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Oxidation of Wine Phenolics:
A Critical Evaluation and Hypotheses

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Abstract: Oxidation reactions involving phenolics might change the chemical and sensory profile of wines. While oxidation is a long-standing problem in winemaking, a definitive understanding of its chemical mechanisms is lacking, and such an understanding could allow us to better predict and control wine aging. We briefly summarize and discuss the current knowledge on the chemistry of wine phenolic oxidation and propose, along with other researchers, a new, comprehensive scheme in which the Fenton reaction and hydroxyl radicals have an essential role. This hypothesis suggests that catalytic iron converts wine's hydrogen peroxide into hydroxyl radical. This leads to a much stronger and less selective oxidant that could react with almost all wine components, in proportion to their concentration and with little selectivity for antioxidant properties. This reaction could produce many electrophilic oxidation products, mainly aldehydes and ketones, that could further modify the chemical composition and sensory perception of wine. While the brevity of this report precludes a full review of oxidation, our aim is to stimulate more study and debate on the mechanisms in wine oxidation chemistry.

Key words: wine, phenolic, oxidation, oxygen

Oxidation is the chemical process by which an electron is removed from an atom, or group of atoms, through reactions that may or may not involve oxygen addition or hydrogen loss (Figure 1). With some exceptions, the effects of oxidation in foods are considered detrimental and may include degradation of vitamins or lipids, loss of nutritional value, development of off-flavors, and browning (Lindsay 1996, Bradshaw et al. 2001). Traditionally, wine oxidation has been associated with sensory and/or microbiological degradation, except in products aged under oxidative conditions where the result is essential for the quality of these products, such as Madeiras or Jerez. However, moderate constitutive or induced wine oxidation can impart benefits to a broad range of wines by stabilizing color and reducing astringency (Atanasova et al. 2002, Castellari et al. 1998).

Phenolic compounds are primary reactants that are oxidized in the presence of oxygen, a process which initiates a cascade of chemical transformations that result in the deterioration of foods and wine. The management of these transformations is critical to the production of wine. Because red wines contain more phenolics than white wines, they are better able to tolerate large amounts of oxygen. Although the fundamental chemistry of oxidation reactions has been well characterized in many food systems, the complexity of wine and its many interacting components have prevented a comprehensive understanding. This paper discusses phenolic oxidation in the context of wine and its multiple components. We have compiled available information on wine oxidation, proposed hypotheses on some unresolved matters, suggested areas for further research, and discussed practical enological implications.

Oxygen in Winemaking

The maximum solubility of oxygen in wine, as with other gases, is influenced by the ethanol and solid particulate content, but depends primarily on temperature and the composition of the gas to which the wine is exposed. Values for oxygen solubility in air-saturated wine of 6.0 mL/L (8.6 mg/L) at room temperature and atmospheric pressure have been reported (Singleton 1987). The amount of oxygen that a wine is exposed to is often described in

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$$Fe^{2+} \longrightarrow Fe^{3+} + e^- \quad (a)$$

$$H_2O_2 + 2e^- \longrightarrow 2\,OH^- \quad (b)$$

$$2\,Fe^{2+} + H_2O_2 + 2\,H^+ \longrightarrow 2\,Fe^{3+} + 2\,H_2O \quad (c)$$
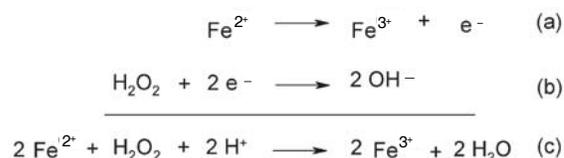
Figure 1 Redox system: oxidation of ferrous to ferric ion (**a**); reduction of hydrogen peroxide to hydroxide (**b**); and overall redox reaction (**c**).

terms of room-temperature air saturations. If wine is exposed to pure oxygen instead, as with the microoxygenation technique, then the higher partial pressure of this gas yields a saturation level about five times higher. This high level could be attained with rapid gas addition, but at typical rates of microoxygenation, the concentration does not approach this level. Compared to room temperature, oxygen solubility increases ~10% at temperatures of about 5°C; thus winemakers trying to avoid oxygen pickup must pay rigorous attention during all practices in which low temperatures are used, such as crushing of white grapes or cold stabilization.

The level of oxygen exposure in the course of winemaking is not trivial. Crushing, pressing, and other practices performed with vigorous agitation in open air lead to saturation. The amount of oxygen entrained during some cellar operations has been determined (Vidal et al. 2001, 2003, 2004, Vivas and Glories 1993). Pumping wine from tank to tank resulted in an average oxygen addition of 0.1 to 0.2 mg/L, while centrifugation added 1.0 mg/L. Values of 2.2 mg/L for protected pump-overs and 7.4 mg/L for those with deliberate aerative splashing were observed. Measurements conducted in Cabernet Sauvignon have shown dissolved oxygen levels between 200 and 250 µg/L after oxygen doses of 5 mL/L/month for 6 months (unpublished data). As expected, this level quickly decreased after the oxygenation ceased.

Measurements by our group and elsewhere (Moutounet and Mazauric 2001) have shown that oxygen is distributed in layers, reaching levels close to saturation at the interface with the tank headspace and down to almost zero oxygen below the first 10 to 20 cm of wine volume. This distribution depends on several factors, including the shape of the tank and the wine's variation in temperature.

To accurately measure meaningful levels of dissolved oxygen in wine, and where in-line measurements are not possible, the following precautions must be taken: (a) samples should be protected from air/oxygen exposure at all times, (b) samples should have the same temperature as the wine being measured, and (c) a sensitive (limit of detection at µg/L) and well-calibrated meter must be used. These conditions are difficult to achieve in a winery setting.

Singleton et al. (1979) estimated that white wines could absorb 10 air saturations before oxidative defects were observed, but he recommended as little oxygen as feasible for best quality. In red wine after saturation, oxygen is reduced to below 1 mg/L in about 6 days at 30°C, mainly because of reactions with phenolics. Singleton (1987) also observed that red wine could tolerate more than 30 saturations (180 mL O_2/L) before showing oxidized character and recommended about 10 saturations to improve quality. The later observations may not reflect the situation of some of today's wines, where higher phenolic levels are observed as a result of the enological practices of maceration before and after fermentation (Ritchey and Waterhouse 1999) and lower iron levels (Sauvage et al. 2002), likely because of the universal use of stainless steel and plastics in modern

facilities. Rossi and Singleton (1966) observed that a wine's oxygen capacity was related to its phenolic concentration. Although moderate air exposure can benefit some wines, especially red, there is no current way to estimate the time or amounts of oxygen required for improvement of specific wines. A better understanding of the chemical reactions involved must be developed before the optimal use of oxygen can be predicted.

Under normal wine conditions of low temperatures and an acidic environment (pH 3 to 4), the slow pace of oxidation reactions hinders measurement of the oxygen consumed per unit and type of phenolic oxidized. The measurement could serve as the basis for predicting the levels of oxygen that a wine can tolerate and could even be used to estimate the amounts of oxygen to which a wine has been exposed. This problem was addressed by Singleton and collaborators (Rossi and Singleton 1966, Singleton et al. 1979), who developed an accelerated test using alkaline conditions and elevated temperatures that allows for a quicker estimate of the oxygen consumption ratio. Unfortunately, as Singleton and colleagues noted, the natural slow oxidation does not exhaust the entire pool of oxidizable phenolics as does the accelerated test. This suggests a regeneration of previously reduced structures during slow oxidation, maintaining at least part of the original pool of oxidizable phenolics.

Phenolics, under high pH conditions, can react directly with oxygen. The weakly acidic character of phenolic compounds (pK_a 9 to 10) allows them to form phenolate anions that can react with oxygen (Figure 2). Removal of one electron from the phenolate anion results in a semiquinone that can disproportionate as described below to yield a quinone and phenol. At moderately high pH (pH 7 to 9), phenolics with pyrogallol units (such as (-)-epigallocatechin) oxidize more readily than those with simple catechol substitutions (Figure 3) (Miura et al. 1998, Inui et al. 2004). At pH >10, substituents on the C ring could be oxidized, equilibrating the reactivity of phenolics with either catechol or pyrogallol units (Mochizuki et al. 2002). However, because wine is acidic, and phenolics have a high
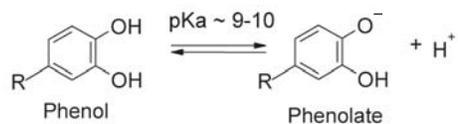
Figure 2 Phenol–phenolate anion equilibrium.

(-)-epicatechin (R=H)

(-)-epigallocatechin (R=OH)
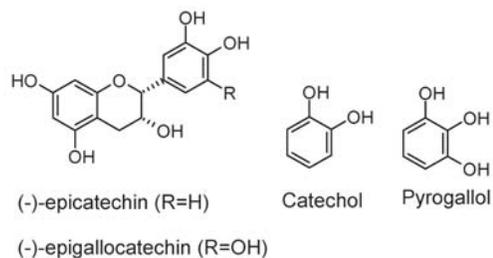
Figure 3 Phenolics with catechol and pyrogallol units.

pK_a, only a small fraction of wine phenolics will be deprotonated, which eliminates this direct oxidation pathway (Singleton 1987, Danilewicz 2003).

The Role of Metals and the Reductive Ladder of Oxidation

Free radicals are very reactive, short-lived fragments of molecules produced by breaking of covalent bonds or abstraction of nonbonding electrons, often in the presence of oxidized metal ions. The characteristic reactivity or instability of these fragments is due to the presence of one or more unpaired electrons. Oxygen has a very limited reactivity toward organic substances in its normal triplet form, which has two unpaired radical electrons in different orbitals, because such reactions are forbidden by quantum rules. Oxygen is more reactive in its singlet form (with no unpaired electrons), but such activation is very unlikely under wine conditions in the absence of light. More likely, it is activated as a result of iron catalysis and reaction with free radicals produced during their interaction.

The role of metals as catalysts or initiators of oxidation reactions has been well studied, although results under wine conditions have been variable. Ribéreau-Gayon (1933) noted a catalytic effect of copper and iron metallic salts but not manganese in wine oxidation, although at that time the means for analyzing the effects produced by the metals was limited. Berg and Akiyoshi (1956) observed that under high concentrations of oxygen (600 mg/L), copper, iron, and their mixtures, added as chloride salts, accelerated wine-browning reactions. Cacho et al. (1995) analyzed the effects of added iron, copper, and manganese sulfate on red wine phenolic oxidation and found that the oxidative process depends on the concentration of these metals in wine. Oszmianski et al. (1996) demonstrated the catalytic effect of iron ions (Fe^{2+}) on oxidation of highly concentrated catechin in a model system, while Makris and Rossiter (2000) observed an increase in the oxidative degradation of quercetin and rutin when cupric and ferrous sulfate (Cu^{2+} and Fe^{2+}) were used as catalysts at 97°C and pH 8.0. Benítez et al. (2002) observed no influence of copper, iron, or manganese sulfate additions (at typical wine concentrations) on browning of Jerez wine. Only higher iron concentrations outside the range found in wine correlated with higher browning levels. Manganese levels below 0.8 mg/L prevented iron from producing an oxidative effect, indicating the importance of interaction between these metals. In wine, metals such as iron and copper can form complexes with proteins, pectins, and phenolics. Evidence of tannin-metal interactions suggests that metal availability in wine could change as oxidation and phenolic polymerization progress.

The oxygen molecule can react with radicals and propagate a chain reaction of oxidation, but there is no evidence that oxygen reacts directly with radicals produced in wine oxidation. In 1989 Singleton stated that autoxidation is autocatalytic (increases as oxidation continues). However, recent reviews of literature (Ribéreau-Gayon et al. 2000, Danilewicz 2003) have concluded that the direct interaction of phenolics and oxygen does not occur unless catalyzed by transition metal ions. We hypothesize that many products resulting from hydrogen abstraction by hydroxyl radicals would react directly with oxygen, supporting Singleton's observation.

Because of the poor direct reactivity of oxygen with organic molecules, the oxidizing potential of molecular oxygen in wine is harnessed by the generation of reactive oxygen species (ROS) that constitute a reductive ladder of oxidation (Figure 4). The initial transfer of an electron leads to formation of superoxide ion, $O_2^{\bullet-}$, which at wine pH exists as the hydroperoxide radical ($\bullet OOH$). This step requires a catalyst, presumably a transition state metal such as iron. The transfer of a second electron would then produce a peroxide, hydrogen peroxide (H_2O_2) being the specific form generated in wine. The next reduction creates an oxidative agent even more reactive than the previous one, namely the hydroxyl radical ($\bullet OH$), via the Fenton reaction between hydrogen peroxide and ferrous iron salts (Figure 5) (Green and Hill 1984, Boulton 2003, Danilewicz 2003). This last reaction produces water, the final product of oxygen reduction.

Both iron reactions require the ferrous form of iron (Fe^{2+}). Phenolics in wine readily reduce ferric (Fe^{3+}) to ferrous; reduction of ferric ion has been used to quantify phenolics, as in the Prussian blue assay (Price and Butler 1977). The other product of this reaction is a quinone, which provides electrophiles for the reactions described.

Primary Oxidation Products from Oxygen Reduction in Wine

The relative concentrations of different antioxidants in wine point to phenolic compounds as the primary substrates for oxidation (Singleton 1987, 1989, Waterhouse 2001). As discussed above, it appears that iron reduces oxygen to the hydroperoxyl radical, or protonated superoxide (Figure 6, reaction 1). The hydroperoxyl radical is
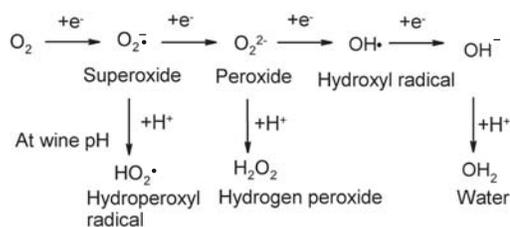
Figure 4 Ladder of oxygen reduction.
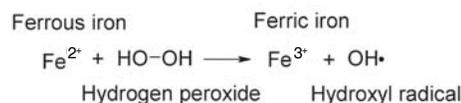
Figure 5 Fenton reaction.

not reactive enough to abstract a hydrogen from many substrates, but the good hydrogen-donating properties of phenolics make them an exception.

When phenolics react with ROS such as the hydroperoxyl radical, the reaction rate of each phenolic depends on its ability to form a stable product radical. Compounds containing a 1,2,3-trihydroxyl group (pyrogallol), a 1,2-dihydroxy aromatic ring (catechol), or a 1,4-dihydroxyl aromatic ring (absent in wine) are easiest to oxidize because the resulting phenoxyl semiquinone radical can be stabilized by a second oxygen atom. Examples of wine phenolics that have these functional groups include caffeic acid, catechin, epicatechin, epicatechin gallate, gallic acid, the proanthocyanidins, hydrolyzable tannins, and quercetin. In short, nearly all wine phenolics are very reactive toward the hydroperoxyl radical. Monophenols and their equivalent meta-di-phenol and substituted phenols (especially methoxy derivatives) are not as readily oxidized because they do not produce stabilized semi-quinone radicals. In fact, studies in model solution have shown that non-catechol phenolics such as phloroglucinol do not consume oxygen unless they form a complex with a "quinine" phenolic such as caffeic acid (Singleton 1989). Similarly, malvidin-3-glucoside, the main anthocyanin present in wine, is not readily oxidized. Oligomeric and polymeric phenolics (procyanidins and condensed tannins) react similarly with ROS as compared to monomeric vicinal dihydroxy phenolics (Lotito et al. 2000).

The catechol functional groups appear to be the primary reacting species with hydroperoxyl radical, and on reacting (Figure 6, reaction 2), they form a semiquinone radical and hydrogen peroxide (pyrogallol species react similarly). The current literature points to a disproportionation that yields quinone and one equivalent of the reduced phenol (Figure 6, reaction 3). Oxygen is reduced to its next oxidation state by donation of a hydrogen radical to the hydroperoxyl radical, forming hydrogen peroxide.

The presence of phenolic radicals in wine has been supported by several electron paramagnetic resonance (EPR) spectroscopy studies. In one, EPR was used to associate the presence of free radicals in wine to the presence of phenolics (Troup et al. 1994). These results were confirmed when the researchers used polyvinylpolypyrrolidone (PVPP) to reduce phenolics from the wine matrix and found a significant reduction of the radical signal measured. In 1995, EPR was used to measure the free radical scavenging capacity of phenolics; superoxide radicals were generated and then trapped with tetramethylpyrrolidine-1-oxide (TMPO) (Glidewell et al. 1995). A reduction in the measured TMPO EPR signals indicated that red and white wines were equally efficient in scavenging the superoxide, while commercial proanthocyanidins and anthocyanidin capsules did not reduce the superoxide free radical signal. Troup and Hunter (2002) used EPR signal intensity to measure aging capacity, presumably an indirect measure of phenolic content. While phenolic radicals seem to be present in wine, the specific phenolic radicals need to be clarified.
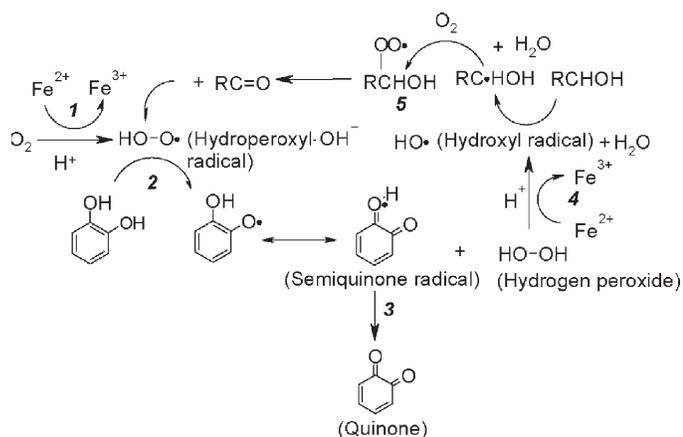
When hydrogen peroxide reacts with ferrous iron to yield hydroxyl radical (Figure 6, reaction 4), this highly unstable radical reacts almost immediately. Thus, it does not react selectively with antioxidants such as phenolics, but instead reacts with all substances present in solution, nearly in proportion to their concentration. Expected products in wine would be oxidation of alcohol to yield acetaldehyde and of organic acids to yield keto acids (Figure 6, reaction 5; Figure 7). This reaction mechanism leads to the supposition that many other products are also formed, in particular products of abundant components of wine such as glycerol, acids, and sugars. As with ethanol, many expected products would be electrophilic ketones and aldehydes. For instance, malic acid forms pyruvic acid, while tartaric acid forms numerous small aldehydes (Fenton 1894). Sugars are present at relatively high levels and would be oxidized to keto and acid-functionalized sugars. Glycerol is an alcohol with a concentration in wine of 5 to 20 g L^{-1} (Ribéreau-Gayon et al. 2000); its wine oxidation products may be important and should be determined. Numerous other products of such a reactive substrate as the hydroxyl radical are possible and should be explored.

Figure 6 Reductive oxidation ladder and primary oxidation products.
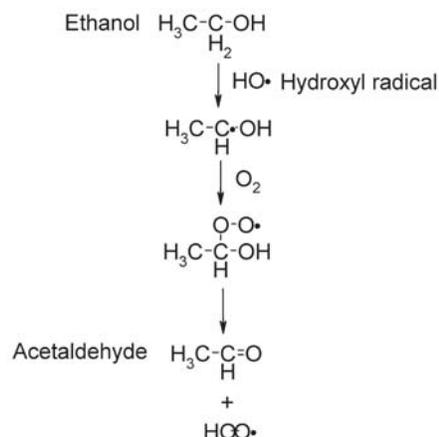
Figure 7 Oxidation of ethanol by hydroxyl radical.

The reactions outlined in Figures 6 and 7 probably explain the observation that oxidation of ethanol to acetaldehyde was dependent on hydrogen peroxide (Wildenradt and Singleton 1974). Recent reviews have also suggested that hydroxyl radical might be of importance in wine oxidation (Boulton 2003, Danilewicz 2003).

New studies of the chemistry and mechanisms of wine oxidation are necessary to fully understand the role of this step in wine oxidation. By comparison, the role of the hydroxyl radical in oxidative flavor stability and the effects of several brewing operations on free radical formation in beer have been studied extensively (Kaneda et al. 1994, 1998, Uchida and Ono 1996, 2000a,b, Andersen and Skibsted 1998).

In the last reduction step, the hydroxyl radical is reduced to water. However, carbon radicals formed (from ethanol) can react with a new oxygen molecule, if present, creating a hydroxylperoxyl form that will decompose to oxidized alcohol (e.g., acetaldehyde) and a hydroperoxyl radical, regenerating initial ROS and allowing oxidation of more phenols to quinones in a cyclic chain of radical reactions.

Secondary Oxidation Products in Wine

Quinone. Most quinone products fall into two well-described categories: reactions with thiols and reactions with phenolics. However, reactions with other nucleophiles in wine may also occur. Their importance depends on the relative concentration and reactivity of the nucleophile.

The first category is reactions with thiols, a functional group with well-established nucleophilicity toward many electrophiles. Singleton observed the reaction of the caftaric acid quinone with the tripeptide thiol glutathione to produce what he called "grape reaction product (GRP)" (Singleton 1985) (Figure 8). This reaction has several important consequences. First, it regenerates a phenolic species from the quinone, which then has the capacity to absorb another equivalent of oxidation. Second, the colorless catechol product of this reaction is not a substrate for enzymatic oxidation, thus this reaction captured oxidation in a product that had no browning potential. Singleton studied the reaction of this quinone with several other thiols. Nearly all reacted, and the reaction was not reversible under wine conditions.
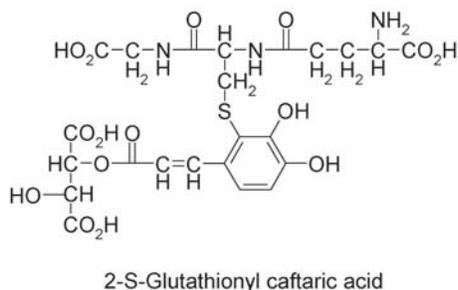
Another reaction of thiols with quinones was reported by Blanchard et al. (2004), who showed that when catechin is oxidized, it reacts with 3-mercaptohexanol, an important factor in the fruity aroma of Cabernet Sauvignon, Merlot, and Cabernet Franc. The result was a loss of fruity varietal character.

Hagerman recently reported the formation of covalent bonds between tannins and proteins, a reaction stimulated by oxidation and dependent on tannin structure (Hagerman 2005). Such reactions have important implications for the sensory attributes of wine tannins. There are many studies on tannins and proteins that show strong noncovalent interactions, but the sensory effect of these complexes, much less a covalent product, has not been tested.

Another well-known reaction of quinones is with other phenolics, especially the electron-rich A ring of flavanols. When this reaction occurs, a new bond forms between two phenolic substances. When those two substances are on condensed tannin chains, the result is a new, larger tannin molecule, joined by a bond that cannot be broken by the acid present in wine. These reactions could lead to the formation of polymeric phenolic structures (Singleton 2001, Cheynier et al. 2002, Danilewicz 2003).

Aldehyde. Acetaldehyde combines with flavanols in a number of well-described reactions. Nucleophilic addition by electron-rich flavonoids to the protonated aldehyde results in a benzylic alcohol. Benzylic alcohol is prone to protonation, which yields water and a benzylic cation, a charged intermediate easily attacked by other nucleophiles. When the nucleophile is another phenolic ring, the reaction yields an "ethyl-linked" product (Figure 9).

Evidence of ethyl-linked anthocyanins-flavanols was first reported by Timberlake and Bridle (1976). Acetaldehyde-flavanol condensation products were later observed in model solution and in red wine (Fulcrand et al. 1996, Saucier et al. 1997). Also confirmed are direct reactions of acetaldehyde with malvidin-3-glucoside to produce vitisin-B (Bakker and Timberlake 1997), the flavanol association cross-linked by glyoxylic acids (Es-Safi et al. 2000), and ethyl-bridged anthocyanins (Atanasova et al. 2002). The relative importance of these different polymerization alternatives to wine context has yet to be evaluated.

Figure 8 Generation of grape reaction product.

2-S-Glutathionyl caftaric acid
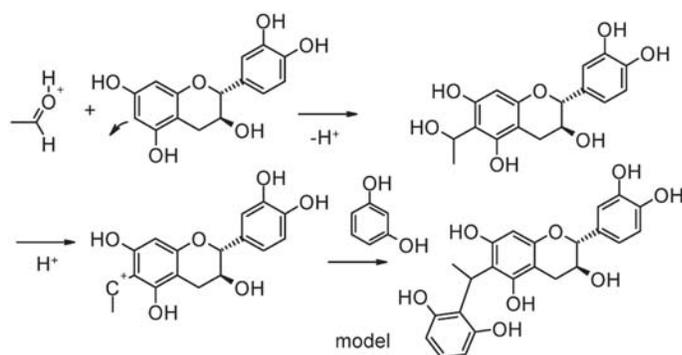
Figure 9 Reaction between a flavanol and acetaldehyde, forming a model ethylene bridged product with resorcinol.

Keto acids. Pyruvic acid is the best-described keto acid in wine. It is known to be formed by yeast activity, but would also be a product of malic acid oxidation by the hydroxyl radical as proposed by Danilewicz (2003). Whether this occurs under wine conditions is unknown, and future studies should address the issue. Pyruvic acid reacts with anthocyanins to form vitisins, or pyranoanthocyanins. These are modified anthocyanins that have an additional conjugated 6-membered ring and that resist SO_2 bleaching as well as color changes with pH shifts, properties of wine pigments (Fulcrand et al. 1998).

There has been no systematic search for products created by oxidation of glycerol or other "alcohols" in wine. These would lead to many aldehydes and ketones, which could be involved in reactions similar to those above. These may be important to color development and other changes to tannin structure.

Figure 11 Wine phenolic oxidation pathway and subsequent hydroxyl radical oxidation of major wine compounds.

Other Antioxidants:
Sulfur Dioxide and Ascorbic Acid

At wine pH, the reaction of oxygen with SO_2, as its sulfite ion, is very slow and essentially irrelevant (Boulton 2003). In 1978, Laszlo and collaborators observed a good correlation between wine oxidation in bottles and the fate of SO_2 (Laszlo et al. 1978). Researchers later recognized that another important role of SO_2 in chemical oxidation is to bind, reversibly, acetaldehyde, other aldehydes, and ketones (Boulton 2003). In addition, SO_2 is very important because it reduces and recycles the quinone product back to phenol, eliminating a reactive electrophile. However, John Danilewicz (personal communication, 2006) observes that one of the most important effects of SO_2 in wine is to react with hydrogen peroxide, reducing the oxidation potential because of the presence of this oxidant. In a similar way, ascorbic acid can also recycle quinones back to phenols (Figure 10), but it can also have the opposite effect depending on its concentration. Under oxygen-rich conditions, ascorbic acid will quickly oxidize, producing peroxide in the same fashion as catechols (Bradshaw et al. 2001). The possible scavenging effects of sulfites on ROS, such as hydroperoxyl radical and hydrogen peroxide, thus limiting the amount of hydroxyl radical formed, should also be investigated.

Figure 10 Protective effect of sulfur dioxide and ascorbic acid.

Conclusions

A summary of wine oxidation is shown in Figure 11. The practical effect of controlled oxidation in winemaking and aging is explained by the reactions between oxygen and phenolics described above. An example of particular importance includes the elimination of "reduced" character by aeration of wine, as by splashing wine in air during a pump-over. This treatment probably stimulates the formation of quinones, which in turn react with reduced sulfur volatiles like hydrogen sulfide and alkyl thiols like ethane thiol. This is a more likely explanation for the loss of reductive character aroma during aeration than evaporation of these volatiles, which is likely to be very slow because of their low concentration. Also, since other sulfides are important to varietal aroma, oxidation can detract from varietal character.

An important implication of the presence of hydroxyl radical is the formation of numerous aldehydes and ketones from oxidation of alcohols in wine; many of these have not yet been discovered. These alcohol derivitives will react with flavonoids, creating linkages that may stabilize color when anthocyanins are involved. The aldehydes and ketones formed by the hydroxyl radical could also create bonds between tannins, or between tannins and proteins or polysaccharides. Such reactions could change hydrogen bonding and van der Waals properties of the tannins, possibly leading to changed sensory attributes.

Reactions between phenolics, whether direct or via aldehyde bridging, may reduce the total concentration of phenolics via precipitation and polymerization during aging. As phenolics polymerize, anthocyanins can be incorporated into these larger phenolic structures, often resulting in color stabilization. These reactions may change the flavor of the wine by reducing astringency.

Ultimately, control of oxidation in winemaking must address several key questions: What exactly is the role of iron and other transition metals in the rate and outcome of wine oxidation? How do sulfites intervene? It is likely that the concentration of iron, oxygen, and sulfites and the concentration and nature of the phenolics present all affect the result, as does pH. After the basic reactions are well understood, the interaction of oxidized species with other wine components must be quantified in order to provide winemakers with predictive tools for improving wine quality.

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