JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Defining the Ascorbic Acid Crossover from Anti-Oxidant to Pro-Oxidant in A Model Wine Matrix Containing (+)-Catechin

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An examination of the ascorbic acid-induced oxidation of (+)-catechin was carried out. Using varying concentrations of ascorbic acid in a model white winebase, it was observed that there are at least two distinct steps in its oxidation process. The first step involves the formation of species that absorb in the visible region of the spectrum, while the second step generates species of less or no absorbance in the visible region. The first step reaches an absorbance maximum when ascorbic acid is completely oxidized. In winebase solutions containing both ascorbic acid and (+)-catechin, the lag period prior to the onset of (+)-catechin oxidation was dependent on the concentration of ascorbic acid. It was also observed that the end of the lag period corresponds to the complete oxidation of ascorbic acid. Xanthylium cations were identified as a species responsible for the increase in absorbance at 440 nm post lag period. The implication of the results, for establishing a chemical basis to the ascorbic acid crossover from antioxidant to pro-oxidant, is discussed.

KEYWORDS: (+)-Catechin; ascorbic acid; white wine; phenolic oxidation; wine polyphenolics; xanthylium cations

INTRODUCTION

The ideology of ascorbic acid as an antioxidant in wine production has varied considerably during the past 10 years, and in Australia, at least, there is an apparent movement back toward its use in white wine vinification. There has been extensive discussion concerning the advantages or disadvantages of ascorbic acid employment as an antioxidant. For example, Bauernfeind and Pinkert (*1*) claim that ascorbic acid favorably affects the flavor, taste, and clarity of wines, and Kielhofer and Wurdig (*2*), along with Vecher and Loza (*3*, *4*), report a fresher and livelier bouquet. However, Marks and Morris (*5*) found that no benefit was gained from adding ascorbic acid to wines already containing sulfur dioxide, the main preservative used in white wine making, with accelerated trials demonstrating that the addition of ascorbic acid increased the potential for browning, a result similarly reported by Peng et al. (*6*). Buettner and Jurkiewicz (*7*) claimed that ascorbic acid may exhibit a "crossover" effect that was dependent upon the level of available ascorbic acid. At greater concentrations, an antioxidant effect was observed, while at lower levels, a proxidant influence resulted. Buettner and Jurkiewicz (*7*) proposed that the switch in reactivity is dependent upon the ratio of ascorbic acid to catalytic metal ions (such as iron and copper), the catalytic metal ions inducing radical chain oxidation reactions.

We have demonstrated previously (*8*) that the addition of ascorbic acid to a solution of (+)-catechin, a wine polyphenolic compound, prepared in a model winebase, results in an initial decrease in the color of the system, as measured by the absorbance at 440 nm. This initial decrease in absorbance, termed the "lag period", was followed by an enhanced rate of browning. This preliminary work (*8*) suggested that it is a degradation product of ascorbic acid that induces the browning, and not ascorbic acid itself.

It has been suggested that one species contributing to the enhancement of browning is hydrogen peroxide − a known strong oxidant and an oxidation product of ascorbic acid (*6*, *9*). However, hydrogen peroxide did not induce (+)-catechin browning to the extent observed for ascorbic acid in conditions similar to that of white wine (*8*). Although dehydroascorbic acid is generally referred to as the major oxidation product derived from ascorbic acid (*7*, *9*, *10*), other additional products reported under wine-like conditions include acetaldehyde (*9*, *11*, *12*), 2,3-diketo-L-gulonic acid (*1*, *13*−*16*), L-threonic acid (*1*, *15*, *17*), oxalic acid (*1*, *15*), L-threo-2-pentulosonic acid (*15*), 4,5,5,6-tetrahydroxy-2,3-diketohexanoic acid (THDH) (*17*), and furfural (*1*, *18*−*20*). Furfural has been found to react with (+)-catechin leading to xanthylium pigments (*21*).

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10.1021/jf034139f CCC: $25.00
Published on Web 06/10/2003

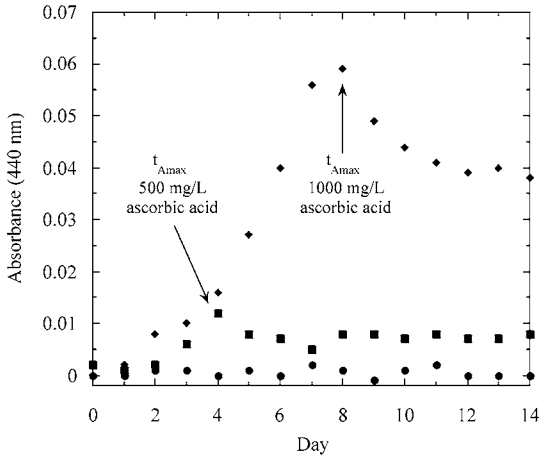


Figure 1. Variation in absorbance at 440 nm over 14 days for solutions of ascorbic acid in a model winebase. ●, 200 mg/L; ■, 500 mg/L; ◆, 1000 mg/L.

The intention of this work was to investigate further the role of ascorbic acid and its degradation products on the oxidative browning of (+)-catechin under winelike conditions. Emphasis was placed on understanding the reactions that occur during the lag period as precursor reactions to the onset of oxidative browning of (+)-catechin.

METHOD AND MATERIALS

Reagents and Chemicals. Grade 1 water (ISO3696) purified through a Milli-Q (Millipore, Bedford, MA) water system was used for all solution preparation and dilutions. (+)-Catechin (AR grade) and potassium bitartrate (AR grade) were purchased from Sigma. Ascorbic acid (AR grade) was obtained from Prolabo. Nitric acid (AR grade, 70%) was purchased from Univar and potassium hydroxide (AR grade) came from BDH. Ethanol (AR, 99.7−100%, BDH) was used without further purification.

Model Winebase. The model winebase was prepared as described previously by Bradshaw et al. (*8*, *22*).

Solutions for Browning Experiments. A 10-mg sample of (+)-catechin (100 mg/L; 3.45×10^{-4} M) and/or 20-mg sample of ascorbic acid (200 mg/L; 1.14×10^{-3} M) were dissolved with stirring in 100 mL of model winebase in a 250-mL Schott bottle. Until required for analysis, the samples were maintained in the dark in a water bath at 45 °C. Flasks were exposed to the air with stirring on a daily basis to replenish the molecular oxygen content. In some studies, additional solutions were prepared with either 25 mg of (+)-catechin (250 mg/L; 8.62×10^{-4} M) and/or 50 mg of ascorbic acid (500 mg/L; 2.84×10^{-3} M), or 50 mg of (+)-catechin (500 mg/L; 1.72×10^{-3} M) and/or 100 mg ascorbic acid (1000 mg/L; 5.68×10^{-3} M). All experiments were performed in duplicate.

Solutions for Monitoring Decay of Ascorbic Acid. Ascorbic acid solutions were prepared in 100-mL model winebase as described by Bradshaw et al. (*22*), with 20, 50, and 100 mg of ascorbic acid, giving resultant concentrations of 200, 500, and 1000 mg/L, respectively. The decay reactions were performed in the same manner as the browning reactions. All experiments were performed in duplicate.

Absorbance Measurements. Absorbance measurements at 440 nm were conducted as detailed by Bradshaw et al. (*8*).

Square Wave Voltammetric Measurements of Ascorbic Acid. Ascorbic acid concentrations were determined by square wave voltammetry as described by Bradshaw et al. (*22*). Dehydroascorbic acid is not electrochemically active and could not be detected by this technique.

Liquid Chromatography-Diode Array Detector Analyses. Liquid chromatography-diode array detector (LC-DAD) analyses were performed as detailed by Bradshaw et al. (*8*). Chromatograms were recorded for each replicate catechin browning and ascorbic acid decay experiments.

RESULTS AND DISCUSSION

Ascorbic Acid Oxidation. In our previous work, we demonstrated that the lag period observed prior to the onset of (+)-catechin oxidative browning did not occur if a preoxidized sample of ascorbic acid was used (*8*). This implies that the post-lag period reaction may be linked to a product of ascorbic acid rather than ascorbic acid itself. As hydrogen peroxide can be discounted (*8*), initial experiments examined the reactivity of ascorbic acid in this lag phase.

Solutions of ascorbic acid containing 200, 500, and 1000 mg/L were prepared in the winebase and subjected to enhanced oxidation at 45 °C in the absence of light. (+)-Catechin was omitted to identify the ascorbic acid specific reactions. Absorbance spectra for the oxidizing ascorbic acid solutions, at least for the 500 and 1000 mg/L solutions, indicated browning, as determined by measurements at 440 nm (A_{440}), for polyphenolic compounds. From **Figure 1**, it can be seen that there is an increase in the A_{440} values to a maximum before an absorbance decrease. The time to achieve maximum absorbance at 440 nm (t_{Amax}) depended on the concentration of ascorbic acid, with the 500 mg/L sample reaching t_{Amax} at day 4, compared with day 8 for the 1000 mg/L solution.

Comparison of LC/DAD chromatograms (278 nm) for the ascorbic acid solutions over the duration of the experimental period (an example is presented in **Figure 2**) indicated that similar products were generated for the 200, 500, and 1000 mg/L ascorbic acid solutions. It was also observed that several peaks in the 278 nm chromatograms changed markedly in peak area during the 14 day time course of the reaction. This alone indicates the reactivity of ascorbic acid solutions and confirms that dehydroascorbic acid is the initial, and not final, oxidation product of ascorbic acid (*1*, *8*). Three peaks in the 278 nm chromatograms (1, 2, and 3 in **Figure 2**) were selected for further analysis, as these were the most clearly resolved peaks.

Figure 3 illustrates the peak area profiles for peaks 1−3 in the 278 nm chromatogram for the 200, 500, and 1000 mg/L

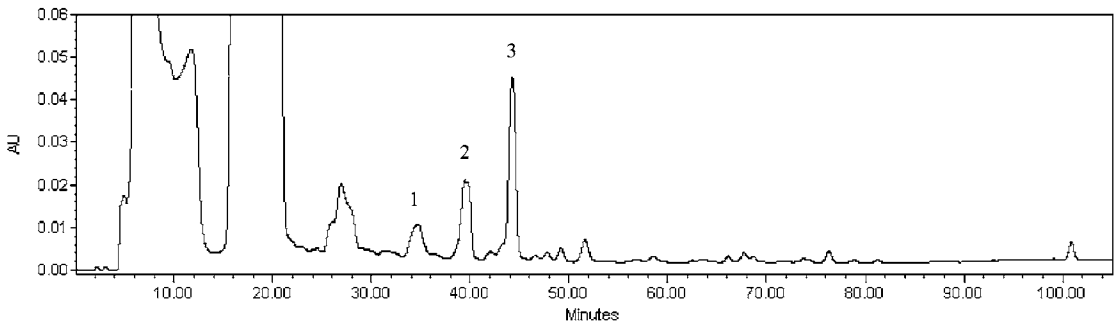


Figure 2. Representative LC/DAD chromatogram (278 nm) for a solution of ascorbic acid (500 mg/L) prepared in a model winebase at t_{Amax} (day 4).

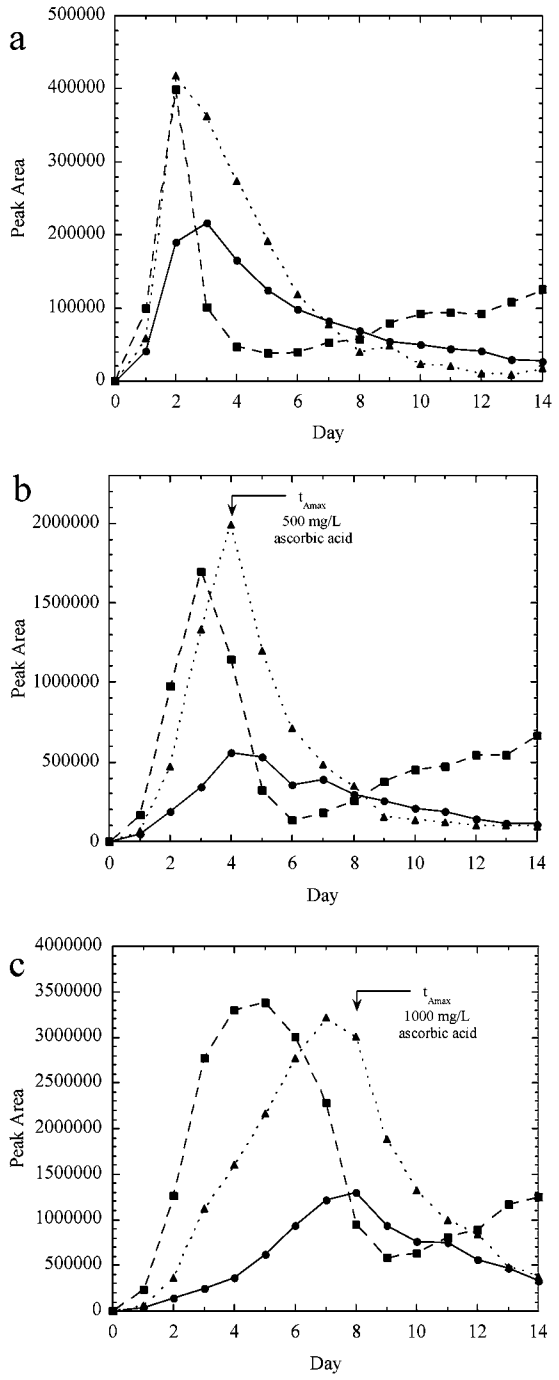


Figure 3. Variation in peak area of three degradation peaks, extracted at 278 nm, over 14 days for oxidation of 200 mg/L (a), 500 mg/L (b), and 1000 mg/L (c) ascorbic acid solutions in a model winebase. (–●–), peak 1; (-■-), peak 2; (···▲···), peak 3.

ascorbic acid solutions. For each ascorbic acid concentration, there is a rapid rise in area for peaks 2 and 3, with peak 2 decreasing rapidly before a further slow rise is observed. The pattern for peak 1 is similar to that for peak 3, except that the peak area maximum is considerably less (**Figure 3**). Intriguingly, the time required for Peaks 1 and 3 to reach maximum absorbance corresponds to t_{Amax} in **Figure 1**. Peak 2, however, attains its maximum absorbance before peaks 1 and 3, and the subsequent slow rise in the area of peak 2 in the latter part of the reaction period (**Figure 3**) implies that it represents the growth and decay of one species, followed by the appearance of one or more new species. The UV/visible spectra for peaks

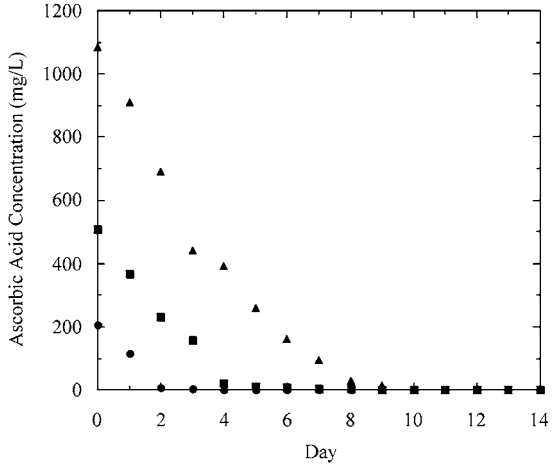


Figure 4. Variation in concentration over time during the oxidation of ascorbic acid, as measured by square wave voltammetry. ●, 200 mg/L; ■, 500 mg/L; ▲, 1000 mg/L.

1−3, extracted from the LC/DAD chromatograms, exhibited λ_{max} values of 300, 275, and 300 nm, respectively. Absorption maxima at wavelengths longer than 300 nm were not found, although tailing into the visible region was observed. This tailing alone is the reason for the observed absorbance at 440 nm (**Figure 1**), the wavelength used for monitoring phenolic browning.

These observations provide support for the production of colored species from the oxidation of ascorbic acid and explain the previously noted absence of an isosbestic point when monitoring the ascorbic acid oxidation process by UV spectrophotometry (*8*). In fact, the data in **Figures 1** and **3** suggest that a two-step process is occurring. The primary step involves the production of colored species, and this is followed by further breakdown to give colorless or less colored species (secondary reaction).

As no additional peaks are observed in the chromatograms at, or after, t_{Amax}, it would appear that the generation of colored species and the subsequent breakdown of the colored products occurs simultaneously. However, the results observed imply that while ascorbic acid is present, the first proposed reaction (i.e., the oxidation of ascorbic acid) predominates with a resultant increase in absorbance at 440 nm. As the primary reaction is dependent upon the presence of ascorbic acid, once the majority of the ascorbic acid is oxidized, the second degradative reaction is able to predominate, with a consequent drop in absorbance observed as the colored products are converted to colorless or less intensely colored products.

To date, it has not been possible to identify the species that give rise to peaks 1−3 in the 278 nm chromatogram (**Figure 3**). LC/MS did not prove successful, and GC/MS was of limited value, as the high concentration of tartaric acid in the medium interfered with the derivatization process. Our studies are continuing to elucidate the structures of these intermediates in the oxidation of ascorbic acid.

Figure 4, which plots the time course for the degradation of ascorbic acid as monitored by SWV for each concentration used, shows that the time required for the oxidation of ascorbic acid is closely related to t_{Amax} and the time that peaks 1 and 3 achieve maximum intensity. The outcome of these SWV experiments, where ascorbic acid concentrations can be measured in-situ, provides a clear indicator of the point at which ascorbic acid is essentially completely oxidized. This point is a critical pre-

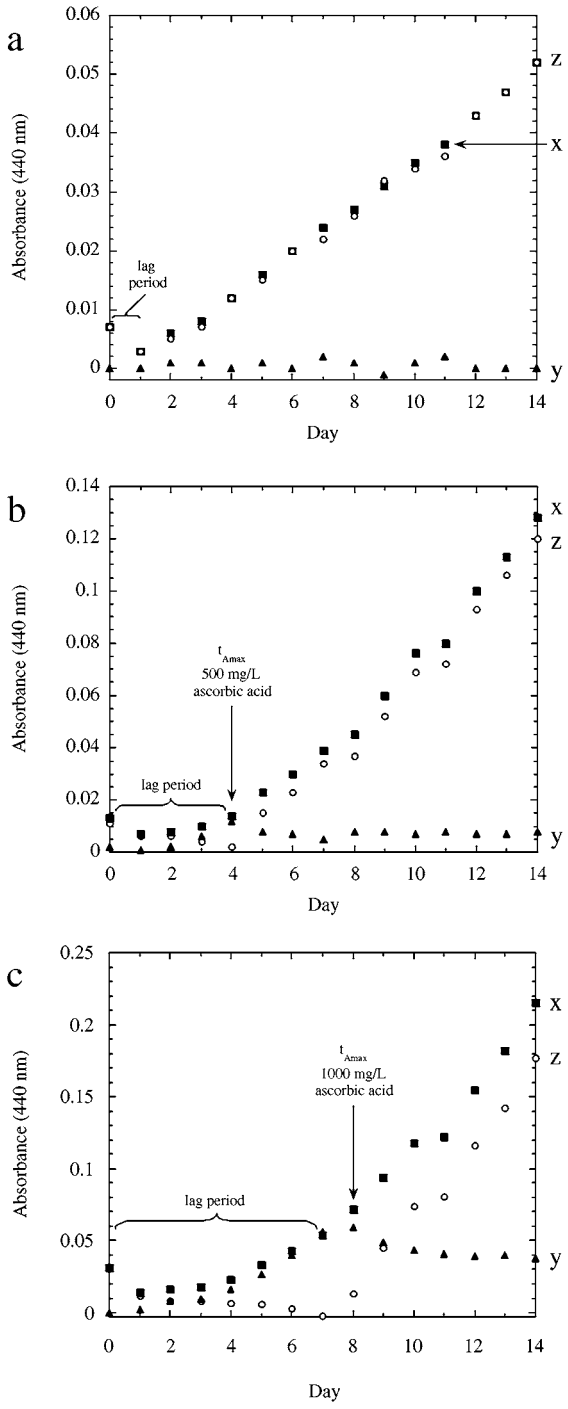


Figure 5. Absorbance/time plots for 200/100 (a), 500/250 (b), and 1000/500 (c) mg/L-ascorbic acid/(+)-catechin respectively, prepared in a model winebase illustrating lag period. ■, ascorbic acid with (+)-catechin (curve x); ▲, ascorbic acid (curve y); ○, normalized ascorbic acid with (+)-catechin (curve z).

requisite for the study of ascorbic-acid-induced oxidation of (+)-catechin.

Ascorbic-Acid-Induced Oxidation of (+)-Catechin. The influence of varying concentrations of ascorbic acid on the oxidative browning of (+)-catechin in a model wine system was followed as previously described (*8*). **Figure 5** presents browning curves for solutions containing 100 and 200 mg/L (**Figure 5a**), 250 and 500 mg/L (**Figure 5b**), and 500 and 1000 mg/L (**Figure 5c**) of (+)-catechin and ascorbic acid, respectively. Also shown in **Figure 5** are changes in the 440 nm absorbance for ascorbic acid alone (data extracted from **Figure 1**).

Table 1. Comparison of Critical Time Events in the Oxidation of Ascorbic Acid and the Formation of Xanthylium Cations from (+)-Catechin

conc of ascorbic acid	t_{Amax}	ascorbic acid oxidn time (days)	conc (+)-catechin	lag period (days)	initial day for xanthylium pigments
200 mg/L		2	100 mg/L	1	3
500 mg/L	4	4	250 mg/L	4	6
1000 mg/L	8	8	500 mg/L	7	10

As we have previously noted (*8*), there is a lag period between 1 and 2 days for the 100 mg/L (+)-catechin/200 mg/L ascorbic acid system (**Figure 5a**) before an increase in A_{440} is observed. For the solutions containing higher concentrations of ascorbic acid and (+)-catechin, there is an increase in A_{440} from Day 0 (**Figure 5,** parts **b** and **c**), although it is evident that this increase closely matches that for the corresponding ascorbic acid alone sample. This suggests that no additional browning due to ascorbic acid/(+)-catechin interactions has occurred in this initial phase. Moreover, after the time where the ascorbic acid solutions reached t_{Amax} (**Figure 1**), the corresponding solutions containing (+)-catechin and ascorbic acid exhibited further A_{440} increases at an enhanced rate (**Figure 5**).

When the A_{440} values for the ascorbic acid/(+)-catechin solutions (curve x in **Figure 5**) are corrected for the absorbance due to ascorbic acid alone (curve y), it is clear (curve z) that there is a well-defined lag period before any (+)-catechin dependent absorbance increase. The lag period obviously depends on the ascorbic acid concentration and ranges from 1 to 7 days. A comparison of values in **Table 1** shows that the lag period correlates well with the time required for the complete oxidation of ascorbic acid.

These observations are consistent with the proposal by Buettner and Jurkiewicz (*7*) of a crossover in the role of ascorbic acid, from an antioxidant effect at higher concentrations to a pro-oxidant role, once the concentration of ascorbic acid had decreased to a suitable level. Significantly, the crossover observed here does not require the need to invoke free radicals as suggested by Buettner and Jurkiewicz (*7*). Rather, the results of this work and our previous studies (*8*) using preoxidized solutions of ascorbic acid clearly demonstrate a direct chemical basis to the crossover.

The LC/DAD chromatograms extracted at 440 nm after 14 days for the three ascorbic acid/(+)-catechin systems show a series of peaks at retention times between 65 and 72 min (**Figure 6a**). A comparison of the peak retention times and profile to those in a chromatogram from Clark and Scollary (*23*), shown in **Figure 6b**, coupled with the UV/visible spectra for peaks 4 and 5 in **Figure 6a** (not shown) indicate that these peaks are due to xanthylium species, examples of which have also been previously identified by Es-Safi et al. (*24*−*26*) as the colored product resulting from the oxidation of (+)-catechin. Dimerization of two catechin moieties through an appropriate bridging agent (e.g., glyoxylic acid) results in the colorless xanthene species, that then undergoes oxidation (**Scheme 1**) to the xanthylium cation (*25*).

The absence of any absorbance at 440 nm in the initial stages of the corrected absorbance/time plots for the three ascorbic acid/(+)-catechin solutions (**Figure 5**, curve z) suggests that

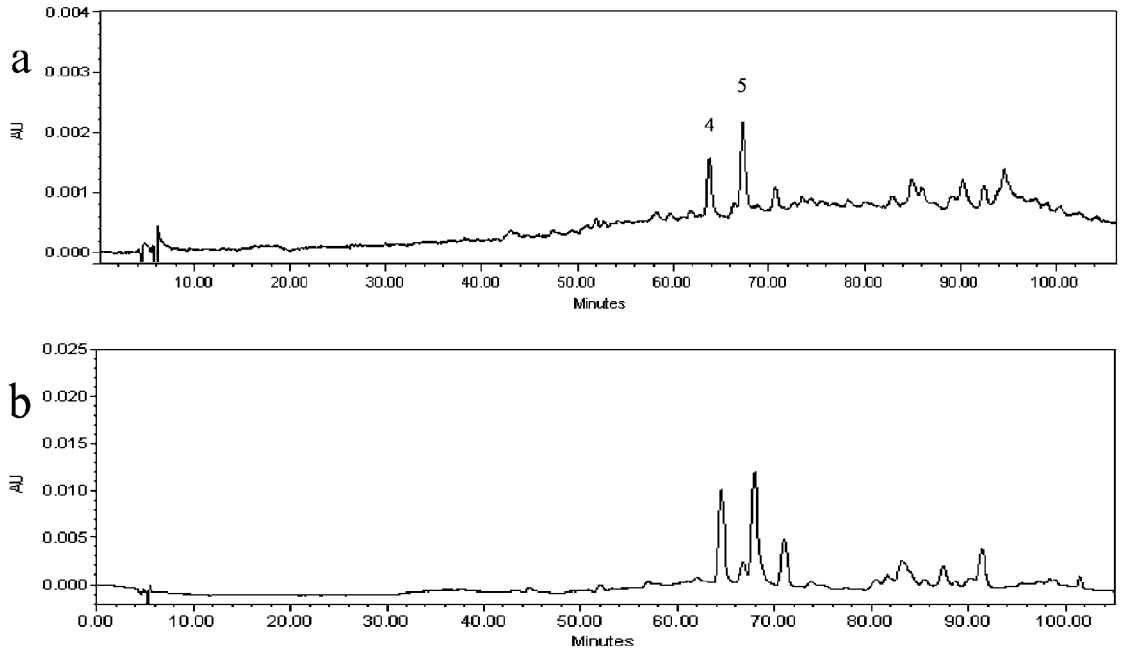
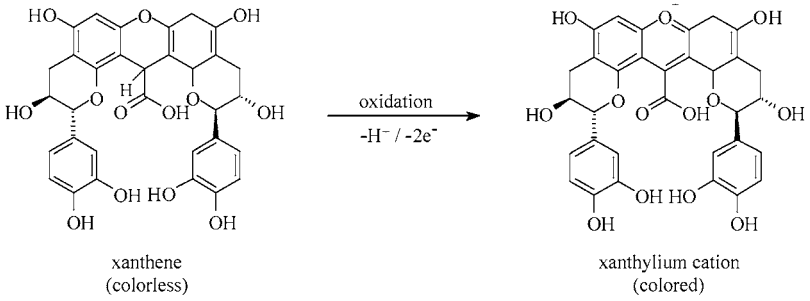


Figure 6. Similarity of LC/DAD chromatograms at 440 nm between 200 mg/L ascorbic acid/100 mg/L (+)-catechin prepared in a model winebase at day 14 (a) and 150 mg/L (+)-catechin/0.6 mg/L copper(II) prepared by Clark and Scollary (b). Peaks 4 and 5 have been identified as being due to xanthylium cations (*23*).

Scheme 1. Schematic for the Oxidation of a Xanthene to a Xanthylium Cation



xanthylium species are not present during the lag period. Indeed, the concentration/time profile of the xanthylium cations for the 200 mg/L ascorbic acid/100 mg/L (+)-catechin solution (**Figure 7a,** corresponding to peaks 4 and 5 in **Figure 6**), whose lag period was 1 day (**Table 1**), demonstrates that this is in fact so, as no xanthylium cations were detected until day 3. Similarly, xanthylium species were not observed in the 500 mg/L ascorbic acid/250 mg/L (+)-catechin solution until day 6 (lag period of day 4), nor in 1000 mg/L ascorbic acid/500 mg/L (+)-catechin solution until day 10 (lag period of 7 days), as **Figure 7**, parts **b** and **c**, respectively, illustrate. It is therefore apparent that, during the lag period, that is, while ascorbic acid remains available, the formation of xanthylium cations is inhibited.

The prevention of xanthylium cation formation during the lag period may be attributed to the low reduction potential, and associated excellent antioxidant ability, of ascorbic acid, whereby the oxidation of the colorless xanthene to the corresponding colored xanthylium species is inhibited by the oxidation of ascorbic acid. This could occur in a fashion similar to the reported prevention of enzymic browning, achieved through a coupled redox reaction involving the reduction of unfavorable *o*-quinones to the *o*-dihydroxyphenolic form by ascorbic acid (*6*). However, once the ascorbic acid has been consumed, the xanthene species is free to be oxidized to its xanthylium counterpart.

Chromatograms for (+)-catechin solutions prepared without ascorbic acid in a model wine system also revealed the presence of xanthylium cation species, although not to the concentration observed in the corresponding samples containing ascorbic acid. It would therefore appear that the enhanced rate of browning observed after the lag period is not due to the formation of any additional products but is instead a result of an accelerated rate of xanthylium cation production (**Figure 7**) brought about by the influence of ascorbic acid degradation products.

It is clear from the data presented here that the crossover from antioxidant to pro-oxidant occurs when all ascorbic acid has been oxidized. Hydrogen peroxide, one well-established product formed from the oxidation of ascorbic acid, has been shown to be incapable of inducing xanthylium cation formation to the extent observed for ascorbic acid (*8*). This in turn implies that one or more breakdown products of ascorbic acid must have an oxidative capacity sufficient to bring about the oxidation of a xanthene to a xanthylium pigment but not sufficient to act oxidatively in the presence of ascorbic acid.

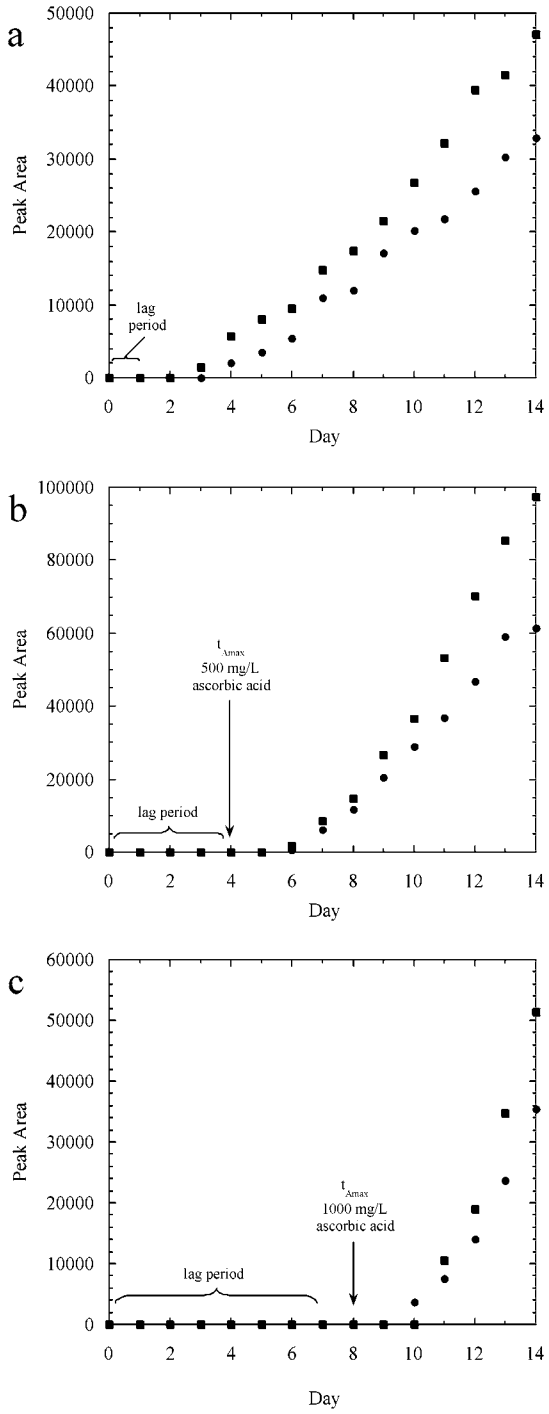


Figure 7. Variation in peak area over 14 days for solutions of 200/100 (a), 500/250 (b), and 1000/500 (c) mg/L ascorbic acid/(+)-catechin respectively, prepared in a model winebase. ●, peak 4; ■, peak 5. Data extracted from chromatograms are equivalent to those shown in **Figure 6a**.

CONCLUSION

The key finding of this work is that ascorbic acid is highly reactive under food and wine conditions and not just under the extreme reaction conditions employed in other breakdown studies. It is clear that the ascorbic acid reaction products are noninnocent and can take part in spoilage reactions. The chemical identity of these ascorbic acid breakdown products is not yet established, and the full understanding of antioxidant to pro-oxidant ascorbic acid crossover must wait until this knowledge is available.

LITERATURE CITED

(1) Bauernfeind, J. C.; Pinkert, D. M. Food Processing with Added Ascorbic Acid. *Adv. Food Res.* **1970**, *18*, 219−315.
(2) Kielhofer, E.; Wurdig, G. The mutual influence of sulfurous and ascorbic acid in winelike synthetic solutions and in wine. *Weinberg Keller* **1959**, *6*, 21−31.
(3) Vecher, A. S.; Loza, V. M. Effect of ascorbic acid addition on the oxidation−reduction potential and quality of champagne prepared by the reservoir method. *Tr. Krasnodarsk. Inst. Pishchevoi Prom.* **1961**, *22*, 139−150.
(4) Vecher, A. S.; Loza, V. M. Effect of ascorbic acid on the wine used in champagne preparation. *Tr. Krasnodarsk. Inst. Pishchevoi Prom.* **1961**, *22*, 151−161.
(5) Marks, A. C.; Morris, J. R. Ascorbic Acid Effects on the Post-Disgorgement Oxidative Stability of Sparkling Wine. *Am. J. Enol. Vitic.* **1993**, *44*, 227−231.
(6) Peng, Z.; Duncan, B.; Pocock, K. F.; Sefton, M. A. The effect of ascorbic acid on oxidative browning of white wines and model wines. *Aust. J. Grape Wine Res.* **1998**, *4*, 127−135.
(7) Buettner, G.; Jurkiewicz, B. A. Chemistry and Biochemistry of Ascorbic Acid. In *Handbook of Antioxidants*; Marcel Dekker: New York, 1996; pp 91−115.
(8) Bradshaw, M. P.; Prenzler, P. D.; Scollary, G. R. Ascorbic-Acid-Induced Browning of (+)-Catechin In a Model Wine System. *J Agric. Food Chem.* **2001**, *49*, 934−939.
(9) Zoecklein, B. W.; Fugelsang, K. C.; Gump, B. H.; Nury, F. S. Sulfur Dioxide and Ascorbic Acid. In *Wine Analysis and Production*; Chapman and Hall: New York, 1995; pp 178−191.
(10) Ball, G. Vitamin C In *Bioanalysis and Analysis of Vitamins in Food*; Chapman and Hall: London, 1998; pp 517−560.
(11) Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. E. The Role of Sulfur Dioxide in Wine. In *Principles and Practices of Winemaking*; Aspen Publishers: Gaithersburg, Maryland, 1998; pp 448−473.
(12) Singleton, V. L. Oxygen with Phenols and related Reactions in Musts, Wines, and Model Systems: Observations and Practical Implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69−77.
(13) Whiting, G. C.; Coggins, R. A. Formation of L-Xylosone from Ascorbic Acid. *Nature* **1960**, *185*, 843−844.
(14) Nath, M. C.; Bhattathiry, E. P. M. Reaction of Alloxan with Ascorbic Acid. *Nature* **1955**, *176*, 787−788.
(15) Niemela, K. Oxidative and Non-Oxidative Alkali-Catalyzed Degradation of L-Ascorbic Acid. *J. Chrom.* **1987**, *399*, 235−243.
(16) Jung, C.; Wells, W. W. Spontaneous Conversion of L-Dehydroascorbic Acid to L-Ascorbic Acid and L-Erythroascorbic Acid. *Arch. Biochem. Biophys.* **1998**, *355*, 9−14.
(17) Deutsch, J. C. Ascorbic Acid Oxidation by Hydrogen Peroxide. *Anal. Biochem.* **1998**, *255*, 1−7.
(18) Vernin, G.; Chakib, S.; Rogacheva, S. M.; Obretenov, T. D.; Parkanyi, C. Thermal decomposition of ascorbic acid. *Carbo. Res.* **1998**, *305*, 1−15.
(19) Tatum, J. H.; Shaw, P. E.; Berry, R. E. Degradation Products from Ascorbic Acid. *J. Agric. Food Chem.* **1969**, *17*, 38−40.
(20) Singh, B.; Dean, G. R.; Cantor, S. M. The Role of 5-(Hydroxymethyl)-furfural in the Discoloration of Sugar Solutions. *J. Am. Chem. Soc.* **1948**, *70*, 517−522.
(21) Es-Safi, N.-E.; Cheynier, V.; Moutounet, M. Study of the Reactions between (+)-Catechin and Furfural Derivatives in the Presence or Absence of Anthocyanins and Their Implication in Food Color Change. *J. Agric. Food Chem.* **2000**, *48*, 5946−5954.
(22) Bradshaw, M. P.; Prenzler, P. D.; Scollary, G. R. Square Wave Voltammetric Determination of Hydrogen Peroxide Generated from the Oxidation of Ascorbic Acid in a Model Wine Base. *Electroanalysis* **2002**, *14*, 546−550.

(23) Clark, A. C.; Scollary, G. R. Copper(II)-Mediated Oxidation of (+)-Catechin in a Model White Wine System. *Aust. J. Grape Wine Res.* **2002**, *8*, 186−195.

(24) Es-Safi, N.-E.; Le Guerneve, C.; Labarbe, B.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Structure of a New Xanthylium Salt Derivative. *Tetrahedron Lett.* **1999**, *40*, 5869−5872.

(25) Es-Safi, N.-E.; Le Guerneve, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. New Polyphenolic Compounds with Xanthylium Skeletons Formed through Reaction between (+)-Catechin and Glyoxylic Acid. *J. Agric. Food Chem.* **1999**, *47*, 5211−5217.

(26) Es-Safi, N.-E.; Le Guerneve, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Xanthylium salts formation involved in wine colour changes. *Int. J. Food Sci. Technol.* **2000**, *35*, 63−74.

Received for review February 13, 2003. Revised manuscript received April 16, 2003. Accepted May 11, 2003. This project was supported by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funding from the Australian Government.

JF034139F