Copper(II)-mediated oxidation of (+)-catechin in a model white wine system

ANDREW C. CLARK[1] **and GEOFFREY R. SCOLLARY**[1,2]

[1] National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga NSW 2678, Australia

[2] Corresponding author: Professor Geoffrey Scollary, facsimile +61 2 69334068, email gscollary@csu.edu.au

Abstract

This study was undertaken to establish the role played by copper(II) in enhancing the rate of oxidation of flavanols. A model white wine system consisting of 12% (v/v) aqueous ethanol saturated with potassium hydrogen tartrate and adjusted to pH 3.2 was used to allow experimentation under well-defined conditions. (+)-Catechin was the oxidisable substrate and copper(II) concentrations up to 0.6 mg/L were employed. The model white wines were maintained at 45°C to induce the browning process. Under these conditions an increase in absorbance at 440 nm occurred provided the copper(II) concentration was 0.3 mg/L or greater. The coloured species responsible for the increase in absorbance were identified as xanthylium cations, formed by linkage of two (+)-catechin molecules. Glyoxylic acid acted as the bridge between the phloroglucinol-type moiety of the (+)-catechin molecules. The production of the xanthylium cations was inhibited by ethanol and also by mannitol and the implications of these observations for a free-radical induced mechanism are discussed.

Abbreviations

HPLC/DAD high performance liquid chromatography/photodiode array detector; **LC/MS** liquid chromatography/mass spectrometry; **PPO** polyphenol oxidase; **UV** ultra violet; **VIS** visible

Keywords: *browning, (+)-catechin, copper(II), model wine, oxidation, xanthylium cation*

Introduction

Enzymic oxidation and autoxidation

Enzymic oxidation of grape juice occurs just after the grape cells are ruptured in the crushing process. The enzymes responsible for this oxidation, often termed polyphenoloxidases (PPO), include tyrosinase and, in mouldy grapes, laccase. Oxidation of flavanoids, as represented by (+)-catechin (Figure 1), in the presence of PPO type enzymes at pH 3 and 6 yielded both coloured and colourless products (Guyot et al. 1996). The first step in the production of these products was the conversion of the B ring of (+)-catechin (Figure 1), that is, the catechol-like moiety of (+)-catechin, to an *ortho*-quinone. The final coloured products contained multiple interflavan linkages whereas the colourless products contained only a single interflavan link. At the wine-relevant pH 3, the colourless compounds were favoured, possibly formed via semi-quinone intermediates as the corresponding *ortho*-quinone is less stable at pH 3 (Kalyanaraman et al. 1984). This work by Guyot et al. (1996) highlights the importance of the correct experimental conditions being used when modelling processes in white wines.

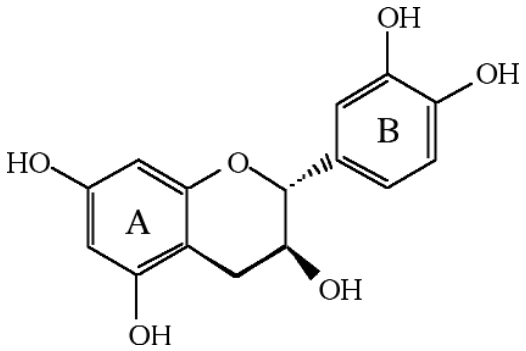

Figure 1. The structure of (+)-catechin. Ring A is of phloroglucinol-type functionality and ring B is of catechol functionality.

In wine, the PPO enzymes are largely absent due to their deactivation or removal with solids in the wine-making process. For this reason, the oxidation process in wine is dominated by indiscriminate chemical reactions rather than the more ordered enzymic oxidation process. In the absence of any catalyst, the non-enzymic oxidation of phenolic compounds is termed autoxidation. Similar to enzymic oxidation, the catechol functionality of phenolic compounds is involved in autoxidation (Figure 2). However, although the overall autoxidation reaction results in an *ortho*-quinone (A in Figure 2), the autoxidation may involve a semiquinone intermediate (B in Figure 2) and under certain conditions the presence of this intermediate species may increase the complexity of the final oxidation products (Singleton 1987).

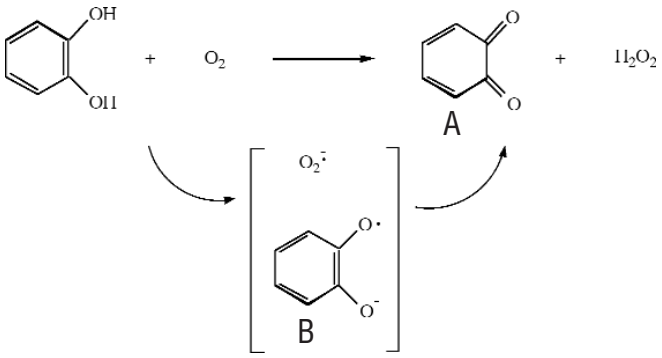

Figure 2. A representation of the autoxidation reaction for catechols to give an *ortho*-quinone (A) and the proposed semiquinone intermediate (B) (Singleton 1987).

Another difference between enzymic oxidation and autoxidation mechanisms is that during the oxidation of catechol-like functional groups a strong oxidant is generated as a byproduct in the latter mechanism (Singleton 1987, Wildenradt and Singleton 1974). Wildenradt and Singleton (1974) assumed that hydrogen peroxide was formed during the autoxidation of (+)-catechin and caffeic acid as they found the presence of acetaldehyde in their reaction systems. That is, they proposed that the generated hydrogen peroxide reacted with ethanol to form acetaldehyde on the grounds that, if the ethanol was replaced by propanol, propanal (the corresponding aldehyde) was formed. The presence of acetaldehyde in wine is known to form methyl-methine interflavan linkages between wine flavanols (Timberlake and Bridle 1976). Later studies proposed that both the superoxide ion and semi-quinone radical were intermediates in the production of hydrogen peroxide and the *ortho*-quinone during the autoxidation of catechol (Figure 2) (Cilliers and Singleton 1992, Singleton 1987). In one study (Singleton 1987), the presence of hydrogen peroxide was confirmed during the autoxidation of gallic acid in water at alkaline pH.

Catechol-like functional groups and metal ions

It has long been assumed that metal ions accelerate autoxidation reactions by increasing the rate of reaction between molecular oxygen and the catecholate functionality of respective phenolic compounds. This is based on the well known ability of catecholate groups to form chelating complexes with metal ions (Avdeef et al. 1978, Carrano and Raymond 1979, Laks et al. 1988, Takeda et al. 1985, van den Berg 1984) and the ability of metal ions to interact directly with molecular oxygen. For example, the copper(II) and copper(I) redox system, in aprotic media, is able to effect a two electron oxidation of catechol-like compounds to the corresponding *ortho*-quinone and finally to the muconic acid ester (Demmin et al. 1981, Rogic and Demmin 1978). The intermediate in both steps was proposed to be a dicopper(II)-catecholate complex. However, the medium, the concentration of copper(II) and copper(I) and the pH in this classical chemistry study were far removed from conditions relevant to wine.

A study more relevant to conditions found in wine was performed on the copper(II) catalysed oxidation of catechol (Balla et al. 1992). This involved an aqueous medium at pH 4.6 to 5.5 with a copper(II) concentration of one to two orders of magnitude higher than those typical of white wine. After 35% completion of the reaction, the overall mechanism was identical to that shown in the top mechanism of Figure 2. Based on kinetic data, the proposed reaction mechanism involved several intermediate copper(II)-catecholate-molecular oxygen complexes and a copper(I)-molecular oxygen species. These intermediates were never identified, however. At the completion of the reaction, the products were mainly unidentified acidic and polymeric species.

It remains to be determined whether similar metal catalysed oxidation reactions will occur in the environment of white wine. Apart from the increased acidity, the matrix of wine is complicated by the number of potential metal ion chelators, such as proteins and organic acids, that will compete with catecholate functional groups. These chelators also may inhibit the redox cycling of copper(II) to copper(I). To date, there has been no published evidence that the presence of metal ions leads to an increase in *ortho*-quinone derived oxidation products in actual or model white wines.

In a study (Oszmianski et al. 1996) conducted on the iron-catalysed oxidation of (+)-catechin in a tartrate buffered model red wine system, the main oxidation products were brown pigments. However, the mechanism for their production was not consistent with either *ortho*-quinone or semi-quinone derived products (Es-Safi et al. 1999a, b). Rather, concentrations of iron(II) ranging from 1–20 mg/L were found to be responsible for the production of a number of coloured xanthylium cations (Figure 3). From the identified mechanism (Es-Safi et al. 1999a, b), it became evident that the reactive site of (+)-catechin was not the catechol functionality but rather the phloroglucinol-type moiety of (+)-catechin (the A ring in Figure 1).

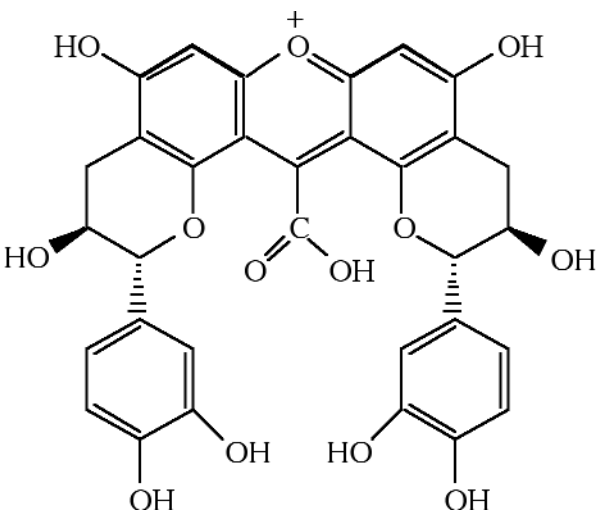

Figure 3. The structure of a xanthylium cation.

The work reported here was conducted to investigate the coloured oxidation products generated in the copper(II)-mediated oxidation of a model white wine system. That is, it was intended to ascertain whether the coloured oxidation products were either *ortho*-quinone or semiquinone derived products, or alternatively, if a mechanism similar to that observed for the iron(II)-mediated oxidation of the model red wine system (Es-Safi et al. 1999a) was dominant. The modelling of white wine was adopted as it is in white wine that oxidative exposure has been attributed with the production of brown pigments (Zoecklein et al. 1995). This process is often referred to as the oxidative browning of a white wine and has been correlated with the concentration of flavanols, such as (+)-catechin (Simpson 1982). Due to the modelling of a white wine, the levels of (+)-catechin adopted were to be lower and the pH more acidic than in the model red wine used previously (Oszmianski et al. 1996). As the average copper(II) concentration found in white wines is generally around or below 0.6 mg/L (Clark and Scollary 2000, Ough and Amerine 1988), depending on the country of origin for the wine, this was the maximum level to be added to the model white wine system. At these concentrations the copper(II) content was to be much lower than the levels of iron(II) used in the previous study (Oszmianski et al. 1996). The effect of some experimental and sample matrix parameters on the production of the coloured pigments was also to be investigated.

Materials and methods

Instrumentation and software

All pH measurements were performed with a TPS pH meter (model 1825 mV) and an Ionode combination pH electrode (IJ40). Buffer solutions (APS Finechem) of pH 7.00 ± 0.05 and pH 4.01 ± 0.05 (at 25°C) were used to calibrate the pH meter before use.

The UV/VIS spectra of samples were recorded on a µQuant Universal Microplate Spectromphotometer that was run by KC4 Software. The single wavelength (440 nm) absorbance of samples was recorded on a Unicam 8625 UV-VIS spectrophotometer.

HPLC/DAD was conducted on a Waters 2690 Separation Module run by Millenium^{32} software and connected to a Waters 996 photodiode array detector. The column used was a reverse phase Wakosil C18RS column of particle size 5 µm and 250 × 2 mm with a guard column of the same type.

LC/MS work was 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.

Reagents

All glassware and plastic ware were soaked for at least 16 hours in 5% Decon90 E-15 and then rinsed with copious amount 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) and iron(II) as iron(II) sulfate heptahydrate (Pronalys, AR). (+)-Catechin monohydrate (Sigma, 98%) was used without further purification.

Model white wine system

The model white wine was prepared by adding approximately 10 g of potassium hydrogen tartrate to aqueous ethanol (12% v/v, 2 L) and stirring overnight at room temperature. Excess potassium hydrogen tartrate was removed from the saturated solution by filtration through a 0.45 µm Sartorius cellulose acetate filter fitted to an all glass filter unit. The pH of the solution was adjusted to pH 3.20 with aqueous tartaric acid (300 g/L). The addition of 150 mg/L (+)-catechin was made immediately prior to the commencement of the induced browning process.

Induced browning process

Samples of 150 mL total volume were prepared in 200 mL Schott bottles with screw top lids. These were placed in a water bath at 45°C which was covered with a lid to minimise ingress of light. Unless mentioned otherwise the samples were opened to the atmosphere at least every second day during the measurement process.

Absorbance measurements

The model white wine without added (+)-catechin was used as the blank solution. To obtain a test portion for absorbance measurement, the Schott bottle was removed from the water bath and the solution stirred while approximately 2.5 mL of sample was taken using a disposable pasteur pipette. This test portion was transferred to a disposable plastic cuvette (Sarstedt) of 10 mm path length and the absorbance measured at 440 nm. The solution in the cuvette was then discarded, the lid replaced on the Schott bottle and the remaining sample returned to the water bath.

Analytical HPLC/DAD analyses

The HPLC/DAD work used a flow rate of 0.15 mL/min and sample injection of 100 µL. A gradient elution was adopted which consisted of solvent A, Grade 1 water, and solvent B, methanol, both containing 0.5% acetic acid. The composition of the mobile phase during the analysis was taken from Saucier et al. (1997):

Time(min)	0	1	59	74	75	88	112	120
A (vol%)	100	95	62	56	48	45	0	100

The UV-VIS spectra were recorded from 250–500 nm. The chromatography column was left at room temperature during the analysis.

Analytical LC/MS analyses

LC/MS work was conducted both in the positive ion mode, with an ion spray voltage of +4 kV and orifice voltage of +30 V, and in the negative ion mode, with an ion spray voltage of –4 kV and orifice voltage of –30 V. Simultaneous wavelength detection at 278 nm and 440 nm was performed. The same column, solvent conditions and flow rate were used as for HPLC/DAD experiments. The sample injection was 20 µL.

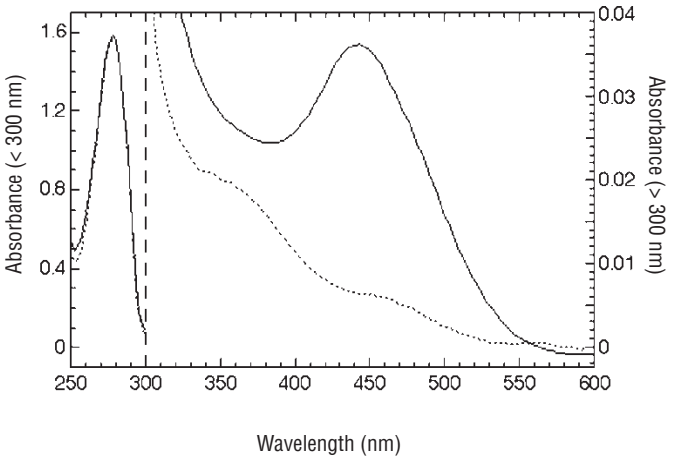

Figure 4. The UV/VIS spectrum of the model white wine system both before (·····) and after (——) 20 days of the induced browning process. The concentration of copper(II) is 0.6 mg/L.

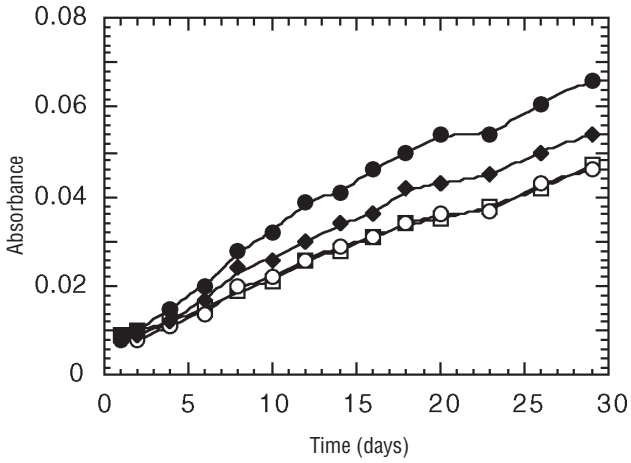

Figure 5. Change in absorbance at 440 nm with time for the model white wine at 0 (□), 0.1 (○), 0.3 (◆) and 0.6 (●) mg/L copper(II) during the induced browning process.

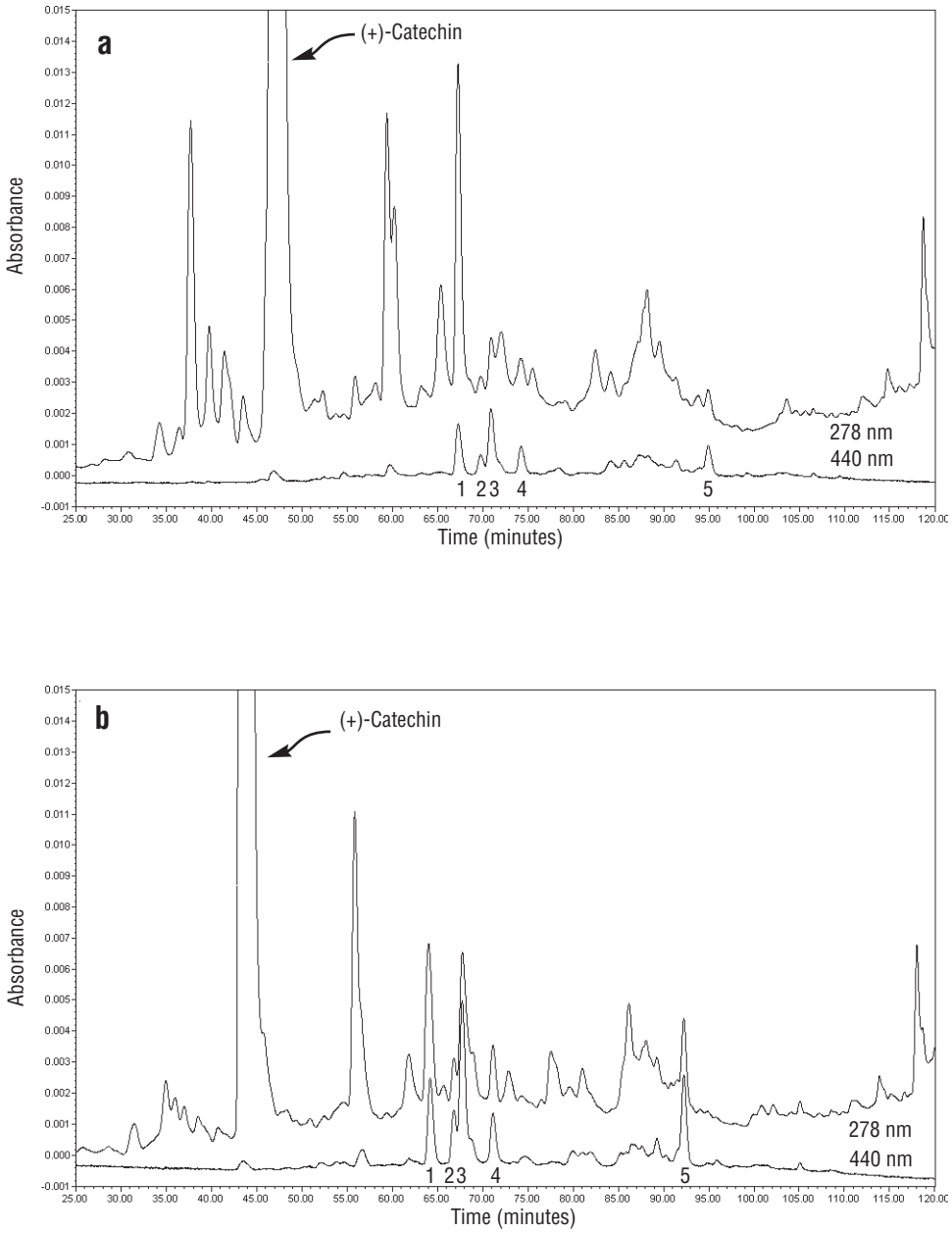

Figure 6. HPLC/DAD chromatograms at 440 and 278 nm of the model white wine with 0 (**a**) and 0.6 (**b**) mg/L copper(II) after 29 days of the induced browning process.

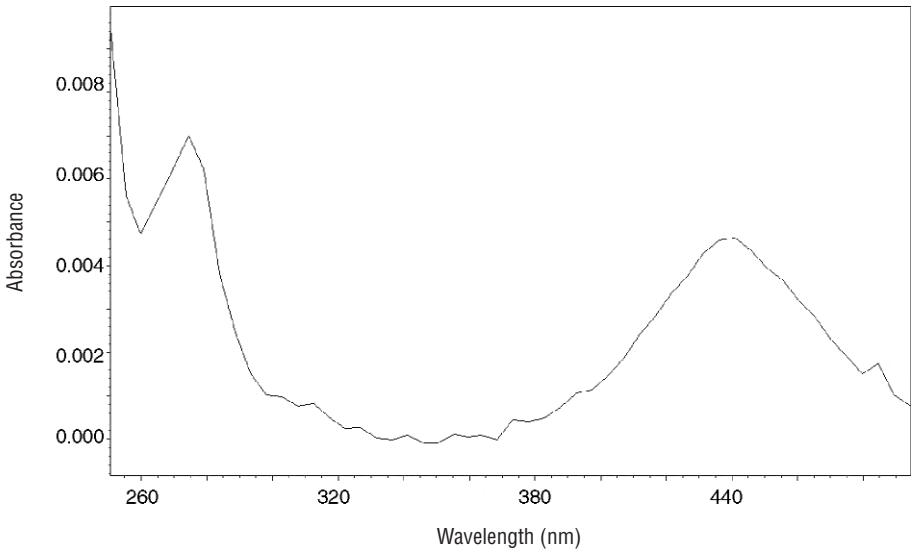

Figure 7. The UV-VIS spectrum of peak 3 in Figure 6b.

Iron(II)-mediated browning of (+)-catechin

The conditions used for the iron(II)-mediated browning of (+)-catechin were identical to those of Osmianski et al. (1996) with 20 mg/L iron(II), added as iron(II) sulfate, except that the sample was incubated at 39°C as performed in the work of Fulcrand et al. (1997).

Reaction between (+)-catechin and glyoxylic acid

Glyoxylic acid (Aldrich, 98%) was added to the model white wine system (150 mL) giving a (+)-catechin to glyoxylic acid ratio of 2:1 (0.50 : 0.25 mM). The sample was stored in darkness at ~ 10°C.

Results and discussion

Influence of Cu(II) concentration on the induced browning process

When the model white wine system underwent the induced browning process, that is, heating at 45°C in darkness, there was an increase in absorbance at wavelengths within the range of 400 to 500 nm (Figure 4). As the absorbance maximum in this region was around 440 nm, all model white wines were measured at this wavelength.

When the model white wine system, with differing concentrations of added copper(II), underwent the induced browning process, the relationship between the rate of browning and the copper(II) concentration was not directly proportional to the copper(II) concentration (Figure 5). Following a lag period, the browning accelerated in all samples and after 29 days it reached an appreciable absorbance of 0.047 to 0.066, depending on the added copper(II) concentration. Negligible difference in the rate of browning occurred in the absence of added copper(II) and with 0.1 mg/L added copper(II), but on the addition of 0.3 mg/L copper(II) (and above) the rate of browning increased considerably. After 29 days of the induced browning process the model white wine solution was coloured but not cloudy, implying that no precipitation had occurred.

All samples were analysed by HPLC/DAD after 29 days of the induced browning process (Figure 6). The photodiode array detector on the HPLC/DAD enabled the chromatograms to be generated simultaneously at 278 nm, a wavelength at which (+)-catechin absorbs, and 440 nm, the wavelength at which the coloured compounds absorb. In the 278 nm chromatograms, the large peak at a retention time of approximately 45 minutes was assigned to (+)-catechin with verification by mass spectral data and from the retention time of a (+)-catechin standard. Throughout the browning process of the model white wine system, both in the presence and absence of copper(II), the peak area of (+)-catechin decreased by less than 5.5%, suggesting that only a minimal fraction of (+)-catechin had been involved in the browning process. It was also apparent that there was some variability in the retention time of peaks between the different sample chromatograms. However, by comparing the retention time of (+)-catechin in different chromatograms and observing the general pattern of eluting peaks, the identification of equivalent peaks in two different chromatograms was easily achieved. This variability was most likely caused by the complex multiple steps in the HPLC/DAD solvent gradient and the lack of temperature control over the chromatography column.

Xanthylium cation formation during the induced browning process

Several peaks were present in the chromatograms at 440 nm with longer retention times than (+)-catechin. Given the nature of the column used in the separation, the elution pattern of these products suggested that they were less polar than (+)-catechin. The profiles of the chromatograms at 440 nm in the absence and presence of added copper(II), and at all different copper(II) concentrations, were identical with only the absorbance intensity of peaks varying, reflecting the differences recorded in Figure 5. The peaks in the 440 nm chromatograms had a corresponding peak in the 278 nm chromatogram.

The UV/VIS spectrum of the compound responsible for the most intense peak in the chromatogram at 440 nm (Figure 6b, peak 3) is shown in Figure 7. The com-

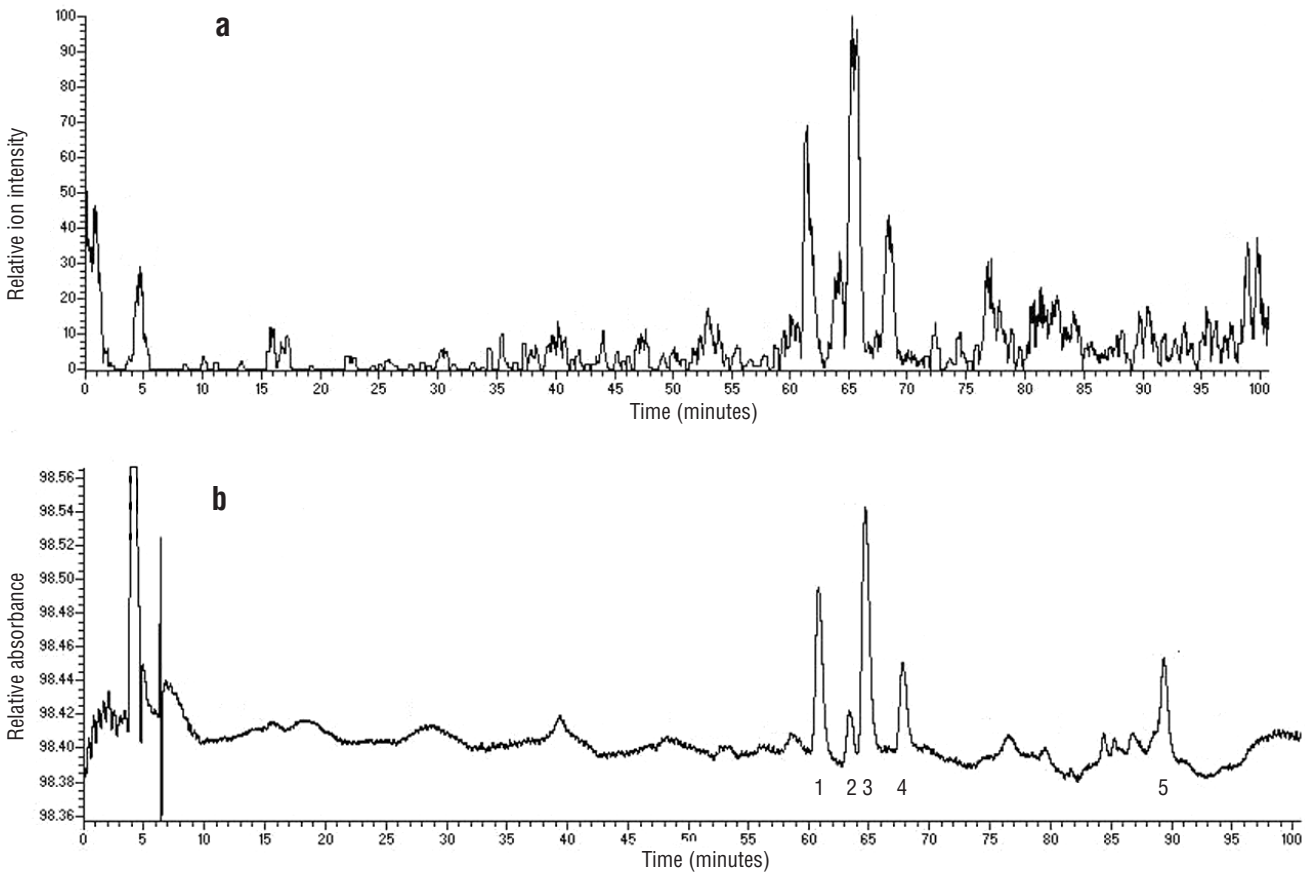

Figure 8. The LC/MS chromatograms generated in the analysis of the model white wine system containing 0.6 mg/L copper(II). The plot of the ion at 617 m/z as monitored by the electrospray mass spectrophotometer detector is shown in (**a**) and the simultaneous plot of absorbance at 440 nm as monitored by the UV detector is shown in (**b**).

pound has absorbance maxima at 280, 310 and 440 nm. The absorbance maximum at 280 nm suggested that this coloured compound had retained some character of (+)-catechin. In fact, peaks 1–4 in the 440 nm chromatogram (Figure 6b) gave identical UV/VIS spectra, while peak 5 (Figure 6b) had a 10 nm bathochromic shift of the maximum in the visible region.

Peak 3 (Figure 6b) was found by LC/MS to have a mass spectrum with a significant ion at 617 m/z. The assignment of this ion as either a fragment or pseudomolecular ion, such as $M+H^+$ or $M+K^+$, was not possible due to the lack of significant ions in the negative ion mass spectrum of peak 3. However, the ion at 617 m/z in the positive ion mode was consistent with the presence of xanthylium cations (Figure 3), analogous to those observed in work published by Cheynier and co-workers (Es-Safi et al. 1999a). The xanthylium cations generated in the iron(II)-mediated oxidation of (+)-catechin (Es-Safi et al. 1999a, Oszmianski et al. 1996) had identical UV/VIS spectra to peaks 1–4 (Figure 7) and were detected by LC/MS (Es-Safi et al. 2000) at 617 (M^+) and 615 ($M^+–2H^+$) m/z in the positive and negative ion modes respectively. Under the LC/MS conditions utilised for the analysis of the model white wine system, the charged xanthylium cations were expected to be more easily detected in the positive rather than negative ion mode.

It had also been shown by Es-Safi et al. (2000) that six different isomeric forms of this specific xanthylium cation are possible. When the mass chromatogram was searched for ions with a m/z value of 617 (positive ion mode) the result in Figure 8a was generated. From the matching profiles it was evident that peaks 1–4 (Figure 8b) had mass spectra with a significant ion at m/z 617 (Figure 8a). Consequently, it was likely that peaks 1–4 were due to the different isomers of the xanthylium cation that had been observed previously (Es-Safi et al. 2000). As only four possible xanthylium cation peaks were found in this study, it was likely that the separation between all isomers had not been achieved and/or not all of the isomers were formed at sufficiently high concentrations to be detected.

To confirm that peaks 1–4 (Figure 6b) were due to xanthylium cations, two samples known to contain xanthylium cations were analysed using the identical chromatographic method as in Figure 6. The first of these samples, containing 20 mg/L iron(II) and 1.16 g/L (+)-catechin, was prepared in a manner identical to that described by Osmianski et al. (1996) with heating at 39°C (Fulcrand et al. 1997) for 12 days. The second sample consisted of glyoxylic acid and (+)-catechin (Es-Safi et al. 1999a) prepared in the model white wine system at a ratio of 1:2 (0.25:0.50 mM). The comparison of 440 nm chromatograms (Figure 9) between these samples and the model white wine system with 0.6 mg/L copper(II)

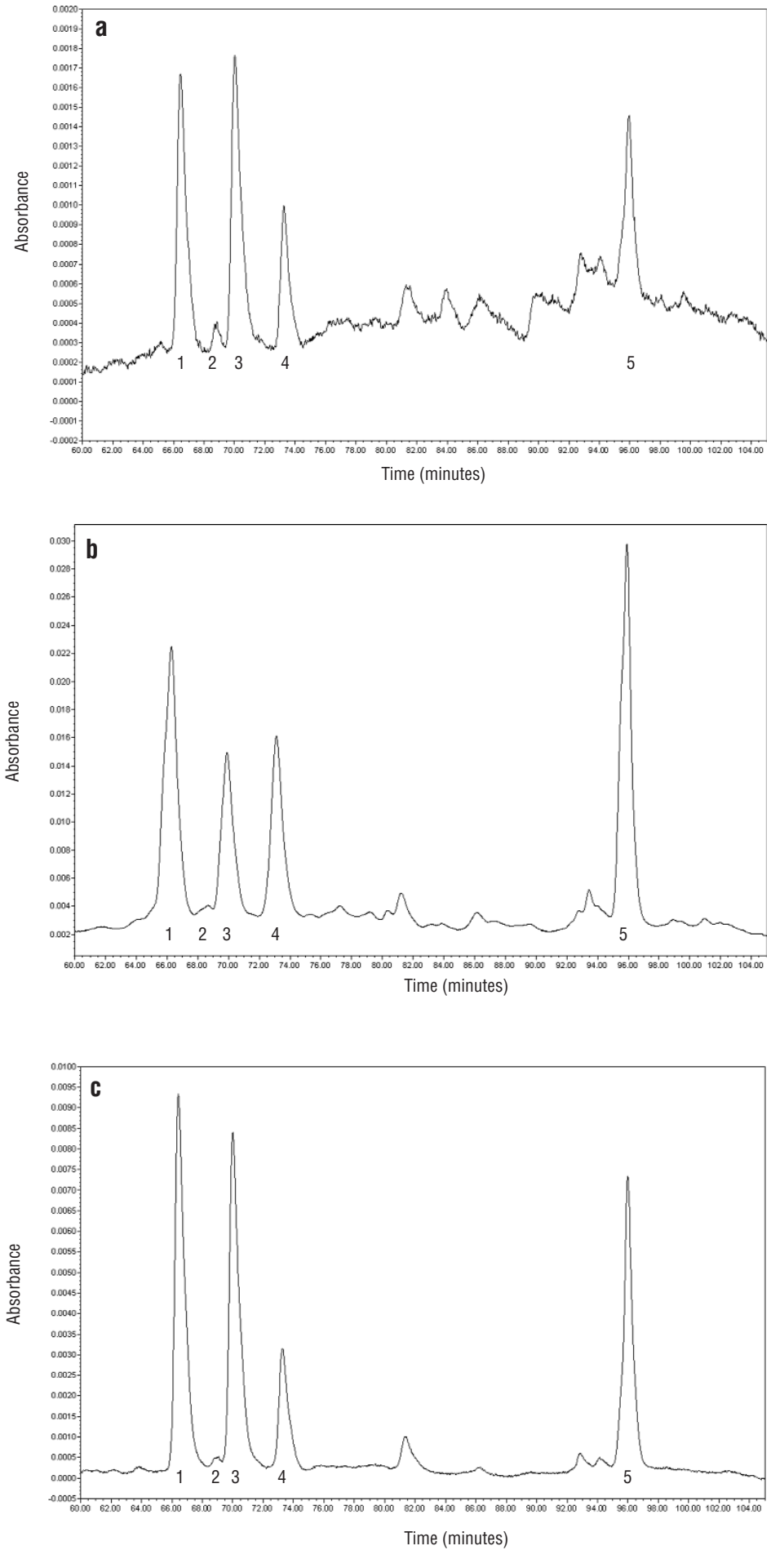

Figure 9. Comparison of the 440 nm chromatograms obtained from the analysis of various (+)-catechin solutions. The solutions are the model white wine system (150 mg/L (+)-catechin) with 0.6 mg/L copper(II) after 12 days at 45°C (**a**), the model wine system (1.16 g/L (+)-catechin) of Osmianski et al. (1996) with 20 mg/L iron(II) after 12 days at 39°C (**b**), and the model white wine system (150 mg/L (+)-catechin) with 0.25 mM glyoxylic acid after several months at 10°C (**c**).

shows that peaks 1–4 are present at identical retention times in all the samples. The LC/MS analysis of all the samples confirmed the presence of a 617 m/z ion for each of the four peaks 1 to 4 (Figure 9a–c). The different conditions used for the copper(II), iron(II) and glyoxylic acid induced reactions most likely contribute to the different peak intensity ratios observed in the chromatograms (Figure 9a–c). Irrespectively, these results confirm that the main coloured pigments generated in the model white wine system both in the absence and presence of copper(II) are xanthylium cations.

Peak 5 in the 440 nm chromatogram of the model white wine system (Figure 6) was observed in all the analysed samples in Figure 9 and its corresponding mass spectrum in the positive ion mode gave a significant ion at 645 m/z. Once again no significant ions were observed in the negative ion mode. The UV/VIS spectrum corresponding to this peak and the ion detected at 645 m/z were both in agreement with the published characteristics of the ethyl ester of the xanthylium cation (Es-Safi et al. 1999a). Similar to the original xanthylium cation, the ethyl ester would also be expected to have six isomeric forms despite the presence of only one peak in the HPLC/DAD.

Role of Cu(II) in xanthylium cation formation

The mechanism for the formation of the xanthylium cations has been described by Es-Safi et al. (1999a, b) as a four-step sequence, involving the oxidative coupling of (+)-catechin via a bridging group. The production of glyoxylic acid, presumably through the oxidative cleavage of tartaric acid (Fulcrand et al. 1997), initiates a reaction between glyoxylic acid and (+)-catechin generating a carboxy-methine linked dimer. After a dehydration step, a xanthene is produced followed by its oxidation to give the xanthylium cation. As all intermediates involved in production of the xanthylium cation are colourless, it is likely that the lag period (Figure 5) involves the initiation steps for the xanthylium cation formation.

Xanthylium cation formation is enhanced by the presence of copper(II) at elevated concentrations in the model white wine system, but copper(II) is clearly not a component of the final product. Rather, of the two 'reactive' rings in (+)-catechin, all chemical modification has occurred at the phloroglucinol-type ring (A ring, Figure 1). This is somewhat surprising as there is considerable published work suggesting the catechol moiety, or B ring, should be capable of complexing with copper(II) ions. The *ortho*-arrangement of the two hydroxy groups in the B ring presents an ideal metal chelation site and catechol itself is an analytical reagent for the determination of copper(II) (van den Berg 1984). Of course, the pH used in previous studies (pH 7–8) was always more basic than that of wine and deprotonation of the hydroxy groups of the B ring would be significant, leading in turn to a greater metal ion chelating propensity.

Irrespective of the absence of copper(II) in the final product, it is apparent (Figure 5) that at concentrations of 0.3 mg/L and higher, copper(II) ions are capable of influencing favourably at least one of four steps in the mechanism for xanthylium cation formation. It has not yet been possible to identify which step or steps the copper(II) may be accelerating. Intriguingly, at a copper(II) concentration of 0.1 mg/L, no enhancement of the browning process was observed (Figure 5). It is not yet certain whether this is due to the low level of copper(II) becoming unreactive, possibly through complex formation, or alternatively, if copper(II) is able to inhibit a step in the browning process and this inhibition is more dominant at lower copper(II) concentrations. The influence of copper(II) on the distribution of reaction products is evident when comparing the 278 nm chromatograms of samples with and without copper(II) (Figure 6). In the 278 nm chromatogram of the copper(II)-free sample (Figure 6a) there are four main peaks with retention times between 35 to 45 minutes that are diminished in the copper(II) containing samples. This suggests that copper(II) is either inhibiting the formation of these particular compounds or is accelerating their degradation. A more detailed study of the role of copper(II) in the mechanism of xanthylium cation formation is presently under way. Importantly our results show that copper(II) is not required for the formation of xanthylium cations from (+)-catechin in a system modelling white wine. Rather, at concentrations resembling those found in white wine, copper(II) accelerates xanthylium cation formation.

These results have illustrated for the first time that xanthylium cations are able to be produced in a system modelling white wine. The critical parameters in this study are the use of lower concentrations of both (+)-catechin and metal ion, the use of copper(II) as the metal ion and a more acidic pH compared with previously reported studies (Oszmianski et al. 1996). Furthermore, there was no evidence for the existence of any coloured compounds that may have been formed from a (+)-catechin derived *ortho*-quinone compound, as occurs in enzymic oxidation. Rather, in chemical, or non-enzymic oxidation, as described here, it is the A-ring that undergoes chemical change. These observations are important in understanding the different types of oxidative browning processes that have been described for white wine. Enzymic oxidation leads to *ortho*-quinones that subsequently react with other wine components to give brown polymers (Singleton 1987). Both non-flavanoids and flavanoids can be involved. However, non-flavanoids do not contain the phloroglucinol-type moiety, as do flavanoids (represented in this work by (+)-catechin) and it is this phloroglucinol unit that is essential for xanthylium cation formation. The work described here, therefore, provides some insight for the observations of Simpson (1982) on the relationship between white wine browning propensity and the concentration of (+)-catechin type compounds.

Effect of oxygen ingress on xanthylium cation formation

Preliminary experiments were conducted on the effect of oxygen ingress on xanthylium cation formation. Two identical model white wine samples were prepared, both with 0.6 mg/L copper(II); one sample was aerated twice

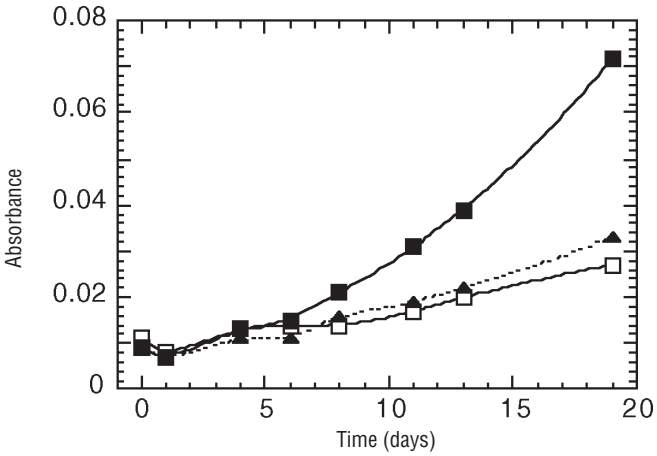

Figure 10. Change in absorbance at 440 nm with time for three different styles of the model white wine (all without copper(II)) during the induced browning process. Model white wine with 12% (v/v) (2.03 M) ethanol (□), 0% (v/v) ethanol (■) and 0% (v/v) ethanol with 2g/L (0.01 M) mannitol (▲).

daily for 24 days while the second sample was only aerated a total of four times in the 24-day period (viz. days 1, 6, 14 and 24). Comparison of the effect of aeration frequency on the absorbance at 440 nm showed that extensive aeration (twice daily) did not markedly influence the absorbance compared with infrequent aeration. The difference in absorbance intensity at 440 nm between the two samples at day 24 was less than 2%. The effect of headspace to sample volume ratio on the rate of browning was examined by comparing absorbance/time plots for two model white wine samples, one with a 200 mL sample volume and 50 mL headspace and the other with a 150 mL sample volume and 100 mL headspace. Both samples were only aerated on absorbance measurement days (i.e. days 1, 6, 13 and 20). As the absorbance difference between the samples was not more than 3% at any stage of the browning process, the results suggest that the variation of the headspace volume did not impact on the 440 nm absorbance reading. In all these experiments, a relatively large headspace to solution volume was used and it is possible that the system was always saturated with respect to its requirements for molecular oxygen. In work with ascorbic acid induced browning of (+)-catechin, Bradshaw et al. (2001) observed that a small headspace to solution volume affected the lag phase only. Further, exclusion of air after the end of the lag phase did not affect the final absorbance reading (Bradshaw et al. 2001), suggesting that oxygen ingress is required only to initiate the browning process. This issue is being refined further in the case of copper(II)-mediated browning.

Influence of ethanol and mannitol on xanthylium cation formation

An investigation into the presence of ethanol on the rate of browning was expected to provide some insight into the possible mechanism of the browning process. To assess the effect of ethanol on the rate of browning in the model white wine, samples without addition of Cu(II) were prepared with and without ethanol (Figure 10). The overall rate of browning increased dramatically in the absence of ethanol. The 440 nm chromatogram and LC/MS data of the ethanol-free model white wine confirmed the production of xanthylium cations in greater amounts than found with a sample containing 12% (v/v) ethanol (Figure 10), implying ethanol is able to inhibit the production of xanthylium cations.

Ethanol is known to react with the hydroxyl radical with a rate constant of 1.9 to 2.2×10^9 (depending on the reaction conditions) (Buxton et al. 1988, Motohashi and Saito 1993). If the inhibitory role of ethanol was via the scavenging of hydroxyl radicals then the addition of mannitol, a known scavenger of this radical (Buxton et al. 1988, Motohashi and Saito 1993), in the absence of ethanol, was expected to also decrease the rate of browning. As shown in Figure 10, the presence of mannitol did decrease the rate of browning. Given the ability of both mannitol and ethanol to suppress the browning process, it is reasonable to assume that the suppression is due to hydroxyl radical scavenging. Ethanol would appear to be a less efficient hydroxyl radical scavenger than mannitol as the extent of suppression is approximately the same (Figure 10), even though ethanol was present in much higher concentration (2.03 M ethanol compared with 0.01 M mannitol). Metal ions, including copper(II), can induce hydroxyl radicals through Fenton type chemistry (Wardman and Candeias 1996) and such a process has been invoked to explain oxidative processes occurring in beer (Chapon and Chapon 1979).

As ethanol is capable of scavenging the hydroxyl radical, a highly complex reaction system must be involved, possibly involving a radical cascade system. It must be pointed out that the xanthylium cation formation is not a chaotic process, as the degree of reproducibility in the absorbance values for equivalent experimental systems is quite high (RSD 1.5%, n = 3). We are presently examining the possible free radical mechanisms that could be involved in this copper(II)-mediated browning reaction.

Conclusions

The main products identified during the browning of the model white wine were xanthylium cations. The existence of xanthylium cations in the model white wine were observed in the absence of copper(II), however the presence of copper(II) above a threshold level led to an increase in concentration of the xanthylium cations. Based on the identification of these products it was concluded that the generation of the xanthylium cations occurred through the reaction of glyoxylic acid with (+)-catechin as proposed by the work of Cheynier's group (Es-Safi et al. 1999a, Oszmianski et al. 1996) on the iron(III)-mediated browning of a model red wine system. There was no clear evidence for the interaction of copper(II) with the catechol moiety of (+)-catechin in the oxidative browning associated with the generation of xanthylium cations.

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