JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 1699–1707 1699
DOI:10.1021/jf903127r

Controlling the Fenton Reaction in Wine

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The fate of hydrogen peroxide in a model wine system was studied under a competitive scenario in the presence of ferrous ions and sulfur dioxide. The metal-catalyzed reduction of hydrogen peroxide (H_2O_2), referred to as the Fenton reaction, yields hydroxyl radicals capable of oxidizing ethanol to acetaldehyde and is now thought to be a key step in nonenzymatic wine oxidation. It appears that sulfur dioxide (SO_2) exerts its protective function in wine by scavenging hydrogen peroxide in oxidizing wine, thereby diverting peroxide from the Fenton route. In this study, the factors affecting the rate and outcome of hydroxyl radical-mediated ethanol oxidation were examined under wine conditions. The exclusion of oxygen in the model wine led to conditions wherein ferric ions (50 μM) were rapidly reduced, presumably by 1-hydroxyethyl radicals. This resulted in the complete stoichiometric conversion of H_2O_2 (300 μM) to hydroxyl radicals, giving an equimolar concentration of acetaldehyde ($\sim$300 μM). Surprisingly, the yield of acetaldehyde was markedly depressed in the presence of oxygen. The addition of a model phenol, 4-methylcatechol (4-MeC; 12 mM), did not protect the ethanol from hydroxyl radical-mediated oxidation under the conditions tested but rather appeared to slightly increase the rate of the Fenton reaction, perhaps by forming a complex with the added iron. The competition for H_2O_2 in the presence of Fe(II) ions and SO_2 was also examined, and the effect of added 4-MeC, as well as dissolved oxygen, was investigated. Higher concentrations of 1-hydroxyethyl radicals, which were trapped by *N-tert*-butyl-α-phenylnitrone (PBN) and detected by electron paramagnetic resonance spectroscopy, were observed when oxygen was excluded and when 4-MeC was included.

KEYWORDS: Wine; oxidation; Fenton reaction; EPR; spin trapping; hydroxyl radicals; hydroperoxyl radicals; 1-hydroxyethyl radicals; sulfur dioxide; spin adducts; metal catalysis; iron

INTRODUCTION

With relatively few exceptions, the oxidation of food constituents has a deleterious effect on food quality. This is especially true of the major macromolecules, most notably unsaturated lipids, the oxidation of which leads to foods with serious sensory flaws and of depleted nutritional value. Wine can be an exception to this rule, however, particularly in the case of maderized wines (e.g., tawny ports, sherries) wherein extensive oxidation is critical to the flavor and aroma characteristics of these products (*1*). Many red wines also benefit from a certain degree of oxidation to reduce astringency and enhance color, although white wines are usually damaged upon exposure to oxygen (*2*). The chemistry of wine oxidation and aging has been the subject of research for centuries, dating back to Pasteur's early studies (*3*), yet only in recent decades has the mechanistic basis of these reactions been investigated. To date, there remain large gaps in our understanding of nonenzymatic wine oxidation. This is unfortunate because wine quality is greatly affected by oxidation, yet the number of resources available for controlling oxygen in winemaking is extremely limited.

Recent findings in our laboratory and elsewhere have demonstrated that nonenzymatic wine oxidation is strongly influenced by the presence of endogenous transition metals (*2*, *4*). The oxidation of wine's major constituents (e.g., ethanol, tartaric acid) is thought to be coupled to the reduction of dioxygen to water (**Scheme 1**). These reactions appear to involve free radical intermediates (*5*), with oxygen being reduced via the stepwise transfer of electrons from wine's constituents. Because the direct reaction between these organic compounds (singlet state) and dioxygen (triplet state) is spin forbidden and, thus, kinetically restricted, transition metals are thought to catalyze these processes due to their ability to redox cycle (i.e., readily donate and accept electrons) (*6*, *7*). It is also important to note the role that the polyphenolic fraction of wine plays in nonenzymatic oxidation, without which the system is apparently unable to oxidize even in the presence of trace metal catalysts (*1*, *2*). As the primary substrates for oxygen in wine, polyphenols are the major targets of hydroperoxyl radicals, which derive from the reaction between dioxygen and ferrous and/or cuprous ions. This reaction is known to yield hydrogen peroxide (H_2O_2), the fate of which is dependent on a number of factors. H_2O_2 is expected to react with a ferrous or cuprous ion (the Fenton reaction) to give a hydroxyl radical, an exceedingly potent species capable of nonspecifically oxidizing virtually all organic constituents in proportion to their abundance (*8*). However, in the presence of excess SO_2, H_2O_2 appears to react swiftly and irreversibly by a two-electron reaction (nonradical) with bisulfite ions (HSO_3^-) under acidic conditions to yield sulfate (HSO_4^-/H_2SO_4) and water, thereby leaving the

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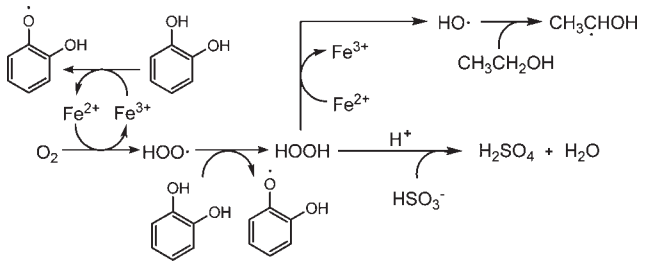
Published on Web 01/04/2010

Scheme 1. Proposed Metal-Catalyzed Wine Oxidation Scheme



organic fraction of wine protected. The ability of SO_2 to inhibit oxidation has been attributed to its reactivity with H_2O_2 (*9*), although some have argued that SO_2 exerts its antioxidant function by reacting directly with oxygen (*10*), but this reaction appears to be prohibitively slow and, therefore, inconsequential under wine conditions (*2*). Nevertheless, the SO_2/H_2O_2 reaction has been studied previously under acidic conditions (pH 0.0−4.5) because it is a key factor in acid (i.e., sulfuric acid) rain production in the atmosphere (*11*).

Given the presence of H_2O_2, SO_2, and transition metals in wine, we hypothesize that the fate of H_2O_2 is dictated by the competition between SO_2/H_2O_2 and $Fe(II)/H_2O_2$ reactions, with the latter reaction resulting in the oxidation of major wine constituents. However, an investigation of these competitive reactions is not straightforward in a wine system due to a number of reasons. For example, the rate of the Fenton reaction is known to be affected by the presence of metal ligands. Wine contains a number of compounds (e.g., organic acids, polyphenols) that are capable of complexing metal ions, which may ultimately affect its reactivity with H_2O_2 (*12*). The pH of the solution may also affect the rate of the SO_2/H_2O_2 reaction (*11*) and, in turn, may affect the amount of H_2O_2 available to the Fenton reaction. The concentration of dissolved oxygen may also affect the Fenton reaction, as has been previously reported (*12*). In addition, one must take into consideration that the relative concentrations of reactants (SO_2 and H_2O_2) are not static. The formation of H_2O_2 in wine at any given time is a function of the rate at which hydroperoxyl radicals react with polyphenols; the kinetics of this reaction are at the mercy of a number of factors (e.g., the composition of the polyphenolic fraction, pH, temperature, dissolved oxygen concentration). Furthermore, the concentration of available HSO_3^- ions is dynamic in wine and is known to decrease steadily as it is consumed by H_2O_2 and/or trapped by quinones (*13*). Clearly, the chemistry of wine oxidation in the presence of SO_2 is complex.

This objective of this study was to investigate, for the first time, the relative rates and outcomes of the SO_2/H_2O_2 and $Fe(II)/H_2O_2$ reactions under a competitive scenario in a model wine system. The effect of dissolved oxygen and a model phenolic compound (4-methylcatechol, 4-MeC) on the reactions is also reported.

MATERIALS AND METHODS

Materials. The spin traps *N-tert*-butyl-α-phenylnitrone (PBN) and 5,5-dimethylpyrroline-*N*-oxide (DMPO) were obtained from Alexis Biochemicals (Lausen, Switzerland) and used as received. Iron(II) sulfate heptahydrate, iron(III) chloride hexahydrate, copper(II) sulfate pentahydrate, (+)-tartaric acid, acetaldehyde, ascorbic acid, and 4-MeC (95%) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide (30% w/w solution) and ethanol were obtained from EMD Chemicals (Gibbstown, NJ). Potassium metabisulfite and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Alfa Aesar (Ward Hill, MA). Acetaldehyde hydrazone standards were prepared as described previously (*26*) and recrystallized from acetonitrile. All other chemicals and solvents were of analytical or HPLC grade. Water was purified through a Millipore Q-Plus (Millipore Corp., Bedford, MA) purification train.

Reactions in Model Wine Solution. A model wine consisting of 12% (v/v) ethanol and phosphoric acid or (+)-tartaric acid (53 mM), adjusted to pH 3.60 with 5 N NaOH, with or without 4-MeC (12 mM), was prepared as described previously (*14*). For reactions carried out under aerial oxygen saturation conditions, the model wine solution (200 mL) was first saturated with medical grade compressed air for 15 min using a medium fritted glass gas dispersion tube (8 mm o.d. tube, CG-203, Chemglass, Vineland, NJ). The model solution (200 mL) was purged with ultrahigh-purity nitrogen gas using the same apparatus for reactions requiring deaerated (anoxic) conditions. Experiments were carried out in a cylindrical jacketed reaction vessel (500 mL capacity, CG-1926, Chemglass) equipped with a magnetic stir bar. The temperature was maintained at 20 °C using a refrigerated circulating water bath. Reactants were introduced to, and aliquots removed from, the vessel by syringe through a rubber septum. Delivery of gases (air or nitrogen) through the dispersion tube was stopped immediately before the reactions were initiated to minimize the volatilization of acetaldehyde, at which point the model solution was blanketed with its respective gas for the duration of the experiment. Stock solutions of Fe(II) (0.25 M) and SO_2 (2.5 M) were prepared immediately before use in either air-saturated or deoxygenated water. The Fe(II) and SO_2 were introduced to the reaction vessel by syringe 1 min before the start of the reaction. It was important to add the Fe(II) in advance to prevent the dynamic formation of ligand-bound (e.g., acids, catechol) iron from affecting reaction rates. In all cases, reactions were initiated upon addition of hydrogen peroxide to the model wine by syringe. The peroxide was added from a stock solution (1.5 M) that was prepared freshly in either air-saturated or deoxygenated water. Hydrogen peroxide stock solutions were confirmed optically using an extinction coefficient of 40 M^{-1} cm^{-1} at 240 nm (*15*). All experiments were performed in triplicate.

Acetaldehyde Analysis. Acetaldehyde in model wine solutions was detected as its 2,4-dinitrophenylhydrazone derivative by high-performance liquid chromatography (HPLC) using a C18 LiChrospher column (4 mm × 250 mm, 5 μm particle size; Merck, Whitehouse Station, NJ) on an Agilent Technologies (Palo Alto, CA) 1100 series reverse phase HPLC with a photodiode array UV−visible detector, as described previously (*14*). Acetaldehyde was quantified on the basis of an external calibration curve prepared in model wine solution.

Fe(II) Oxidation and Fe(III) Reduction Kinetics. The oxidation of ferrous ions to ferric ions in aerated solution was followed by measuring the decline of Fe(II) concentration over time. The Ferrozine method was used to determine Fe(II) concentrations, as described previously (*12*), using an Agilent 8453 UV−vis spectrophotometer. This method was also used to measure the kinetics of Fe(III) reduction to Fe(II). Briefly, model wine was dispensed to a quartz cuvette, to which Fe(III) (50 μM final concentration; added as a stock solution of $FeCl_3$ in water) and the Ferrozine reagent were added, as described previously (*12*). The reaction was initiated by addition of the 4-MeC (0.68 mM final concentration; added as a stock solution of 4-MeC in water). Reaction kinetics were measured by following the increase in absorbance at 562 nm, corresponding to the Ferrozine−Fe(II) complex (*16*). All experiments were run in triplicate.

EPR Spin Trapping. The PBN spin trap was dissolved directly into the model wine solution, giving a final concentration of 30 mM. DMPO was added to samples from a stock solution prepared in water to give a final concentration of 50 mM. In both cases, the spin traps were added before the Fe(II) and H_2O_2. Samples (~1 mL) were loaded into a multiple-bore quartz cell (AquaX 19-bore cell; Bruker BioSpin, Billerica, MA), and the EPR spectra were recorded on a Bruker eScan R X-band spectrometer at room temperature. Samples were analyzed 10 min after the start of each experiment. The EPR microwave power was set to 37.86 mW, the modulation frequency was 86 kHz, and a sweep time of 2.62 s was used. Each sample was scanned a total of 20 times. A sweep width of 50 G was used for experiments with PBN, and 100 G was used for experiments with DMPO. The receiver gain was set to 4.48×10^3. EPR calibration was performed using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) (5 μM). Simulation and fitting of the EPR spectra were performed using the PEST WinSIM program (*17*). All EPR experiments were performed in duplicate.

RESULTS AND DISCUSSION

Factors Affecting Acetaldehyde Formation Rates and Yield in Model Wine. The Fenton reaction was investigated in a model

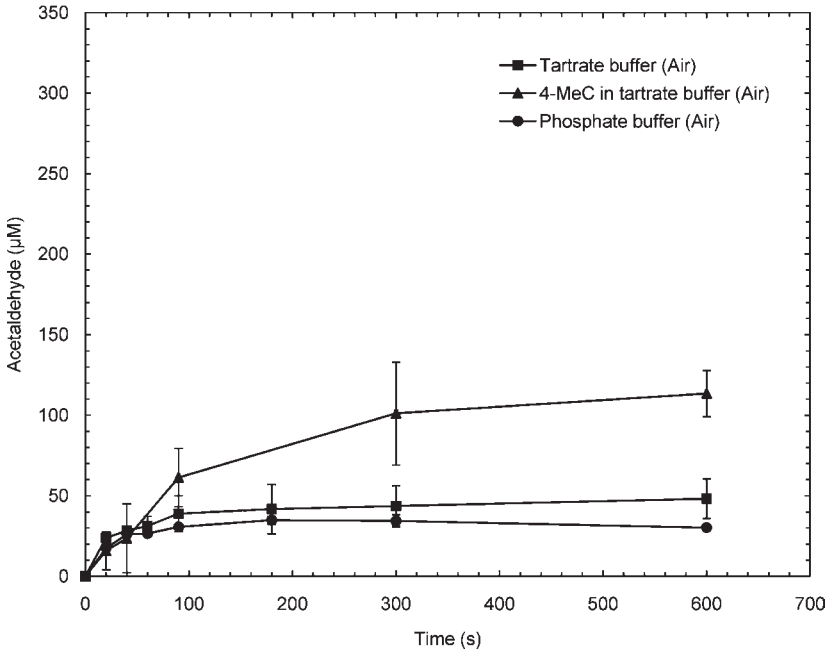


Figure 1. Rate of acetaldehyde formation by hydroxyl radical-mediated ethanol oxidation (50 μM Fe(II); 300 μM H_2O_2) in the presence of air.

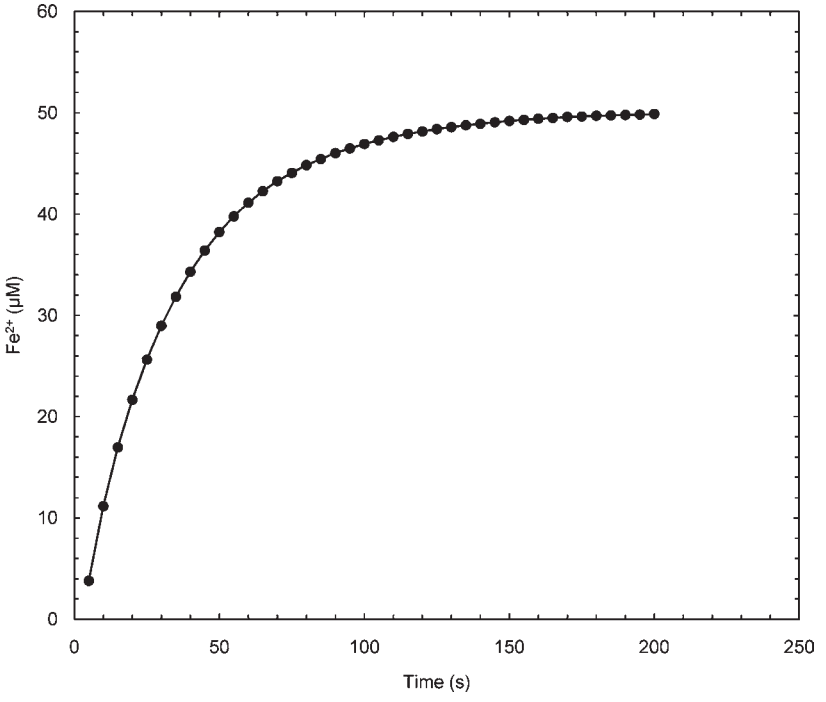


Figure 2. Rate of Fe(III) reduction by 4-methylcatechol (0.68 mM) in model wine solution.

wine solution in which a 6× molar excess of H_2O_2 was used with respect to iron ([H_2O_2] = 300 μM; [Fe(II)] = 50 μM). When the reaction was carried out in an air-saturated model wine made with phosphoric acid, acetaldehyde levels were observed to increase to a maximum concentration of 34 μM within 180 s (**Figure 1**), apparently due to the depletion of Fe(II). Phosphoric acid was selected for this particular experiment because it is not readily oxidized and, thus, should not affect the final yield of acetaldehyde. Surprisingly, the substitution of tartaric acid for phosphoric acid did not decrease the overall yield of acetaldehyde, as was originally expected, as tartaric acid was thought to be a good substratre for oxidation, but it led to an increase in the apparent rate of acetaldehyde production under the same conditions. The oxidation of tartaric acid to glyoxylic acid has been reported previously under wine conditions (*18*, *19*). However, given that the concentration of ethanol is ca. 38 times greater than that of the acid ([EtOH] = 2 M; [tartaric acid] = 53 mM), the concentration of tartrate may simply be too low to effectively compete with ethanol for hydroxyl radicals. The net increase in acetaldehyde yield in this experiment may be explained by considering the reduction potential of the Fe(III)/Fe(II) couple in the presence of tartaric acid versus phosphoric acid. As a strong Fe(III) ligand (*20*, *21*), tartaric acid would lower the reduction potential of the Fe(III)/Fe(II) couple (*2*) and, therefore, increase the rate of the Fenton reaction by facilitating electron transfer from Fe(II) in the H_2O_2 complex.

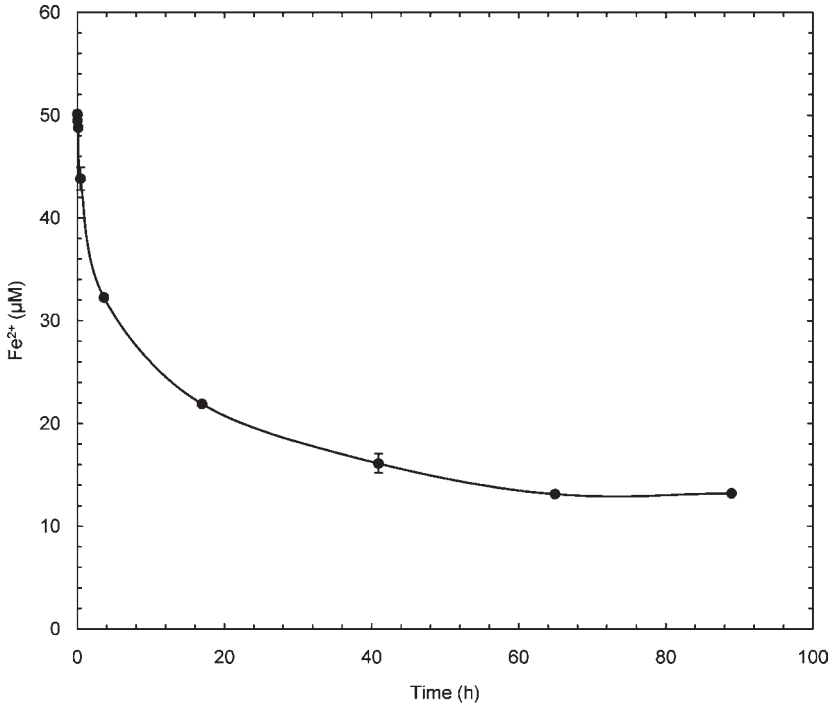
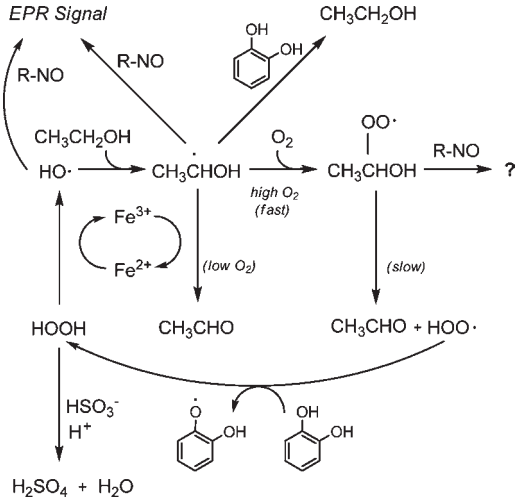


Figure 3. Rate of Fe(II) autoxidation in model wine solution without 4-methylcatechol.

The effect of phenolic compounds on the Fenton reaction in the wine system was investigated using 4-MeC at a concentration ([4-MeC] = 12 mM) resembling that of a red wine with respect to total polyphenols. The phenol was dissolved directly into the model wine prepared with tartaric acid and, again, the reaction was run under aerial conditions. No appreciable change in the initial rate of the reaction could be discerned in the 4-MeC system under these conditions; however, the final yield of acetaldehyde (113 μM) was significantly greater at the end of the experiment (600 s) compared to the model systems without 4-MeC. These results show that a greater amount of H_2O_2 was converted to acetaldehyde during this time. We believe this occurs because the 4-MeC serves to regenerate the catalytically active form of the metal, Fe(II). As noted above, in the absence of a Fe(II) reducing agent, the final yield of acetaldehyde could not exceed that of the initial Fe(II) concentration, as 1 mol of Fe(II) gives 1 mol of hydroxyl radicals, which oxidizes 1 mol of ethanol to 1 mol of acetaldehyde. This appears to be the case in experiments run without 4-MeC: the final yield of acetaldehyde is slightly less (30 and 48 μM for the phosphate and tartrate model systems, respectively) than the initial Fe(II) concentration (50 μM). In the presence of 4-MeC, however, some of the Fe(III) that is produced by the Fenton reaction is reduced to the Fe(II) state, which then goes on to reduce more H_2O_2 to ultimately give more acetaldehyde.

The ability of 4-MeC to reduce Fe(III) to Fe(II) in the model system was subsequently investigated and was found to be a relatively fast reaction (**Figure 2**). Essentially all of the added Fe(III) (50 μM) was reduced by 4-MeC (12 mM) within 200 s. The rate of Fe(II) autoxidation in the model system without added 4-MeC was also measured (**Figure 3**) and was observed to be relatively slow compared to the reaction between Fe(III) and 4-MeC. It was found that only 5.1 μM Fe(II) was oxidized to Fe(III) within 600 s in model wine containing tartaric acid but without 4-MeC under aerial conditions, representing ~10.2% of the initial Fe(II). With such a rapid reduction of the iron, one may wonder why the added 4-MeC did not increase the yield further, as it should have been able to reduce the Fe(III) to Fe(II) multiple times during the reaction period of 600 s. However, it is possible

Scheme 2. Proposed Scheme Depicting the Pathways and Products of Nonenzymatic Metal-Catalyzed Wine Oxidation under High and Low Dissolved Oxygen Concentrations[a]



[a] Spin traps are denoted R-NO.

that oxygen may be trapping the intermediate ethoxyl radical as noted below in more detail (**Scheme 2**).

The effects of oxygen on the rate and yield of final products of the Fenton reaction in wine were subsequently investigated. To examine this effect, the same experiments as described above were performed in a deoxygenated system. The removal of dissolved oxygen from the model wine was expected to depress the final acetaldehyde yield; however, this was not the case, as a significantly higher yield of acetaldehyde was observed in all deoxygenated systems (**Figure 4**). The Fenton reaction in model wine containing tartaric acid resulted in a maximum yield of 308 μM acetaldehyde in 180 s, suggesting a stoichiometric conversion of H_2O_2 (300 μM) to acetaldehyde, despite the fact that Fe(II) was limiting (50 μM). Clearly, the Fe(III) produced by the Fenton reaction is being quickly reduced to Fe(II), which would explain

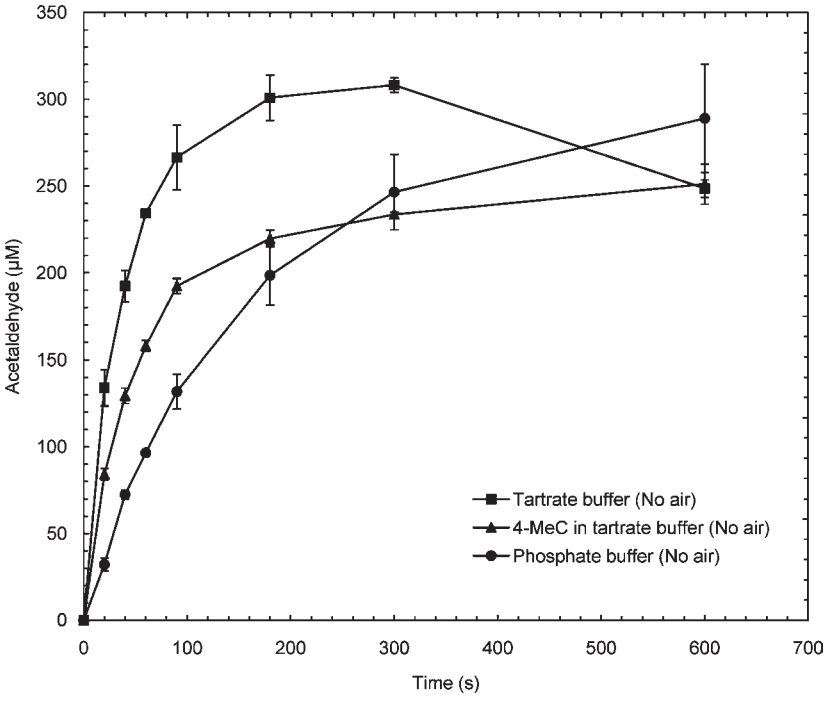


Figure 4. Rate of acetaldehyde formation by hydroxyl radical mediated ethanol oxidation (50 μM Fe(II); 300 μM H_2O_2) in the absence of air.

why Fe(II) is apparently not limiting in this system. It is likely that the 1-hydroxyethyl radical is responsible for reducing Fe(III) in the absence of oxygen (**Scheme 2**). We have recently identified this radical species in both model and real wines (*5*), and the 1-hydroxyethyl radical is known to be highly reducing and capable of rapidly reducing Fe(III) to Fe(II) (*2*). In comparison, the rate of acetaldehyde formation via the Fenton reaction was slower when tartaric acid was substituted with phosphoric acid, with 248 μM acetaldehyde observed after 600 s. This is consistent with the results obtained in oxygenated solution and with previous studies demonstrating the accelerating effect of iron-complexing organic ligands on the Fenton reaction rate (*12*).

When 4-MeC (12 mM) was added to the model wine containing tartaric acid, a decrease in both the reaction rate and acetaldehyde yield at 300 s was observed compared to the system without the phenol. It is possible that a fraction of the hydroxyl radicals or ethoxyl radicals was quenched by 4-MeC, thereby depressing acetaldehyde yield. The rate difference might be attributable to Fe(II) stabilization by the catechol, and this merits further investigation. The loss of acetaldehyde in the tartaric acid solution between 300 and 600 s may be explained by the reductive reactivity of Fe(II) toward the aldehyde, perhaps enhanced by tartrate ligands (*22*).

Identification of the Hydroxyethyl Radical. The spin trap PBN was used to quantify the 1-hydroxyethyl radical in all experiments; however, DMPO was first used to confirm the identity of the various radical species generated in the model wine system. This step was important to establish that ethanol was the major target of hydroxyl radicals in the model solution, as evidenced by the observation of 1-hydroxyethyl radical ($MeCH^{\bullet}OH$) spin adducts as the dominant species. It was also important to rule out the possibility that other radicals might contribute to the signal derived from the PBN/$MeCH^{\bullet}OH$ spin adducts, as it is often difficult to distinguish radicals using PBN alone. For example, the PBN/$MeCH^{\bullet}OH$ and PBN/$^{\bullet}OH$ adducts give rise to a triplet of doublets in the EPR spectrum with similar hyperfine coupling constants (PBN/$MeCH^{\bullet}OH$, a_N = 15.2 G, a_H = 3.34 G; PBN/$^{\bullet}OH$, a_N = 15.3 G, a_H = 2.75 G, respectively) (*23*). When the Fenton reagents ([H_2O_2] = 300 μM; [Fe(II)] = 50 μM) were added to water containing DMPO (50 mM), a spectrum due solely to the DMPO/$^{\bullet}OH$ adduct was observed (a_N = 14.8 G, a_H = 14.5 G) after 10 min under aerial conditions (**Figure 5a**). The identical spectrum was observed when the experiment was repeated in the presence of tartaric acid (53 mM) with the final pH of the solution adjusted to 3.6 (**Figure 5b**), indicating that the acid did not give rise to a product radical capable of reacting with the spin trap. However, the intensity of the signal was suppressed by 54.5% when tartaric acid was present. Such an observation is expected given the relative concentrations of DMPO (50 mM) and tartaric acid (53 mM) in solution. In the absence of tartaric acid, virtually all of the hydroxyl radicals generated by the Fenton reaction are able to react with the spin trap, whereas the addition of tartaric acid depresses the ability of DMPO to trap the radical through competition, although attempts to detect glyoxylic acid, the expected product, were not successful. However, there have been no direct reports of glyoxylic acid since 1894 (*24*). The DMPO/$^{\bullet}OH$ signal intensity was further suppressed upon addition of 4-MeC (12 mM) to the tartaric acid solution (**Figure 5c**), presumably due to hydroxyl radical "scavenging" by the phenol. No additional radicals were observed other than the DMPO/$^{\bullet}OH$ spin adduct in this system.

The same Fenton system was also established in a simple ethanol solution (2 M) containing DMPO in which, predictably, the DMPO/$MeCH^{\bullet}OH$ and DMPO/$^{\bullet}OH$ spin adducts were observed to be the major and minor species, respectively (**Figure 5d**). Again, this is expected due to the large excess of ethanol relative to the spin trap and is consistent with previous studies (*25*). Finally, Fenton reagents were added to the same model wine system as described above, and the resulting spin adducts were compared in the presence of either DMPO (50 mM) or PBN (30 mM) (**Figure 6**). In the presence of DMPO, the 1-hydroxyethyl radical adduct was the only species identified (hyperfine coupling constants: a_N = 15.7 G, a_H = 22.9 G). The EPR spectrum observed in the PBN system is also consistent with the 1-hydroxyethyl radical adducts (a_N = 15.5 G, a_H = 3.3 G) and, again, no species other than the PBN/$MeCH^{\bullet}OH$ adduct

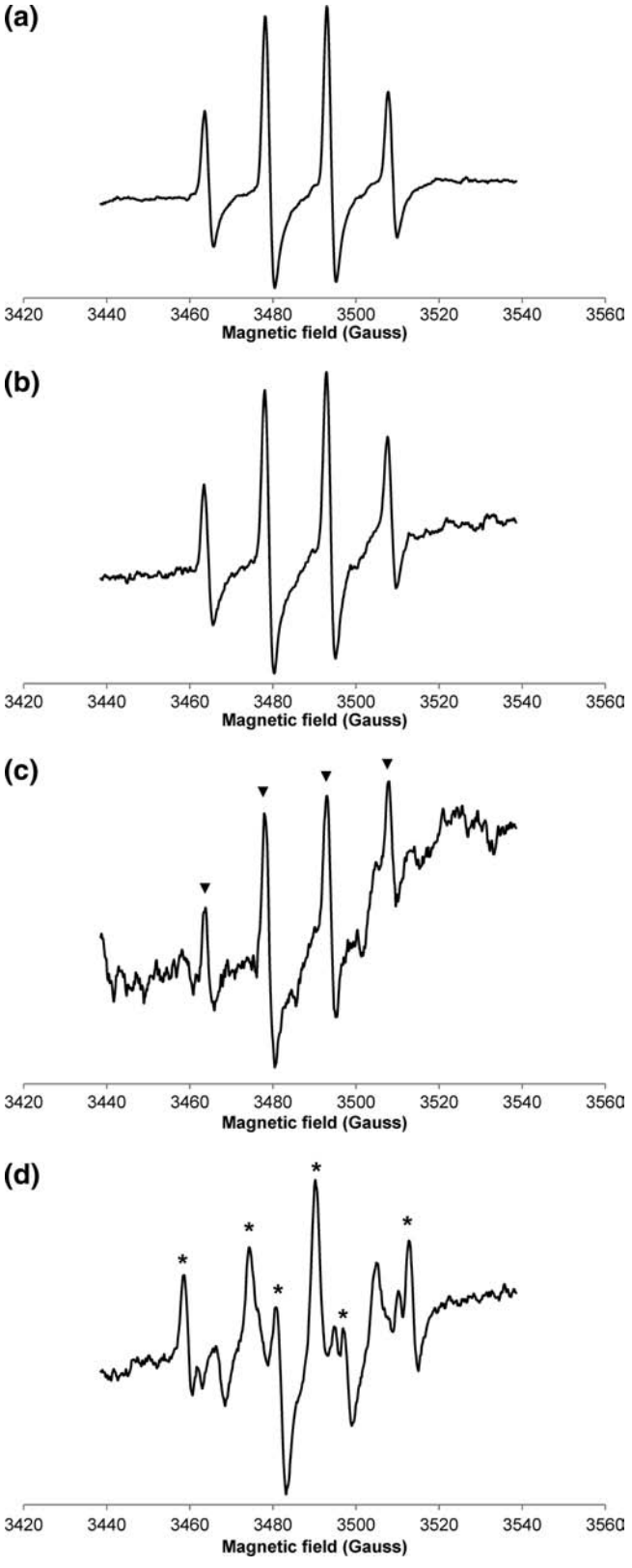


Figure 5. Experimental spin pattern of DMPO/•OH spin adduct in (**a**) water, (**b**) tartaric acid solution (53 mM, pH 3.6), or (**c**) tartaric acid solution (53 mM, pH 3.6) containing 4-MeC (12 mM) following the addition of Fenton reagents (50 μM Fe(II); 300 μM H_2O_2). Peaks assigned to the DMPO/•OH adducts are denoted (▼). (**d**) Experimental spin pattern of DMPO/$CH_3C^{\bullet}HOH$ spin adduct in ethanol solution (2 M) following the addition of Fenton reagents (50 μM Fe(II), 300 μM H_2O_2). Peaks assigned to the DMPO/$CH_3C^{\bullet}HOH$ adducts are denoted (∗).

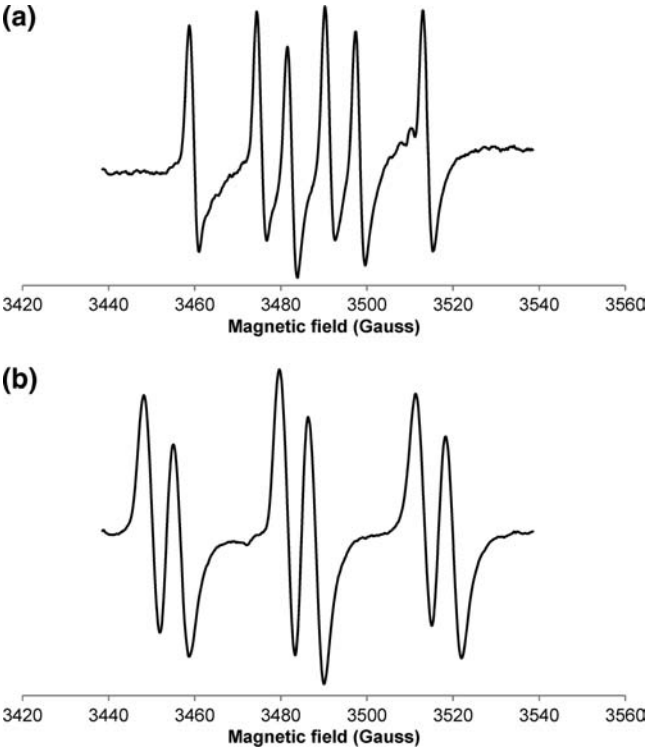


Figure 6. (**a**) Experimental spin pattern of DMPO/$CH_3C^{\bullet}HOH$ spin adduct in simple model wine solution (2 M ethanol, 53 mM tartaric acid, pH 3.6) following the addition of Fenton reagents (50 μM Fe(II), 300 μM H_2O_2). (**b**) Experimental spin pattern of PBN/$CH_3C^{\bullet}HOH$ spin adduct in simple model wine solution (2 M ethanol, 53 mM tartaric acid, pH 3.6) following the addition of Fenton reagents (50 μM Fe(II), 300 μM H_2O_2).

could be identified, demonstrating that the high concentration of ethanol makes it the overwhelming reactant for the nondiscriminating hydroxyl radical generated in the Fenton reaction.

Effect of Sulfur Dioxide on the Fenton Reaction in Model Wine. It is now widely accepted that a key role of sulfur dioxide in preserving finished wine is to inhibit nonenzymatic oxidation by reacting with hydrogen peroxide (*2*, *4*, *9*). The reaction is known to occur at wine pH and most likely proceeds by nucleophilic attack of the bisulfite ion (HSO_3^-) by H_2O_2 to form a peroxymonosulfurous acid intermediate, which subsequently rearranges to give sulfuric acid (*11*). The reaction does not involve free radicals and is an effective means of disabling the oxidation potential of H_2O_2 in wine. Without added sulfur dioxide, any H_2O_2 in wine would be consumed by the Fenton reaction and would lead to wine oxidation. Put into this context, sulfite performs its function in wine by competing with Fe(II) for H_2O_2. However, the nature of this competition is potentially complex, as it is dependent on a number of factors (e.g., coordination of metal catalysts that could affect their reactivity, the pool of "free" versus "bound" sulfur dioxide).

The ability of SO_2 to competitively inhibit the formation of 1-hydroxyethyl radicals in model wine was investigated using PBN (30 mM). As noted above and previously (*5*), the Fenton reaction in wine yields the 1-hydroxyethyl radical as its major product, and this has also been demonstrated in beer (*25*). Sulfur dioxide has been shown to suppress 1-hydroxyethyl radical production in both beer (*26*, *27*) and wine systems (*28*), presumably by scavenging H_2O_2 before it is able to react with Fe(II). Therefore, EPR spin trapping is well-suited to investigate the effect of sulfites on the fate of H_2O_2 in wine.

A range of SO_2 concentrations (25−1000 μM) was used while the concentrations of Fenton reagents were held constant

([H_2O_2] = 300 μM; [Fe(II)] = 50 μM) in the absence of 4-MeC. In air-saturated solution, sulfur dioxide levels exceeding 250 μM were found to inhibit the formation of PBN/MeCH$^{\bullet}$OH adducts most effectively (**Figure 7a**). The basis of this inhibition is the ability of SO_2 to compete with Fe(II) for H_2O_2, thereby protecting the ethanol from hydroxyl radicals. SO_2 concentrations above 250 μM were also found to suppress ethanol oxidation in deoxygenated model solutions; however, at SO_2 levels at 250 μM and below, higher concentrations of PBN/MeCH$^{\bullet}$OH adducts were observed compared to the oxygenated system. This is consistent with the results reported above wherein the absence of oxygen led to higher acetaldehyde yields. Again, these data seem to indicate that the Fe(III) produced by the Fenton reaction is being reduced by 1-hydroxyethyl radicals at a rate higher than the reaction between Fe(II) and H_2O_2. It is expected that both the spin trap and Fe(III) compete for 1-hydroxyethyl radicals in this system. When oxygen is introduced, it appears the 1-hydroxyethyl radical preferentially reacts with dioxygen instead of Fe(III) to form the 1-hydroxyethylperoxyl radical (**Scheme 2**). This reaction between 1-hydroxyethyl radicals and oxygen has been studied previously, and its rate is thought to be diffusion-limited (*25*, *29*). The 1-hydroxyethylperoxyl radical is assumed to react further to give acetaldehyde and the hydroperoxyl radical as products, although the data suggest this reaction is relatively slow within the time frame of the experiment. The rate constant for the spontaneous, unimolecular production of hydroperoxyl radicals and acetaldehyde from 1-hydroxyethylperoxyl radicals is reported to be 50 s^{-1} (*29*), although it is possible that other components of this solution could accelerate the decomposition. It is also conceivable that acetaldehyde is rapidly oxidized to acetic acid in the aerated treatments or that some products react further with hydrogen peroxide, thus diverting the oxidation to other pathways.

The effect of added 4-MeC (12 mM) was evaluated in the same system. Whereas the catechol reduced the formation of acetaldehyde, its presence led to conditions that favored the formation of PBN/MeCH$^{\bullet}$OH adducts. Starting at low levels of SO_2, the concentration of spin adducts was at least twice as high in the presence of 12 mM 4-MeC (**Figure 8**). The production of 1-hydroxyethyl radicals could only be completely stopped when SO_2 was present at 1000 μM (64 mg L^{-1}), although 500 μM SO_2 did inhibit the production of PBN/MeCH$^{\bullet}$OH adducts by 87.2% compared to the control. Deoxygenating the solution resulted in higher observed concentrations of spin adducts in all treatments, as was also the case with model wine without 4-MeC (**Figure 9**). Although dissolved oxygen results in line width broadening or

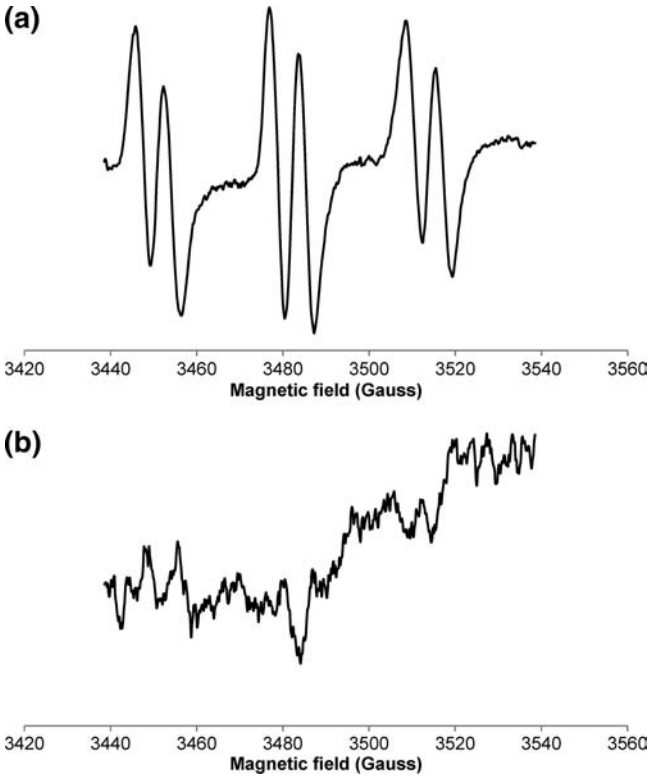


Figure 7. (**a**) Experimental spin pattern of PBN/$CH_3C^{\bullet}HOH$ spin adduct in model wine solution (2 M ethanol, 53 mM tartaric acid, pH 3.6) containing 4-methylcatechol (12 mM) following the addition of Fenton reagents (50 μM Fe(II), 300 μM H_2O_2). (**b**) Experimental EPR spectrum in model wine solution (2 M ethanol, 53 mM tartaric acid, pH 3.6) containing 4-methylcatechol (12 mM) following the addition of 50 μM Fe(II).

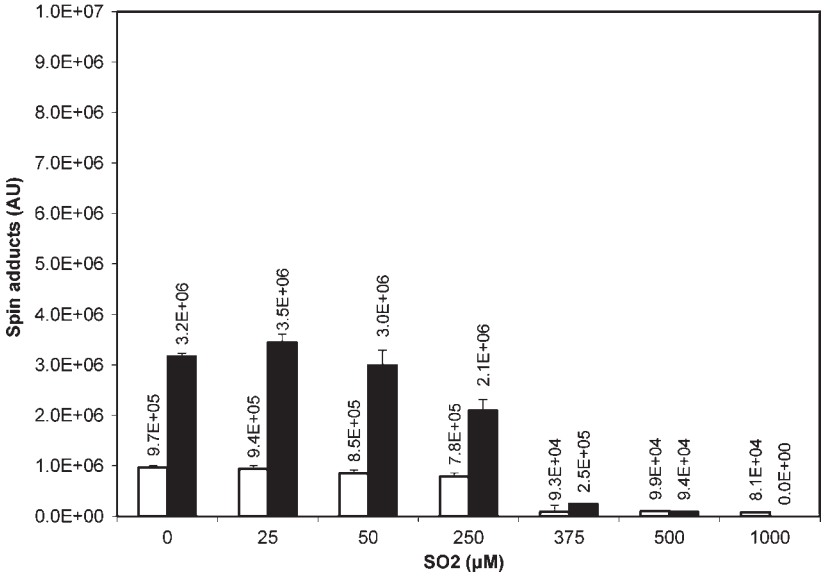


Figure 8. Observed PBN/$CH_3C^{\bullet}HOH$ spin adducts in aerated model wine solution with increasing levels of SO_2 (2 M ethanol, 53 mM tartaric acid, pH 3.6) in the presence (black bars) or absence (white bars) of 4-methylcatechol (12 mM) following the addition of Fenton reagents (50 μM Fe(II), 300 μM H_2O_2). Samples were analyzed after 10 min at 20 °C.

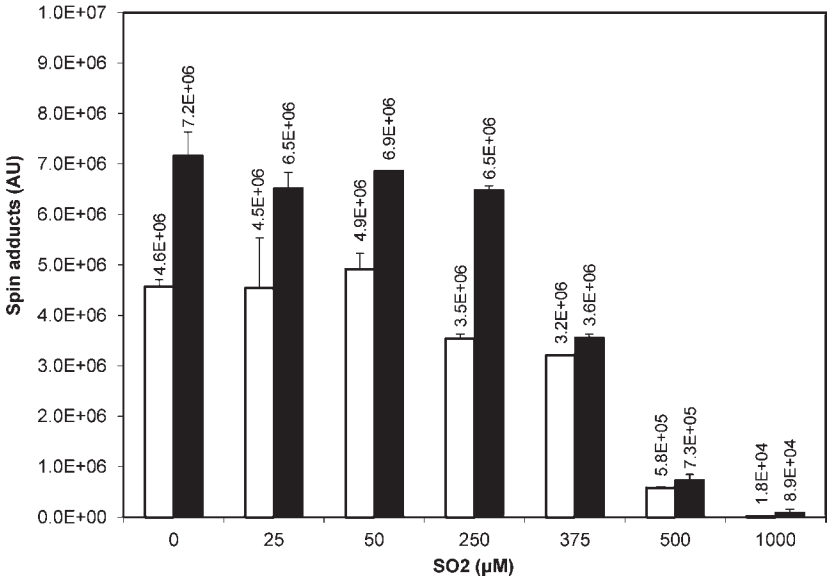


Figure 9. Observed PBN/$CH_3C^{\bullet}HOH$ spin adducts in deaerated model wine solution with increasing levels of SO_2 (2 M ethanol, 53 mM tartaric acid, pH 3.6) in the presence (black bars) or absence (white bars) of 4-methylcatechol (12 mM) following the addition of Fenton reagents (50 μM Fe(II), 300 μM H_2O_2). Samples were analyzed after 10 min at 20 °C.

spin−spin coupling in EPR experiments, which is the basis of EPR oximetry, this could not account for the differences in signal intensities between the oxygenated and deoxygenated treatments in our study. One explanation of why more radicals were formed in the presence of 4-MeC is that oxidation of the phenol could lead to H_2O_2 production during the course of the experiment, which would subsequently be reduced to hydroxyl radicals in the presence of Fe(II) and result in artificially high levels of PBN/$MeCH^{\bullet}OH$ adducts. To explore this possibility, Fe(II) (50 μM) and 4-MeC (12 mM) were added to model wine containing PBN (30 mM) and allowed to stand for 10 min. No PBN/$MeCH^{\bullet}OH$ spin adducts were observed (**Figure 7b**), indicating that H_2O_2 is not produced in significant yield during time frame of the experiment.

It is possible that 4-MeC (12 mM) competes with 1-hydroxyethyl radicals (≤0.3 mM) for Fe(III) ions. Such competition would effectively lead to higher concentrations of the radical available for reaction with PBN (**Scheme 2**). However, the same 4-MeC can also quench the 1-hydroxyethyl radical before it reacts with Fe(III) ions, reducing it to ethanol, thus lowering the yield of acetaldehyde. This may explain the higher observed yield of PBN/$MeCH^{\bullet}OH$ spin adducts but lower yield of acetaldehyde (**Figure 4**). In the case of the oxygenated system, the Fenton pathway is starved for Fe(II), which 4-MeC replenishes by fast reduction, in addition to the above-described effect.

It is clear that the Fenton reaction in wine is dramatically affected by variables of importance in wine processing, during which the levels of phenolics vary widely, especially between white and red wines, and the level of oxygen can vary from saturation to virtually zero. Future work should address the different products yielded at various levels of oxygen, as this has such a dramatic effect on the radical species formed and the reaction outcome. For instance, is it possible to detect the hypothetical hydroxyethylperoxyl radical, and does it decompose efficiently into acetaldehyde, or are other products formed? Such questions may help to address questions related to the impact of different oxygen treatments in winemaking, in which, for example, air saturation is sometimes employed in barrel racking, but microoxygenation treatments achieve 100−200 μg/L of O_2 over sustained periods (*30*), and bottled wine consumes a few milliliters per liter of oxygen per year.

ABBREVIATIONS USED

4-MeC, 4-methylcatechol; PBN, *N*-*tert*-butyl-α-phenylnitrone; DMPO, 5,5-dimethylpyrroline-*N*-oxide; $MeCH^{\bullet}OH$, 1-hydroxyethyl radical.

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Received for review September 5, 2009. Revised manuscript received November 19, 2009. Accepted December 2, 2009.