JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Effect of Metal Chelators on the Oxidative Stability of Model Wine

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ABSTRACT: Oxidation is a major problem with respect to wine quality, and winemakers have few tools at their disposal to control it. In this study, the effect of exogenous Fe(II) (bipyridine; Ferrozine) and Fe(III) chelators (ethylenediaminetetraacetic acid, EDTA; phytic acid) on nonenzymatic wine oxidation was examined. The ability of these chelators to affect the formation of 1-hydroxyethyl radicals (1-HER) and acetaldehyde was measured using a spin trapping technique with electron paramagnetic resonance (EPR) and by HPLC-PDA, respectively. The chelators were then investigated for their ability to prevent the oxidative loss of an important aroma-active thiol, 3-mercaptohexan-1-ol (3MH). The Fe(II)-specific chelators were more effective than the Fe(III) chelators with respect to 1-HER inhibition during the early stages of oxidation and significantly reduced oxidation markers compared to a control during the study. However, although the addition of Fe(III) chelators was less effective or even showed an initial pro-oxidant activity, the Fe(III) chelators proved to be more effective antioxidants compared to Fe(II) chelators after 8 days of accelerated oxidation. In addition, it is shown for the first time that Fe(II) and Fe(III) chelators can significantly inhibit the oxidative loss of 3MH in model wine.

KEYWORDS: *wine oxidation, 3-mercaptohexan-1-ol, 1-hydroxyethyl radicals, chelators, electron paramagnetic resonance*

INTRODUCTION

The oxidation of wine constituents often results in deleterious quality defects, including browning reactions,[1] loss of characteristic aroma compounds,[2] and the production of carbonyls associated with undesirable aromas.[3,4] Nonenzymatic wine oxidation is thought to be catalyzed by trace quantities of transition metals, in particular, iron and copper.[5,6] Whereas the direct reaction between molecular dioxygen and the organic fraction of wine is, in fact, thermodynamically favorable, it is kinetically restricted. The reaction between triplet (e.g., dioxygen) and singlet species (e.g., phenolics, alcohols, acids, etc.) is spin forbidden by Pauli's exclusion principle. As such, ground state oxygen must be excited to the singlet state before it can react with other organic molecules in wine. This is possible via phytochemical excitation,[7] but the most likely mechanism under wine conditions is the one-electron reduction of oxygen by transition metal catalysts.[5]

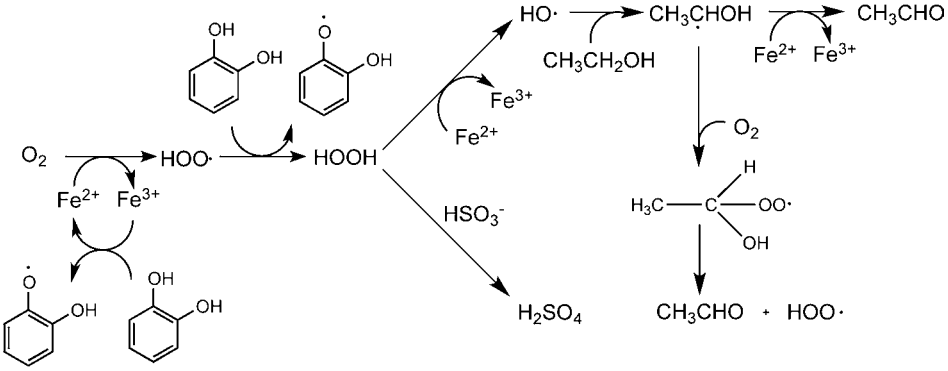
In the presence of Fe(II), oxygen is reduced by a sequential one-electron reduction to yield a superoxide anion radical, which is quickly converted to a hydroperoxyl radical ($HOO^{\bullet}$) under acidic wine conditions[5] followed by its reaction with phenolics and subsequent reduction to hydrogen peroxide (H_2O_2) (Scheme 1). H_2O_2 is thought to react quickly with either bisulfite[8] (when present) or reduced transition metals[9] (e.g., Fe(II) or Cu(I) ions), the latter being a relatively slower reaction. The metal-catalyzed reduction of H_2O_2, known classically as the Fenton reaction, yields highly oxidizing hydroxyl radicals ($HO^{\bullet}$) [10] (Scheme 1). As extremely reactive species, $HO^{\bullet}$ radicals are capable of reacting at diffusion-limited rates with organic components in wine. Due to the fact that ethanol is the major organic component in wine, it is thus the principal target for these radicals,[5,11] which has been shown previously to yield 2-hydroxyethyl radicals (~15%) and 1-hydroxyethyl (HER) radicals (~85%).[11] 1-HER can be further oxidized to acetaldehyde[9,12] or, perhaps, react with other wine components.[13] For example, we have recently demonstrated that wine thiols, such as 3-mercaptohexan-1-ol (3MH), are a target of 1-HER in wine.[14]

In addition to the reactive radical species formed during nonenzymatic wine oxidation, phenolics bearing catechol or pyrogallol groups can be oxidized to quinones.[15] These catechol and pyrogallol moieties can be quickly oxidized by reacting with $HOO^{\bullet}$ or Fe(III) to form semiquinone radicals; upon the loss of another electron by reaction with a radical or Fe(III), a quinone is formed[5] (Scheme 2). These quinones are responsible for many defects in wine, such as browning due to polymerization,[1,16] or Michael-type addition reactions, which can lead to the loss of important aroma-active thiols.[2,17,18]

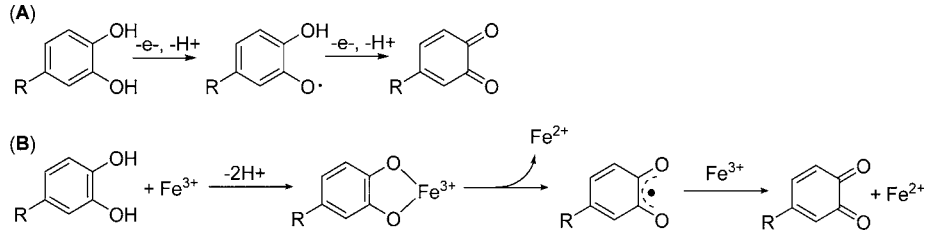
Fe(II) and Fe(III) ions represent the dominant two oxidation states of iron in wine. Iron speciation in wine has been measured in various studies, and it has been reported that the majority of free iron is present as Fe(II),[9,19] although a large fraction of iron is bound to tartrate[20] and tannins.[21] The reason for the dominance of the ferrous species is likely due to the acidic, reducing environment of wine. In addition, wine phenolics are thought to be able to maintain iron in its reduced state.[9] Danilewicz argued that the equilibrium of Fe(II):Fe(III) is important with respect to oxygen consumption and phenolic oxidation.[22] In a simple model wine system, it has been shown that oxidation reactions effectively cease once an equilibrium between Fe(II) and Fe(III) is established. However, once other compounds are introduced that are capable of disrupting this equilibrium, such as sulfites and other nucleophiles, the equilibrium is disrupted and allows for oxidation reactions to progress.[22] The importance of the

Received: June 11, 2013
Revised: August 29, 2013
Accepted: September 4, 2013
Published: September 4, 2013

Scheme 1. Proposed Nonenzymatic Oxidation of Wine via Metal-Catalyzed Reduction of Oxygen and Subsequent Oxidation of Ethanol



Scheme 2. (A) Formation of Quinone upon the Oxidation of Catechol by Loss of Two Electrons and Two Protons; (B) Metal-Catalyzed Oxidation of Catechol and Regeneration of Fe(II)



Fe(II):Fe(III) ratio has also been argued in lipid peroxidation, yet remains controversial.[23,24]

Clearly, transition metals are responsible for catalyzing a number of reactions in wine, often leading to many undesirable effects. The most effective way to completely prevent metal-catalyzed oxidation processes would be to completely remove all trace iron and copper from the juice, must, or wine. In previous studies, the progressive removal of transition metals from wine slowed and eventually shut down oxidation reactions;[25] however, the complete removal of transition metals is not practical, and many of these methods lead to the unintentional stripping of polyphenolic species as well as important aroma-active components.[26] There have been some recent attempts by Trela to remove iron using phytic acid which have proven fairly successful; however, this practice led to an increase in calcium levels, which could lead to stability issues post-bottling.[27] An alternative approach would be to bind metals by chelation in a way that disrupts their ability to redox cycle, which, in turn, would disable their catalytic activity.

A chelator's ability to disrupt iron's redox cycling is key to its efficacy as an inhibitor of oxidation. Wine already contains many small molecules that are able to complex Fe(III) and Fe(II) and effectively alter the reduction potential (E_0) of this redox couple. The more positive the E_0, the greater the oxidizing power of Fe(III); the lower the E_0, the greater the reducing power of Fe(II).[28] Tartaric acid, a hydroxy acid that is present at high concentrations in wine, can bind to Fe(III) at an acid-to-metal ratio of 1:1 as a metal complex of 2:2 at wine pH.[29] This effectively lowers the E_0 of the Fe(III)/Fe(II) couple,[5] making Fe(II) more reducing and increasing the rate of the reduction of O_2 and H_2O_2 to $HOO^{\bullet}$ and $HO^{\bullet}$, respectively. Phenolics containing either catechol or pyrogallol groups can effectively bind to Fe(III) ions upon their deprotonation. At physiological pH, this yields a relatively stable complex[30] and can retard metal-catalyzed oxidation reactions. However, under the acidic conditions of wine, these complexes are less stable, which results in the polyphenol quickly reducing Fe(III) ions to Fe(II) ions[31,32] (Scheme 2). This can effectively reinitiate the metal-catalyzed reduction of oxygen.

The potential application of selective metal chelators capable of strongly binding iron, and inactivating all of its binding sites, could be an effective tool for inhibiting wine oxidation. In this work, various chelators with high affinities for Fe(II) and Fe(III) were used to assess their effect on the rate of oxidation in model wine solutions. The rate of oxidation was tested by measuring the kinetics of 1-HER and acetaldehyde formation, which are two major markers for wine oxidation. In addition, the ability of test chelators to prevent the oxidative loss of 3MH was investigated.

MATERIALS AND METHODS

Materials. Iron(II) sulfate heptahydrate, 4-methylcatechol (4-MeC), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid monosodium salt hydrate (Ferrozine), 2,2′-bipyridyl (BiPy), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and acetaldehyde−DNPH analytical standard were obtained from Sigma-Aldrich (St. Louis, MO, USA). Phytic acid (50% w/w in water) was obtained from TCI America (Portland, OR, USA). (Ethylenedinitrilo)tetracetic acid (EDTA) was purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). 2,4-Dinitrophenylhydrazine (DNPH) was purchased from MCB Laboratory Chemicals (Norwood, OH, USA). L-Tartaric acid, 3-mercaptohexan-1-ol (3MH), 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr), and 3-mercapto-1,2-propanediol (mercaptoglycerol, 90%) were obtained from Alfa Aesar (Ward Hill, MA, USA). Hydrogen peroxide (30% w/w; H_2O_2) was obtained from EMD Chemicals (Gibbstown, NJ, USA). The spin trap phenyl-*N*-*tert*-butyl nitrone (PBN) was purchased from GeroNova Research Inc. (Reno, NV, USA). Water was purified via a Millipore Q-Plus system (Millipore Corp., Bedford, MA, USA). All other chemicals and solvents were of analytical or HPLC grade. The wine used in this study was vinified from mechanically harvested Pinot gris and was generously donated by

dx.doi.org/10.1021/jf4024504 | J. Agric. Food Chem. 2013, 61, 9480−9487

Mazza Vineyards (2010; North East, PA, USA). The endogenous concentrations of Fe and Cu in the wine were 17.9 μM Fe and 1.73 μM Cu as determined by inductively coupled plasma mass spectrometry. The pH of the wine was 3.22, and the total SO_2 concentration was 98 mg/L at the start of all experiments, as measured by the aeration/oxidation method.[33]

EPR Spin Trapping. PBN (30 mM) was dissolved directly into the model wine solution, which contained 12% v/v EtOH and 8.0 g/L tartaric acid with the pH adjusted to 3.6 with NaOH and shaken to achieve air saturation. Stock solutions (20 mM) of each chelator (Ferrozine, BiPy, EDTA, DFO, and phytic acid) were made in water in advance and stored at −80 °C until needed. Stock solutions of Fe(II) (9 mM) and 4-methylcatechol (4-MeC) (100 mM) were freshly prepared in water acidified with HCl (pH 2). The model wine (0.955 mL) was added to 1.8 mL capacity microcentrifuge tubes. Each given chelator treatment (25 μL; 500 μM final concentration), 4-MeC (10 μL; 1 mM final concentration), and Fe(II) (10 μL; 90 μM final concentration) was added to the model wine. Samples were mixed by vortex, and a 50 μL aliquot was immediately withdrawn for measurement by EPR (as described below). Samples were stored in the dark at ambient temperature, and subsequently aliquots were taken at various time intervals.

The wine's pH was adjusted to 3.6 to match that of the model wine system. The sulfur dioxide level was reduced by three consecutive additions of H_2O_2 (3% v/v) spaced at 20 min intervals under constant agitation and headspace blanketing with nitrogen gas, as described previously.[11] The wine was allowed to sit overnight at room temperature, and the final total SO_2 measured was 30 mg/L. PBN (30 mM) was dissolved directly into the wine and was subsequently saturated with air. The wine (0.967 mL) was then added to 1.8 mL capacity microcentrifuge tubes. To the wine was added the given chelator treatment (25 μL; 500 μM final concentration) in addition to Fe(II) (8 μL; 90 μM final concentration) to achieve the same concentrations used in model wine experiments. Samples were stored in the dark at ambient temperature for the duration of the study.

All samples were saturated with air throughout the experiment, and as such, oxygen was not limiting over the time frame of the reaction. Wine or model wine samples (50 μL) containing PBN were loaded into 50 μL borosilicate micropipets. The 1-HER/PBN adduct was quantified, and the EPR spectra were recorded on a Bruker eScan R (Bruker BioSpin, Rheinstetten, Germany) spectrometer operating in X-band at room temperature. The sweep width was set to 50 G, and the microwave power was set to 37.86 mW. Modulation frequency and modulation amplitude were set to 86.00 kHz and 2.45 G, respectively. The receiver gain was set to 4.48×10^3. The conversion time and sweep time were set to 20.48 ms and 10.49 s, respectively. The total number of scans per sample was 10.1. HER adducts produced a triplet of doublets (hyperfine coupling constants: a_N = 15.7 G, a_H = 3.3 G) as observed in previous studies.[9,11] The intensity was quantified by adding the maximum and minimum values of the central doublet.

Acetaldehyde Analysis. Model wine (9.55 mL) was added into a 20 mL capacity headspace vial (23 × 46 mm, 20 mm clear crimp). To the model wine were added each chelator treatment (250 μL; 500 μM final concentration), 4-MeC (100 μL; 1 mM final concentration), and Fe(II) (100 μL; 90 μM final concentration). The sample was then capped with a red crimp cap with a blue silicone/PTFE septum. The sample was mixed by vortex, and 100 μL sample aliquots were withdrawn at room temperature using a Hamilton 100 μL capacity syringe and dispensed directly into DNPH reagent (procedure described below). Samples were stored in the dark at either ambient temperature or 50 °C.

Acetaldehyde was measured in model wine solutions as its DNPH derivative by HPLC according to previous methods.[13,34] DNPH reagent solution was prepared by dissolving DNPH (200 mg) in acetonitrile (100 mL), followed by acidification with 70% w/w perchloric acid (4 mL). Sulfuric acid (25% w/w; 40 μL) and DNPH reagent (240 μL) were added to a 100 μL aliquot of model wine in a 1.8 mL capacity microcentrifuge tube. The derivatization reaction was carried out at ambient temperature for 3 h, at which point 60:40 acetonitrile/water (480 μL) was added to the sample. The sample was then filtered over a PTFE syringe tip filter (0.45 μm; 13 mm). Chromatographic separation was achieved isocratically using a ZORBAX Eclipse Plus C18 column (4.6 × 150 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) with a mobile phase consisting of 70:30 methanol/water. The acetaldehyde−DNPH derivative was detected using a diode array detector at 365 nm and quantified on the basis of an external standard curve prepared with an authentic acetaldehyde standard, which was reacted with DNPH as described in the procedure.

3MH Analysis. Sample preparation was performed in a manner similar to the acetaldehyde analysis (described above) with the following modification: 3MH was added from a freshly made stock solution (100 μg/L) to achieve a final concentration of approximately 1 μg/L. Samples were stored in the dark at 50 °C. The whole sample (10 mL) was used per time point, as described below. Extraction and derivatization were performed as described for previous methods[35,36] with the following modifications: Solid-phase extraction was performed using an Agilent SampliQ 12-position SPE vacuum manifold. All of the samples and solutions were poured into a reservoir on the top of the cartridge and left to pass through the cartridge with a pressure of 0.17 bar. The wine sample (10 mL) was passed through a 50 mg Bond Elut-ENV cartridge conditioned with 1 mL of dichloromethane, methanol, and water. Phosphate buffer (6 mL; 0.2 M 40% methanol) was then passed, followed by water (1 mL), DBU (1 mL; 6.7% in water), and PFBBr (100 μL; 2 g/L in hexane), and allowed to react at ambient temperature for 20 min. Mercaptoglycerol (100 μL; 2 g/L in 6.7% DBU) was then passed through and allowed to react for an additional 20 min. The Bond Elut-ENV cartridge was rinsed with phosphate buffer (6 mL; 0.2 M 40% methanol) and water (1 mL). The samples were eluted into a 13 × 100 mm glass culture tube containing sodium sulfate with hexane/ether solution (1 mL; 1:3 ratio). Samples were mixed by vortex, and 600 μL was carefully withdrawn using a 1 mL Hamilton syringe and placed in a 10 mL screw cap vial; they were then evaporated to dryness under nitrogen and sealed with a silver screw cap with a silicone/PTFE septum.

Chromatographic separation and quantification were achieved using HP 6890/5972 GC/MSD. The capillary column was a DB-FFAP (30 m × 0.25 mm × 0.25 μm) from Agilent Technologies (Santa Clara, CA, USA). The sample was extracted for 30 min at 110 °C with a SPME fiber (DVB/CAR/PDMS) using GERSTEL MPS2 (Linthicum, MD, USA). The thiol adduct was desorbed from the fiber directly in the GC injector for 10 min using splitless mode. Helium was used as carrier gas at a constant flow of 1.2 mL min−1, and injector temperature was set at 250 °C. The column oven temperature was as follows: 80 °C for 10 min, then heated to 220 °C at 5 °C min−1. Detection was performed by negative electrochemical ionization in selective ion monitoring (SIM) mode. m/z 133, 181, and 314 were monitored, and m/z 314 was used as the quantification ion as it had the least noise in the spectra. After each run, the fiber was baked out for 25 min at split mode 1:100. Helium was used as carrier gas at a constant flow of 1.2 mL min−1, and the injector temperature was set at 270 °C. The column oven temperature ramp was as follows: 220 °C for 5 min, heated to 245 °C at 10 °C min−1 for 17.5 min. An external standard curve was prepared in model wine containing 4MeC (0.25−5.0 ppb) with good linearity (R = 0.9942).

Statistical Analysis. Data were analyzed using two-way ANOVA and Bonferroni's post-test to determine differences between treatments (Minitab 16 Statistical Software, State College, PA, USA). Treatments were considered statistically significant when $p < 0.05$. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Effect of Chelators on 1-HER Formation. 1-HER has been shown to be a key radical intermediate of wine oxidation and, as such, was used to monitor the progress of the oxidation in model wine.[11,12] This radical is sufficiently stable to be trapped using a nitrone spin trap (e.g., PBN) and quantified by measuring the intensity of the EPR spectrum corresponding to

the spin adduct (Figure 1). In a model wine system, PBN/1-HER adduct formation was observed within the first 4 h in the chelator-free control (Figure 2).

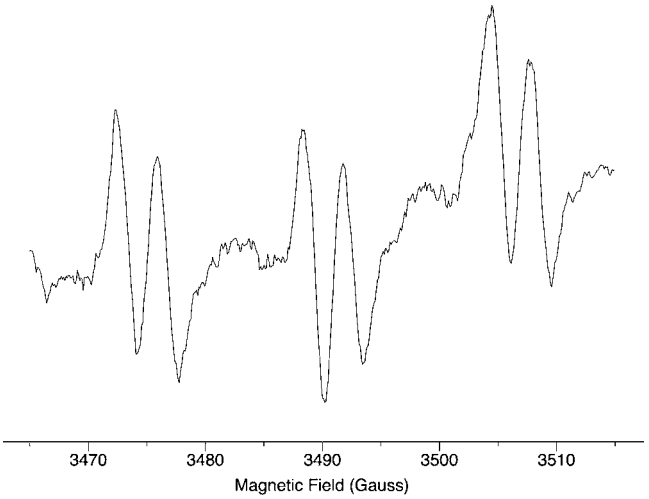


Figure 1. Representative experimental EPR spectrum corresponding to the PBN/1-HER spin adduct intensity in 2010 Pinot gris stored under air at ambient temperature.

The addition of each chelator to model wine was observed to significantly influence the rate of PBN/1-HER formation. The addition of EDTA resulted in an immediate increase in spin adducts, demonstrating pro-oxidant activity (Figure 2). EDTA is known to lower the Fe(III)/Fe(II) reduction potential (E_0) at low pH, thereby increasing the reducing power of Fe(II).[37] In addition, whereas EDTA is capable of binding both Fe(II) and Fe(III) ions, it forms a more stable complex with Fe(III), and results in the rapid oxidation of Fe(II), while, in the process, reducing O_2 and H_2O_2 to $HOO^{\bullet}$ and $HO^{\bullet}$, respectively.[38] Interestingly, phytic acid, which like EDTA preferentially binds Fe(III) and complexes with all coordination sites of iron, did not appear to cause a pro-oxidant effect over the time frame of the reaction and, in fact, inhibited 1-HER formation compared to control (Figure 2). This observation warrants further study.

The Fe(II) chelators, BiPy and Ferrozine, were able to completely inhibit the formation of 1-HER over the time frame of the reaction (Figure 2). The nitrogen ligands on both Ferrozine and BiPy are thought to preferentially bind to Fe(II) and significantly increase the E_0 by stabilizing the low oxidation states of metals.[39] This, in effect, is thought to stabilize Fe(II) and thus limit its reactivity with O_2 and H_2O_2.

The same reaction was also carried out in Pinot gris with reduced SO_2 levels. The same general trend that was observed in model wine was observed in this system, although an initial lag phase was seen, likely due to the quenching of H_2O_2 by residual SO_2 (Figure 3). This system likely had an Fe(II):Fe(III) ratio representative of white wine and indicates that the chelator treatments work in a similar manner as they did in the model wine, despite the fact that only Fe(II) was initially present. Interestingly, whereas the EDTA-containing treatment produced the highest spin adduct intensity, indicating a higher rate of radical formation, it produced a slightly longer lag phase as compared to the control. However, unlike the initial results seen in model wine for phytic acid, 1-HER formation for this treatment was not significantly different from control (except for 47.5 h). As was seen in the above-described experiments in model wine, no evidence of PBN/1-HER radical adduct formation was observed for either BiPy or Ferrozine treatment over the course of the study (Figure 3).

Effect of Chelators on Acetaldehyde Generation. 1-HER is thought to be oxidized to acetaldehyde under wine conditions (Scheme 1), although there is evidence that it can react with other wine components. Acetaldehyde generation rates were monitored to corroborate EPR spin trapping data. This was deemed important as the PBN/1-HER spin adducts are known to degrade over time and can be reduced or oxidized to EPR silent species. During the first 24 h of the study, acetaldehyde analysis showed a similar trend as was observed by

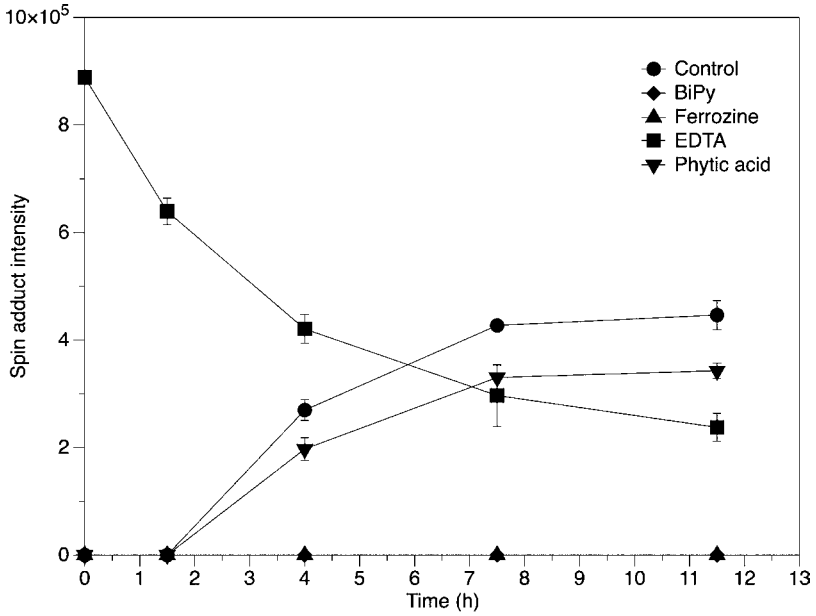


Figure 2. Intensity of PBN/1-HER spin adducts (arbitrary units) in the presence of various iron chelator treatments in model wine (12% EtOH, 8 g/L tartaric acid, pH 3.6) and 4-MeC under air at ambient temperature.

dx.doi.org/10.1021/jf4024504 | J. Agric. Food Chem. 2013, 61, 9480−9487

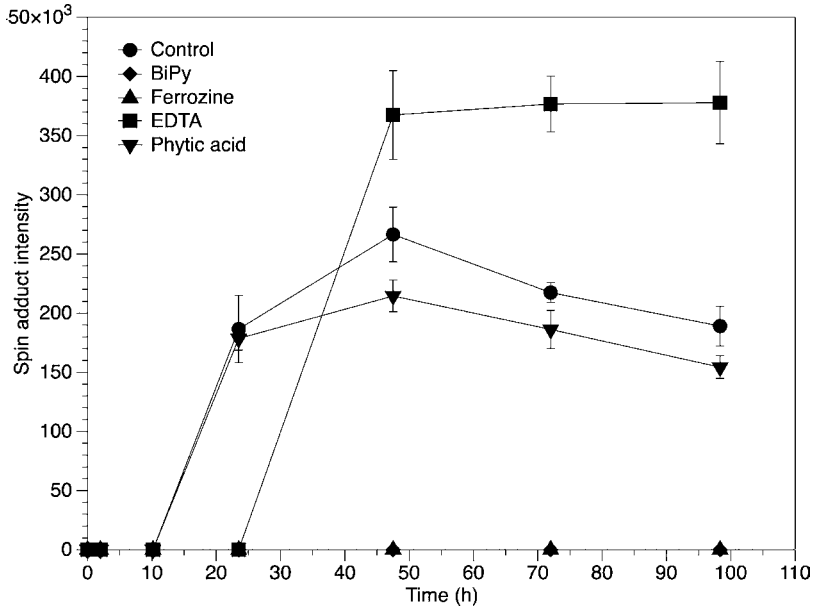


Figure 3. Intensity of PBN/1-HER spin adducts (arbitrary units) in the presence of various iron chelators in Pinot gris adjusted to pH 3.6 under air at ambient temperature.

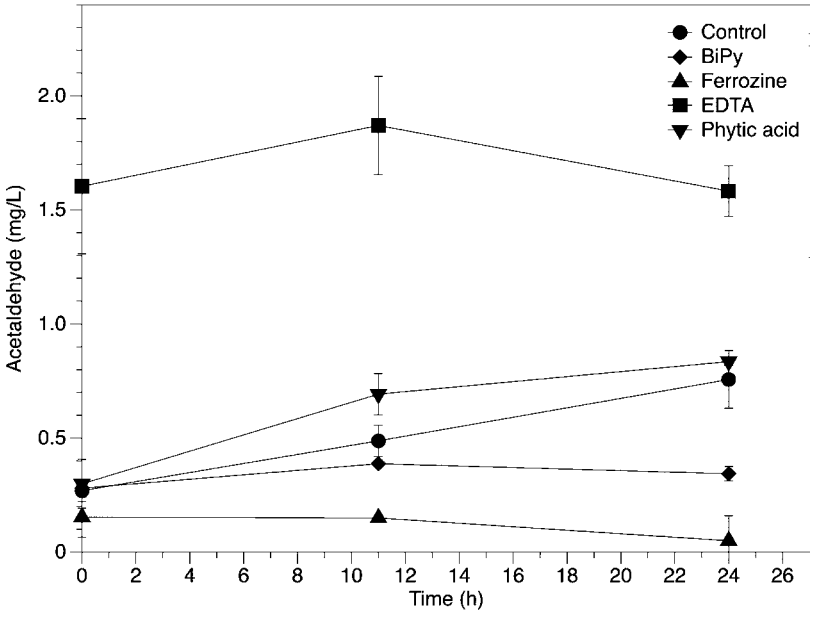


Figure 4. Acetaldehyde formation over time in the presence of various iron chelator treatments in model wine (12% EtOH, 8 g/L tartaric acid, pH 3.6) and 4-MeC under air at ambient temperature.

EPR spin trapping experiments (Figure 4). EDTA-containing treatments showed an immediate pro-oxidant effect as demonstrated by EPR experiments. Phytic acid-containing trended toward higher acetaldehyde levels; however, this was not statistically significant. As of the study's final time point (24 h), EDTA had 108% more acetaldehyde compared to the control. Although the Fe(II) chelator treatments did not completely inhibit oxidation, as observed by the EPR experiments, they did significantly reduce total acetaldehyde formation compared to control, with BiPy and Ferrozine treatments inhibiting acetaldehyde by 55% and 94% at 24 h, respectively. However, the readings for acetaldehyde were lower than expected given the amount of Fe(II) added, indicating the degree of oxidation was minimal.

To be representative of a longer wine oxidation process, acetaldehyde formation was measured over a longer time frame at 50 °C. Although the very initial time points show the same trend as observed in the previous experiments, the roles of Fe(III) and Fe(II) chelators appeared to be reversed. Whereas all chelators significantly inhibited acetaldehyde formation rates compared to the control (Figure 5), treatments containing BiPy or Ferrozine resulted in significantly higher acetaldehyde formation rates compared to the EDTA and phytic acid treatments.

Both BiPy- and Ferrozine-containing treatments yielded similar effects throughout the experiment. Whereas initially these treatments inhibited acetaldehyde formation, the most compared to EDTA and control treatments, by days 5 and 8

dx.doi.org/10.1021/jf4024504 | J. Agric. Food Chem. 2013, 61, 9480−9487

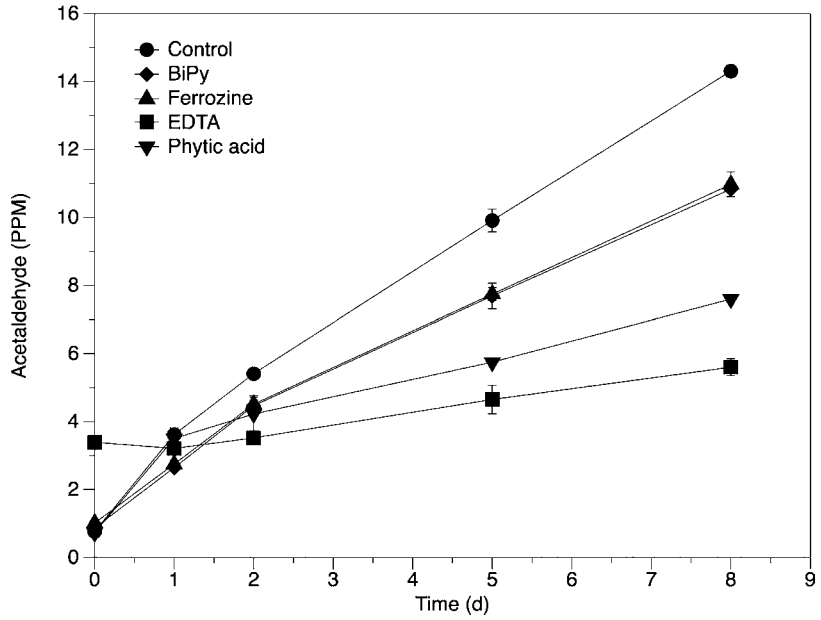


Figure 5. Acetaldehyde formation over time in the presence of various iron chelator treatments in model wine (12% EtOH, 8 g/L tartaric acid, pH 3.6) and 4-MeC under air at 50 °C.

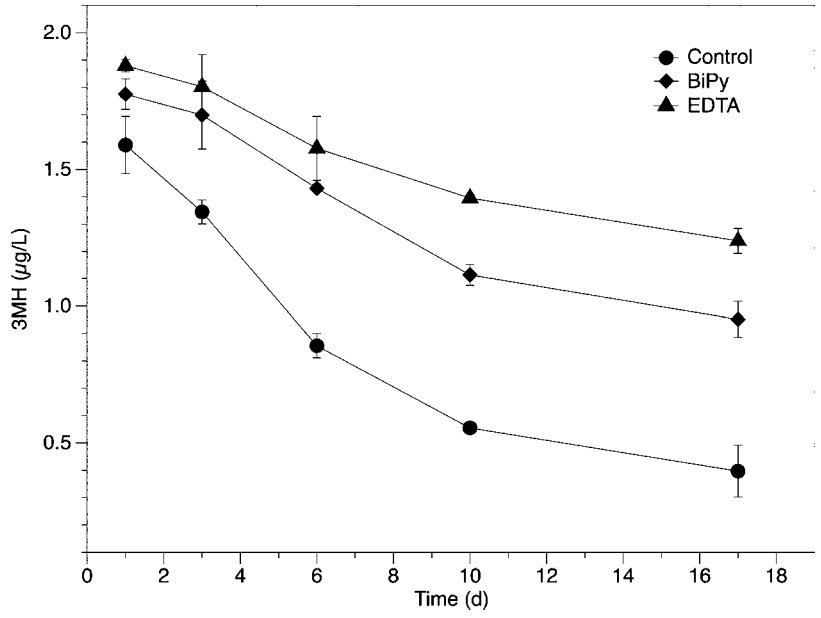


Figure 6. 3MH loss over time in the presence of various iron chelator treatments in model wine (12% EtOH, 8 g/L tartaric acid, pH 3.6) and 4-MeC under air at 50 °C.

they had significantly higher acetaldehyde formation compared to the Fe(III) chelators, but still significantly lower than the control treatment. By day 8, BiPy- and Ferrozine-containing treatments inhibited acetaldehyde formation by 24 and 23%, respectively, relative to the control. EDTA treatment showed an initial pro-oxidant activity, but interestingly, the reaction drastically slowed at the subsequent days, and after just one day, it was at the same acetaldehyde levels as the rest of the treatments. The phytic acid treatment did not show the same pro-oxidant activity as EDTA, but after day 1 they did not significantly differ from each other throughout the rest of the experiment. By day 8, the Fe(III) chelator proved to be the most effective oxidation prevention treatments, with EDTA and phytic acid treatments inhibiting acetaldehyde formation by 61% and 59%, respectively, compared to the control treatment (Figure 5).

The data appear to suggest that EDTA, an Fe(III) chelator, promotes the oxidation of Fe(II) to Fe(III), thus causing a short-term pro-oxidant effect, as evidenced by an initial burst of 1-HER. However, once all iron was bound by EDTA, the complexed metal appears to be relatively stable and limit further oxidation reactions. As the redox cycling ability of iron is not completely prevented upon binding to EDTA, catechols could potentially reduce ferric ions while complexed with EDTA by outer-sphere electron transfer,[40,41] albeit at a much slower rate. Phytic acid, on the other hand, did not show a significant pro-oxidant effect as would be expected as it preferentially binds to Fe(III) and warrants further studies. The

dx.doi.org/10.1021/jf4024504 | J. Agric. Food Chem. 2013, 61, 9480−9487

Fe(II) chelator treatments do not completely inactivate iron either, as O_2 and H_2O_2 can still occasionally bind to iron leading to the eventual oxidation of ethanol to acetaldehyde (Scheme 1). The high concentrations of tartaric acid and catechol may shift the equilibrium of iron speciation to Fe(III) as they preferably bind to Fe(III), allowing the oxidation of Fe(II) and reduction of O_2 and H_2O_2.

Effect of Chelators on Oxidative Stability of 3MH. For simplicity, two representative chelator treatments were investigated compared to a chelator-free control: BiPy was selected as a representative Fe(II) chelator and EDTA as a Fe(III) chelator (Figure 6). Interestingly, the initial pro-oxidant activity of EDTA as observed by 1-HER formation and acetaldehyde yield did not translate to 3MH loss. In fact, at no point did the EDTA-containing treatment have a lower concentration of 3MH compared to control and BiPy. At day 1, the EDTA-containing treatment had a significantly higher concentration of 3MH than control, and the BiPy treatment was not significantly different from control. Up until day 6, EDTA and BiPy treatments were not significantly different from each other, but had significantly higher concentrations of 3MH compared to control. By days 10 and 17, the EDTA treatment had significantly preserved a higher concentration of 3MH than the BiPy treatment; by day 17, there was 75% loss of 3MH for control, 46% loss for BiPy, and 34% loss for EDTA (Figure 6).

We propose two mechanisms by which these chelators prevent the loss of 3MH in wine. The first mechanism involves preventing the formation of quinones that can react with 3MH via Michael-type addition reactions to form thiol−catechol adducts, effectively causing a loss of 3MH.[17,18]The second mechanism involves preventing the formation of 1-HER, which is capable of directly oxidizing the thiol, followed by subsequent dimerization or potentially scavenging of the thiyl radical by a catechol and forming subsequent semiquinone radicals and quinones in close proximity to the thiol.[14]

This first part of the present study focused primarily on a model wine system containing only the major components of wine (i.e., ethanol, tartaric acid, and phenolics); however, there are other components capable of complexing transition metals in wines. Whereas 4-MeC was used at a concentration representative of the phenolic content of white wines, in reality, there are a variety of polyphenolic compounds capable of forming a diverse set of ferric complexes. In addition, the presence of various nucelophiles including sulfites, thiols, and amino acids, as well as other transition metals that were not added in this system, can interact with iron and greatly affect oxidation rates.

Several of the chelator treatments used in this study were observed to inhibit oxidation reaction (e.g., Ferrozine, BiPy); however, the obvious downside is that these compounds are either toxic and/or not permitted as food additives. Future work should focus on food grade Fe(II) chelators. For example, phytic acid or its derivatives are permitted for use in wine in some regions and warrants further study as a preventative antioxidant in wine.

In the present study, we have shown a clear inhibition of 1-HER formation in model wine through the use of exogenous metal chelators. This inhibition of radical formation should, theoretically, inhibit the formation of quinones as well; however, this requires further study. In addition, we show for the first time that chelators can be used as an effective treatment for preventing the loss of an important aroma-active thiol (3MH) in model wine, which should translate to other varietal thiols. Further work should focus on real wine and, perhaps most importantly, the effect of chelator interventions on the sensory attributes of wine.

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Notes

The authors declare no competing financial interest.

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