JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

The Role of Copper(II) in the Bridging Reactions of (+)-Catechin by Glyoxylic Acid in a Model White Wine

ANDREW C. CLARK,*,† PAUL D. PRENZLER,†,‡ AND GEOFFREY R. SCOLLARY†

National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, New South Wales 2678, Australia, and School of Science and Technology, Charles Sturt University, Locked Bag 588, Wagga Wagga, New South Wales 2678, Australia

The influence of copper(II) on the bridging reactions between (+)-catechin and glyoxylic acid was studied in a white winelike medium. When the reaction was performed in darkness at 45 °C, copper(II) increased the maximum levels of carboxymethine-linked (+)-catechin dimer and xanthylium cation pigment as monitored by high-performance liquid chromatography/photodiode array detection (HPLC/DAD) and liquid chromatography/mass spectrometry (LC/MS). At 10 °C, similar results were observed except that the xanthene intermediate was monitored and found to also increase in concentration at higher copper(II) concentrations. The kinetics for the formation of these species suggested that copper(II) accelerated the bridging of two (+)-catechin units by glyoxylic acid. The acid group of glyoxylic acid allowed copper(II) to influence this reaction, as no copper(II) enhancement was observed when acetaldehyde was used in place of glyoxylic acid.

KEYWORDS: Copper(II); white wine; (+)-catechin; xanthylium cation; model wine; browning; glyoxylic acid; acetaldehyde; oxidation

INTRODUCTION

The quality of a white wine is in part based on its color; therefore, excessive coloration can often be an indicator of spoilage. Such color changes may often occur well after bottling of the wine; consequently, the implications are not only costly product losses but also compromised consumer confidence.

Metal ions have been observed to accelerate changes in the coloration of model wines. The increased coloration of (+)-catechin-containing model wines, buffered at pH 3.20−3.70 with tartaric acid, in the presence of either added iron(II) or copper(II), has been attributed to the production of xanthylium cation pigments, of which **5** (**Table 1**) is an example (*1*, *2*). The role of the metal ions in the production of xanthylium cations is not entirely understood, although it has been suggested that iron(III), formed from oxidation of iron(II), and copper(II) are involved in the oxidation of tartaric acid to produce glyoxylic acid that can bridge two (+)-catechin units and result in xanthylium cation formation (*3*, *4*).

In the reaction between (+)-catechin **1** and glyoxylic acid **2** (**Scheme 1**), a (+)-catechin/glyoxylic acid adduct initially formed reacts with a further (+)-catechin unit to produce a carboxymethine-linked (+)-catechin dimer (*4*). The carboxymethine bridge may form at positions 8-8 (as shown by **3**), 6-6, or 8-6 between the (+)-catechin units (*5*). Therefore, as the latter isomer has two diastereoisomers, the total number of carboxymethine-linked (+)-catechin dimer isomers formed is four. Each of these isomers may undergo dehydration to form xanthenes that subsequently oxidize to form xanthylium cations. In the case of **3**, the associated xanthene cation and xanthylium cation are **4** and **5**, respectively (*6*). From the four carboxymethine-linked (+)-catechin dimers, a total of six xanthene and xanthylium cation isomers may be formed (*7*).

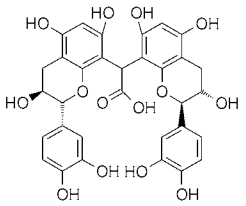
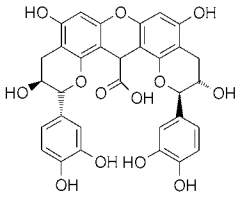
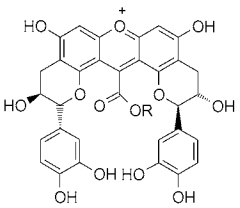
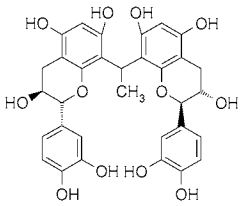
Glyoxal can also react with (+)-catechin to produce identical xanthylium cations to those formed from (+)-catechin and glyoxylic acid (*3*). However, only the reaction between (+)-catechin and glyoxylic acid produced the carboxymethine-linked (+)-catechin dimer, the major intermediate observed in the copper(II) and iron(III)-mediated oxidation of model wines (*3*, *4*).

In model wines containing (+)-catechin, tartaric acid, and copper(II), the production of xanthylium cations was shown to depend on the concentration of copper(II) present (*2*, *3*). However, this influence of copper(II) was only suggested to be a consequence of copper(II) promoting the production of glyoxylic acid from tartaric acid (*3*). Yet, as **Scheme 1** shows, the reaction between (+)-catechin and glyoxylic acid to form the xanthylium cation is a multistep reaction, and copper(II) could influence any one or all of these steps, not just the oxidation of tartaric acid to glyoxylic acid. Preliminary studies have shown that glyoxylic acid can be detected in tartaric acid solutions that are exposed to sunlight (*8*) and that with subsequent addition of (+)-catechin and varying levels of copper(II) to these solutions the formation of xanthylium cations increases significantly with copper(II) concentration (*8*). Al-

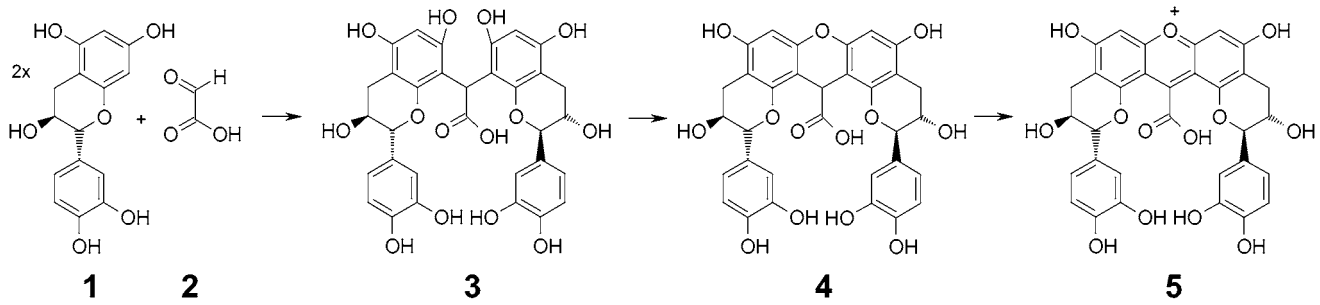
* To whom correspondence should be addressed. Tel: +61 2-69334181. Fax: +61 2-69334068. E-mail: aclark@csu.edu.au.
† National Wine and Grape Industry Centre, Charles Sturt University.
‡ School of Science and Technology, Charles Sturt University.

10.1021/jf034566t CCC: $25.00
Published on Web 09/06/2003

Table 1

Compound (one structural isomer shown)	 **3** Carboxymethine-linked (+)-catechin dimer	 **4** Xanthene	 **5** Xanthylium cation	 **7** Methylmethine-linked (+)-catechin dimer
Uv-visible spectra characteristics (maxima and *shoulder* nm)	280	279	R = H: 277, 438-444 and *310* R = CH_2CH_3: 272, 463 and *310*	276-281
LC/MS signal (negative, positive m/z)	635, 637	617, 619	615, 617	605, 607
Peak assignments	**a**, **b**, **c** and **d**	**j**	R = H: **e**, **f**, **g** and **h** R = CH_2CH_3: **i**	**k** and **l**

Scheme 1. Reaction between (+)-Catechin **1** and Glyoxylic Acid **2** To Generate the Carboxymethine-Linked (+)-Catechin Dimer **3** and Xanthene **4** Intermediates as Precursors to the Formation of the Xanthylium Cation **5**



though the initial concentration of glyoxylic acid in these samples was not quantified, this result provides tentative evidence that copper(II) may influence at least one step in **Scheme 1**. Other work in red wine (*9*) has suggested that copper does not catalyze reactions between acetaldehyde and phenolic compounds, and these reactions are similar to the first step in **Scheme 1**.

Therefore, this study was undertaken to more clearly define the role of copper(II) in the reactions between glyoxylic acid and (+)-catechin. The concentrations of (+)-catechin, the xanthylium cation, and intermediates (**Scheme 1**) were followed by HPLC/DAD and LC/MS in the reaction between (+)-catechin and glyoxylic acid at variable copper(II) concentrations. To assess the importance of the acid moiety of glyoxylic acid on the copper(II)-mediated bridging of (+)-catechin, the reaction between acetaldehyde and (+)-catechin was also followed at variable copper(II) concentration.

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. Copper(II) was added as copper(II) sulfate pentahydrate (BDH, AR). Potassium hydrogen tartrate (>99%) and L-(+)-tartaric acid (>99.5%) were obtained from Sigma. (+)-Catechin monohydrate (Sigma, 98%) was used without further purification. The single wavelength (440 nm) absorbance of samples was recorded on a Unicam 8625 UV−visible spectrophotometer.

HPLC/DAD was conducted on a Waters 2690 Separation Module run by Millenium[32] software and connected to a Waters 996 DAD. The column used was a reverse phase Wakosil C18RS column of particle size 5 μm and 250 mm × 2 mm with a guard column of the same type. The HPLC/DAD analyses were carried out as described previously (*2*).

LC/MS experiments were conducted on a SpectraSYSTEM LC run by Xcalibar software with a P4000 sample pump, UV6000LP UV detector, and a Finnigan AQA quadrapole MS with an electrospray source. The same column was used as in HPLC/DAD experiments. The LC/MS experiments were carried out as published previously (*2*).

Reactions. The model white wine 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 stirring overnight at room temperature. (+)-Catechin was added to this solution immediately prior to the preparation of an experiment. The pH of the model white wine was 3.2 ± 0.1. The addition of 0.25 mM glyoxylic acid (Aldrich, 98%) or 1.5 mM acetaldehyde (BDH, >99.5%) was made to the model white wine (150 mL) in 200 mL Schott bottles with screw top lids. The samples were then held in darkness at either 45 or 10 °C, and the sample bottles were only opened on measurement days.

RESULTS AND DISCUSSION

Influence of Copper(II) on the Reaction between Glyoxylic Acid and (+)-Catechin at 45 °C. Glyoxylic acid (0.25 mM)

was added to model white wine solutions containing variable copper(II) concentration, and the resulting solutions were maintained in darkness at 45 °C, a temperature similar to that used in many accelerated oxidation studies (*2*, *7*, *10*, *11*). The copper(II) concentrations adopted in this study, 0, 0.1, 0.3, and 0.6 mg/L, encompassed the levels of copper generally found in white wines (*12*, *13*). The concentration of glyoxylic acid, which provided a (+)-catechin to glyoxylic acid ratio of 2:1, was expected to be sufficient to ensure that the amount of added glyoxylic acid was in excess of that spontaneously generated from the oxidation of tartaric acid in the model white wine (*8*). Separate experiments showed that even though formation of glyoxylic acid is critical to **Scheme 1**, it could not be detected by HPLC/DAD in a 12% aqueous ethanol tartaric acid solution after heating at 45 °C for 3 days, regardless of the presence of copper(II). On the other hand, the addition of 0.25 mM glyoxylic acid to the model white wine was easily detected by HPLC/DAD (peak area, 14.8×10^3). Hence, the addition of 0.25 mM glyoxylic acid would enable a reaction between (+)-catechin and glyoxylic acid that was pseudo-zero order with respect to the formation of glyoxylic acid. Moreover, if copper(II) influenced the production of xanthylium cations or their intermediates under these conditions, then this would provide evidence that copper(II) was accelerating at least one step in **Scheme 1**.

The reaction solutions were analyzed by HPLC/DAD and LC/MS over the 10 day reaction period. In the 278 nm chromatograms of all samples, there were four peaks (peaks a−d, **Figure 1A**) that were observed to increase in peak area over the first few days of the reaction period. However, after 10 days, the 278 nm chromatogram of the samples became much more complicated (**Figure 1B**) and the accurate integration of peaks b−d became difficult. At day 10, well-defined peaks were observed in the 440 nm chromatogram (**Figure 1C**) with corresponding peaks in the 278 nm chromatogram (**Figure 1B**).

On LC/MS analysis of the copper-free sample, each of the four peaks a−d (**Figure 1A**) had associated with them significant *m*/*z* signals at 635 and 637 in the negative and positive ion modes, respectively. These *m*/*z* signals, including a sole maximum at 280 nm in the UV−visible spectrum for each of the peaks, are consistent with the assignment of these peaks as carboxymethine-linked (+)-catechin dimers (**Table 1**). Interestingly, despite the different chromatography conditions utilized in this study, the peak elution pattern and relative peak intensities observed for peaks a−d are similar to those observed for the isomers of the carboxymethine-linked (+)-catechin dimer identified in past studies (*3*, *7*). In all of the remaining samples of varying copper concentration, peaks a−d were assigned as carboxymethine-linked (+)-catechin dimers based on the agreement of their respective retention times, UV−visible spectra, and both negative and positive ion mode LC/MS data with the copper-free sample (**Table 1**).

The peaks e−h in the 440 nm chromatograms were consistent with the profile already observed for xanthylium isomers examined with identical chromatographic conditions (*2*) to that used in this study. The assignment of these peaks (**Table 1**) was confirmed with detection (LC/MS) of ions with *m*/*z* values of 615 and 617 in the negative and positive ion modes, respectively, at the retention times of peaks e−h. The UV−visible absorption spectra of these peaks, all with maxima at 277 nm, another between 438 and 444 nm, and a shoulder at 310 nm, were also consistent with that for the xanthylium cation (i.e., maxima at 273, 308, and 444 nm (*6*)). Peak i had a retention time, UV−visible spectra (i.e., maxima at 272 and 463 nm and

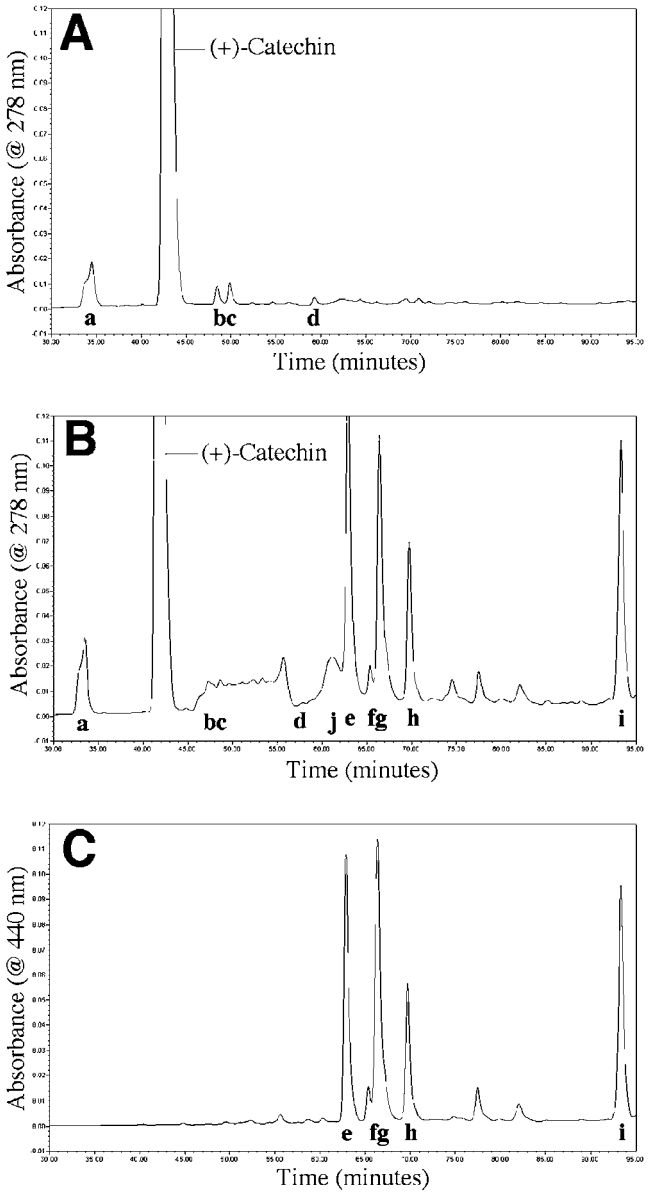


Figure 1. HPLC/DAD chromatograms of the model white wine with 0.25 mM glyoxylic acid and 0.6 mg/L copper(II) at day 1 (**A**; 278 nm) and day 10 (**B**; 278 nm and **C**; 440 nm) of the induced browning process.

shoulder at 310 nm), and LC/MS data (i.e., *m*/*z* signal at 643 and 645 in the negative and positive ion modes, respectively) consistent with an ethyl ester of the xanthylium cation (*2*).

Peak j (**Figure 1B**), with a broad appearance, was observed to have significant ions at 617 and 619 *m*/*z* in the negative and positive ion modes, respectively, and a sole maximum at 279 nm in the UV−visible spectrum. These data suggested that peak j was due to at least one of the six expected xanthene isomers (**Scheme 1**). Unfortunately, peak j was not fully resolved from peak e. The broad appearance of peak j (**Figure 1B**) as well as the peaks in the LC/MS ion chromatogram at 617 and 619 *m*/*z* (negative and positive ion modes, respectively; data not shown) suggested that peak j is most likely due to coelution of more than one xanthene isomer. The LC/MS data also suggested that other xanthene isomers might have been eluting between peaks c and d in an unresolved portion of the 278 nm chromatograms (**Figure 1B**).

The peak area due to (+)-catechin and the peaks a−j in the 278 nm chromatogram (**Figure 1A**) were followed over the 10 day reaction period to define a role of copper(II) in the time−

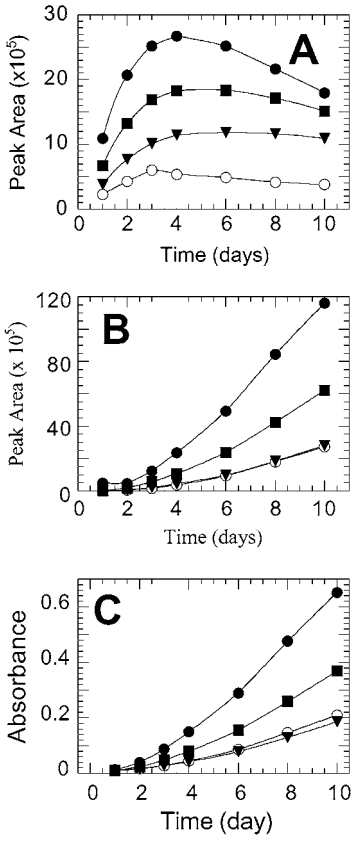


Figure 2. Change in peak areas of peak a (**A**) and peaks e–i (**B**) in the chromatograms of the model white wine with 0.25 mM glyoxylic acid and the change in absorbance at 440 nm (**C**). The copper(II) concentrations were 0 (○), 0.1 (▼), 0.3 (■) and 0.6 (●) mg/L. All samples were held at 45 °C for 10 days.

course dependence of these compounds. The amount of (+)-catechin utilized after the 10 days was 14, 15, 21, and 27% of the original (+)-catechin concentration in the 0, 0.1, 0.3, and 0.6 mg/L copper(II)-containing samples, respectively. This was high as compared to a 1% loss of (+)-catechin observed previously (*2*) in the 0.6 mg/L copper(II)-containing model white wine system without added glyoxylic acid after heating for the same period. The large decrease in (+)-catechin further confirmed that 0.25 mM added glyoxylic acid was in excess of the levels of glyoxylic acid spontaneously produced in the model white wine with 0.6 mg/L copper(II) (*2*). Most importantly, the ability of copper(II) to accelerate the loss of (+)-catechin under these conditions suggested that the consumptive reactions of (+)-catechin were copper(II)-dependent.

The areas of peaks a−d, associated with the carboxymethine-linked (+)-catechin dimer, could be accurately integrated over the first 3 days of the induced browning process, but after this stage, peaks b−d became unresolved from the surrounding peaks. Over the first 3 days of the reaction period (**Figure 2A**), it was evident that peak a increased with the increased concentration of copper(II) in the reaction mixture. Peaks b−d behaved similarly to peak a over this period (data not shown). During days 4−10, it was observed that the area of peak a either leveled out or decreased depending on the concentration of copper(II) in the sample (**Figure 2A**). Considering that the carboxymethine-linked dimer and its subsequent products are the major products formed from (+)-catechin in this work (**Figure 1A**,**B**), the kinetics of (+)-catechin and the carboxymethine-linked (+)-catechin dimer suggest that copper(II) accelerates the reaction between glyoxylic acid and (+)-catechin to form the dimer.

Accurate integration could not be obtained for peak j, which was assigned as a xanthene, throughout the 10 day analysis period, as peak j was not fully resolved from peak e. However, it appeared that peak j was generally larger with higher copper(II) concentrations (data not shown).

The summed intensity of peaks e−h (**Figure 2B**), combined isomers of the xanthylium cation, behaved similarly to the absorbance of the model white wine system at 440 nm (**Figure 2C**). The copper(II) concentrations of 0.3 and 0.6 mg/L copper(II) accelerated both the intensity of absorbance at 440 nm and the summed area of peaks e−h, while at copper(II) concentrations of 0 and 0.1 mg/L, the absorbance values and summed peak areas were similar. This pattern of copper(II) dependency was consistent with that observed for these parameters in the model white wine without added glyoxylic acid (*2*). The area of peak i (ethyl ester of the xanthylium cation) also behaved in a fashion similar to peaks e−h. Therefore, the results show that copper(II) can influence the formation of the xanthylium cation despite this reaction being pseudo-zero order with respect to the formation of glyoxylic acid.

These results demonstrate that copper(II) can influence at least one step of those illustrated in **Scheme 1** for the production of xanthylium cation from (+)-catechin and glyoxylic acid, a role different to the production of glyoxylic acid from tartaric acid (*3*). Although it is clear that copper(II) is promoting the reactions responsible for the production of the carboxymethine-linked (+)-catechin dimer from glyoxylic acid and (+)-catechin, further work is required to assess if copper(II) is influencing the remaining steps of xanthylium cation production (**Scheme 1**).

Influence of Copper(II) on the Bridging of (+)-Catechin by Glyoxylic Acid at 10 °C. The addition of glyoxylic acid to the model white wine at variable copper(II) concentrations was repeated at 10 °C rather than 45 °C. This was performed in an attempt to stabilize the xanthene and allow further insight into the role of copper(II) on the latter stages of **Scheme 1**. Previous work (*3*) had shown decreased reaction rates for **Scheme 1** when solutions of (+)-catechin, tartaric acid, and copper(II) were stored at 20 °C rather than 40 °C.

Throughout a period of 40 days, the model white wine samples were analyzed by HPLC/DAD and LC/MS. After 40 days, the 278 nm chromatograms provided much improved resolution for peaks a−d (**Figure 3A**) as compared to the results obtained using a reaction temperature of 45 °C after 10 days (**Figure 1B**). Once again, peaks a−d provided LC/MS data and UV−visible spectra characteristic of the carboxymethine-linked (+)-catechin dimer (**Table 1**). Peak j also had improved resolution under these conditions as compared to those of **Figure 1B**, mainly due to the smaller intensity of peak e, and the LC/MS data and UV−visible spectra were again consistent with its assignment as a xanthene (**Table 1**). At this lower reaction temperature, the production of the xanthylium cation from the xanthene was slowed considerably with peaks e−h being much less intense than those generated in the experiment at 45 °C. The peaks between c and d in **Figure 1B** were not observed when the reaction was performed at 10 °C (**Figure 3A**).

At 10 °C, copper(II) increased the concentrations of both the carboxymethine-linked (+)-catechin dimer (**Figure 3B**) and xanthene (**Figure 3C**) and also increased the consumption of (+)-catechin from 12 to 19% in the 0−0.6 mg/L copper(II)-containing samples, respectively. The small levels of xanthylium cations also showed copper(II) dependency with the summed areas of peaks e−h having 0.14, 0.21, 0.62, and 1.12 × 10^5 for

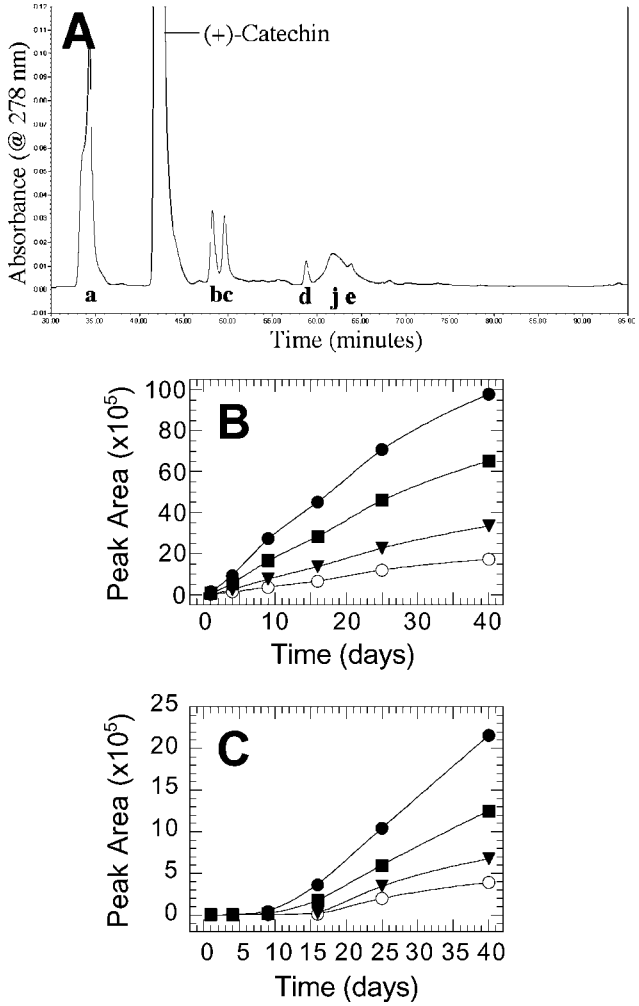
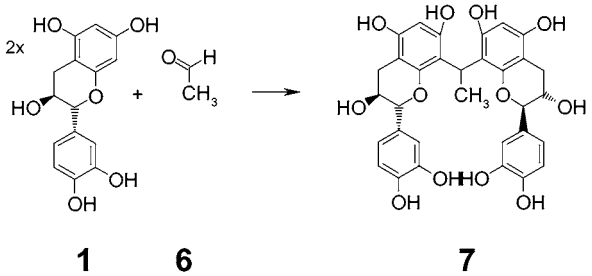


Figure 3. The HPLC/DAD chromatogram (278 nm) of the 0.6 mg/L copper(II)-containing model white wine with 0.25 mM glyoxylic acid (**A**) and the change in peak areas of peaks a–d (**B**) and peak j (**C**). The copper(II) concentrations were 0 (○), 0.1 (▼), 0.3 (■) and 0.6 (●) mg/L. All samples held at 10 °C for 40 days.

Scheme 2. Reaction between (+)-Catechin **1** and Acetaldehyde **6** Resulting in the Formation of a Methylmethine-Linked (+)-Catechin Dimer **7**



the 0, 0.1, 0.3, and 0.6 mg/L copper(II)-containing samples, respectively. Therefore, these results show that copper(II) can influence at least one step in **Scheme 1** at 10 °C as well as at 45 °C. Furthermore, it is evident from the copper(II)-dependent levels of (+)-catechin and the carboxymethine-linked (+)-catechin dimer that the first step in **Scheme 1** is also influenced by copper(II) at 10 °C. As the copper(II) dependency for the concentrations of the carboxymethine-linked (+)-catechin dimer and xanthene were similar (**Figure 3B**,**C**), it is likely that copper(II) accelerated the production of the former, and with

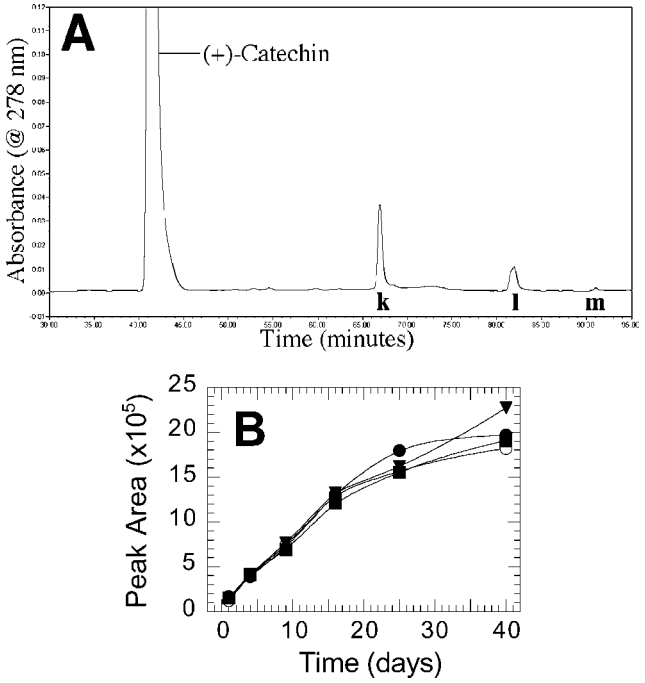


Figure 4. The 278 nm chromatogram of the 0.6 mg/L copper(II)-containing model white wine with 1.5 mM acetaldehyde (**A**) and the change in summed area of peaks k and l (**B**). The copper(II) concentrations were 0 (○), 0.1 (▼), 0.3 (■) and 0.6 (●) mg/L and the samples were held at 10 °C.

subsequent formation of the latter, the copper(II) dependency became common for both (**Scheme 1**). Consequently, there does not appear to be any gross effect of copper(II) on the second step of **Scheme 1**. Given the slow reaction rates and small concentrations of xanthylium cations generated, the influence of copper(II) in the last step of the **Scheme 1** cannot be established with any degree of certainty.

Influence of Copper(II) on the Bridging of (+)-Catechin by Acetaldehyde at 10 °C. The reaction between acetaldehyde and (+)-catechin was monitored at variable copper(II) concentration to allow comparison with the results obtained for the (+)-catechin and glyoxylic acid reaction. In the reaction of acetaldehyde with (+)-catechin, various isomers of a methylmethine-linked (+)-catechin dimer are obtained and the formation of one isomer is shown in **Scheme 2**. However, unlike the carboxymethine-linked (+)-catechin dimer, no evidence has been forthcoming to suggest that the methylmethine-linked (+)-catechin dimer can readily undergo dehydration and oxidation to form equivalent xanthylium cations. The four different isomers of the methylmethine-linked (+)-catechin dimer, including **7**, have been previously studied (*14*).

The reaction between acetaldehyde and (+)-catechin was conducted at 10 °C rather than at 45 °C to minimize loss of the volatile acetaldehyde. As some loss of acetaldehyde was envisaged during sampling, the concentration of acetaldehyde used was considerably higher than that described above for glyoxylic acid (1.5 and 0.25 mM, respectively). In previous studies, the production of methylmethine-linked (+)-catechin dimers has also involved an excess of acetaldehyde (*14*, *15*).

Figure 4A shows the 278 nm chromatogram generated after the model white wine, with 1.5 mM acetaldehyde and 0.6 mg/L copper(II), held at 10 °C for 40 days. Peaks k−m were all observed to increase during the 40 day reaction period in the absence and presence of copper(II) (0.1, 0.3, and 0.6 mg/L).

From the work of Saucier et al. (*14*), whose identical chromatographic conditions were used in this study, the methylmethine-linked (+)-catechin dimers were observed to elute after (+)-catechin with similar intensities as peaks k−m (**Figure 4A**). From the work of Saucier et al. (*14*), it was evident that peaks k and m were due to single isomers, respectively, while peak l was due to two isomers, explaining the broadened profile of this peak. From LC/MS data, peaks k and l were observed to have significant ions at 605 and 607 *m*/*z* (negative and positive ion modes, respectively) and these are characteristic of the methylmethine-linked (+)-catechin dimers (**Table 1**). The ion signal associated with peak m (**Figure 4A**) was not detected by LC/MS. Because of both the inability to confirm the identity of peak m and its comparatively low intensity, only the peak areas of peaks k and l were used to assess the effect of copper(II) on the production of the methylmethine-linked (+)-catechin dimers.

From the results in **Figure 4B**, it is clear that copper(II) does not have any effect on the production of the methylmethine-linked (+)-catechin dimers. Although there is some small difference in the levels of the methylmethine-linked (+)-catechin dimers between the different samples after days 25 and 40, this difference is not related to the concentration of copper(II) in the reaction system. Therefore, this result is consistent with a study performed in red wine (*9*), where the presence of copper(II) in the red wine was suggested to be unable to accelerate the combination of acetaldehyde with phenolic compounds. However, in this red wine study (*9*), the measurements of acetaldehyde, total phenolic compounds, total tannins, and degree of tannin condensation were conducted rather than the identification of specific products, as described here.

A comparison of the data in **Figure 4B** with that in **Figure 3B** suggests that the acid functionality of glyoxylic acid is important in enabling copper(II) to promote the production of the bridged (+)-catechin/aldehyde species. This in turn implies that a copper(II)−glyoxylate complex is probably involved in the production of the carboxymethine-linked (+)-catechin dimers.

CONCLUSION

The role of copper(II) in the production of xanthylium cations from (+)-catechin in winelike conditions has been more clearly defined. Copper(II) is not just a possible oxidation catalyst in the conversion of tartaric acid to glyoxylic acid (*3*), but it is actively involved in the condensation reactions of glyoxylic acid and (+)-catechin. More specifically, it has been demonstrated that the role of copper(II) is important in the formation of carboxymethine-linked (+)-catechin dimer from glyoxylic acid and (+)-catechin; therefore, this implies that copper(II) accelerates the reaction of (+)-catechin with either glyoxylic acid and/or the (+)-catechin/glyoxylic acid adduct. Copper(II) is less important in the conversion of the carboxymethine-linked (+)-catechin dimer to the xanthene, while further work is required to establish the effect of copper(II) on the oxidation of the xanthene to the xanthylium cation.

The acid moiety of glyoxylic acid is crucial for copper(II) to exert an influence on the formation of the carboxymethine-linked (+)-catechin dimer, and consequently, a mechanism involving the complex formation between copper(II) and glyoxylic acid is likely. The results confirm for the first time the ability of a metal ion to accelerate the bridging reaction between (+)-catechin and glyoxylic acid in a winelike medium.

The results of this study allow further insight into reactions that may allow copper(II) to contribute to wine coloration. Tartaric acid additions are frequently made during the production of white wines, and it is not inconceivable that such tartaric acid may have trace amounts of glyoxylic acid impurities, generated either through the storage conditions of tartaric acid (*8*) or during the production of tartaric acid itself. If trace levels of glyoxylic acid are added to wine, then the level and speciation of copper(II) in the wine may be critical with regard to coloration of the wine when compared to wines that have negligible levels of copper(II). The ability of iron(III) to facilitate the bridging reactions of glyoxylic acid with (+)-catechin is not known, but it is expected that iron(III) would behave similarly to copper(II).

ABBREVIATIONS USED

HPLC/DAD, high-performance liquid chromatography/photodiode array detector; LC/MS, liquid chromatography/mass spectrometry; UV, ultraviolet.

ACKNOWLEDGMENT

The work was carried out at the National Wine and Grape Industry Centre in Wagga Wagga.

LITERATURE CITED

(1) Es−Safi, N.; Le Guernevé, 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.

(2) Clark, A. C.; Scollary, G. R. Copper(II)-mediated oxidation of (+)-catechin in a model white wine system. *Aust. J. Grape and Wine Res.* **2002**, *8*, 186−195.

(3) Es−Safi, N.; Cheynier, V.; Moutounet, M. Effect of copper on oxidation of (+)-catechin in a model solution system. *Int. J. Food Sci. Technol.* **2003**, *38*, 153−163.

(4) Fulcrand, H.; Cheynier, V.; Oszmianski, J.; Moutounet, M. An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry* **1997**, *46*, 223−227.

(5) Es−Safi, N.; Le Guernevé, C.; Cheynier, V.; Moutounet, M. 2D NMR analysis for unambiguous structural elucidation of phenolic compounds formed through reaction between (+)-catechin and glyoxylic acid. *Magn. Res. Chem.* **2002**, *40*, 693−704.

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

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

(8) Clark, A. C.; Scollary, G. R. Influence of light exposure, ethanol and copper(II) on the formation of a precursor for xanthylium cations from tartaric acid. *Aust. J. Grape and Wine Res.* **2002**, *9*, 64−71.

(9) Cacho, J.; Castells, J. E.; Esteban, A.; Laguna, B.; Sagrista, N. Iron, copper, and manganese influence on wine oxidation. *Am. J. Enol. Vitic.* **1995**, *46*, 380−384.

(10) 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.

(11) Simpson, R. F. Factors affecting oxidative browning of white wine. *Vitis* **1982**, *21*, 233−239.

(12) Ough, C. S.; Amerine, M. A. *Methods for analysis of musts and wines*; Ed.; John Wiley & Sons: New York, 1988; 277−278.
(13) Clark, A. C.; Scollary, G. R. Determination of total copper in white wine by stripping potentiometry utilising medium exchange. *Anal. Chim. Acta* **2000**, *413*, 25−32.
(14) Saucier, C.; Guerra, C.; Pianet, I.; Laguerre, M.; Glories, Y. (+)-Catechin-acetaldehyde condensation products in relation to wine-ageing. *Phytochemistry* **1997**, *46*, 229−234.
(15) Fulcrand, H.; Doco, T.; Es−Safi, N.; Cheynier, V.; Moutounet, M. Study of the acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry. *J. Chromatogr. A* **1996**, *752*, 85−91.

Received for review May 29, 2003. Revised manuscript received August 1, 2003. Accepted August 10, 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 funds from the Australian Federal Government.

JF034566T