The Influence of Stereochemistry of Antioxidants and Flavanols on Oxidation Processes in a Model Wine System: Ascorbic Acid, Erythorbic Acid, (+)-Catechin and (−)-Epicatechin

Andrew C. Clark,*[†] Jochen Vestner,[†,‡] Célia Barril,[†] Chantal Maury,[§]
Paul D. Prenzler,[†] and Geoffrey R. Scollary[‖]

[†]National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588,
Wagga Wagga 2678, Australia, [‡]University of Applied Sciences Wiesbaden, Department Geisenheim,
Von-Lade-Strasse 1, 65366 Geisenheim, Germany, [§]UMT Vinitera, Ecole Supérieure d'Agriculture,
Laboratoire GRAPPE, 55 rue Rabelais, BP 30748, 49007 Angers Cedex 1, France, and
[‖]School of Chemistry, The University of Melbourne, 3010, Australia

The stereochemical influence of antioxidant and flavanol compounds on oxidation processes in a model wine system was studied. The diastereoisomers, ascorbic acid and erythorbic acid, were used as antioxidants in a model wine system containing either (+)-catechin or (−)-epicatechin as the oxidizable flavanol compound. Samples were stored at 45 °C for a period of 14 days and analyzed by UV/visible spectrometry, CIELab, UPLC-PDA, and LC-MS. The results showed that less brown oxidative coloration occurred for samples with erythorbic acid for a given flavanol compound, while (+)-catechin provided less yellow coloration for a given antioxidant. Although erythorbic acid was degraded faster than ascorbic acid, it was associated with less decay in the accompanying flavanol compound. Xanthylium cation pigments were identified as the major contributor to color development. Furthermore, the production of pigment precursors, previously identified as furanone-substituted flavanols, was confirmed in all cases and their corresponding xanthylium cation pigments were lower in the presence of erythorbic acid than ascorbic acid. The results demonstrate that erythorbic acid is more efficient at minimizing oxidative color development than ascorbic acid in the model wine system.

KEYWORDS: Browning; white wine; (+)-catechin; xanthylium cation; (−)-epicatechin; model wine; ascorbic acid; erythorbic acid

INTRODUCTION

The oxidation of white wine is a well-known spoilage phenomenon. With sufficient oxygen ingress, a white wine will eventually change from a faint yellow color to a brown color (*1, 2*), and will also have a dramatic change toward negative aroma and palate notes (*3, 4*). The change in color is linked to the presence of skin and seed-derived flavanol-type phenolic compounds in the white wine, such as (+)-catechin or (−)-epicatechin (**Figure 1**) (*2*). The total flavanol concentration in white wines is highly variable, ranging from trace levels (< 5 mg/L) to levels upward of 200 mg/L, and depends on the grape variety, production techniques adopted and measurement techniques utilized (*5−10*). The majority of dry table white wines have flavanol levels in the lower ranges (i.e. < 30−50 mg/L), and those with more than 50 mg/L flavanols are often prepared from juice with extended skin and seed contact.

Despite the common occurrence of white wine oxidation, the actual pigments responsible for the brown coloration in white wine have not yet been fully characterized. This is mainly due to the diverse range of phenolic compounds present in white wine (*11*), their variable and often low concentrations, and the complexity involved in both oxidation and the subsequent polymerization reactions (*12−15*).

For this reason, model wine systems have commonly been used to study oxidation reactions that have some relevance to white wine (*12−14, 16*). This generally involves ethanol concentrations in the range of 10−14% (v/v), pH buffering between 3.0 and 3.7, and the presence of at least one phenolic compound. Such a model wine system, with saturated oxygen levels, has led to the identification of yellow xanthylium cation pigments (**Figure 1**) that are formed from the reaction of (+)-catechin with a degradation product of tartaric acid, one of the two major organic acids of grapes (*17*). It has been subsequently shown that similar xanthylium cations can be generated from the diastereoisomer of (+)-catechin, namely, (−)-epicatechin (**Figure 1**), albeit with molar absorptivity coefficients approximately twice that of the (+)-catechin-derived xanthylium cations (*18*). This means that, for a

*Corresponding author. Mailing address: National Wine and Grape Industry Centre, Locked Bag 588, Wagga Wagga, NSW, 2678 Australia. Tel: +61 2-69334181. Fax: +61 2-69334068. E-mail: aclark@csu.edu.au.
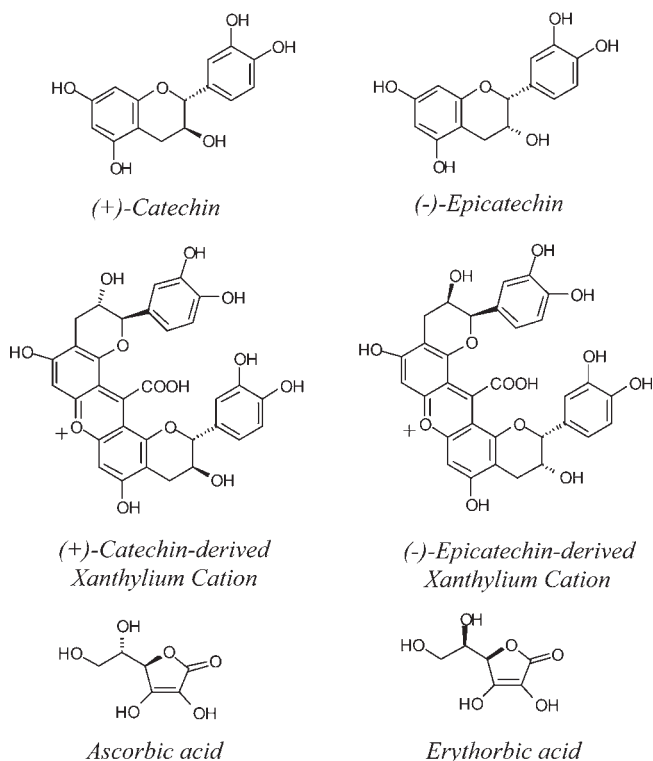
Figure 1. Structures of (+)-catechin, (−)-epicatechin, their respective xanthylium cations, ascorbic acid, and erythorbic acid.

similar initial flavanol concentration in a model wine system, (−)-epicatechin is capable of producing a color that is about twice as intense as that observed with (+)-catechin in the final oxidized model wine. This highlights the importance of stereochemistry in determining the molar absorptivity coefficients of xanthylium cations. The presence of these same yellow xanthylium cation pigments has been confirmed in white wines with elevated flavanol levels and after oxidative storage conditions (19).

In order to prevent the oxidative spoilage of white wine, the traditional antioxidant utilized is sulfur dioxide (15, 20). However, sulfur dioxide is not efficient at preventing the oxidation of phenolic compounds in the presence of dissolved oxygen, especially at the sulfur dioxide concentrations typically adopted in white wine (i.e., 20−35 mg/L free sulfur dioxide) (20). Instead, the main antioxidant role of sulfur dioxide is to react with the products formed from the oxidation of phenolic compounds and the reduced molecular oxygen. This mainly includes *o*-quinone compounds and hydrogen peroxide (15), but may also include acetaldehyde if the reaction between sulfur dioxide and hydrogen peroxide is not sufficiently rapid (21). For this reason, the complementary use of the antioxidant ascorbic acid has added benefits due to its efficient ability to react directly with molecular oxygen, albeit mediated by metal ions, and thereby prevent the oxidation reactions of wine components at the earliest possible stage. The timing and concentration of ascorbic acid addition are variable, but it is typically added at rates ranging from 50 mg/L to 150 mg/L (22−25). Wine trials have clearly shown that the addition of ascorbic acid as well as sulfur dioxide to Chardonnay and Riesling at bottling resulted in wines able to maintain their color and flavor longer than those wines that only adopted a sulfur dioxide addition (26). On the other hand, the use of ascorbic acid without hydrogen sulfite has been demonstrated to be detrimental (27, 28), with its degradation products inducing oxidative coloration after the ascorbic acid is near depleted (i.e., >90% consumed). In this sense, ascorbic acid is still acting as an antioxidant while present, but its degradation products exert their pro-oxidative effects after the sufficient decay of ascorbic acid.

However, ascorbic acid is not the only complementary antioxidant to sulfur dioxide on offer, as erythorbic acid is also a permissible wine additive in many countries. Given that erythorbic acid and ascorbic acid are diastereoisomers, it is reasonable to expect that they should both have antioxidant activities. Erythorbic acid is commonly described as having identical antioxidant capability to ascorbic acid in white wine (21, 29), while in other foods or biological systems, their comparative antioxidant capability is variable (30−32) but in some instances erythorbic acid shows superior activity to ascorbic acid (33−35). There are also several anecdotal observations in the Australian wine industry that erythorbic acid may have some improved abilities over ascorbic acid to prevent the production of dominant red tints, or pinking, in white wine. However, despite this, ascorbic acid is still the main complementary antioxidant to sulfur dioxide used in white wine.

Based on potential for improvement in antioxidant capabilities, this study was undertaken to compare the role of erythorbic acid and ascorbic acid as antioxidants using a model wine system containing the isomeric flavanol compounds (+)-catechin and (−)-epicatechin. The work was conducted in the presence of elevated oxygen and the absence of sulfur dioxide in order to determine the role of the antioxidants in a worst-case scenario for white wine. The impact of the antioxidant and flavanol compound combinations on the oxidative coloration, degradation kinetics and products were followed by UV/visible spectrophotometry, CIELab, UPLC-PDA and LC−MS.

MATERIALS AND METHODS

Reagents and Apparatus. All glassware and plasticware were soaked for at least 16 h in 10% nitric acid (BDH, AnalaR) and then rinsed with copious amounts of grade 1 water (ISO 3696). Solutions and dilutions were prepared using grade 1 water. Potassium hydrogen L-(+)-tartrate (>99%) was obtained from Aldrich (USA) and L-(+)-tartaric acid (>99.5%) from Sigma-Aldrich (USA). L-Ascorbic acid (>99%, Sigma, USA), erythorbic acid (98%, Aldrich, USA), (+)-catechin monohydrate (98%, Sigma, USA) and (−)-epicatechin (95%, Sigma, USA) were used without further purification. To determine the concentration of metal contamination in the flavanol and antioxidant reagents, concentrated solutions (130 g/L) of each reagent, as well as a blank, were acid-digested with concentrated nitric acid and analyzed by inductively coupled plasma with optical emission spectroscopy (ICP-OES). The only contaminant found greater than the detection levels of the ICP-OES method was 1.2 ± 0.1 ng/kg iron ($n = 4$, 95% confidence limits) in the ascorbic acid standard, corresponding to an iron concentration of 0.48 ± 0.04 μg/L in the final model wine system. For this reason, 100.0 μg/L iron(II) was added to all samples in the form of iron(II) sulfate heptahydrate (98%, Ajax Chemicals, Australia) to limit the impact of the iron contamination on oxidation reaction rates.

Absorbance measurements and spectra were recorded on a μQuant Universal Microplate Spectrophotometer (Biotek Instruments, New York, NY) with the software KC4 v3.0 (Biotek Instruments). The absorbance measurements were recorded at 440 nm and spectra recorded from 200 to 600 nm. CIELab measurements were conducted on a Shimadzu (Kyoto, Japan) UV-1700 UV/visible spectrophotometer with UVPC Color Analysis software (version 3.00). The L^*, a^* and b^* CIELab values were calculated at the daylight illuminant D65 and with a 10° observer angle. The transmission was scanned over the range 380−780 nm with samples in 10 mm quartz cuvettes. The model wine solution without additives was used as the blank solution.

Liquid chromatography was conducted with an ultraperformance liquid chromatography (UPLC) system consisting of a Waters (Milford, MA) Acquity binary solvent manager connected to a sample manager and a PDA detector all run by Empower[2] chromatography manager software. The column was a Waters Acquity BEH C18 (2.1 × 50 mm) with 1.7 μm particle diameter. Injection volume was 7.5 μL, and the elution gradient consisted of solvent A (0.5% acetic acid in water) and B (0.5% acetic acid

in methanol), as follows (expressed in solvent A and cumulative time): 100% at 0 min, 100% at 1 min, 95% at 1.31 min, 62% at 5.25 min, 56% at 6.27 min, 48% at 6.34 min, 45% at 7.22 min, 0% at 8.85 min, 0% at 9.4 min, 100% at 9.74 min, and 100% at 10.76 min. The flow rate was 0.45 mL/min. Chromatograms and UV/visible spectra were recorded over the range 200−700 nm.

LC−MS experiments were conducted on a SpectraSYSTEM Thermo Scientific-LC (Waltham, MA) run by Xcalibur software (Waltham, MA) with a P4000 sample pump, UV6000LP UV detector and a Finnigan AQA quadrapole MS with an electrospray source. The column used was a reverse phase Wakosil C18RS (5 μm packing, 250 mm × 2 mm) from SGE (Ringwood, Australia) with a guard column of the same type. The flow rate, solvent composition, solvent gradient and injection volume were the same as described previously (*14*). The LC−MS experiments were carried out in the positive ion mode, with an ion spray voltage of +3 kV and orifice voltage of +10 V, and in the negative ion mode, with an ion spray voltage of −3 kV and orifice voltage of −30 V. In experiments investigating fragmentation products, the orifice voltages were increased to +80 and −80 V in the positive and negative ion modes respectively. Simultaneous wavelength detection at 278 and 440 nm was performed.

The ratio of 280 nm molar absorptivity coefficients for the (+)-1″-methylene-6″-hydroxy-2H-furan-5″-one-flavanol isomers was determined by LC−MS according to a variation of the method of Labrouche et al. (*18*), and outlined further below (see Calculation of Relative 280 nm Molar Absorptivity Coefficients). The MS ion chromatogram, corresponding to the parent ion for each isomer (403 *m/z* in the positive ion mode, 401 *m/z* in the negative ion mode), was obtained in duplicate at each cone voltage of −30 V, +30 V and +60 V, and the 280 nm chromatogram was obtained (*n* = 6) from the photodiode array detector.

Reactions. The model wine solution was prepared by adding 0.011 M potassium hydrogen tartrate and 0.008 M tartaric acid to aqueous ethanol (12% v/v, 2 L) and stirred overnight at room temperature. The pH of the model wine solution was 3.2 ± 0.1. The majority of the studies were conducted with (+)-catechin or (−)-epicatechin (0.69 mM, 0.20 g/L), and either ascorbic acid or erythorbic acid (2.3 mM, 0.40 g/L) which were added to this solution and stirred until dissolved. Iron(II) (100 μg/L), in the form of iron(II) sulfate heptahydrate, was added immediately prior to the commencement of an experiment. Samples (100 mL) were placed in "250 mL" Schott Duran reagent bottles and had a measured headspace volume of 220 mL. Samples were stirred vigorously to vortex for 3.0 min at the start of the experiment and during sampling to replenish the oxygen in the bottles. The total available oxygen at the start of the experiment and after sampling was calculated to be around 19×10^{-4} mol, composed of 8.0 mg/L (0.25×10^{-4} mol) dissolved oxygen in the 100 mL sample, assuming saturation (*1*), and 18.8×10^{-4} mol in the 220 mL headspace. Given that the maximum drop in ascorbic acid was 120 mg/L (or 0.68×10^{-4} mol) between sampling, and the reaction between oxygen and ascorbic acid is 1:1, the amount of oxygen available to the sample was in excess of that consumed by ascorbic acid or erythorbic acid. The samples were held in darkness at 45 °C, and the sample bottles were only opened on measurement days. All samples were prepared in triplicate and the plotted data are the means of the replicates with the error bars representing the 95% confidence limits.

Calculation of Relative 280 nm Molar Absorptivity Coefficients. The ratios of the 280 nm molar absorptivity coefficients for (−)-MHF-8-epicatechin to (+)-MHF-8-catechin, and for (−)-MHF-6-epicatechin to (+)-MHF-6-catechin, were determined from LC−MS data using a modified method of Labrouche et al. (*18*). Labrouche et al. (*18*) calculated the overall ratio of molar absorptivity coefficients for xanthylium cations, such as those shown in **Figure 1**, in the positive ion mode at a single cone voltage. For the MHF-flavanols in this current study, the ratio of 280 nm molar absorptivity coefficients were calculated at three different cone voltages (−30 V, +30 V and +60 V), covering both positive and negative voltages, in order to limit any impact of stereochemical structural differences on ionization efficiency.

The ratio of the relative molar absorptivity coefficients of the (+)-catechin-derived and (−)-epicatechin-derived MHF-flavanols were calculated using the Beer−Lambert Law as

$$\varepsilon_e/\varepsilon_c = (A_e \times c_c)/(A_c \times c_e)$$

where A, ε and c refer to the absorbance, molar absorptivity coefficients and concentration, and the subscripts c and e refer to (+)-catechin-derived
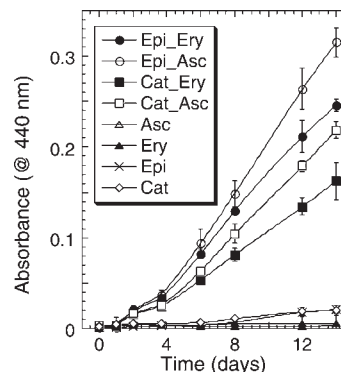

Figure 2. Change in 440 nm absorbance during the storage of samples at 45 °C in darkness. Error bars indicate the 95% confidence limits (*n* = 3).

and (−)-epicatechin-derived MHF-flavanols respectively. From the mass chromatograms, the concentration of MHF-flavanol, *c*, will be proportional to the total ion current, *I*,

$$c = r \times I$$

where *r* is a constant; then,

$$\varepsilon_e/\varepsilon_c = (A_e \times I_c)/(A_c \times I_e)$$

The ratio of molar absorptivity coefficients for MHF-8-catechin to MHF-8-epicatechin was found to be 1.08 ± 0.09. The ratio of molar absorptivity coefficients for MHF-6-catechin to MHF-6-epicatechin was found to be 1.1 ± 0.2.

RESULTS AND DISCUSSION

Oxidative Coloration of Model Wine Systems. The model white wine system was prepared with the different combinations of flavanol and antioxidant compounds, namely, (+)-catechin/(−)-epicatechin and ascorbic/erythorbic acids, respectively. The concentrations of antioxidant (400 mg/L) and flavanol (200 mg/L) were at high levels for white wine, but allowed sufficient time to analyze the kinetics of reagent decay and for appreciable concentrations of reaction products to form. It has been previously shown that higher concentrations of ascorbic acid/(+)-catechin did not affect the species formed but only their rate of formation (*27*).

All the samples were initially a similar pale yellow color and consequently of low 440 nm absorbance (day 0, **Figure 2**). After 14 days of incubation in darkness at 45 °C the binary combinations of flavanol and antioxidant constituents increased in 440 nm absorbance intensity, much more rapidly than for the samples with a single component (**Figure 2**). This was consistent with past reports for the ascorbic acid and (+)-catechin combination in model wine systems (*27, 28*) but demonstrated that erythorbic acid also had the potential to induce coloration. Of the binary systems, those with ascorbic acid as the antioxidant were of higher 440 nm absorbance, as were those with the flavanol compound, (−)-epicatechin. Consequently, the ascorbic acid/(−)-epicatechin pair resulted in the highest 440 nm absorbance after 14 days while the erythorbic acid/(+)-catechin combination gave the lowest 440 nm absorbance. The presence of a lag period before the accelerated coloration was evident in all the binary samples (**Figure 2**), and duration of the lag period appeared similar in length regardless of the antioxidant present.

Although the data in **Figure 2** provide an insight into the 440 nm absorbance change in the samples, it does not provide a measure of the overall color of the samples. To achieve this, the samples were also analyzed by CIELab, which provides an analytical description of sample color with respect to three
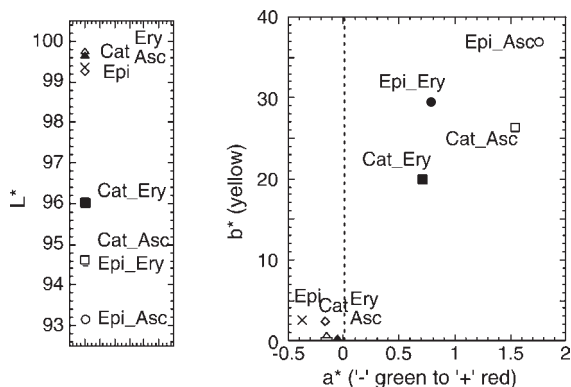
Figure 3. CIELab analysis of samples after 14 days of storage at 45 °C in darkness. The results are significantly different (*P* = 0.05) for any samples whose symbols are not in contact for a given parameter. Error bars indicate the 95% confidence limits (*n* = 3).

parameters: *a** indicating green to red coloration, *b** indicating blue to yellow coloration, and *L** indicating transparent (100) to opaque (0) coloration. At day 0, the samples were of either little or no difference to each other as described by the CIELab parameters (data not shown). However, by day 14 a divergence in the CIELab data for the samples was apparent (**Figure 3**). The description of the samples by the *b** and *L** parameters provided an ordering of the samples consistent with the 440 nm data (**Figure 2**): epicatechin/ascorbic > epicatechin/erythorbic > catechin/ascorbic > catechin/erythorbic. Intriguingly, the CIELab analysis also showed that the samples with ascorbic acid had higher *a** values than those with erythorbic acid. Therefore, these CIELab data demonstrate that not only can erythorbic acid provide lower intensity of yellow color in the oxidizing samples compared to ascorbic acid but it also can limit the amount of red coloration. Such an outcome is consistent with the anecdotal observations by winemakers that erythorbic acid is more effective than ascorbic acid against pinking, or development of red tints, in white wines. In the samples with ascorbic acid, the increased production of the primary yellow and red colors meant that the samples appeared more orange than the samples with erythorbic acid, which was in agreement with the visual assessment of the samples (data not shown).

Change in Flavanol and Antioxidant Concentrations. During the oxidative coloration of the samples, the flavanol and antioxidant concentrations were also monitored. Consistent with past studies (*28*), the decay of ascorbic acid was not influenced by the presence of flavanol compounds. The same was observed in this study for erythorbic acid. Consequently, the data shown for ascorbic acid and erythorbic acid in **Figure 4a** are the average (*n* = 9) of all samples containing either ascorbic acid or erythorbic acid regardless of the presence of the supporting flavanol compound. The rate of erythorbic acid degradation, 11.9 mg/h from 0 to 32 h, was significantly greater (*P* = 0.05) than that of ascorbic acid, 9.6 mg/h over the same period of time. The degradation of these antioxidants was repeated at room temperature (20 °C) at which the difference in degradation rate became more apparent (**Figure 4b**). The time for depletion of the antioxidant is important as the protective effect of such antioxidants in wine often only occurs while at least some of the antioxidant still remains in solution (*28*).

The oxidation rate of ascorbic acid (and presumably erythorbic acid) is known to be critically dependent on trace metal concentrations (*36*). In the model wine system adopted here, iron ions at a concentration of 100 μg/L are most probably chelated by the chiral pH buffer L-tartrate (*37*). An explanation as to the different

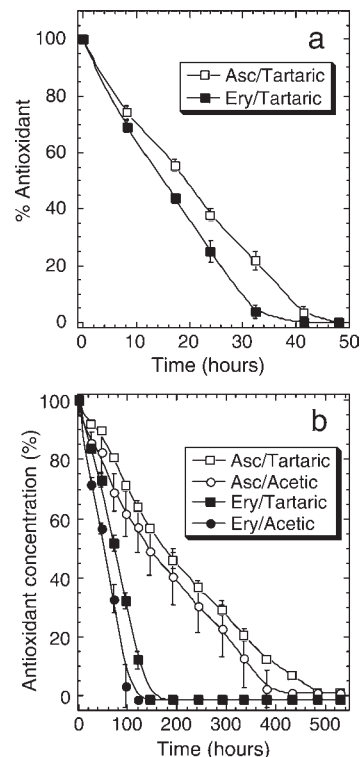**Figure 4.** Decay in antioxidant during the storage of samples at (**a**) 45 °C or (**b**) room temperature. The samples contain either ascorbic acid or erythorbic acid in tartaric acid or acetic acid buffer. Error bars indicate the 95% confidence limits (*n* = 9 in (**a**) and *n* = 3 in (**b**)).

rate of antioxidant degradation may be that it was due to the chirality of the metal−buffer complex. To test this hypothesis, L-tartaric acid was substituted by acetic acid, an achiral organic acid, and the antioxidant decay monitored during storage at 20 °C. Despite the change in supporting buffer, the decay rates for ascorbic acid and erythorbic acid were still significantly different from each other at 1.6 mg/h and 3.9 mg/h respectively from 0 to 96 h, and only slightly different from those with the tartrate buffer (**Figure 4b**). Based on these results, it can be concluded that the rates of decay of the antioxidants are not related to the chirality of the supporting buffer.

Consideration also must be given to the ability of both ascorbic acid and erythorbic acid to undergo nonoxidative degradation. This degradation is often termed "anaerobic degradation" despite still occurring during the oxidative degradation of the antioxidants (*38*). However, based on the kinetic and activation energy data of Anmo et al. (*39*), the first order nonoxidative decay constants (45 °C) for ascorbic acid and erythorbic acid were calculated to be 2.6×10^{-4} h^{-1} and 9.6×10^{-4} h^{-1}, respectively. This corresponds to negligible antioxidant decay (i.e., 1.3% and 3.8% of the initial concentration, respectively), in the time scale of **Figure 4a**. At 20 °C the rate constants are 10-fold lower (i.e., 0.28×10^{-4} h^{-1} and 0.60×10^{-4} h^{-1}, respectively) and correspond to a loss of less than 1.5% the initial concentration of the antioxidants. Based on these low rates of anaerobic degradation, it was apparent that nonoxidative decay mechanisms were not responsible for the different rates of antioxidant decay between antioxidants in **Figure 4**.

Consequently, it seems that erythorbic acid is intrinsically capable of reacting more rapidly, compared to ascorbic acid, with either molecular oxygen, hydrogen peroxide and/or some other degradation product of the antioxidant. Therefore, although ascorbic acid may protect the model wine for a longer
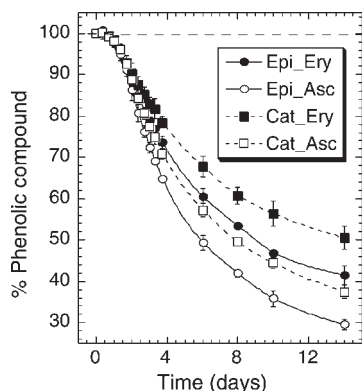
Figure 5. Decay in flavanol compound concentration during the storage of samples at 45 °C. The concentrations for (+)-catechin or (−)-epicatechin alone did not significantly ($P = 0.05$) deviate from their starting concentration. Error bars indicate the 95% confidence limits ($n = 3$).

period of time, due to its slower reaction in the presence of oxygen, erythorbic acid may offer a greater capacity for protection, and less possibility of oxidative side-reactions, due to its faster reaction in the presence of oxygen.

A comparison of the rate of loss of each flavanol compound (**Figure 5**) demonstrates that erythorbic acid, like ascorbic acid (*28*), accelerates the consumption of the accompanying flavanol. However, at day 14, the loss of the flavanol compound is significantly less ($P = 0.05$) in the samples with erythorbic acid compared to those with ascorbic acid, and the difference is evident well after the depletion of both antioxidants in the samples (i.e., 48 h, **Figure 4a**). Such results support the above proposition that erythorbic may offer a greater capacity for protection of wine components, such as flavanols, against oxygen than ascorbic acid. As far as the flavanol compounds are concerned, the loss of (−)-epicatechin is significantly faster than (+)-catechin, and this is consistent with the respective reaction rates of these compounds with aldehydes such as acetaldehyde and glyoxylic acid (*18, 40*).

Production of Colorless Pigment Precursors. During the incubation of a flavanol with an antioxidant, the major products generated in the first two days were peaks a to h (**Figure 6**) in the 280 nm chromatograms. Samples containing (+)-catechin with either ascorbic acid or erythorbic acid showed colorless products with the same retention times (peaks a with c, and b with d, **Figure 6**), UV/visible spectra (λ_{max} 284 nm), and LC−MS data (403 m/z, positive ion mode), and hence were identical products. These colorless products have already been identified in the (+)-catechin and ascorbic acid system (peaks a and b, **Figure 6a**) as (+)-1″-methylene-6″-hydroxy-2*H*-furan-5″-one-6-catechin ((+)-MHF-6-catechin, **1** in **Figure 7**) and (+)-MHF-8-catechin (**2** in **Figure 7**) isomers respectively (*12*), and both have the ability to evolve into yellow xanthylium cation pigments (*12*). Their formation involves the degradation of ascorbic acid to form the aldehyde, ʟ-xylosone, which then undergoes an electrophilic addition to the phloroglucinol ring of (+)-catechin at either carbons-8 or -6, followed by rearrangement to form the (+)-MHF-catechin. Given that the MHF moiety has no chiral carbons, and that erythorbic acid presumably can form either identical degradation products to ascorbic acid or related stereoisomers (*41, 42*), it is understandable that erythorbic acid and ascorbic acid provide the same substituted-(+)-catechin compounds.

Equivalent products (peaks e to h in **Figure 6**, **3** and **4** in **Figure 7**) were also identified for (−)-epicatechin during its incubation with either ascorbic acid or erythorbic acid. This identification was based on the identical UV/visible spectra

(λ_{max} 284 nm), LC−MS parent ion data (403 m/z, positive ion mode) and LC−MS fragmentation data as for the (+)-MHF-catechin isomers. The fragmentation ions included the retro Diels−Alder cleavage of the dihydropyran moiety in the isomers to provide an ion at 251 m/z, the loss of the (+)-catechin or (−)-epicatechin unit to leave the methylenehydroxyfuranone unit at 113 m/z, or a combination of both fragmentation mechanisms to yield an ion with m/z of 139. Another fragment ion at 151 m/z appeared to be the 139 m/z ion with a −CH$_2^+$ group attached, hence the cleavage of the hydroxyfuranone unit with the methylene group remaining behind.

During the reaction of (+)-catechin and ascorbic acid, the concentration of MHF-addition isomers increases to a maximum and then begins to decrease upon depletion of ascorbic acid in solution (*12*). The results in **Figure 8** confirm that this is also the case for the (−)-MHF-epicatechin isomers. In fact, the decrease in the erythorbic acid-derived MHF-addition products (**Figure 8**) occurs earlier than for the equivalent ascorbic acid products, coinciding with the different decay rates for the respective antioxidants (**Figure 4a**).

The 280 nm chromatogram peak areas for the MHF-addition products generated by erythorbic acid are substantially lower in intensity than those generated by ascorbic acid. In order to convert chromatographic peak areas into concentrations of the MHF-addition products, it is necessary to know the molar absorptivity coefficients of these species. Although the molar absorptivity coefficients for these compounds are yet to be reported, relative concentrations of the various MHF-addition products (**1−4**, **Figure 7**) can be calculated based on the ratio of their 280 nm molar absorptivity coefficients. Using the method reported by Labrouche et al. (*18*) (see Material and Methods), the ratio of 280 nm molar absorptivity coefficients for MHF-8-catechin (**1**) to MHF-8-epicatechin (**3**) was 1.08 ± 0.09. For the 6-substituted isomers **2** and **4** the ratio was 1.1 ± 0.2. Therefore, the molar absorptivity coefficient between the different MHF-flavanols is not significantly different ($P = 0.05$) and hence the peak areas shown in **Figure 8** allow the relative concentrations of the different species to be compared. That is, erythorbic acid produces a maximum concentration for the MHF-flavanols that is around half that of those generated from ascorbic acid. Furthermore, the type of flavanol appeared to have little influence on the maximum MHF-flavanol concentration reached.

Production of Colored Products. In all model wines, the main colored compounds detected in the solutions were the xanthylium cations, in both their acid and ester forms, as reported by Labrouche et al. (*18*). The xanthylium cations were identified by matching their UPLC retention times with xanthylium cations synthesized by published methods (*17*), UV/visible spectra (λ_{max} 280, 440, or 460 nm, with shoulder at 310 nm), and LC−MS parent ion data (617 m/z acid form, 645 m/z ester form, positive ion mode). Therefore, regardless of whether ascorbic or erythorbic acid is present in solution, identical xanthylium cations were formed for a given flavanol. However, peak areas from the 440 nm chromatograms (**Figure 9**) show that the (−)-epicatechin-derived xanthylium cations provide over twice the intensity of the corresponding (+)-catechin-derived pigments. This is consistent with the known extinction coefficient data for the corresponding xanthylium cations (*18*), as well as the increased rate of loss of (−)-epicatechin compared to (+)-catechin (**Figure 5**). Furthermore, there is good consistency between the 440 nm absorbance data in **Figure 2** and the xanthylium peak area data in **Figure 9**, indicating that xanthylium cations make the predominant contribution to absorbance at 440 nm. In the samples with ascorbic acid, the higher concentration of the yellow xanthylium cations led to increased tailing of their absorbance into the 500 nm region
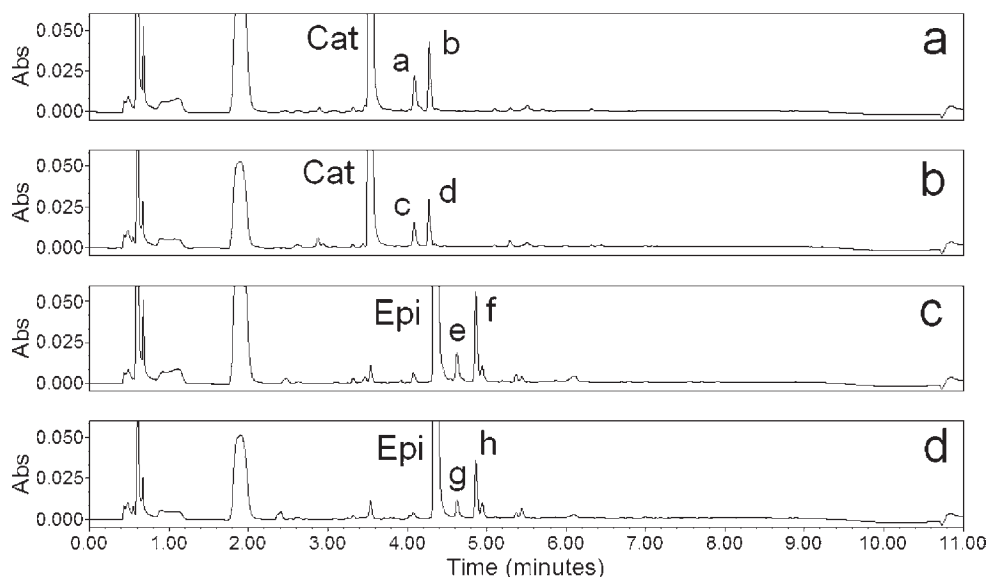
Figure 6. 280 nm chromatograms of the (**a**) (+)-catechin/ascorbic acid, (**b**) (+)-catechin/erythorbic acid, (**c**) (−)-epicatechin/ascorbic acid, and (**d**) (−)-epicatechin/erythorbic acid samples after 32 h of storage at 45 °C. The peaks eluting before the flavanol are due to the antioxidant and its degradation products.
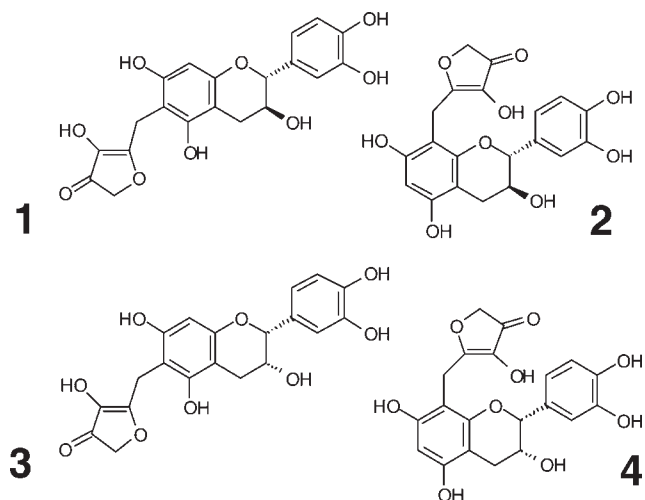

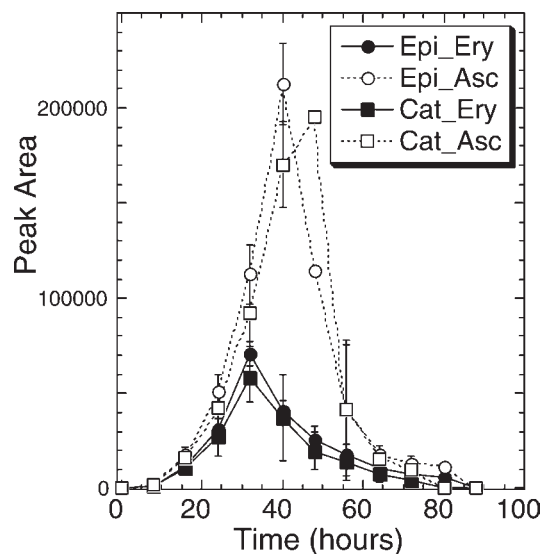**Figure 7.** MHF-flavanol compounds identified in the model wine systems.

Figure 8. Peak area of the MHF-8-flavanols during the storage of samples at 45 °C. Error bars indicate the 95% confidence limits ($n = 3$).

of the spectrum and this accounted for the increased yellow and red coloration in the samples with ascorbic acid. This is consistent with the CIELab data reported above. Given that the xanthylium cations were the main pigments generated in all samples, it is apparent that the (−)-MHF-epicatechin isomers were also precursors to xanthylium cation pigments, as is known to be the case for (+)-MHF-catechin (*12*).

After day 7, it is evident that the rate of xanthylium cation production for the erythorbic acid-derived xanthylium cations has slowed compared to that of the ascorbic-acid derived xanthylium cations (**Figure 9**). This is consistent with the lower maximum concentrations reached for the MHF-flavanols in the case of erythorbic acid compared to ascorbic acid (**Figure 8**). It must be noted that the MHF-flavanols are not the only precursors for the xanthylium cation production as these pigments can also form via the oxidative degradation of tartaric acid in the presence of hydrogen peroxide and metal ions, or via exposure to light (*12*, *43*). The source of hydrogen peroxide in the model wine system may be from the oxidation of flavanol compounds and/or the oxidation of the antioxidant compounds (*37*). However, based on the studies of Barril et al. (*12*, *44*), the MHF-flavanol pathway

would appear to make the more significant contribution to xanthylium cation production in samples containing ascorbic acid, and therefore by implication, also samples containing erythorbic acid.

The results of this study have shown that on deliberate oxidation of a model wine system the stereochemical impacts of antioxidants and flavanols are significant. In the presence of either of the flavanols (+)-catechin or (−)-epicatechin, erythorbic acid produces less oxidative coloration, at wavelengths corresponding to yellow and red, than the equivalent solutions containing ascorbic acid. Stereochemistry was also important in the oxidative degradation of both antioxidants and flavanols. Erythorbic acid was oxidized faster than ascorbic acid, and (−)-epicatechin was oxidized faster than (+)-catechin. This difference in reaction rates has significant implications in binary mixtures. First, the decomposition of flavanols did not occur as rapidly in the presence of erythorbic acid as it did in the presence of ascorbic acid. Second, this translated into lower production of colorless
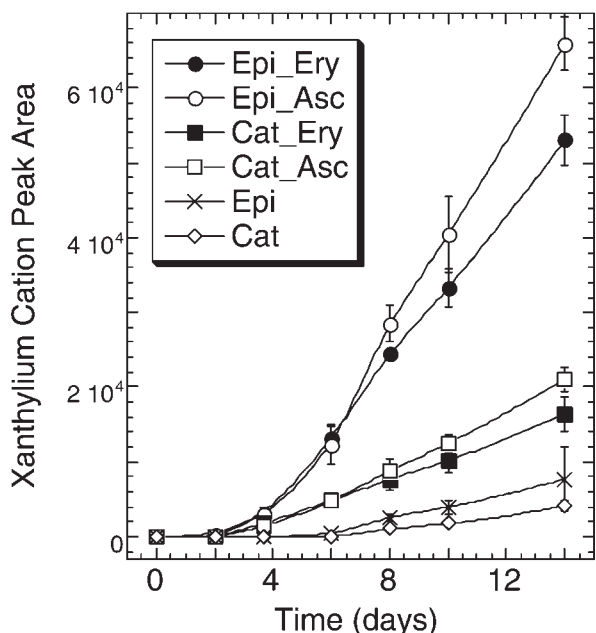
Figure 9. Comparison of the xanthylium cation peak areas, in the 440 nm chromatograms, formed during the storage of samples at 45 °C. Error bars indicate the 95% confidence limits (*n* = 3).

MHF-flavanol adducts, which, although not yet detected in white wine, are known precursors to yellow xanthylium cation pigments. Hence solutions containing erythorbic acid develop less color than those of ascorbic acid for a given flavanol. As reported previously (*18*) xanthylium cations derived from (−)-epicatechin are more intensely colored than those derived from (+)-catechin because the molar absorptivity coefficient is greater. Based on these results, erythorbic acid has certain advantages over ascorbic acid as an antioxidant and further studies are required to assess whether such advantages still exist during the complementary role of erythorbic acid to sulfur dioxide in wine media and under less oxidative storage conditions.

ABBREVIATIONS USED

UPLC-PDA, ultraperformance liquid chromatography with photodiode array detector; LC−MS, liquid chromatography with mass spectrometry; UV, ultraviolet; CIE, International Commission on Illumination; ICP-OES, inductively coupled plasma with optical emission spectroscopy; MHF, (+)-1″-methylene-6″-hydroxy-2*H*-furan-5″-one.

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