. In very dry wood of 20 mm thickness it can be up to 0.1 ppm/h, which can lead to oxidation (Vivas *et al*., 2003). Wine is, however, seldom saturated with O_2, due to insufficient contact or the exclusion of air during the production process. The temperature of the wine also influences the dissolved O_2 saturation level, with higher concentrations dissolving at lower temperatures. At temperatures of between 5 and 35°C the amount of O_2 necessary to saturate wine drops from 10.5 mg/L to 5.6 mg/L. The rate of quinone formation, however, increases with an increase in temperature, although the kinetics of this reaction is temperature independent (Margalit, 1997; Ribéreau-Gayon *et al*., 2000b; Vivas de Gaulejac *et al*., 2001). Oxygen can also be introduced in a controlled manner to wine by a process called micro-oxygenation, which will be discussed later in more detail. The contact of wine with O_2 can be minimised by the use of inert gasses, such as N_2, CO_2 and even argon gas, which can displace the air in a tank or barrel.

The addition of SO_2 can also influence the rate of O_2 consumption. The free sulphur dioxide in wine comprises the molecular, bisulphite and sulphite forms. The O_2 consumption rate in must declines drastically with the addition of SO_2. This is because the SO_2 does not have an anti-oxidative effect in must, but rather inhibits oxidation enzymes. In wine, however, chemical oxidation occurs, and it is mainly the sulphite form of SO_2 that can react with O_2, but it is still slow under winemaking conditions such as low pH and high ethanol levels. The ascorbate-oxygen reaction is almost 1700 times faster than that between SO_2 and O_2. First-order kinetics suggests that 4 mg/L SO_2 reacts with 1 mg/L O_2. The molecular form of SO_2 can also react with H_2O_2 that is formed from the oxidation of phenolic molecules. There seem to be surprisingly few kinetic studies on the interaction between O_2, H_2O_2 and SO_2 (Boulton *et al*., 1996; Ribéreau-Gayon, 2000a).

pH

Wine phenols exist in either the phenol or phenolate anion forms. The negative charge of the phenolate anion is delocalised via the benzene ring from the oxygen atom to the ortho and para posi-

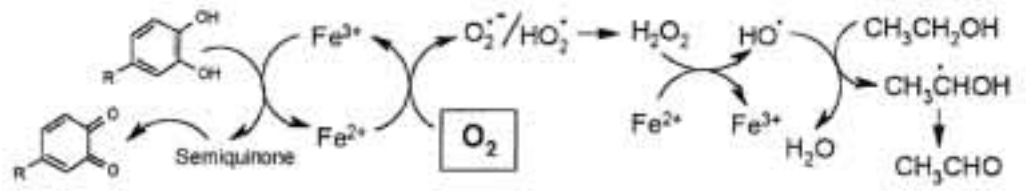

FIGURE 1

The overall oxidative process in wine and the central role of iron (Danilewicz, 2003).

tions, lending 8 kcal resonance stabilisation to the phenolate anion compared to the phenol. However, at wine pH (pH 3-4), very few of the phenolic molecules, with a pK_a value of 9-10, are in the phenolate form, but the major influence that pH has on this is clear, with 10 times more phenolate existing at pH 4 than at pH 3. During oxidation, removal of the phenolate anion will lead to its replacement due to equilibrium. Oxidation, when an electron is removed, is much easier from the phenolate anion than from the protonated phenol.

Phenolic molecules also differ in their susceptibility to high pH, with caffeic acid and gallic acid becoming less stable towards degradation at high pH, and with (-)-epicatechin and (+)-catechin being much more resistant. The structures of the latter two molecules are not planar and the p electrons of the two benzene rings cannot interact with one another due to conjugation. The spatial arrangements of the -OH groups and the p electrons influence the extent of p orbital overlap and consequently its susceptibility to chemical change. Care should therefore be taken especially when handling white wines with high pH because these are more susceptible to oxidation. They contain caffeic acid derivates as the main phenolic molecules (Cilliers & Singleton, 1990b; Cilliers & Singleton, 1990c; Boulton *et al*., 1996; Friedman & Jürgens, 2000). Cilliers and Singleton (1989) found that the amount of phenol consumed per phenol unit at wine pH was about 1.4 to 18 times higher than under alkaline conditions. The rate of the non-enzymatic auto-oxidation of caffeic acid is also enhanced by increasing pH and temperature. Although the oxidation of ascorbic acid by O_2, which is catalysed by Fe^{3+}, increases with an increase in pH, the reduction of Fe^{3+} by ascorbic acid decreases, with the reaction ceasing at neutral pH (Danilewicz, 2003). Wine thus consumes much more O_2 under slow, acidic conditions than under fast, alkaline conditions.

Phenolic concentration and composition

The phenolic concentration of wine is an indication of its capacity for O_2, with higher phenol-content wines being able to accommodate higher concentrations of O_2. The removal of phenolic compounds from wine with fining reduces the wine's capability to react with O_2. It is calculated that a young, full-bodied red wine can consume 2.4 g or more O_2 under slow acidic conditions (as would happen during barrel ageing when O_2 is added over a long period of time), which is more than its own volume in O_2 or 5-10 L of air (Singleton, 1987; Boulton *et al*., 1996). Winemaking practices that lead to higher phenolic concentrations, such as skin contact, hard pressing and barrel ageing of wine, should lead to a higher capacity of this wine for O_2.

Numerous studies have reported on the autocatalytic effect of forced oxidation in a wine based medium, with O_2 consumption increasing when two different types of phenolic molecules are involved. The process of regenerative polymerisation, where slow oxidation leads to previously non-oxidisable moieties being incorporated into a re-oxidisable hydroquinone, also leads to an increase in the oxidisable substrates of a wine. However, this seems to be a relatively slow process. The resulting dimeric product has a lower redox potential than its original constituents, and thus buffers the latter against oxidation. At lower pH levels the lower concentrations of phenolate anions will all have time to participate in the regenerative polymerisation reaction to form re-oxidisable hydroquinones with quinones. At high pH levels and when O_2 is added at a fast rate it is not long before no phenols remain to react. This is reflected in the fact that when forced oxidation of wine takes place, browning of the wine follows an autocatalytic pattern, with an initial lag phase. This is due to the dimeric product having a lower redox potential, as mentioned earlier, with two dimeric oxidised semi-quinones reacting with each other to form a tetramer, etc. This process can take place until the molecule becomes too large, and precipitates (Singleton, 1987; Boulton *et al*., 1996). Cilliers and Singleton (1990a) reported that one molecule of caffeic acid consumed 3.4 atoms of O_2, and this increased to 4.9, 5.5 and 8.5 when phloroglucinol, cysteine and glutathione were added, respectively. The association between catechin and caffeic acid and the addition of cysteine and glutathione increased this further to 13.2 and 19.2 after 9 h. Both cysteine and glutathione act in generating a re-oxidisable product by reducing the quinone back to a caffeic acid and by substituting the quinone to regenerate the hydroquinone form of 2-S-cysteinyl caffeic acid or 2-S-glutathionyl-caftaric acid (Bassil *et al*., 2005). Depletion of glutathione and cysteine leads to quinone formation and browning. The addition of ferrous sulphate and Fe^{2+} to a model wine solution increased the oxidation of (+)-catechin by O_2 and the rate of O_2 consumption (Vivas *et al*., 1993; Oszmianski *et al*., 1996). Ellagic tannins have a much higher capacity for O_2 consumption than condensed tannins do. The rate of O_2 consumption is also faster in the case of ellagitannins, due to more vicinal ortho -OH groups. When ellagic tannins and condensed tannins are added together, the O_2 consumption rate initially increases dramatically, possibly indicating a competition for the O_2 (Vivas & Glories, 1996b).

Desirable levels of oxygen in different wines

Boulton *et al*. (1996) reported on different levels of O_2 required for certain wine styles. In white wine, about 10 saturations led to the wine becoming oxidised, but it is well known that even fewer additions may lead to a reduction in the fruitiness of wine. Ten saturations led to the minimum concentration necessary to obtain a standard sherry. Red wines differ widely in their capacity, but normally improve with up to 10 saturations (60 mL/L), with others showing improvement even up to 25 saturations (150 mL/L). A value of 10 saturations (or about 60-70 mg/L) is in line with the total amount of O_2 that a red wine can receive in a year because a few rackings and other winemaking procedures can contribute about 20 mg/L, and the barrel ageing regime about 40 mg/L O_2 per year (Vivas *et al*., 1999a; 1999b; 2003).

Oxygen addition in must, enzymatic oxidation and hyperoxidation

During the crushing, pressing and other processing steps, O_2 comes into contact with the grape juice, leading to the enzymatic oxidation of phenolic molecules. For this to occur the oxidation enzyme, O_2 and the phenolic substrate must be present. The polyphenol oxidases of healthy grapes are known as tyrosinase, cresolase and catechol oxidase, with laccase occurring in *Botrytis* infected grapes. The latter enzyme is considered a more dangerous enzyme by the winemaker because it is more resistant to SO_2 and has a wider substrate oxidation spectrum. It is not inhibited to the same degree by its oxidation products, as is tyrosinase. Laccase is more active at the low pH values of must and alcohol levels in wine, than tyrosinase. The rate of browning and O_2 consumption is, however, not much different in juices prepared from healthy or rotten grapes (Schneider, 1998; Ribéreau-Gayon *et al*., 2000a).

The main substrates for these oxidation enzymes are the cinnamic acid derivatives, with caftaric acid and coutaric acid occurring at an average of 106 and 10 mg/L respectively in protected white juices (Singleton *et al*. 1984; Cheynier *et al*., 1989b). Caftaric acid concentration can also differ dramatically between cultivars, ranging from 40 to 400 mg/L (Singleton *et al*., 1986). These derivatives occur mainly in the liquid part of the grapes, with flavanoid based phenolics (mainly catechin and condensed tannins) being dominant in the skins, stems and pips. When juice and wine contain higher concentrations of these flavanoids, they also become more susceptible to oxidation and subsequent browning (Schneider, 1998). During oxidation, caftaric acid is oxidised to its corresponding quinone by tyrosinase. Glutathione, with a mercapto group, has a nucleophilic centre to substitute into the electrophilic ring of the quinone, leading to regeneration of the vicinal dihydroxy ring of the caffeic acid (Singleton *et al*., 1985). The product, 2-S-glutathionyl-caftaric acid or Grape Reaction Product (GRP) is no longer a substrate for further oxidation by tyrosinase. Laccase can, however, due to its wider substrate specificity, further oxidise the GRP, with a second addition of glutathione, if available, leading to the formation of GRP2. It does not seem as if laccase can further oxidise the GRP2 under winemaking conditions (Singleton *et al*., 1985; Cheynier *et al*., 1986; Cheynier & Van Hulst 1988; Boulton *et al*., 1996). Depletion of glutathione and other nucleophiles, which can serve the same role, leads to browning, and the use of cysteine to protect against oxidation should be investigated further. The glutathione to caffeic acid ratio should give an indication of the susceptibility of a certain cultivar to oxidation. This ranges from 1.3 to 12.7 and 0.6 to 10.5 in berries and musts, respectively. Musts can also be divided into three groups according to their hydroxycinnamic acid content, with higher concentrations leading to browner colour. A hydroxycinnamic acid to glutathione ratio of 0.9 to 2.2, which leads to lightly coloured oxidised must, causes the rapid formation of GRP and high levels of GRP, due to the availability of sufficient glutathione. In medium coloured juices (with a ratio of 1.1 to 3.6) GRP is formed with caftaric acid, and GRP-o-quinone reacts further when glutathione exhaustion has taken place. Small amounts of GRP2 are also formed here. A ratio of 3.8 to 5.9 leads to dark coloured musts, due to glutathione being depleted by the high caftaric acid o-quinone concentration before GRP2 can be formed. This could explain the difference in sensitivity of different musts to oxidation. No correlation between sugar concentration and the ratio could be found (Singleton *et al*., 1985; Cheynier *et al*., 1989a; Boulton *et al*., 1996; Margalit, 1997). After depletion of the glutathione, the caftaric acid quinone can oxidise GRP and flavanols, and be reduced back to caftaric acid. It can also polymerise with caftaric acid to regenerate a re-oxidisable phenol. The kinetics of degradation differs between flavanoids, with procyanidin B2 disappearing relatively quickly compared to catechin, but the rate of oxidation between laccase and catechol oxidase did not differ significantly (Oszmanski *et al*. 1985; Schneider, 1998). Cheynier *et al*. (1988) found caftaric acid, catechin, epicatechin and epicatechin gallate undergo 70, 50, 46 and 46% decreases respectively after 2 h of oxidation by grape polyphenol oxidase. When the flavanoids were oxidised with caffeic acid their oxidation rate increased, but the condensation reaction of catechin with caftaric acid was still slower than when trapped by glutathione. Catechin also increases the oxidation rate of procyanidin dimers and GRP, but not to the same degree as caftaric acid. Caftaric acid is thus enzymatically oxidised to its quinone, with the consumption of half an atom of O. Catechin is either oxidised to its corresponding quinone in the same manner, with the consumption of one O atom, or by coupled oxidation, by reducing the caftaric acid quinone. The caftaric acid o-quinone with catechin or the catechin o-quinone with caftaric acid can then form a condensation product with a lower redox potential than its monomer constituents and can hence be further oxidised (Cheynier *et al*., 1988). In a subsequent study, however, Cheynier and Ricardo da Silva (1991a) found that polyphenol oxidase did not degrade procyanidins alone but, in the presence of caftaric acid, the oxidative condensation of the galloylated procyanidins proceeded more quickly than the oxidative condensation of non-galloylated procyanidins. This degradation was also influenced by pH, with the nucleophilic addition of a phenolic ring on a quinone occurring between (+)-catechin and its oxidation products occurring at high pH, and semi-quinone radical coupling occurring at low pH. The colour of these products differed; they were colourless at pH <4 and yellow at high pH. Their interflavanic bonds also differed from the original monomer (Guyot *et al*., 1995; Monagas *et al*., 2005).

During red winemaking, when an oxidative environment may prevail, and under low glutathione and high hydroxycinnamic acid concentrations, anthocyanins can react with caftaric acid quinones, leading to oxidation of the latter through coupled oxidation or condensation reactions. The latter reaction takes place when the nucleophilic C6 or C8 carbon undergoes a condensation reaction with the electrophilic quinone. o-Diphenolic anthocyanins, like delphinidin and petunidin-3-glucoside, usually react rapidly, but malvidin-3-glucoside reacts more slowly due to its condensation with quinones (Monagas *et al*., 2005).

These phenomena of regenerative polymerisation contribute to the ability of the must to accommodate higher concentrations of O_2 than expected, but the O_2 accommodation of different musts can differ drastically, ranging from 0.5 to 5 mg/L/min. The consumption of O_2 by tyronase is very fast, ranging from 30 to 200 mg/L, with 10-15 mg/L being taken up during whole-bunch crushing. The uptake is also faster initially, but decreases as the phenolic substrate is depleted, with laccase, if present, increasing the total uptake further (Cheynier *et al*., 1993; Schneider, 1998; Ribéreau-Gayon *et al*., 2000a).

The winemaker must apply certain winemaking techniques to prevent oxidation of must during the production process. Oxygen can be excluded by using inert gasses such as N_2 or CO_2 in presses, pipes and tanks. Oxidation enzymes can also be inhibited by the addition of SO_2. Up to 90% decrease in the activity of tyrosinase has been observed upon the addition of 50 mg/L SO_2, but higher dosages are necessary to effectively inhibit laccase. SO_2 also reduces caftaric acid quinone and enhances the solubility of phenolic molecules. Settling of juice decreases the activity of tyrosinase because it is largely associated with the solid parts of the grape berry. Bentonite fining has also been found to do this, with 100g/hL leading to a 30% loss in activity, but it also removes glutathione. Heating of the must to 45 and 65ºC will destroy tyrosinase and laccase respectively (Schneider, 1998; Ribéreau-Gayon *et al*., 2000a).

Another strategy to prevent oxidation is to limit the phenolic substrates available for oxidation, especially the flavanoid con-

tent, by soft pressing, no skin contact and removal of stems. A process called hyperoxidation, where large quantities of O_2 are added to the must, can also achieve this (Schneider, 1998). The latter leads to the oxidation of phenolic molecules, which settle, and the juice can then be removed from the precipitate by racking, with no SO_2 added to the must at crushing. To achieve this, O_2 is pumped either in line, while the juice is circulated in the same tank, pumped from tank to tank, added with a diffuser in the juice, or used instead of N_2 when using flotation. Juice that did not receive any skin contact can thus be treated with one saturation, but up to three saturations are necessary to remove sufficient flavanoid molecules from juice that did have skin contact. It is imperative that the subsequent clarification is done efficiently before fermentation starts because the precipitate can redissolve in alcohol. The reductive conditions during alcoholic fermentation and adsorption to yeast cells reduce the brown colour further (Schneider, 1991, 1998).

It is unknown whether must hyperoxidation contributes to the quality of wine. It is however clear that bitterness and astringency decrease markedly with O_2 addition, and that this difference becomes greater during ageing of the wine. These wines are obviously also less susceptible to unwanted browning. In different studies, the aromas of Chardonnay, Riesling, Faberrebe and Parellada were considered more intense in the treated wines. This was more pronounced in the juices that received skin contact before the treatment. This could be due to an increase in fatty acids and esters. Other studies, however, showed a decrease in aroma quality, with more vegetative aromas being formed, possibly due to C6 aldehydes and alcohols being formed under these conditions. The addition of even H_2O_2 to wine did not decrease the methoxypyrazine level of white wine (Singleton *et al*., 1980; Cheynier *et al*., 1991b; Marais, 1998; Schneider, 1998). Non-volatile flavonoids can, however, indirectly influence the aroma of wine, by yielding acetaldehyde from ethanol during coupled oxidation (Schneider, 1998).

Phenolic molecules can also be removed with fining agents such as PVPP, gelatine and activated charcoal. Charcoal-treated juice made from very rotten Sauvignon blanc grapes had a less intense brown colour than the control (Du Toit, 2003).

Oxygen addition during alcoholic fermentation and malolactic fermentation

Completion of the alcoholic fermentation is a crucial step in the winemaking process. During this process yeast transforms sugar into alcohol, CO_2 and energy, and produces flavour compounds such as fatty acids, esters, higher alcohols etc. If this fermentation is not completed successfully then spoilage micro-organisms can use residual sugar to spoil it. Numerous causes for stuck/sluggish alcoholic fermentation have been identified, which include high sugar, low nitrogen, thiamine depletion, excessive clarification, pesticides and a lack of O_2 (Bisson, 1999). Yeast cells need O_2 to produce sterols and unsaturated fatty acids that play a key role in the fluidity and activity of membrane associated enzymes, which influence ethanol tolerance, fermentative capability and viability of yeast (Valero *et al*., 2001). A dosage of 5 mg/L O_2 is optimal to achieve this when added at the end of the cell growth phase, but when 1 mg/L was added the relative increase in CO_2 production ranged from 10 to 41% between strains. By combining this addition with ammonia addition at the halfway mark of fermentation, it reduced the fermentation by up to 50% in problem fermentations. The maximum O_2 consumption rate was also found to take place at this time (Sablayrolles *et al*., 1996; Julien *et al*., 2000). Yeast also assimilates more nitrogen when it is supplied with O_2, but strain differences in fermentation efficiency exist in the absence of O_2. When added before the halfway mark of fermentation, O_2 is assumed to be used in mitochondrial development, ring cleavage of proline and respiration, despite the high sugar content of must. Salmon *et al*. (1998), however, found that the superfluous O_2 consumption rate during the growth phase of yeast was probably due to mitochondrial alternative respiratory pathways and that O_2 dependent ergosterol biosynthesis accounted for less than 15% of the total O_2 consumption at the beginning of the stationary phase. Blateyron *et al*. (2003) found that the addition of 5 mg/L O_2 to fermenting must did not affect the sensory characteristics of the wine compared to the control, but the addition of an excess (50 mg/L) did decrease the quality, with an increase in brown colour. In the absence of O_2 medium chain fatty acids, especially hexanoic, octanoic and decanoic acids, accumulate in the yeast and can be secreted into the wine, contributing to sluggish/stuck fermentations (Bardi *et al*., 1999). Oxygen has also been found to be depleted from different musts within 2.75 to 4.25 h from the start of fermentation. Its addition may in future serve as a means of proline utilisation by yeast under fermentative conditions (Poole *et al*., 2002). Buescher *et al*. (2001) were able to induce *S. cerevisiae* strain L2226 to produce up to 20.96% alcohol when the yeast fermentation was supplied with O_2 during the first 48 h and nutrients were added together at the start of fermentation. Only 17.89% alcohol was produced when no O_2 was added. Non-*Saccharomyces* yeast strains can also contribute to the complexity of the wine, by producing certain metabolites. *Torulaspora delbrueckii* and *Kluyveromyces thermotolerans* survived longer during fermentation with *S. cerevisiae* in O_2 rich must (Holm Hansen *et al*., 2001). The addition of O_2 can be used by the winemaker to ensure a complete fermentation, especially in countries such as South Africa, where grapes have relatively high sugar concentrations. The addition of O_2 to the must also leads to the production of higher concentrations of esters and higher alcohols by *S. cerevisiae* and *S. capensis* (Valero *et al*., 2002). Oxygen can be supplied in large-scale fermentations by sparging air through the tank. This will also help to keep the yeast in suspension.

Certain wines, especially certain white varieties from the Loire valley, Burgundy and Champagne in France and other wine producing countries, are often matured on the yeast lees after fermentation. During this period of time the inactivated yeast undergoes a process called autolysis, which is defined as the hydrolysis of intracellular endohydrolases activated upon cell death. During autolysis the yeast releases different nitrogenous compounds, lipids and polysaccharides into the wine. This process is believed to contribute to the fuller mouth feel and aroma of these wines, while absorbing volatile thiols and anthocyanins. Autolysis can also contribute to a wine's protein and tartaric stability by releasing mannoproteins. O_2 can be introduced during this period by opening the barrels, transfer of the wine and through a process called battonage, where the lees is stirred periodically in order to mix it uniformly (Fornairon-Bonnefond *et al*., 2003). It has been observed that yeast lees has a capacity to con-

sume this O_2, with rates ranging from 3 to 11 µg O_2 h^{-1} 10^{-9} from the second month to the sixth month of lees contact. Specific uptake rates also differ between strains, with 100, 50, 42 and 11% of initial O_2 concentrations remaining in white wines for strains Su6, Uvaferm, L2898 and VL1, respectively, after 3000 h of yeast lees contact. Production of biomass peroxydes is directly linked with O_2 consumption by yeast lees, with Cu^{2+} additions, serving as auto-oxidation catalysts, increasing this rate. Cell viability of yeast lees decreased faster in the presence of O_2, but it did not affect the release of amino acids. These reactions lead to ergosterol levels being reduced in the yeast cell walls, with the formation of 9(11)-dehydro-ergosterol, 5α,6α-epoxy(22E)-ergosta-8,22-diene-3β,7α-diol or ergosterol epidioxide (Salmon *et al*., 2000; Fornairon-Bonnefond & Salmon, 2003). Yeast has a stronger capacity for absorbing O_2 than polyphenols, in the same order as 9 g/L of polyphenols, which is higher than the polyphenol concentrations normally found in wine. However, yeast lees and polyphenols in combination had a much lower capacity of O_2 consumption than the theoretical sum of this capacity when tested alone. This is due to the capacity of the yeast lees being reduced drastically after contact with polyphenols. This is probably because of a collapse of cytoplasmic intermembrane space, which lowers the accessibility and reactivity of O_2 towards the sterols and unsaturated fatty acids of the membranes. The initial slight decrease and later increase in the capacity of the polyphenols could be due to adsorption on the lees yeast, with gradual release from the lees. The adsorption by the lees of polyphenols follows biphasic kinetics, with no preference for low or high polymeric-size tannins, although epigallocatechin units were adsorbed more by the yeast (Salmon *et al*., 2002; Mazauric & Salmon, 2005). Therefore yeast lees plays a very important role in the reduction/oxidative potential of wine.

During red wine production the effective mixture of skins with the must is required for extraction of anthocyanin and tannins from the skins. Pre-fermentative O_2 addition to red must during skin contact resulted in lower concentrations of red pigments, anthocyanins, caftaric acid and total phenols. The concentrations of total tannins and anthocyanins after six months' storage were 1220 and 192 mg/L, respectively, in the control, compared to 679 and 150 mg/L in the must, to which most O_2 was added. Wines made from the treated musts had more aged characteristics, such as more polymerised colour and a higher colour hue (Castellari *et al*., 1998). Pumping over in comparison to punch down and rotor tanks may also lead to lower extraction of polyphenols (Marais, 2003), although this could be due purely to this being a softer extraction technique, as Italian researchers did not find any significant difference in polyphenol concentrations after O_2 addition during fermentation. It is not known how much O_2 is taken up by the yeast, reacts with polyphenols, or simply evaporates with the CO_2 during a red wine fermentation. More research on this is clearly necessary.

The addition of O_2 during fermentation has also been found to affect the subsequent malolactic fermentation. Aeration led to a hundred-fold lower level of lactic acid bacteria than in the anaerobic control after alcohol fermentation, but the former lactic acid bacteria numbers increased more rapidly to 10^8 cfu/mL, compared to 10^7 cfu/mL in the anaerobic treatment. In the aerated treatment where no temperature control was induced during alcoholic fermentation malic acid was consumed the fastest. This could be ascribed to differences in alcohol levels after fermentation (12 and 13% for the aerobic and anaerobic treatments, respectively) that led to different cell counts (Reguant *et al*., 2005). Such a significant difference in alcohol levels is uncommon because high concentrations of O_2 should be sparged off during fermentation by the resulting CO_2 release. Oxygen during malolactic fermentation can also influence the sensory characteristics of wine, especially in Chardonnay where diacetyl contributes to the typical buttery aroma of these wines. Oxygen enhances the conversion of a-acetolactate to diacetyl, with 12 mg/L being produced under semi-aerobic conditions compared to 2 mg/L under anaerobic conditions, however, this was consumed again by the bacteria. Cell growth, malic and citric acid degradation did, however, differ significantly between the semi-aerobic and anaerobic conditions. Limited exposure to air during malolactic fermentation could thus enhance diacetyl production, but this should be followed by SO_2 addition and filtration to avoid subsequent consumption by yeast and lactic acid bacteria (Nielsen & Richelieu, 2000; Bartowsky & Henschke, 2004). The general effect of O_2 on lactic acid bacteria during commercial winemaking is, however, not well understood and should be investigated further.

Effect of oxygen during ageing of wine

Effect of oxygen on white wine colour

The colour of white wine is an important quality parameter. The colour of a young white wine normally has a slight yellow or greenish tint, with white wine that has been aged in barrels achieving a deeper yellow. A brown colour is normally unwanted because this indicates oxidation in white table wine. Brown colour is normally measured at 420 nm in white wine. As previously discussed, brown colouration can be induced by enzymatic oxidation. These enzymes are however normally not very active in wine because their precipitation during alcoholic fermentation occurs and alcohol inhibition of these enzymes takes place in wine. Hence, browning in white wine is a chemical process that is slower than enzymatic-induced oxidation. Browning in white wine can be due to three mechanisms. The first is the oxidation of phenolic molecules to their corresponding quinones, in varying degrees of polymerisation, producing a yellow-brown colouration. This oxidation reaction is influenced by the copper and iron concentrations. The second mechanism is the oxidation of tartaric acid to glyoxylic acid, which leads to the condensation of phenolic molecules due to the glyoxylic acid acting as a bridge between phenolic molecules. Varying degrees of polymerisation of the latter can also contribute to the yellow-brown spectrum. Acetaldehyde, produced during coupled oxidation or fermentation, can also enhance the yellow colour by inducing the condensation of phenolic molecules (Es-Safi *et al*., 1999c; Lopez-Toledano *et al*., 2004; Monagas *et al*., 2005).

The chemical mechanisms involved in the oxidation of phenolic molecules to quinones have been discussed earlier, and only those involved in the oxidation of white wine *per se* will be mentioned. The main phenolic molecules occurring in white wine that do not receive extensive skin contact or are not aged in oak barrels are the hydroxycinnamic acid derivatives. However, caftaric, coutaric, ferulic and caffeic acid do not seem to play a major role in the browning of white wine because there is little correlation

between their concentrations in white wine and susceptibility to browning. A good correlation does however exist between flavanols and browning sensitivity, especially with (+)-catechin, (-)-epicatechin and dimeric procyanidins B1-B4. The hydroxycinnamic acids may, however, contribute to the browning by being involved in coupled oxidation reactions with these flavanols, as discussed earlier (Simpson, 1982; Fernández-Zurbano *et al*., 1995). Flavanols also differ in their sensitivity to oxidative degradation. Jorgensen *et al*. (2004) found that skin procyanidins degraded faster than those from seeds, with flavan-3-ol monomers slowing the degradation of seed procyanidins. After 21 h of oxidation under mildly basic conditions, skin procyanidins, seed procyanidins alone, and seed procyanidins with the added monomers declined to 11.8%, 25.1% and 28.2% respectively. This was also reflected in the rate of degradation of these three substrates. The degradation rates of individual subunits also differ, with (-)-epigallocatechin being degraded faster than (-)-epicatechin. The former constitutes the major part of skin procyanidins, which explains the faster degradation of the skin fraction. It is clear that winemaking techniques that influence the procyanidin concentration in wine also affect sensitivity to browning. The pressing method, skin contact, skin contact time, pasteurisation of the juice and cultivar affect the procyanidin concentration of grape juice. Elvira and Chardonnay were found to have high concentrations of catechins and Seyval and Niagara to have high procyanidin concentrations, especially of B1 and B2 (Fuleki & Ricardo-da-Silva, 2003).

The second pathway of browning, relatively recently described, is that induced by the oxidation of tartaric acid, which yields glyoxylic acid. This acts as a bridging mechanism between flavanol molecules. The oxidation takes place in the presence of catechin and either Fe^{3+} or Cu^{2+}. The resulting colourless or yellow products absorb at a maximum between 440 and 460 nm. In these reactions (+)-catechin reacts with glyoxylic acid to produce a (+)-catechin/glyoxylic acid adduct, which reacts with a further (+)-catechin molecule to form a carboxymethine-linked (+)-catechin dimer. This carboxymethine bridge can form between C8-C8, C8-C6 or C6-C6 of the (+)-catechin units. Dehydration of the dimers forms xanthenes, which can undergo oxidation to form xanthylium salts. These salts have a yellow colour and a maximum absorption at 460 and 440 nm for the esterified and non-esterified salts, respectively. Copper and Fe^{3+} catalyse this reaction. Copper enhances the condensation reaction between (+)-catechin and glyoxylic acid and/or the reaction of a (+)-catechin with the (+)-catechin/glyoxylic acid adduct. However, the acid moiety of glyoxylic acid seems crucial for this reaction because Cu^{2+} did not enhance the acetaldehyde induced addition when it was used instead of glyoxylic acid. Iron probably exerts the same type of mechanism. In many wine countries tartaric acid can be added to wine as an acid supplement and it can contain trace amounts of glyoxylic acid, which can influence the colour of white wine in the presence of especially higher concentrations of Cu^{2+} (Es-Safi *et al*., 2000; Clark *et al*., 2003; Monagas *et al*., 2005).

The oxidation of a phenolic molecule produces H_2O_2, which in turn oxidises ethanol to form acetaldehyde. This can also be produced by yeast during alcoholic fermentation. Acetaldehyde can form ethyl bridges between two (+)-catechin molecules, with carboxymethine-bridged dimers being formed due to the oxidation of tartaric acid, as mentioned earlier. This reaction takes place faster in the case of (-)-epicatechin than with (+)-catechin when each is added alone with acetaldehyde. The degradation product of (+)-catechin has a more reddish hue than (-)-epicatechin. When the two flavanols occur together, (-)-epicatechin also disappears faster than (+)-catechin, with both homo- and heterogeneous ethyl-linked oligomers being formed. The reaction is also faster at lower pH levels, due to more acetaldehyde carbocations, but the faster reaction of (-)-epicatechin compared to (+)-catechin is enhanced by a pH increase (Es-Safi *et al*., 1999b; Lopez-Toledano *et al*., 2002a). Glyoxylic acid or acetaldehyde can be protonated to form an electrophilic C+ carbocation (R1), which undergoes a nucleophilic attack by the C6 or C8 of (+)-catechin to form the corresponding benzylic alcohol (Fig 2). Subsequent protonation, with the loss of H_2O, leads to an electrophilic benzylic carbocation being formed, which can undergo nucleophilic attack from (+)-catechin to form a dimer. This leads to C6-C6, C6-C8 or C8-C8 interactions between two (+)-catechin molecules, with the latter forming at the highest concentrations and the C6-C6 forming at very low concentrations, probably due to steric hindrance. Drinkine *et al*. (2005) investigated the effect of adding glyoxylic acid and acetaldehyde to (+)-catechin. They found that glyoxylic acid alone led to a three times faster disappearance of (+)-catechin than acetaldehyde alone ($t_{1/2}$= 2.3+/-0.2 h for glyoxylic acid and $t_{1/2}$= 6.7+/-0.2 h for acetaldehyde). This was due to structural differences, with glyoxylic acid having both an aldehyde and carboxylic acid group, which has some conjugation associated with its structure, leading to higher aldehyde polarisability. Acetaldehyde, with aldehyde and methyl functional groups, has no conjugation, which thus favours the faster reactions R1 and R2 in glyoxylic acid. However, the rate of the dimer formation was similar, implying that the reaction rate of R3 and R4 is faster with acetaldehyde. For $RI_1(G)$, as indicated in Fig. 2, the intramolecular hydrogen bonds between the carboxyl functional group and the -OH group of the benzylic alcohol may not favour its protonation and dehydration. The same applies to the carboxylic group and the -OH group of C7, which may hinder nucleophilic addition of the second (+)-catechin. When mixed together, ethyl-bridged dimers appeared and disappeared sooner than carboxymethine-bridged dimers. Polymerisation proceeded further, up to tetramer units, with polymers containing both ethyl and carboxymethine-bridges (Saucier *et al*., 1997; Drinkine *et al*., 2005). These reactions were, however, executed in the absence of metal catalysts, which would have induced the formation of xanthylium salts. The brown colour also increased linearly with an increase in polymerisation (Lopez-Toledano *et al*., 2004). These interactions between acetaldehyde and glyoxylic acid might influence the colour, state of polymerisation of flavanoids, and ultimately the taste of wine, and they should be investigated further.

An interesting observation made by Bonilla *et al*. (2001) is that yeast reduces the brown colour of oxidised white wine. The brown colour of an oxidised white wine was reduced with a higher yeast dosage (ranging from 1-5 g/L), which was compatible to PVPP or activated charcoal fining. HPLC analysis revealed that vanillic, syringic, coutaric acids, and especially flavan-3-ol derivatives, were significantly reduced by the yeast addition. Yeast prevents the degradation of (-)-epicatechin and (+)-catechin, exhibiting a stronger inhibition of the degradation of the latter com-

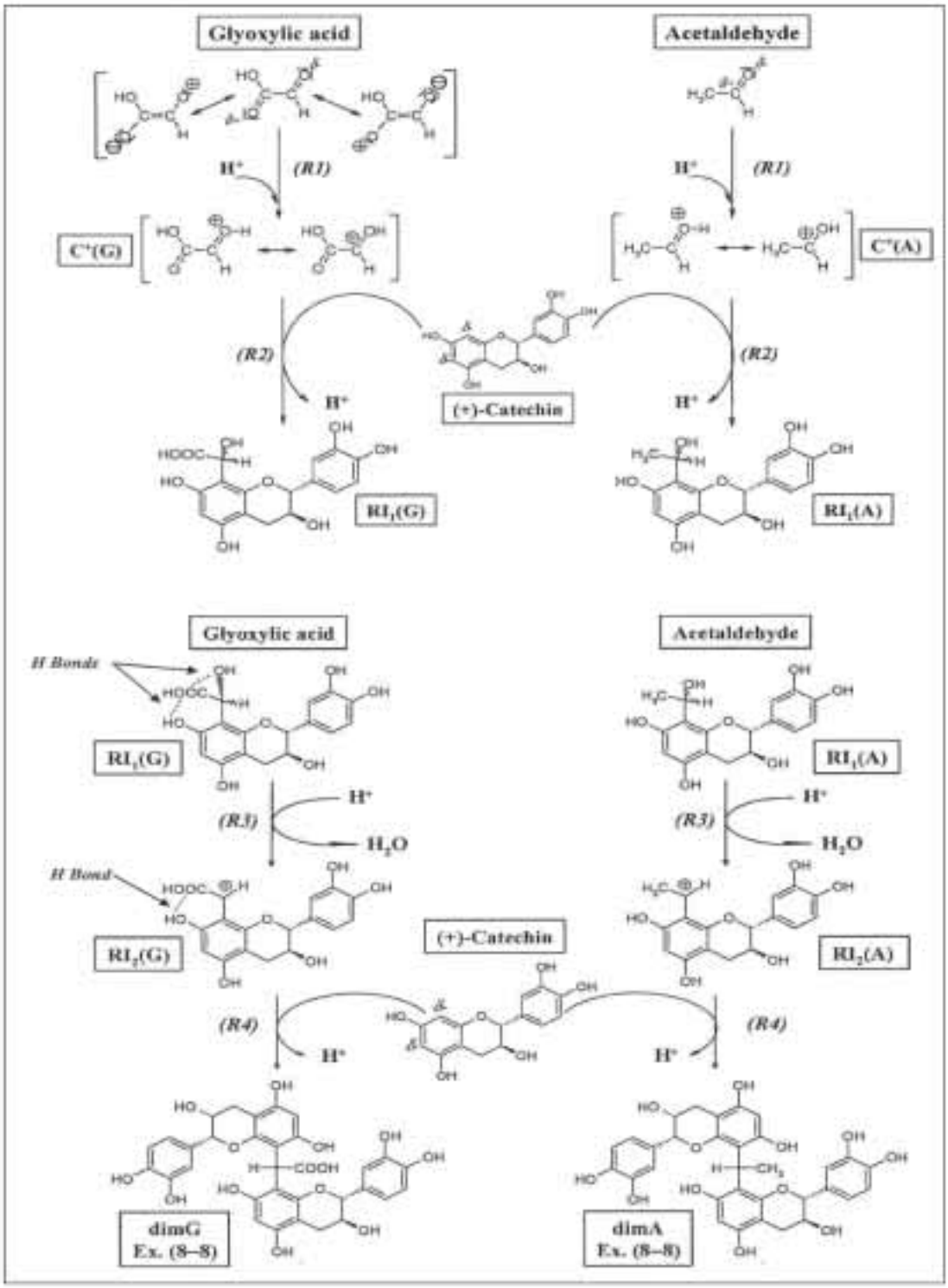

FIGURE 2

The formation of dimers from glyoxylic acid and acetaldehyde (Drinkine *et al*., 2005).

pound. This explains the prevention of brown colouration of sherry with flor yeast. The yeast prevents flavanol degradation rather than protecting the wine from air, by growing on its surface. An increase in polymerisation leads to the resulting oxidative degradation product absorbing more in the brown spectrum. Yeast also seems to prefer association with the browner, more polymerised flavanols, compared to monomeric flavanols. This is reflected in 7.7, 36.4, 53.7 and 64.6% of the (+)-catechin, dimer, trimer and oligomers, respectively, being removed by the yeast in a model solution. The yeast thus seems to have a preference for these compounds, which absorb in the yellow/brown spectrum, with the cell walls being the active absorbing area. These reactions proceed very slowly under the acidic conditions in wine and probably play more of a role in browning if the wine contains higher levels of flavanols, and after it has been racked from the yeast lees (Bonilla *et al*., 2001; Lopez-Toledano *et al*., 2002b; Lopez-Toledano *et al*., 2004). The addition of yeast at lower concentrations also improved the aroma of the oxidised wine.

Pinking of certain white wines remains a problem in the wine industry. This happens when wine is made reductively, i.e. when O_2 is kept to a large degree from coming into contact with it by use of inert gases, such as N_2 and CO_2. These wines can then become pink when exposed to small amounts of air, often during bottling, and will become brown when further exposed to O_2 later. Although only aesthetically unacceptable, these bottled wines often have to be opened and treated. Possible compounds responsible for pinking have not been identified but are thought to be phenolic chromophore compounds. Wines made from Sauvignon blanc, Albarino, Garnatxa blanc and Verdejo have been found to be pinking sensitive, although this did not happen in successive vintage years. An assay in which H_2O_2 is added to wine has been developed to test for potential pinking. PVPP alone, PVPP plus bentonite, and PVPP plus ascorbic acid were found to be 74%, 90% and 98% effective, respectively, in reducing tendencies of pinking. These combinations are equally effective in removing already developed pinking. Ascorbic acid alone was also found to be effective in preventing pinking, but new evidence suggests that it can enhance the formation of brown colouration under certain circumstances (Simpson, 1977; Lamuela-Raventó *et al*., 2001).

Ascorbic acid can also serve as a substrate for oxidation in wine. In the past this anti-oxidant has been used in many wineries for this purpose, especially in white wine, due to its O_2 scavenging ability. The products of ascorbic acid oxidation, dehydro-ascorbic acid and H_2O_2, necessitate the use of SO_2 in combination with ascorbic acid in order to prevent further oxidation by H_2O_2. In recent years evidence has accumulated showing that ascorbic acid can be a pro-oxidant rather than an anti-oxidant in wines under certain conditions, with white wine becoming browner when an air headspace is left in combination with ascorbic acid. Also, sulphur dioxide does not seem to minimise this browning effect (Peng *et al*., 1998; Bradshaw *et al*., 2001). When Bradshaw *et al*. (2001 & 2003) oxidised ascorbic acid alone, two phases were observed. The first was the complete oxidation of ascorbic acid, with species being generated that absorb in the visible spectra. The second generated species with a lighter colour, or no colour. They also found that ascorbic acid enhanced the extent of browning in a model wine solution containing (+)-catechin. The onset of browning was, however, first preceded by a 'lag phase' when a decrease in browning was observed in comparison with the control containing no ascorbic acid. Pre-oxidised ascorbic acid did not exhibit this lag phase, but O_2 is required only for the initiation of the oxidation of ascorbic acid, with higher concentrations of initial O_2 shortening the lag phase. Higher concentrations of ascorbic acid enhanced the brown colour observed (0.06 for 1000 mg/L and 0.015 for 500 mg/L, at 440nm), as well as extending the lag phase and time to reach maximum brown colour. Oxidation of tartaric acid yields glyoxylic acid, generating a colourless xanthene species, which can undergo oxidation to form coloured xanthylium salts. The effective anti-oxidant activity of ascorbic acid initially prevents formation of the coloured xanthylium salts, explaining the initial decrease in colour during the lag phase. As mentioned previously, H_2O_2 is one of the oxidation products of ascorbic acid, but yields were only 21%, as one would expect from a 1:1 production ratio from ascorbic acid. Furthermore, the addition of H_2O_2 to the model solution did not elicit the same extent of browning as ascorbic acid, suggesting that other oxidation products induce the browning. Other oxidation products of ascorbic acid under wine conditions include acetaldehyde, diketo-L-gulonic acid, L-threonic acid, oxalic acid, L-threo-2-pentulosonic acid, 4,5,5,6-tetrahydro-2,3-diketohexanoic acid and furfural. Dehydroascorbic acid is one of the initial oxidation products. After depletion of ascorbic acid during oxidation its oxidation products can then accelerate the formation of the coloured xanthylium salts, explaining the rapid increase in colour during this period. These oxidation products are not oxidative enough to elicit this in the presence of ascorbic acid. This "cross over" of ascorbic acid as anti-oxidant to pro-oxidant thus depends on the concentration present.

Addition of SO_2 increases the lag phase mentioned above. A decrease was seen in the absence SO_2, the absence of ascorbic acid and formation of the brown xanthylium salts. At an ascorbic acid to SO_2 molar ratio of 0.8:1 the SO_2 increased the lag period to four days, but with a considerable loss in the SO_2 concentration; there was a 100% loss in the SO_2, ascorbic acid, (+)-catechin combination, compared to a 43% loss when the ascorbic acid was omitted. Increasing the SO_2 to a 3:1 ratio inhibited this over the 14-day time period of the evaluation. This ratio, which is 200 mg/L for ascorbic acid and SO_2 in the presence of 100 mg/L (+)-catechin, is quite high in winemaking terms. The ratio of SO_2 consumed to ascorbic acid was 1.7:1, which is higher than the expected 1:1. This is even more surprising considering that the oxidation of ascorbic acid yields only 21% of the expected H_2O_2. Sulphur dioxide seems to bleach the coloured xanthylium salt, but does not, contrary to popular belief, reduce dehydro-ascorbic acid back to ascorbic acid (Bradshaw *et al*., 2001; 2004). Flamini and Dalla Vedova (2003) found that *Oenococcus oeni* reduces glyoxal to glycolaldehyde, which has a 10 times higher browning capacity than ascorbic acid. In the light of these findings wine producers should reconsider the use of ascorbic acid during the wine production process. This is especially relevant for white wines that have higher concentrations of flavanoids and to which tartaric acid, possibly containing glyoxylic acid impurities, has been added. Ascorbic acid has been hailed as a replacement for SO_2, but when it is added to wine it necessitates higher SO_2 additions that can prevent it from turning into a pro-oxidant after exposure to O_2.

Effect of oxygen on red wine colour

Red wine obtains its colour from anthocyanins, which are normally extracted from the grape skins during the alcoholic fermentation. Different anthocyanins exist in grapes and wine, as mentioned earlier. The red colour can also be an indication of quality, with deep red wines normally judged as being of superior quality, depending on the other characteristics of the wine. In a young red wine up to 50% of anthocyanins can exist in the colourless carbinol pseudobase. During red wine ageing, the colour of red wine changes from red, in a young red wine, to mauve to brown/red, in the barrel, to eventually brown/orange after prolonged ageing in the bottle (Ribéreau-Gayon *et al*., 2000b). Different chemical reactions induce these changes in colour. These are:

1. Direct anthocyanin-tannin condensation reactions (A-T product). These reactions take place between the nucleophilic C6 or C8 carbons of (+)-catechin, (-)-epicatechin or procyanidins and the electrophilic C4 carbon of the anthocyanin molecule.

The products are colourless flavenes, which can be oxidised to the corresponding flavylium ions, finally developing into yellow xanthylium salts. These reactions take place during fermentation, with subsequent racking from the yeast or lactic acid bacteria lees introducing O_2. This increases the wine's colour density when the flavene is oxidised (Liao *et al*.; 1992; Santos-Buelga *et al*., 1999; Ribéreau-Gayon *et al*., 2000b).

2. Electrophilic carbocations, formed from procyanidins in a low pH medium such as wine, can react with nucleophilic C6 or C8 carbons of the anthocyanin in its hydrated hemi-acetal form (T-A product). The products are colourless, but are rapidly dehydrated into a reddish-orange form. This reaction is stimulated by higher temperatures, and O_2 is not required. It occurs predominantly during bottle ageing. Although the addition of oligomeric procyanidins with the anthocyanins in both A-T and T-A products seems to occur more in wine than anthocyanin polymer additions do. A-T and T-A polymers of up to octamers have been detected (Remy *et al*., 2000; Ribéreau-Gayon *et al*., 2000b; Hayaska and Kennedy, 2003).
3. Vinyl phenols, normally associated with *Brettanomyces* spoilage, can also associate with anthocyanins. This is due to an electrophilic cyclo-addition of the ethylenic bond of the 4-vinylphenol molecule with the C4 and C5 carbons of the anthocyanin, with subsequent oxidation leading to the formation of a pyrane ring. In aged Pinotage wines the pigment Pinotin A has been discovered. This is formed between the anthocyanin and a hydroxycinnamic acid moiety, especially caffeic acid in Pinotage, with oxidation leading to its formation. Anthocyanin-vinylcatechin products have also been identified, which possibly form from the reaction between a flavylium ion and a catechin molecule with a vinyl group on its C8 carbon, with oxidation leading to pigments having a red-orange colour. These molecules are also more resistant to SO_2 bleaching and pH changes, and they also contribute to the red to tawny change in colour of an older red wine. They can then act as a co-pigment, resulting in higher colour stability (Schwarz *et al*., 2003; Monagas *et al*., 2005).
4. The origin of acetaldehyde in wine has been discussed earlier. In wine, which is an acid medium, acetaldehyde can be carbocated by the addition of a proton. This electrophilic moiety will then react with the C6 or C8 positions on a flavanol molecule, which, after the loss of H_2O, undergoes nucleophilic attack of the electrophilic C8 position of a colourless carbinol pseudobase anthocyanin molecule. The resulting product, with an ethyl bond, can be protonated to form a coloured compound. This reaction has been confirmed for malvidin-3-glucoside with different procyanidins, and evidence suggests that the same reaction takes place with cyanidin, delphinidin, peonidin and petunidin (Alcade-Eon *et al*., 2004; Monagas *et al*., 2005). (+)-Catechin, (-)-epicatechin and epigallocatechin have all been shown to react in this way with malvidin-3-monoglucoside. Trimeric and tetrameric pigments have been identified, but only position C8 of the anthocyanin molecule can be involved in this reaction, with the polymerisation ceasing when the anthocyanin forms the two terminal products of the chain. However, recent evidence suggests that the C6 position of the anthocyanin can also be reactive, as anthocyanins in the absence of flavanols formed dimers, trimers and tetramers via ethyl bonds with each other when acetaldehyde was added (Es-Safi *et al*., 1999a; Atanosova *et al*., 2002b). This reaction, which is faster than the previous two, takes place during barrel ageing when controlled oxygenation takes place. Oxygen can come into contact with the wine at this stage through wine-making actions, such as racking or topping up barrels. Oxygen also permeates through the staves of the barrel, with tight-grain oak wood allowing higher O_2 concentrations in the wine. Anthocyanins involved in these polymerisation reactions are less prone to SO_2 bleaching and colour changes due to pH changes. The bisulphite ion, which decolourises the anthocyanin molecule, cannot associate that easily with the polymerisation product due to stearic hindrance. In model solutions containing (+)-catechin, malvidin-3-glucoside, glyoxylic acid and the colourless (+)-catechin dimer, with a carboxymethine bridge in coloured carboxymethine-bridged dimers, resulted, although model solutions containing the anthocyanin, (+)-catechin, tartaric acid and ethanol yielded only the flavanol dimer. Clearly, additional research is needed to evaluate the contribution of this to the changes observed in the evolution of red wine colour during ageing (Santos-Buelga *et al*., 1999; Monagas *et al*., 2005).

During barrel ageing, the colour intensity (the sum of the brown, red and violet colours) increases. In South African Pinotage and Shiraz wines it was found that the origin of the barrel (American, French or Russian) did not affect the difference in colour intensity, colour hue or total red pigments. The colour density increased from 8-10 to 12-16 between 3-6 months after barrelling. Such a difference in colour density can be observed visually. During this time period the total red pigments decreased, but the percentage of pigments in the red form increased from 15 to 45%. A drop in free and total anthocyanins was thus observed, with the concentration of anthocyanins dropping from about 850 mg/L to 400 mg/L within six months. This transformation of colourless anthocyanins into the coloured form compensates for their loss and leads to the increase in colour density. Oxygen does not seem to influence the total concentration of pigment colour, but does increase the proportion in the red form, as well as increase pigments resistant to SO_2 bleaching (Atanosova *et al*., 2002a; Fourie, 2005). Colour density can also decrease during ageing in a steel tank over a few months, but O_2 addition prevents this. The storage of red wine in non-aerated vats also leads to lower concentrations of coloured anthocyanins. Temperature plays a very important role in these reactions, as high storage temperature in combination with high O_2 concentration can lead to anthocyanin and tannin breakdown reactions, which can increase the yellow hue of the wine. The oxygen addition should be done in a controlled manner, and not in excessive amounts, because this can lead to excess acetaldehyde formation, excessive polymerisation and precipitation of colour matter. A favourable tannin to anthocyanin ratio is apparently also required, namely in the order of 4:1. Too low a ratio may lead to anthocyanin breakdown reactions and too high a ratio to over-polymerisation and precipitation. These ratios need to be investigated further, under different wine-making conditions (Singleton, 1987; Ribéreau-Gayon *et al*., 2000b; Atanosova *et al*., 2002a).

During oak ageing, ellagitannins, such as vescalagin and castalagin, are extracted from the wood. Vivas and Glories (1996a) and

Vivas (1999a) found that when these tannins oxidise, due to their greater oxidising capacity, they produce larger amounts of acetaldehyde than condensed tannins. This leads to higher levels of polymerisation, which induces tannin-anthocyanin polymerisation, leading to higher colour density. This does not happen in all red wines, and tannin addition can lead to certain wines becoming too astringent. These tannins also seem to buffer catechins from oxidation, by being oxidised themselves, and thus preventing formation of a brick-yellow colour. In these experiments the ellagic tannins were, however, added at 300 to 1000 mg/L to the model solution or wine, and it is not certain if they will react during oak ageing to the same degree because they were isolated at only a few mg/L from oak-aged wines**.** More of these tannins could probably end up in the wine during ageing because they are easily hydrolysed (Puech *et al*., 1999). In our own laboratory we found that exposure of a young red wine to O_2 *per se* had a larger influence on colour development than the addition of commercial tannins according to the supplier's recommendations (unpublished data).

Effect of oxygen on red wine taste

Different phenolic molecules are involved with the bitterness, astringency and fullness of red wine, but it is mainly the flavanols that are responsible for these tastes and flavours. A very young red wine might be harsh, course, very astringent and even bitter. During ageing of red wine in barrels the wine becomes softer and less astringent. The different methods of polymerisation of (+)-catechin and (-)-epicatechin were discussed in the previous section under white wine oxidation, and basically the same reaction mechanisms occur for these compounds in red wine. Polymerisation leads to these molecules becoming less reactive towards mouth proteins, with the wine being perceived as less astringent (Nikfardjam & Dykes, 2003). During ageing, it is mainly the acetaldehyde-induced polymerisation that contributes to the polymerisation of flavanols. The resulting products are not as reactive towards proteins as their constituents. However, direct C4-C8 and C4-C6 polymerisation reactions between procyanidin molecules produce products that are more reactive towards proteins and are hence more astringent than those formed from acetaldehyde-induced condensation reactions. In the case of flavanols, where the C6 and C8 positions can be occupied, polymers larger than trimers have been isolated. Both types of reactions produce procyanidins with a limit of 8 or 10 flavan units. The interaction of anthocyanin molecules with procyanidins can also influence the taste of wine because they can form the terminal subunits, thus preventing further polymerisation (Ribéreau-Gayon *et al*., 2000b; Monagas *et al*., 2005).

Effect of oxygen on wine aroma

The addition of O_2 to white wine is normally undesirable. Even small additions of O_2 to white wine can lead to loss of aroma, especially fruitiness, although the quality of some white wines may increase with a little O_2 contact. Periodical O_2 addition has led to decreasing fruitiness and general quality, with a correlating increase in oxidation character in Sauvignon blanc and Chardonnay wines. In the Sauvignon blanc however, low initial concentrations of O_2 actually enhanced the quality score, by contributing to the complexity of the wine. The addition of H_2O_2 to a neutral Chenin blanc spiked with 2-methoxy-3-isobutylpyrazine did not lead to degradation of this important flavour compound of Sauvignon blanc over a three-month period (Marais, 1998). It thus seems that this compound is resistant to oxidative degradation in wine. At high O_2 additions the formation of unwanted off-flavours will take place, with oxidised white wines being described as caramel, overripe fruit, crushed apple, acetaldehyde, woody, rancid, farm-feed, honey-like and cooked vegetables (Escudero *et al*., 2002; Silva Ferreira *et al*., 2003b). After ten O_2 saturations white table wine normally becomes completely brown and maderised, and aromatic degradation can occur before it can be identified by colour change (Singleton *et al*., 1979; Boulton *et al*., 1996; Silva Ferreira *et al*., 2003a).

Simpson (1978) reported on the effect of O_2 addition and enhanced ageing at 50°C for 28 days on the composition of Riesling. The concentrations of ethyl n-hexanoate, hexyl acetate, acetic acid, ethyl n-octanoate, vitispirane, 1-hexanol, ethyl furonate and ethyl lactate did not differ significantly between the treatments. Benzaldehyde, diethyl succinate and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), however, increased from 0, 3.8, 0.066 mg/L to 0.18, 4.4 and 0.09 mg/L, respectively. The concentration of 2-phenylethanol was however lower in the oxidised wine. Enhanced ageing under anaerobic conditions increased ethyl n-octanoate, vitispirane, ethyl furonate, ethyl n-decanoate, TDN and 2-phenethanol concentrations. Marais *et al*. (1992) found that TDN, trans-vitispirane, 2,6-dimethyl-7-octen-2,6-diol and trans-1,8-terpin concentrations, and the intensity of the bottle-aged kerosene-like character, increased significantly with ageing in Weisser Riesling wines. However, decreases were observed in diendiol-1, linalool, isoamyl acetate, ethyl caproate, hexyl acetate, 2-phenethyl acetate, hexanol, 2-phenyl ethanol, and in the intensity of young wine character, with higher storage temperatures accelerating these changes. This study clearly showed that lower storage temperatures (15°C) were more favourable for the sensory development of Weisser Riesling wines during ageing.

Ferreira *et al*. (1998) developed a GC-MS method to simultaneously measure compounds in oxidised wine, including t-2-hexenal, t-2-octenal, t-2-nonenal, furfural, 5-methyl-furfural, hexenal, benzaldehyde, furfurol and eugenol. In subsequent studies phenylacetaldehyde, TDN, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), methional (see Table 1) and other compounds were also isolated from oxidised white wines. The cooked vegetable aroma could especially be predicted from the t-2-nonenal, eugenol, benzaldehyde and furfural concentrations. The honey-like and boiled potato aromas could be predicted from the phenylacetaldehyde and methional concentrations. The woody aroma of oxidised wines, even in those that did not receive any wood contact, could be attributed to an increase in eugenol. Surprisingly, acetaldehyde did not seem to play a role in oxidised white wine aroma because its concentration did not vary significantly during the oxidation process, but other unidentified compounds probably influenced the aroma (Escudero *et al*., 2002; Silva Ferreira *et al*., 2002).

The oxidative aroma formation of white wine is dependent on several parameters, which include: O_2 concentration, pH, storage conditions, SO_2 concentration, phenolic composition and ascorbic acid concentration. The floral aroma of white wine seems to degrade faster at higher temperatures, with O_2 additions, and with lower pH values increasing this trend. Sulphur dioxide additions decrease this degradation. At lower temperatures (15°C), however,

TABLE 1

Certain compounds isolated from oxidised white wine (Compiled from Simpson, 1978; Margalit, 1997; Silva Ferreira *et al.*, 2002; Silva Ferreira *et al.*, 2003a).

Compound	Associated flavours	Concentration (µg/L)
Furfural	Woody, cooked vegetables	90.2
t-2-nonenal	Cooked vegetables, woody	62.6
Eugenol	Cooked vegetables, woody	6.2
5-M-furfural	Woody	40.5
Benzaldehyde	Cooked vegetables, woody	165
Hexenal	Pungent	62.3
Phenylacetaldehyde	Honey-like	80-120
Methional	Farm-feed	30-40
TDN	Spicy, kerosene-like	90
Vitispirane	Camphor, eucalyptus	360

degradation proceeds faster at pH 4 than pH 3, and the addition of O_2 has an even more dramatic effect, with the floral aroma almost disappearing after a single saturation. This correlated with the high concentration of phenylacetaldehyde and methional (about 6 and 9 times higher) found in oxygenated wines stored at 45°C, compared to other treatments. The formation of linalool oxide and especially 2.2-dimethyl-5-(1-methylpropenyl)-tetrahydrofuran is enhanced by high temperatures and low pH values. Evidence suggests that the latter compound could impart a rotten food aroma to the wine. TDN and vitispirane are also greatly affected by lower pH values (Silva Ferreira *et al*., 2002). As mentioned earlier, non-volatile flavanoids have also been suggested to indirectly influence the aroma of wine by yielding acetaldehyde from ethanol during coupled oxidation (Schneider, 1998). But, in light of the findings of Escudero *et al*. (2002), namely that acetaldehyde does not seem to influence the oxidised aroma, this should be investigated further. Hoenicke *et al*. (2002) showed that 2-amino-acetophenone, which imparts an untypical aged off-flavour (acacia, soapy) to wine, is formed via the intermediates 2-formamidoacetophenone and 3-(2-formylaminophenyl)-3-oxopropionic acid after oxidative degradation of indole-3-acetic acid. More research is necessary on wine parameters that affect white wine aroma in combination with O_2, especially under South African conditions. This should include reductive and oxidative treatment of juice, and O_2 specifications during different steps of the winemaking process, especially for different cultivars and white wine types.

The effect of O_2 on red wine aroma has not been investigated in detail. According to Blanchard *et al*. (2004) 3-mercaptohexanol, a fermentation product, is an important flavour compound of certain red and Rosé wines made from Cabernet Sauvignon, Merlot and Grenache grapes, imparting fruity aromas to these wines. Dissolved O_2 added to a red wine at 5 mg/L decreased within 48 h to 0.5 mg/L. This also led to a 30% decrease in 3-mercaptohexanol levels, but this decrease only started after 48 h. The addition of anthocyanins and especially catechins led to an even greater decrease, with H_2O_2 also enhancing this decrease, compared to O_2. The quinones generated can easily react with thiols according to a Michael addition reaction or generate H_2O_2, which can further oxidise 3-mercaptohexanol. In a model solution containing (+)-catechin, the 3-mercaptohexanol decreased from 1000 ng/L to 238 ng/L. When 30 g/L SO_2 was added to the sample, the 3-mercatohexanol level fell to only 647 ng/L. Anthocyanins and SO_2, as observed with 3-mercaptohexan-1-ol, seem to play a synergetic role in reducing the concentration of 3-mercaptohexanol. It is thus clear that the transport of wine can also lead to the reduction of the concentration of these odorous thiol compounds and that SO_2 plays a critical role in protecting them from oxidation (Murat *et al*., 2003; Blanchard *et al*., 2004). The oxidative environment inside an oak barrel also stimulates the formation of sotolon, which forms due to the oxidation of threonine. The oxidative degradation of phenylalanine and b-phenylethanol in a barrel also leads to higher concentrations of phenylacetaldehyde, reminiscent of an old oak oxidation flavour (Jarauta *et al*., 2005). In a study conducted by Cerdàn *et al*. (2004), the concentrations of ethyl butyrate and ethyl octanoate decreased in red wine during a period of 18 months in barrels, while those of ethyl hexanoate and ethyl decanoate increased. Du Toit (2006) found that in South African Pinotage wine excessive oxidation led to a decrease in the coffee, fruity and banana aromas. This was also reflected in a decrease in the isoamyl acetate concentration, which imparts the banana character to Pinotage wines. Decreases of these flavours were also correlated with an increase in first a potato skin and later an acetaldehyde-like flavour. It is clear that the effect of O_2 on the aroma composition of red wine should be investigated further.

Port sometimes acquires aromas reminiscent of truffles, quince or metals during ageing, which can be ascribed to dimethyl sulphide. This is due to the formation of this compound with oxidation, with low pH levels accentuating this phenomenon. Dimethyl sulphone follows the same pattern. Methional also decreases in concentration in the presence of O_2, but no methionol is formed. Another off-flavour, 2-mercapto-ethanol, also decreased; it is oxidised to bis(2-hydroxydiethyl) disulphide, which does not have an odour. This explains why older ports never have sulphur off-flavours associated with cauliflower (methionol), rubber/burnt (2-mercapto-ethanol) or cooked potato (methional) (Silva Ferreira *et al*., 2003c). Low pH and high storage temperature are two parameters that increase the formation of TDN in port during ageing, with O_2 additions initially simulating its formation. High O_2 additions lead to its breakdown. Vitispirane, with a camphor or eucalyptus aroma, follows basically the same trend as the other parameters mentioned. Excessive O_2 also leads to the degradation of β-ionone and β-damascenone, which have a flavourful violet and ripe fruit aroma, respectively. The degradation of these molecules can be effectively prevented by the use of SO_2, illustrating this preservative's anti-oxidative effect in even a product such as port, in which concentrations are normally kept low during ageing. Concentrations of SO_2 that are too high can also lead to the degradation of β-damascenone (Daniel *et al*., 2004; Silva Ferreira & De Pinho, 2004). Oxygen exposure in sweet fortified wines leads to the formation of sotolon and 5-(ethoxymethyl)-furfural that impart the characteristic aromas to mature sweet white and red wines, respectively. The presence of polyphenols in fortified red wines also leads to less oxidation of aroma compounds than in sweet white wines, due to the polyphenols' anti-oxidative characteristics and their capability to react with aldehydes (Cutzach *et al*., 1999).

Micro-oxygenation

Micro-oxygenation is the process whereby O_2 is added to wine, by means of an apparatus, in a controlled manner. This is

achieved by filling a known volume with gas at a high pressure. The volume is then transferred via a low-pressure circuit to the diffuser and into the wine. The latter normally consists of a ceramic or stainless steel sparger that produces small bubblesthat can dissolve in the wine. The aim of micro-oxygenation is to introduce O_2 into the wine at a rate equal to or slightly less than the wine's ability to consume that O_2. It has to be managed in such a way that, after addition, all O_2 has been used up, while sufficient SO_2 is still left to protect the wine against oxidation. Different reasons for the application of micro-oxygenation are advocated by the producers of the apparatus. These include: higher colour density, decreases in astringency, sulphur off-odours, green, vegetative aromas, and production costs. Stainless steel tanks in conjunction with micro-oxygenation and alternative wood products can be used to simulate an oak barrel. Wine is also supposed to be market-ready sooner.

Oxygen can be supplied during different stages of the wine-making process. It can be supplied at 1-4 mg/L/day just after malolactic fermentation, especially to press wine fractions, which are rich in polyphenols. The stage at which micro-oxygenation is normally applied is during the ageing period, after malolactic fermentation, when between 1-6 mg/L/month is introduced into the wine (Parish *et al*., 2000; Sullivan, 2002). The wine's temperature must be about 15°C because temperatures that are too high will lead to poor solubility of O_2 and temperatures that are too low to possible accumulation of O_2 in the headspace of the tank. Chemical reactions will also take place too slowly at low temperatures. With certain systems, which dose the O_2 according to mL/L, a tank of at least 2.2 m is required for sufficient pressure on the sparger to operate correctly.

Ribéreau-Gayon *et al*. (2000b) reported on a wine of which the colour intensity increased from 0.82 to 1.67 OD units in 5 months during micro-oxygenation. Anthocyanins and tannins also decreased in concentration, with an increase in the HCl index (polymerisation index), although a control receiving no O_2 was not included in this report. Du Toit (2006) found that the colour density and SO_2 resistant pigments increased in South African red wines when the micro-oxygenation treatment started just after the completion of malolactic fermentation, but this did not happen in wines where it started seven months after malolactic fermentation. Micro-oxygenation and the maturation of Pinotage wines matured in oak barrels also led to lower concentrations of catechin and the procyanidin B1 compared to the untreated control, with a corresponding increase in polymeric pigment and polymeric phenols**.** McCord (2003) found slight increases in the red and brown colour intensity of a Cabernet Sauvignon that received micro-oxygenation with a concurrent increase in polymerised anthocyanin level and a decrease in the free anthocyanin level in the wine that received O_2 compared to the control. The decrease in anthocyanin level was also higher when oak was added to the wine, possibly due to ellagitannin-induced condensation. No conclusive results could be found concerning the effect of micro-oxygenation on oak compounds. The micro-oxygenation did, however, significantly reduce the unwanted sulphur compounds methyl and ethyl mercaptan to below their aroma threshold of 1 part per billion. Dimethyl sulphide concentration did not increase with micro-oxygenation.

The effect of micro-oxygenation on the taste of wine has not been fully elucidated. Wine receiving micro-oxygenation can apparently go through structuring and harmonisation phases. The former leads to the wine actually becoming more astringent and harsh and can last from one to six months. During the subsequent harmonisation phase a decrease in astringency is observed, with the wine becoming more complex, although this has not been fully scientifically elucidated. This is then the ideal time to terminate the micro-oxygenation because over-oxidation might lead to a mean degree of polymerisation of procyanidins that is too high, with a resulting increase in bitterness and with tannins becoming too dry (Parish *et al*., 2000; Nikfardjam & Dykes, 2003). Micro-oxygenation also leads to vegetative, green wines becoming fruitier (Sullivan, 2002). Du Toit (2006) found that a tasting panel statistically preferred micro-oxygenation-treated wines compared to the control in younger red wines, but in older red wines it should be applied with care as prolonged treatment led to the wine becoming over-aged, with an increase in the barnyard/medicinal character, which corresponded with an increase in *Brettanomyces* counts. Micro-oxygenation also led to higher acetic acid bacteria counts, although no increase in volatile acidity was observed in these wines. Taste, however, is still the main parameter by which to measure the progression of micro-oxygenation, but since it is a subjective procedure, it should be supplemented with other techniques. Oxygen can also be introduced by a sparger into barrels, which introduces higher concentrations into the wine. This can lead to savings on racking costs (Vivas, 1999b). It is clear that additional research is needed on micro-oxygenation because little has been published on this process. This is probably due to the relatively large volumes needed for adequate research on micro-oxygenation.

Effect of O_2 on acetic acid bacteria and *Brettanomyces*

Acetic acid bacteria are Gram (+), catalase negative aerobic micro-organisms that occur in wine. They are notorious for wine spoilage due to the conversion of ethanol to acetic acid, which leads to acidification of the wine and an increase in the volatile acidity. Ethanol is transformed into acetaldehyde and the latter to acetic acid by alcohol dehydrogenase and acetaldehyde dehydrogenase, respectively. Low O_2 concentrations lead to the inhibition of acetaldehyde dehydrogenase, resulting in acetaldehyde accumulation in the wine. *Gluconobacter oxydans* normally dominates on healthy grapes, with *Acetobacter aceti* and *A. pasteurianus* dominating in wine. Recently, there is renewed interest in the metabolism and survival of acetic acid bacteria in wine, due to the fact that they survive under the relative anaerobic conditions often found in wine. Acetic acid bacteria have been isolated in high numbers from fermenting must and have been proven to be able to cause sluggish alcoholic fermentation (Du Toit & Lambrechts, 2002; Du Toit & Pretorius, 2002). The transfer and racking of wine after alcoholic fermentation and during ageing introduces O_2 into the wine, that can lead to a 10^2-10^3 cfu/mL increase in acetic acid bacteria numbers. A 30-40-fold increase in acetic acid bacteria numbers was also noticed after the introduction of 7.5 mg/L O_2. Drysdale and Fleet (1989) found rapid growth of *A. pasteurianus* and *A. aceti* from 10^4 to 10^8 cells/mL in wine that was saturated with O_2. At 70% saturation, cell counts increased to 10^6-10^7 cells/mL, with 50% saturation contributing to the survival of the strains. Du Toit *et al*. (2005) found that acetic acid bacteria go into a viable, but non-culturable state in wine, especially under limited O_2 conditions. This can be negat-

ed by the addition of O_2, with plate and epifluorescence microscopy counts being the same after aeration. The O_2 permeating through oak staves during maturation probably also supports the survival of acetic acid bacteria in wine. Certain acetic acid bacteria strains also seem to be resistant to high concentrations of SO_2.

Brettanomyces are yeast able to spoil wine by production of 4-vinylphenol and 4-vinylguaiacol by the enzyme cinnamate decarboxylase from p-coumaric and p-ferulic acids, respectively. The vinyl groups can then be further reduced to 4-ethyl phenol and 4-ethyl guaiacol by vinyl phenol reductase. These volatile phenols have flavours reminiscent of horse sweat, farmyard and medicine. At lower concentrations they can contribute to the complexity of the wine, but become unwanted at excessive concentrations. *Brettanomyces* have been found to survive especially in oak barrels, probably due to the b-glycosidase activity these micro-organisms have, that can release some glucose from the oak for their growth. Oxygen has been found to contribute to the rapid growth of *Brettanomyces* in wine. Oxygen also stimulates the growth of *Brettanomyces* in must and induces acetic acid production. Faster production of 4-ethylphenol in the presence of O_2 has been observed, correlating with cell growth in red wine. The addition of SO_2, however, rapidly kills off *Brettanomyces* cells, with a 45-min exposure of cells to 0.64 mg/L molecular SO_2 being sufficient to achieve this. *Brettanomyces* also seem to be more sensitive to SO_2 than acetic acid bacteria, with only 0.25 mg/L molecular SO_2 inducing rapid cell death in certain strains. Bound SO_2 does not seem to affect *Brettanomyces* (Ciani & Ferraro, 1997; Du Toit *et al*., 2005).

The effect that winemaking practices such as micro-oxygenation have on acetic acid bacteria and *Brettanomyces* numbers should be investigated. It is clear that the wine producer should avoid O_2 pickup and use SO_2 effectively when suspecting possible infection with either acetic acid bacteria or *Brettanomyces*.

Role of oxygen in bottled wine

Oxygen pick-up during bottling

The final step in the winemaking process is that of bottling. During this process the wine must be transferred into the bottles with minimum exposure to micro-organisms and O_2. The choice of bottles as a storage container is probably due to the inert characteristics of glass and it not being permeable to air. Before bottling, the wine should also be in the right state. This includes protein, temperature and SO_2 stability as well as a low dissolved O_2 level (below 0.5 mg/L, but ideally close to 0.3 mg/L). White wine especially should be transferred into the pre-bottling tank under an inert gas. Nitrogen or argon gas can be used, with the latter having a higher specific gravity. Pipes and the filler also need to be filled with an inert gas before bottling starts. The headspace in tanks that is not completely full must be replaced with an inert gas (Allen, 1994). During the bottling process the filler should ideally not add much more than 1 mg/L of O_2 to the wine, but this can go up to 3 mg/L. During filling, a vacuum is often drawn inside the bottles, the wine flows inside and the headspace is filled with CO_2 (Lewis, 1991, Vivas & Glories, 1996b).

Oxygen diffusion as influenced by the bottle closure

The type of closure can play a critical role during the ageing of wine. Oxidative spoilage of bottled wine has been observed in both white and red wines. Corks differ in their permeability to air. Initial studies indicate that compounds within the cork are responsible for this phenomenon (Waters *et al*., 1996; Caloghiris *et al*., 1997). In an in-depth investigation, Godden *et al*. (2001) looked at the performance of a screw cap, two natural corks, two technical corks and nine synthetic corks used to bottle the same Semillon wine. The wines bottled with the screw cap retained the most SO_2 and exhibited the least browning over the 20-months ageing period. Compared to this, the SO_2 loss was highest with most of the synthetic corks, intermediate with the natural corks and least evident with the technical corks. Sulphur dioxide concentrations actually decreased from 7 and 61 mg/L free and total for a synthetic closure, compared to 20 and 84 mg/L and 24 and 90 mg/L for a technical cork and the screw cap, respectively, after six months bottle ageing. This was also reflected in the colour of the wines, with wines closed with synthetic corks generally becoming browner sooner. These wines also had an oxidised aroma and lost a high percentage of fruity character. The wine bottled with the screw cap however developed a higher reduced aroma, reminiscent of struck flint or rubber after 36 months, compared with the other treatments. At this stage, only the screw cap and cork based products had an acceptable aroma (Francis *et al*., 2003).

Lopes *et al*. (2005) measured the amount of O_2 permeating through different types of corks. Technical corks had very low O_2 diffusion after the first month (1.4-2.8 mg/L). Natural corks had rates between 2.3 and 3.8 mg/L and artificial corks 3.6-4.3 mg/L in the fist month, respectively. The diffusion rates then dropped, with technical corks having between 0.01 and 0.10 mg/L O_2 diffusion per month after the first month. Natural corks had 0.24-0.5 mg/L O_2 diffusion/month and synthetic corks 0.85-1.5 mg/L O_2 diffusion/month during this period. Synthetic corks do not absorb wine as cork based products do, which probably contributes to the sealing capability of the latter. The exact method of O_2 diffusion into the bottles is unknown, but it could be that O_2 inside the corks initially permeates into the wine, with atmospheric O_2 permeating later. This may explain the high initial rate of O_2 diffusion in the first month. The large variability observed between natural corks might also explain the random oxidation phenomena of individual bottles. The way bottles are stored also influences air diffusion, with upright bottles, especially those sealed with agglomerated cork stoppers, permitting more O_2 to come into contact with the wine (Godden *et al*., 2001; Mas *et al*., 2002; Lopes *et al*., 2005).

Measuring and evolution of oxidation or redox potential in wine

The measurement of O_2 in wine can be conducted with an O_2 meter. It consists of two electrodes with a membrane permeable to O_2. The O_2 causes a change in potential between the two electrodes that can be measured. The oxidation-reduction potential of wine can also be measured with a similar electrode. The potential is especially influenced by the O_2 concentration, metals, ethanol, phenolic compounds, type of container and temperature, with glycerol having little effect. Winemaking operations, especially those that introduce O_2 such as racking, topping up of barrels, filtration and tannin addition also influence this. The effect of different winemaking operations on this potential can be seen in Fig. 3. It is clear that during alcoholic fermentation the potential drops due to the reductive conditions. It increases again with O_2 pickup

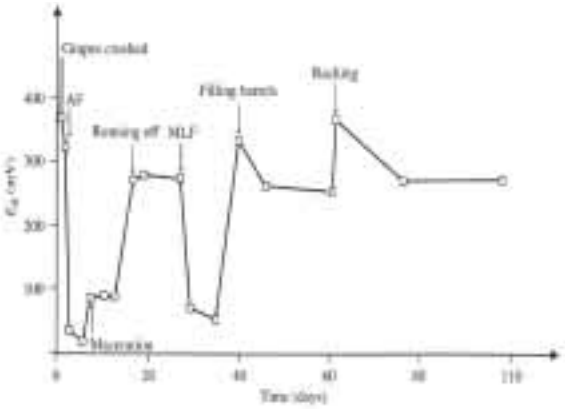

FIGURE 3

Evolution of the oxidation-reduction potential of a red wine during the production process. AF: alcoholic fermentation, MLF: malolactic fermentation (Ribéreau-Gayon *et al*., 2000a).

at racking after malolactic fermentation and racking of the wine (Vivas & Glories, 1996b; Ribéreau-Gayon *et al*., 2000b).

The progression of oxidation can be measured, especially in white wine, with a spectrophotometer. **Absorption** at 420 nm measures the brown spectrum of light and can serve as an indicator of oxidation. However, aromatic degradation can occur before it can be identified, by an intense yellow-brown colour (Silva Ferreira *et al*., 2003b). Other methods of measuring oxidation in wine should thus be developed. This is especially true for red wines, where a colour change is even less indicative of oxidation than in white wines. Du Toit (2006) found that Fourier-transform infrared spectroscopy could distinguish between oxidised and control Pinotage wines, and should be investigated further as a possible means by which to follow oxidation in red wines.

CONCLUSIONS

It is clear that O_2 can play an important role during the winemaking process. The main substrates for oxidation in wine are phenolic compounds, but other compounds such as ascorbic acid and tartaric acid can also be oxidised. These oxidation reactions produce quinones, acetaldehyde, H_2O_2, glyoxylic acid, etc., which can induce polymerisation reactions in the wine. Oxygen can be introduced by the winemaker into the must, during fermentation, and especially during ageing of red wine, to induce favourable chemical changes in the wine. This should be done with care however, as different off-flavours can also be produced in the wine when too high O_2 concentrations are introduced. There are still many aspects regarding the reactions of O_2 in wine that remain unknown. These include the effect of O_2 on red wine aroma and sensory development, and should be investigated in the future.

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