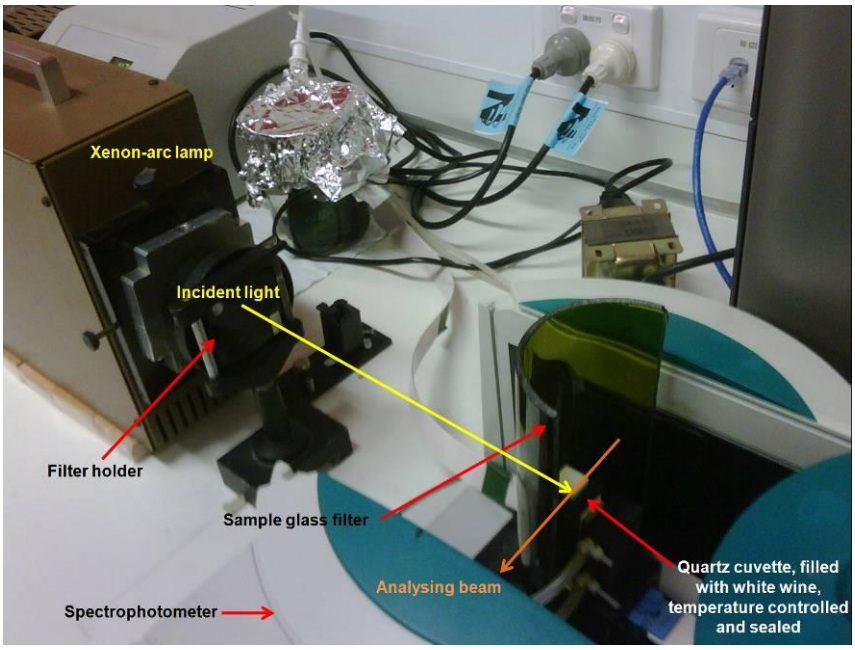
WINE BOTTLE COLOUR AND OXIDATIVE SPOILAGE



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UM 0902**

Principal Investigator: **Dr Geoffrey R. Scollary**

Research Organisation: **The University of Melbourne**

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WINE BOTTLE COLOUR AND OXIDATIVE SPOILAGE

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Date: 22 October, 2010

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ABSTRACT
Significant progress has been achieved in understanding the impact of light on the development of pigmentation in white wine in this pilot project. White wine is more susceptible to the effect of low wavelength light, particularly light less than 400 nm. All wine bottles used in this project, both traditional weight and newer light weight bottles, allow the transmission of the critical wavelengths to some extent. Iron tartrate has been identified as a potential photo-initiator and the presence of molecular oxygen is essential for pigmentation development. This report suggests the potential of riboflavin to act as a protecting agent for wine from light-struck effects, although more work on the sensory impacts is required. Exposure studies under controlled temperature conditions showed that wine in lighter coloured bottles was more susceptible to pigmentation, but darker bottles had higher pigmentation levels when temperature was not controlled. The possibility of identifying marker compounds for both light effects and temperature effects is discussed.

EXECUTIVE SUMMARY

There is environmental pressure on the wine industry to reduce the mass of glass bottles as well as to move away from darker coloured bottles to either 'flint' or 'amber' colours. It is argued that this contribution would ultimately lower energy and recycling costs and at least partially address current issues of sustainability, emissions, energy, packaging and resources. While these arguments are substantially valid, changing the colour and mass of wine bottles tends to be dictated by aesthetics and consumer appeal. The advent of lighter weighted bottles has addressed issues relating to reduced carbon emissions through lower energy costs of transport. However, there is a lack of information regarding the stability of wine in glass bottles that are thinner and lighter coloured than their traditional counterparts. The combination of lighter and/or thinner bottles may offer less light protection and have potential major implications for wine stability. The impact of elevated temperatures to which wine may be subjected during transport and storage, as well as the combination of light, temperature and residual oxygen content from bottling, on wine stability has not been investigated in detail previously.

This pilot project was designed to examine the influence of light, temperature, glass transmission (colour and bottle thickness) which impacts on the development of pigmentation or enhanced colour of white wine. Particular focus was made on the relationship between the wavelength of radiation necessary for the photo-activation process and glass transmissivity. In addition to these environmental variables, the link between various wine components (type of phenolic compound, iron(III) tartrate, riboflavin and molecular oxygen was also investigated).

To achieve these goals, two experimental strategies were used:

- a small scale wine irradiation setup which shows the impact of light (ultraviolet, low visible light wavelengths and specific wavelengths of light) on white wine under controlled/uncontrolled temperature conditions;
- a large scale wine irradiation setup allowed for whole wine bottles to be irradiated for a set-time under controlled/uncontrolled temperature conditions which shows the impact of bottle glass colour.

The main findings of this study were:

- Ultraviolet and, to a lesser extent, low wavelength visible light contributes to pigment production in white wine under controlled temperature conditions
- Ultraviolet light causes greater pigmentation in wine compared to red, orange and yellow light
- Temperature, that is heating wine in darkness at 25°C, 45°C, 50°C and 60°C does contribute to pigment development but not to the extent when UV light is present
- The transmission of ultraviolet light is greatest through Flint > Arctic Blue > French Green and Antique Green
- All glass types allow the transmission of low wavelength ultraviolet light to pass through to the wine, resulting in pigmentation development
- There is no significant difference in light induced wine pigmentation in the visible region between heavy and light weighted bottles of the same colour
- Under controlled temperature and light conditions xanthylium type compounds were identified by UPLC and LC-MS. Greater pigment production was observed in Flint> Arctic Blue > French Green > Antique Green bottles

- Under uncontrolled temperature and light conditions unknown pigments were identified, but not characterized by UPLC and LC-MS. Greater pigment production was observed in Antique Green > French Green > Arctic Blue and Flint bottles
- Aroma (descriptors) and colour profiles (CIELab) validated these findings
- Iron tartrate was found to be the photoactive species responsible for the degradation of tartaric acid to glyoxylic acid;
    - the presence of iron is essential for the production of glyoxylic acid;
    - the presence of light is essential for the production of glyoxylic acid;
    - the absence of ethanol decreased the amount of glyoxylic acid produced.
    - the relationship between wine colour development and bottle colour and thickness showed the highest pigmentation levels were found when wine samples were exposed to light with a Flint glass filter in place and the lowest levels when an Antique Green glass filter was used.
- Riboflavin was found to be a possible 'protecting agent' to prevent or minimise the development of phenolic-derived pigments that are formed during the exposure of white wine to short wavelength radiation.
- Molecular oxygen plays an important role in the production of pigments during irradiation with light. Pigmentation is greater in the bottles that were exposed to a high oxygen environment and xanthylium pigments could only detected in the high oxygen samples.
- The decay rates of ascorbic acid in Flint, Arctic Blue, French Green and Antique Green bottles were found to be identical in all bottle types; that is, there is no influence of the surface of the glass bottle on the solution chemistry oxidative processes.
- Pigment development was also identified in preliminary studies on red wine.

The communication strategies adopted for the dissemination of the research outputs to the wine industry and the scientific community are:

- One published industry technical paper, with a further two in preparation;
- One wine industry technical conference poster presentation and one chemistry conference poster presentation with a second chemistry conference presentation to occur in November 2010;
- Three scientific papers are in preparation;
- Two multiple day programs for Year 10 science students as part of the 'Growing Tall Poppies' initiative.

Collaboration with Dr Andrew Clark and John Blackman of the National Wine and Grape Industry Centre was established during this project, especially through the sharing of equipment and facilities. The outputs of the project will be fed into the oenology teaching program at CSU.

BACKGROUND

There is environmental pressure on the wine industry to reduce the mass of glass bottles as well as to move away from darker coloured bottles to either 'flint' or 'amber' colours (Waste and Resources Action Program 2010). It is argued that this contribution would ultimately lower energy and recycling costs and at least partially address current issues of sustainability, emissions, energy, packaging and resources. While these arguments are substantially valid, changing the colour and mass of wine bottles tends to be dictated by aesthetics and consumer appeal. The advent of lighter weighted bottles has addressed issues relating to reduced carbon emissions through lower energy costs of transport. However, there is a lack of information regarding the stability of wine in glass bottles that are thinner and lighter coloured than their traditional counterparts. The combination of lighter and/or thinner bottles may offer less light protection and have potential major implications for wine stability. The impact of elevated temperatures to which wine may be subjected during transport and storage, as well as the combination of light, temperature and residual oxygen content from bottling, on wine stability has not been investigated in detail previously.

Exposure of bottled wine to light tends to occur in retail outlets or in domestic situations where artificial fluorescent lighting generates energy at shorter wavelengths (low visible and ultraviolet (UV) radiation). In an early study on sparkling wines, exposure to light at wavelengths reported to be less than 400 nanometres (nm) produced detectable sensory changes identified by off-odours that resulted from the degradation of sulfur-containing amino acids in wine (Maujean and Seguin 1983).

In another study, the 'light-struck' aroma produced on exposure of still and sparkling wines to fluorescent lighting was investigated. Wine stored in green bottles showed a significant sensory difference after 31.1 hours (still) and 18 hours (sparkling). These times were reduced to 3.3 hours (still) and 3.4 hours (sparkling) when clear glass bottles were used (Dozon and Noble 1989).

While off-odour production from light exposure has been examined, there has only been minimal work on the link between pigment production and light impact. Generally, oxidative processes are required for pigment production, the most detailed example being xanthylium cation pigments. These pigments are formed from a series of reactions involving catechin-type phenolic compounds and glyoxylic acid, a degradation product of tartaric acid (Clark *et al.* 2007, Clark and Scollary, 2002, 2003, Es-Safi *et al.* 2000, Labrouche *et al.* 2005).

For light-induced changes to occur, a photoactivator is required. Riboflavin was proposed as the photoactivator by Maujean and Seguin (1983) and this was reinforced by Mattivi *et al.* (2000). Riboflavin has been established as the photoinitiator of light-struck effects in beer (Goldsmith *et al.* 2005), but an equivalent detailed study has not been performed on wine. Compounds such as (+)-catechin, (-)-epicatechin, tryptophol (Pozdrik *et al.* 2006) and (–)-epigallocatechin gallate (Becker *et al.* 2005) are efficient quenchers of the triplet-excited state of riboflavin, essentially stopping or at least minimising any photoactive processes.

Oxygen is also a critical parameter and its variable concentration in wine is often overlooked in the assumption of riboflavin effects on wine. On the other hand, it has been proposed that iron(III) tartrate may well be the photoactive species involved in sunlight-induced degradation of tartaric acid (Clark *et al.* 2007). Furthermore, iron(III) citrate is also known to be photoactive at wavelengths around 350-360nm (Abrahamson *et al.* 1994). The critical wavelengths for photo-induced degradation of wine have not been determined.

The colour of the wine bottle will be a crucial factor influencing the photodegradation of wine as different wavelengths of light will be transmitted depending on the colour (composition) of the glass. Maury *et al.* (2010) have recently published a study in which a Sauvignon Blanc wine was exposed to sunlight for 70 days. While the conditions were extreme, the results showed that more colour development occurred in the dark green (Antique Green) bottle than in Flint or French Green. Maury *et al.* (2010) suggested that this may be due to a temperature effect as wine in darker bottles was generally at higher temperatures than in lighter coloured bottles.

A study that separates light effects from temperature effects has not been carried out previously. In the work reported here, the impact of temperature and light (alone and in combination) was examined, followed by a determination of critical wavelengths at which the development of pigmentation occurs. The effect of different bottle types on light effects was then examined.

PROJECT AIMS AND PERFORMANCE TARGETS

The intention of this project is to address the lack of knowledge of light and thermal effects on wine, focussing on the consequent impact on wine quality and consumer acceptance if the environmental expectations of a move to clear glass bottles are to be met. The specific aims for this 1-year pilot project are to:

- Determine the critical wavelengths (action spectrum) at which photodegradation of wines (white, red) occurs
- Identify the active compounds which act as photoinitiators and deactivators
- Determine the influence of bottle colour and bottle thickness (weight) on the extent of photodegradation
- Determine the critical role of oxygen on the photodegradation process
- Determine the influence of temperature on the photoactivation process
- Prepare an information booklet for the wine industry on the critical determinants that can lead to the photodegradation on wine and disseminate this information through the GWRDC web site and industry meetings.

Year 1	Output	Due Date mm/yy	Activities
a	Information on the photodegradation of red and white wines	08/09 08/09	Appoint post-doctoral researcher Initiation of action spectrum studies using solar simulator
b	Information on the relationship between photodegradation and wine bottle colour	12/09 02/10 03/10	Complete action spectrum studies using solar simulator Use bottles of different colours Use bottles of one colour, but of different weights
c	Information on the photoactivity (activators/deactivators) of individual wine components	04/10	Expose model wines with individual wine components to light Evaluate photoactivity or photo deactivation of selected wine components
d	Information on the respective influences of light and temperature on wine degradation	05/10	Using solar simulator, expose wines to different light and temperature regimes
e	Sensory evaluation of wines exposed to light	05/10	Sensory trial completed with participating winemakers
f	Final report, conference presentation, technical and research publications	06/10	Final report completed Presentation at AWITC (July 2010) Technical publication submitted Research publication prepared

The project commenced at the beginning of September, 2009, following the appointment of Dr Daniel Dias as the post-doctoral researcher. By agreement with the GWRDC, the date for completion of the project was extended to 31 August, 2010.

The project focussed on pigmentation development in white wine and only a preliminary investigation on red wine was performed. The sensory trial on light-exposed wines was carried out at the National Wine and Grape Industry Centre, rather than with winemakers as originally proposed. Significant progress was achieved in determining the critical factors influencing light effects on white wine. Within the time constraints of this one-year pilot project, sufficient data to publish an information booklet proposed in the project submission could not be achieved. Irrespective, the recommendations at the end of this report provides a strong scientific basis for winemakers, wine retailers and consumers to make decisions for the safe bottling, display and storage of white wine.

METHOD

Two different exposure systems were used. In one (small scale irradiation setup), the light source was focussed on the sample held in a spectrophotometer cell. This allowed the UV/visible spectrum to be recorded at regular intervals during the exposure period. The second system (large scale irradiation setup) was constructed to allow whole bottle exposure.

Small scale irradiation setup

A sample (2mL) of either a wine solution or wine base solution was added either to 10mm quartz or glass cuvette that was temperature controlled using a CARY single cell Peltier accessory. A high pressure xenon arc-lamp (XBO 150 W1) installed in a Rofin fan-cooled housing fitted with a collimating lens was placed 240mm away from the centre of the cuvette and the light directed through a 1cm x 2cm window onto the cuvette (Figures 1). The total light intensity reaching the cuvette was measured to be approximately 1 Watt. Sections of glass cut from wine bottles were used as filters and were placed directly in front of the cuvette holder window as shown in Figure 2 to simulate a sample of wine in a bottle. Ultraviolet and visible light, and selected visible light filters were used in the light irradiation studies. Exposure and analysis times were recorded at 0, 1, 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300, 360 minutes, unless otherwise stated. UV-Visible (200–800nm) and transmission spectra were recorded on a Varian CARY 50 Bio UV-Visible spectrophotometer.

Figure 1. The small scale irradiation arrangement used in this study.

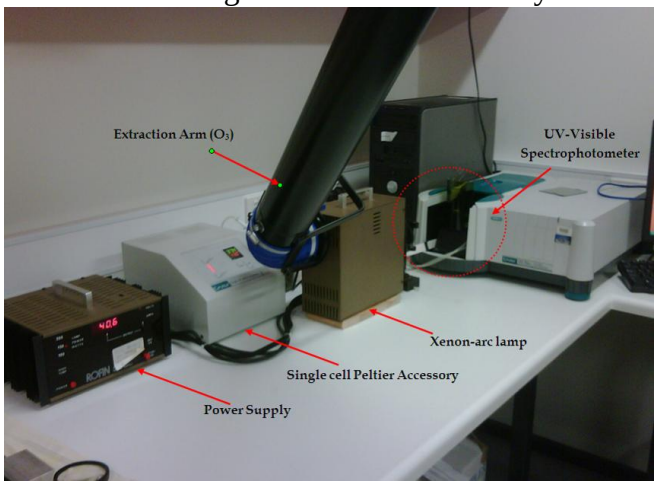
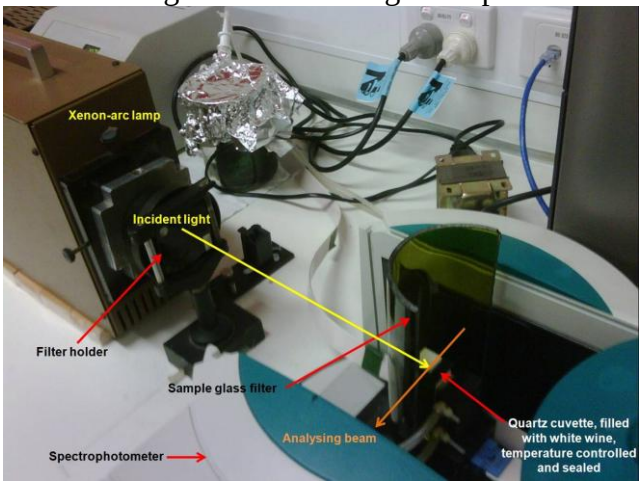


Figure 2. Small scale irradiation arrangement simulating a sample of a white wine in a glass bottle.



A comparison between the solar spectrum (Figure 3) and the output of the Xenon arc-lamp used in the small scale irradiation experiments (Figure 4) shows the similarity of the spectral distribution in the visible and upper UV ranges.

Figure 3 Solar spectrum of sunlight. (visible region)

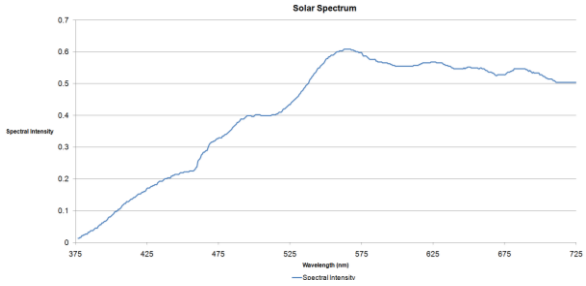
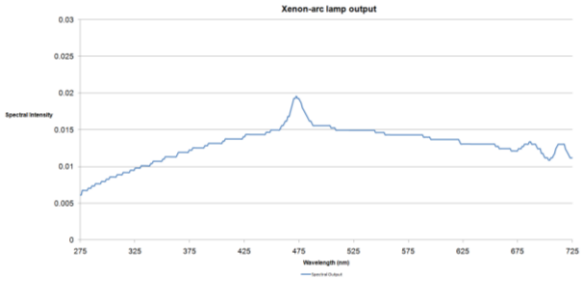


Figure 4 Xenon-arc lamp spectral distribution. (upper UV and visible regions)



Glass bottles

Wine bottles (claret punted, 750mL) were used in this project. The bottles are designated here by their trade names to describe their colour: Flint (clear, FG), Arctic Blue (light blue, AB), French Green (pale green, FRG) and Antique Green (dark brown/green, AG). Both traditional weight and newer lightweight bottles were used. Figure 2 illustrates the experimental set-up in which samples of each bottle type were cut in half lengthwise and used as filters between the xenon-arc light source and the wine sample in the spectrophotometer quartz cell to simulate a sample of wine in a bottle.

Commercial white wine

Various Chardonnay samples [ethanol: 13.0% (v/v)] were purchased commercially in either 750 mL bottles, 3L or 10L casks. The wines were enriched with either (+)-catechin (Sigma, 98%) or (-)-epicatechin (Sigma, 98%) at 100mg/L. This flavan-3-ol concentration was chosen to represent a white wine produced from heavily extracted grapes and would be at the higher end of flavan-3-ol concentration in white wine. This is in line with systems used in other light exposure studies (Clark *et al.*, 2003, Maury *et al.*, 2010).

Model wine base (WB) system

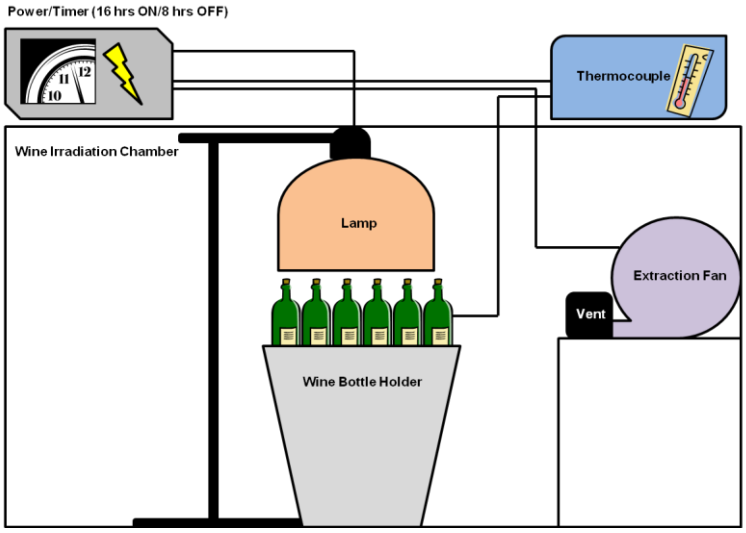
The model wine base was prepared by the addition of 0.011 M potassium hydrogen tartrate (Sigma > 99%) and 0.007 M L(+)-tartaric acid (Sigma > 99.5%) to 12% (v/v) aqueous ethanol and stirring overnight before use. The pH of these solutions was 3.2 ± 0.1 (Clark *et al.*, 2008).

Large scale irradiation setup

Wine bottles of different colours (750 mL) were filled with the Chardonnay wine, enriched with either 100mg/L (+)-catechin or (-)-epicatechin. The headspace of each bottle was flushed for 2 mins with nitrogen (N_2). The bottles were sealed and placed into the wine bottle holder which was able to accommodate up to eight bottles per experiment (Figure 5). Each bottle was placed at a slight angle in a circular manner in the holder to ensure that each bottled received an equal amount of light from the light source.

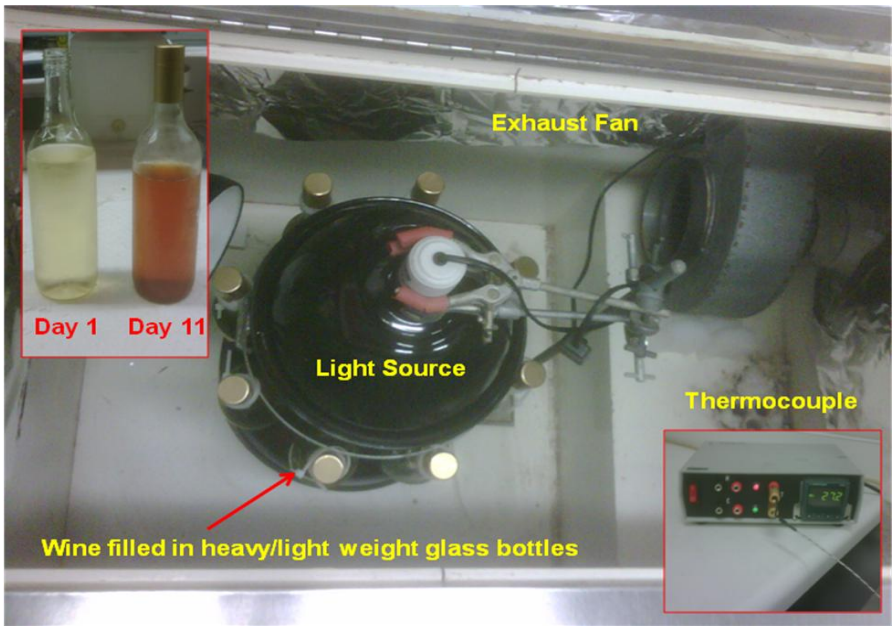
The bottle holder was then placed in a 'light-tight' box. The light source used in the irradiation experiments was a MegaRay® mercury vapour, self ballasted, 160W (high UVA and UVB flood lamp) placed approximately 40 cm above the bottles. The UV output at the working distance was 150 $\mu W/cm^2$, equivalent to full sun. A thermocouple allowed measurement of the ambient temperature inside the chamber and bottle surface temperature during the course of the irradiation.

Figure 5 Schemetic illustration of the large scale wine irradiation arrangement.



An exhaust fan was placed inside the chamber which was vented allowing for a constant temperature of 38 ± 3°C for all bottles. The ambient temperature was 28 ± 2°C. The entire system was controlled by a 24 hr timer. Typically at 17:00 hours, the lamp, extraction fan and thermocouple were turned on for an irradiation time of 16 hrs. At 09:00 hrs, the lamp, extraction fan and thermocouple were turned off automatically. The "off" time of 8 hrs was selected to simulate night time. Figure 6 shows the actual experimental setup.

Figure 6 Large scale wine irradiation arrangement.

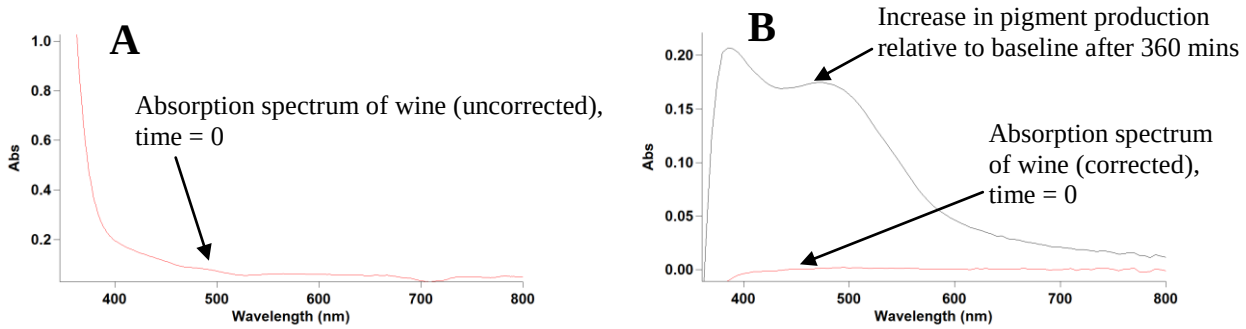


RESULTS AND DISCUSSION

1.0 Differentiating between light and temperature effects on pigment development

The approach adopted here for recording changes in the UV/visible absorption spectrum was to use 'difference spectra'. In essence, this involves recording changes in the UV/Vis region relative to the spectrum of the unexposed wine. Figure 7 demonstrates a typical response. Curve A in Figure 7 illustrates the spectrum of the wine itself, that is for the untreated sample while the two curves in B show the difference spectra for the wine before exposure and after exposure to light at 45^{o}C for 360 mins.

Figure 7. Uncorrected absorption spectrum of the wine (**A**) and the difference absorption spectra after exposure to light and heat (**B**). See text for experimental conditions.



Two absorption bands are apparent for the treated wine sample in curve B (Figure 7). One has its maximum in the low visible region (around 380 nm) while the other maximum is centred around 480 nm. The majority of the results reported here for pigment production are based on changes in the 480 nm absorbance values. This is slightly higher than the 440 nm used in other studies on pigment production (see Maury *et al.*, 2010) and is probably a reflection of using difference, rather than absolute, spectra to monitor absorbance changes.

1.1 Temperature effects

This initial experiment was designed to ascertain the impact of temperature alone on pigment production in a white wine. Samples of Chardonnay with added (-)-epicatechin were degassed with nitrogen and placed in a quartz cuvette and filled to 3mL. The cuvette was placed in the cell holder of the spectrophotometer and held at a constant temperature (25°C, 45°C, 50°C and 60°C) over six hrs in darkness. The only exposure to light occurred during the acquisition of a UV/visible spectrum.

Figure 8 illustrates the changes in the difference spectra over time for the sample maintained at 60^{o}C for 5 hours. The increase in the absorbance at 480 nm is apparent and this absorbance increase was accompanied by detectable sensory effects and clearly visible colour changes. Over the entire exposure period, the colour changed from the original pale yellow to orange/peach and, finally, to orange-brown with sensory changes described as 'sweet' or 'stewed apples', suggesting aldehydic oxidation products.

Figure 8. Chardonnay with added (-)-epicatechin (100 mg/L) heated in darkness at 60°C illustrating increase in pigment production at 480nm.

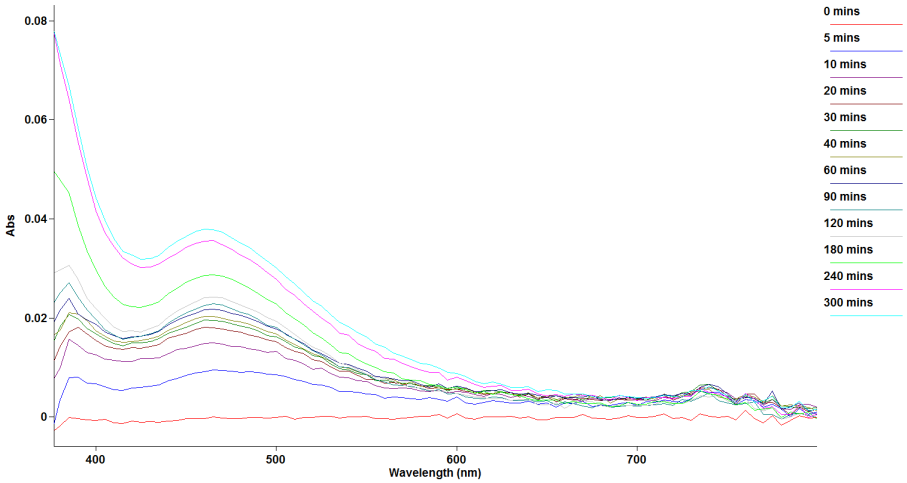
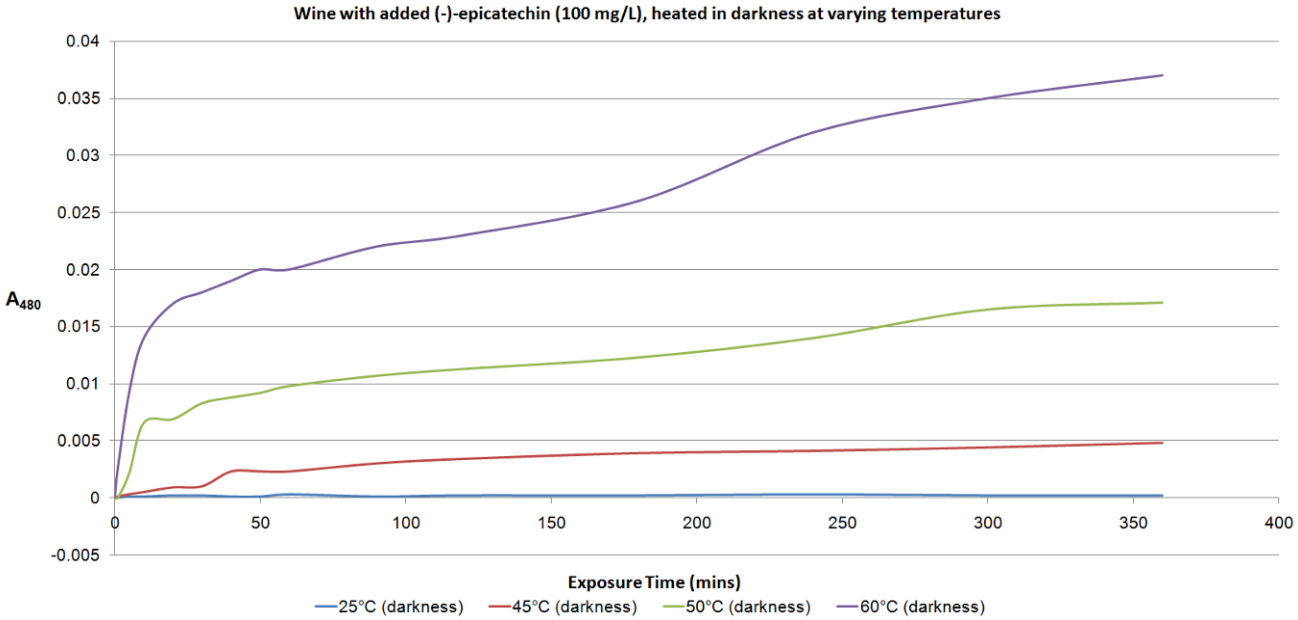


Figure 9 shows the results for the change in absorbance at 480nm over a 6 hr period for each of the temperatures used. It is apparent that after 6 hrs at 25°C, there is no increase in the A_{480} value. A slight increase in the A_{480} value was observed at 45°C with marked increases occurring at 50°C and 60°C. On the basis of these observations, it was decided that 45°C would be used in these experiments as it would reflect typical scenarios where white wine would be subjected to extreme conditions, for example, wine stored in a boot of a car during transportation or a bottle placed directly in the sun on a hot summer's day.

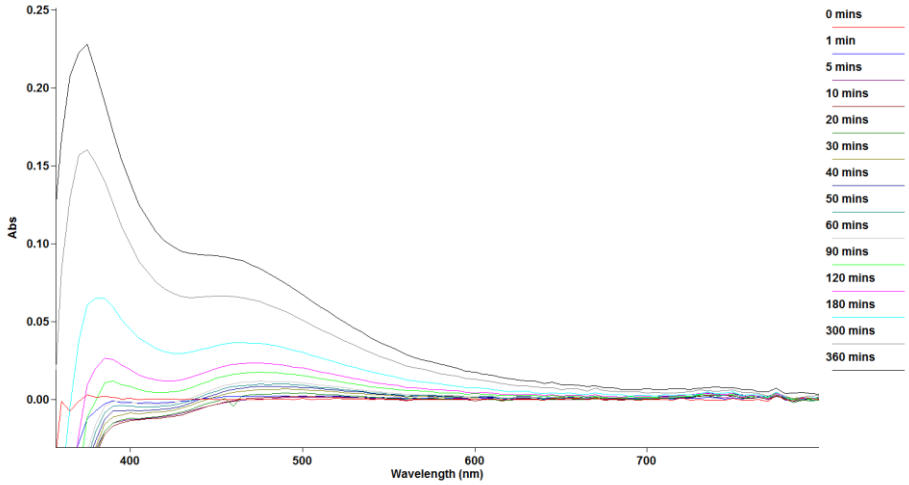
Figure 9. Chardonnay with added (-)-epicatechin (100 mg/L) heated individually in darkness at 25°C, 45°C, 50°C and 60°C.



1.2. Light effect

The same experimental approach was used here as described above for the temperature study except that the sample was maintained at 45°C and exposed to the output of the xenon-arc light source over a 6 hr period. Figure 10 shows the changes in the absorption spectrum when a Chardonnay sample was exposed to radiation from the xenon-arc lamp over a six-hour period.

Figure 10. Chardonnay sample with added (-)-epicatechin (100 mg/Litre) exposed at 45^{o}C to radiation from a xenon-arc lamp over 6 hrs.



The difference spectra presented in Figure 10 illustrates a marked increase in absorbance in the visible region. The A_{480} value after 6 hrs is 0.1 absorbance units compared with 0.05 absorbance units when temperature alone was used as the variable. This result confirms the critical importance of light for inducing changes in the wine's pigment composition. Figure 11 indicates the extent of pigment development.

Figure 11. Photograph of a cuvette filled containing a Chardonnay sample with added (-)-epicatechin at time = 0 (left hand image) and after irradiation for 360 mins (right hand image) showing distinct change in colour.

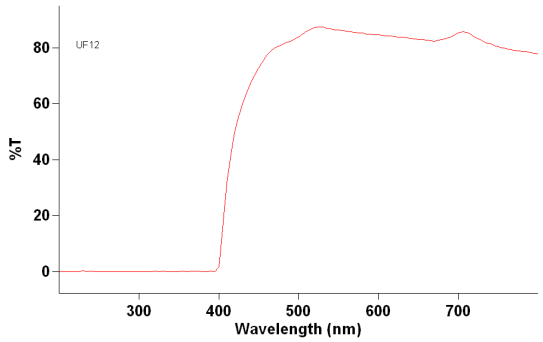


2.0 Determining the critical wavelength region for light activation

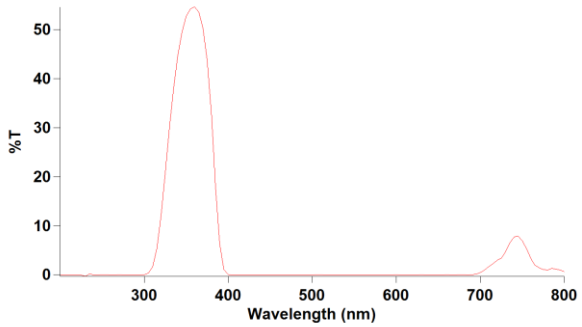
2.1 Description of filters used for wavelength selection

In order to determine the critical wavelengths that contribute to any photochemical process, various cut-off filters that allow specific ranges of light to pass through were placed between the xenon-arc lamp and the wine sample in the cuvette (see Figure 2 for layout). The various filters utilised are shown in Figure 12. The UV filter (5543a) allows a maximum of 50% of the xenon radiation output to reach the sample and this is over a narrow wavelength range. The other filters used transmit about 80% of the xenon lamp's output.

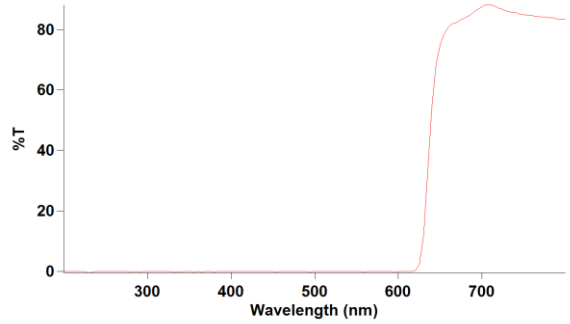
Figure 12. Transmission spectra for various cut off filters used to vary wavelength region reaching the wine sample in the cuvette.



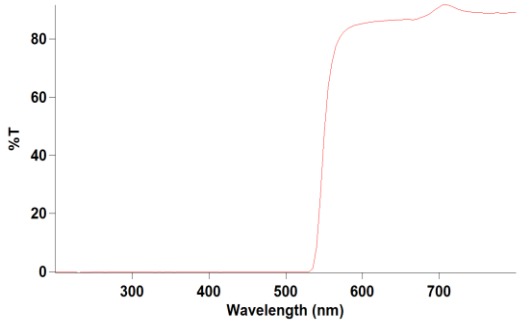
UF12 filter transmits visible light above 400 nm



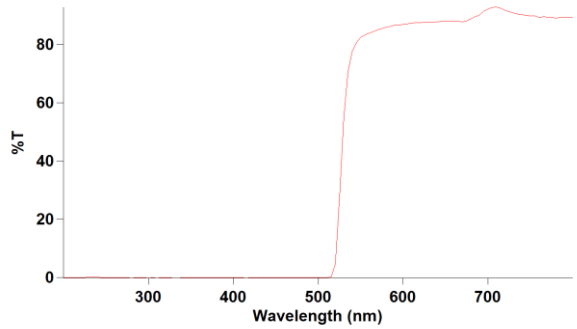
5543a transmits UV light and some infrared



2404 filter transmits red light



Orange filter transmits orange light

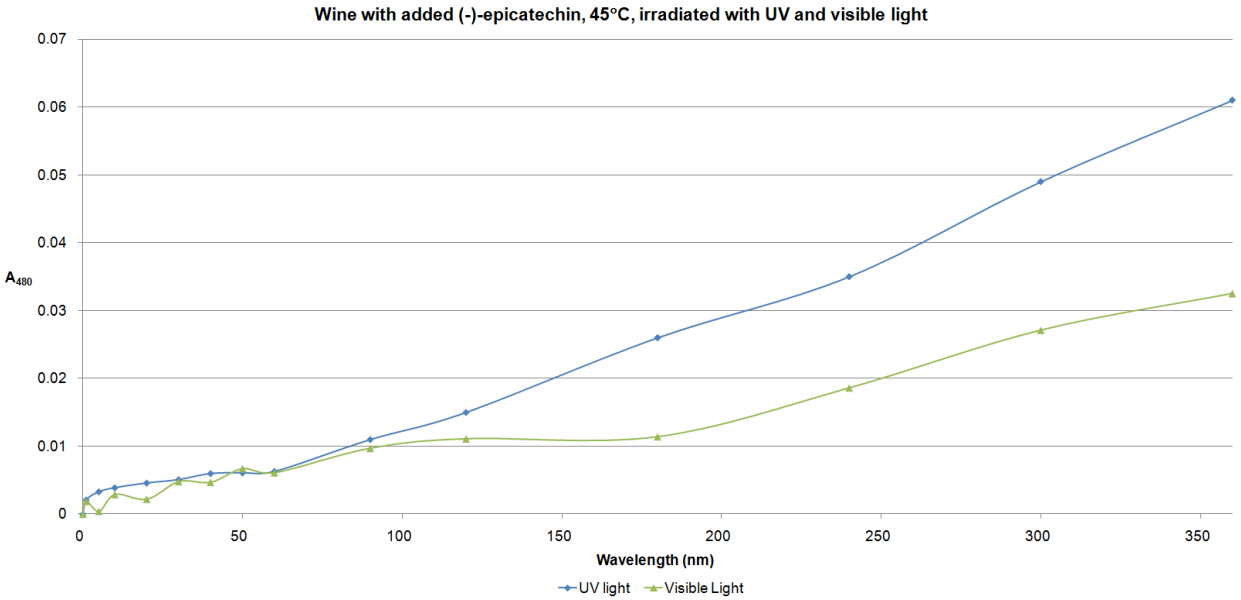


3486 filter transmits yellow light

2.2 Exposure studies at different wavelengths

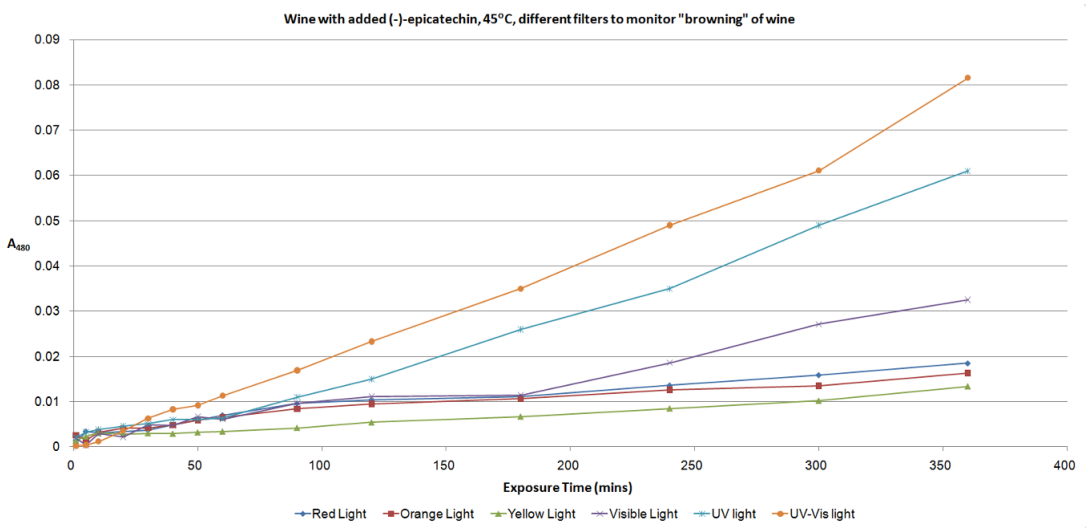
Initially, the impact of a filter that allowed only visible radiation (λ > 400nm, UF 12) was compared with one that allowed low wavelength visible and ultraviolet light (λ < 400nm, 5543a) was examined. The resulting difference absorption spectra indicated that a more significant change in wine composition occurred with low wavelength visible and ultraviolet light; that is, light less than 400 nm (Figure 13).

Figure 13. Comparison of the changes in the absorbance at 480 nm for a Chardonnay sample with added (-)-epicatechin (100 mg/L) at 45°C for light exposed to radiation < 400 nm (blue curve) and radiation > 400 nm (green curve).



This study was then extended using each of the filters shown in Figure 12. With each filter, a fresh sample of the Chardonnay containing 100 mg/L added (-)-epicatechin was exposed for 6 hrs. Figure 14 illustrates for each filter used, the change in absorbance at 480 nm, the visible region wavelength of maximum absorbance in the difference spectra.

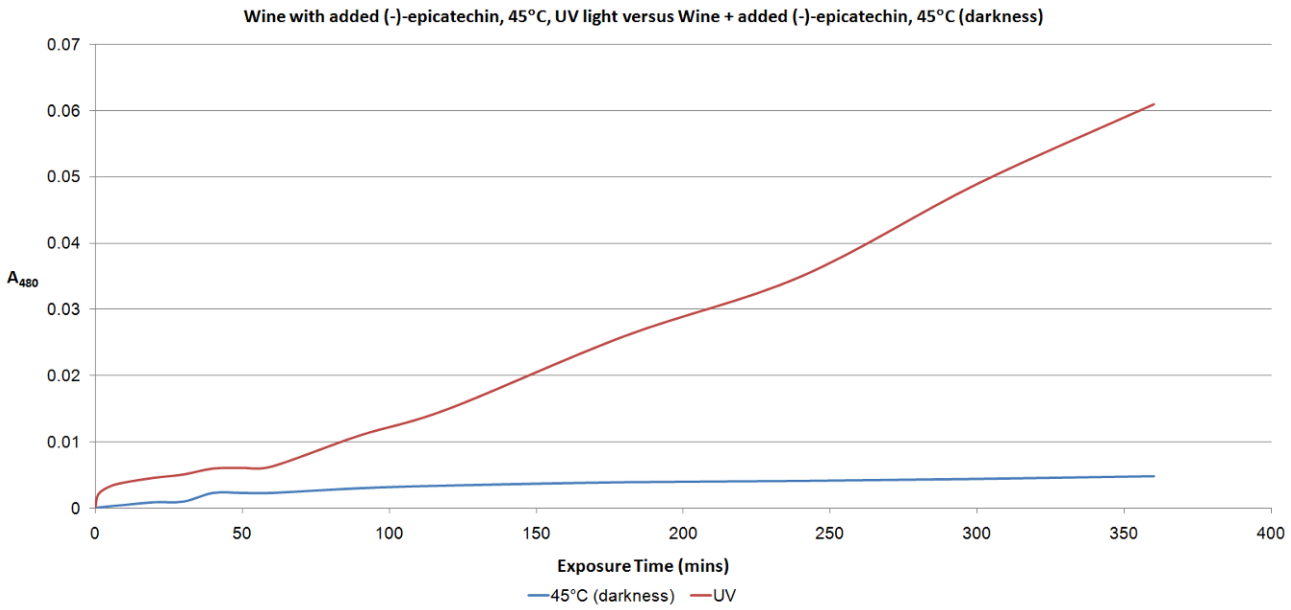
Figure 14. Change in the A_{480} value for a Chardonnay sample with added (-)-epicatechin (100 mg/L) when exposed to xenon radiation at 45°C using different cut-off filters.



It is apparent from the data in Figure 14 that low wavelength visible and near ultraviolet light is required to induce a significant increase in absorbance. Only minor changes in absorbance were observed with the red, orange and yellow filters, not much greater than that induced by temperature alone (see Figure 9). While radiation at wavelengths greater than 400nm did result in an observable increase in absorbance (to 0.03), radiation less than 400 nm produced an increase to 0.06 absorbance units over the time period studied. Moreover, the increase in absorbance was observed to occur after a shorter exposure time when radiation less than 400 nm was used: 90 min compared to 180 min for radiation greater than 400 nm. The combined ultraviolet and visible output of the xenon-arc lamp (UV-Vis light curve in Figure 13) generated the largest increase in the A_{480} value.

To confirm the importance of low wavelength radiation in inducing pigment production, an experiment was performed in which one Chardonnay sample (with added (-)-epicatechin and degassed) was maintained at 45°C in darkness while another was exposed to UV light (λ < 400nm) at 45°C. Figure 15 shows the comparison and confirms the importance of low wavelength radiation, and not temperature, in inducing colour change over the 6 hr exposure period. While these results were obtained at 45°C, the same basic spectral changes were also observed at 25°C, but to a lesser degree.

Figure 15. A Chardonnay sample with added (-)-epicatechin (100 mg/L) heated at 45°C, irradiated with radiation <400 nm versus the same white wine with added (-)-epicatechin (100 mg/L) heated in darkness at 45°C.



These results are of major significance to the wine industry as they demonstrate for the first time that low wavelength radiation is critical for pigment production. They are in agreement with the proposal of Clark *et al.* (2007) that low wavelength radiation may result in the photoactivation of iron(III) tartrate with consequent free radical production. This issue is discussed in more detail in Section 5.2 of this report.

3. 0 Relationship between bottle colour and pigment development

The ability of light to reach wine in a glass bottle is dependent on many factors including bottle colour and composition and glass thickness. The experimental design used in this project allowed sections cut from bottles to be used as 'filters' in exposure experiments. That is, using the system shown in Figure 2, the xenon lamp radiation could pass through a wavelength selection filter (see Figure 12) and a glass bottle filter allowing a study of the effects of both radiation wavelength and bottle type on the development of pigmentation.

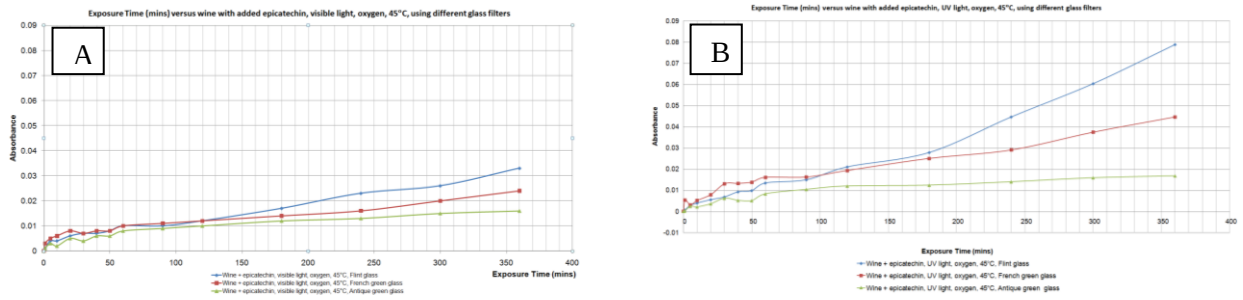
The bottles used in this study were selected to represent those in common use in the Australian wine industry. The traditional 'heavy weight' and the newer 'lightweight' bottles were used. The bottle types and codes used were

- Flint (FG)
- Arctic Blue (AB)
- French Green (FRG)
- Antique Green (AG)

3.1. Initial studies on the impact of UV-light and visible light on white wine using different glass filters

The Chardonnay wine with added (-)-epicatechin (100mg/L) was added to the cuvette and was irradiated using only visible light (>400 nm; UF12 filter) at 45°C and using different glass filters (heavy weight AG, FRG and FG). An analogous exposure experiment was carried out using the same conditions as described but irradiating with UV light alone (<400 nm; 5543a filter). Figure 16 compares the results for the change in absorbance at 480 nm. It is evident that the lighter coloured bottle glass allows the transmission of light resulting in greater pigment development compared to darker glass bottles (A_{480}: FG>FRG>AG). Significantly, radiation less than 400 nm induces greater pigment production compared to radiation above 400 nm. It is also of critical importance that there is an increase in the A_{480} value for the darkest bottle used (AG). That is, even the darkest bottle appears unable to stop photochemically active radiation reaching the wine sample.

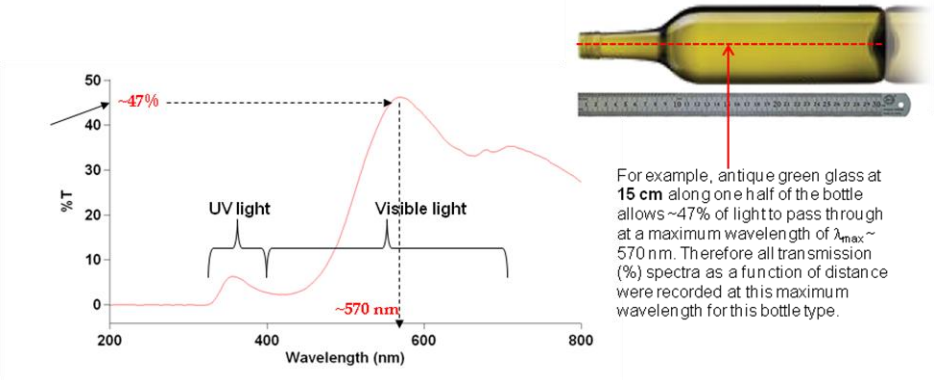
Figure 16. Chardonnay with added (-)-epicatechin exposed at 45^{o}C to radiation using AG, FRG and FG filters. **A**: radiation > 400 nm; **B**: radiation < 400 nm



3.2 Characterisation of bottle transmissivity

The transmission of light for the bottle types used in this work was measured to assess the amount of ultraviolet and/or visible light that could reach the wine sample during storage. Flint (FG), Arctic Blue (AB), French Green (FRG) and Antique Green (AG) bottles were examined. Both the traditional ('heavy') weight and light weighted bottles were cut in half and transmission spectra were recorded in two ways. First, the transmission spectrum was recorded at 15 cm from the opening of the bottle (see Figure 17). Second, transmission values were recorded at 1 cm intervals along the full length of the bottle at 570 nm, the wavelength that corresponds to the maximum transmission value.

Figure 17. Schematic representation of the approach used in recording bottle transmission spectra.



Figures 18 and 19 present the transmission spectra for the heavy- and light-weighted bottles respectively. Figure 18 reproduces Figure 20, but demonstrates the amount of radiation < 400 nm that can be transmitted by all bottle types used in this study.

Figure 18. Transmision (%) spectra for heavy weighted bottles (AG, FRG, AB and FG).

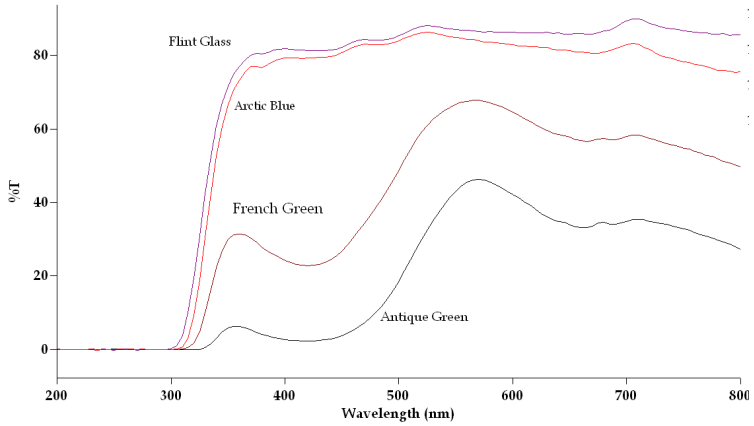


Figure 19. Transmision (%) spectra for light weighted bottles (AG, FRG, AB and FG).

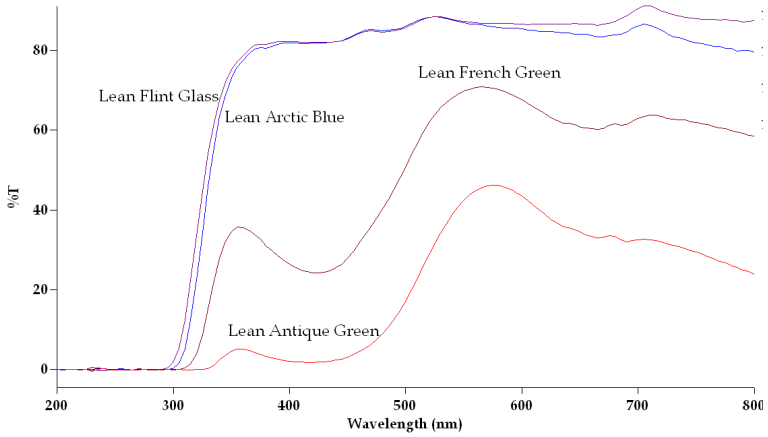
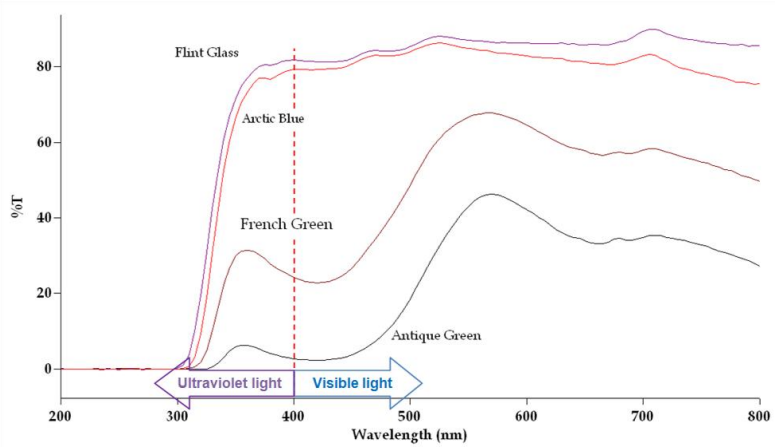


Figure 20. Transmision (%) spectra for heavy weighted bottles (Figure 18), identifying the amount of radiation less than 400 nm that can reach the wine sample.



Flint (FG) and Arctic Blue (AB) glass transmit all visible wavelength radiation and some ultraviolet light. French Green (FRG) and Antique Green (AG) glass transmit most visible wavelengths, but at a lower intensity than either Flint or Arctic Blue (AB) glass. With French Green (FRG) and Antique Green (AG), the percentage transmission in the visible range is noticeably variable, with the greatest transmission at λ~570nm. All glass types allow the transmission of near ultraviolet radiation to pass though the glass in the 300 to 400 nm wavelength range (Figure 20). This observation is significant with respect to the observation pigmentation increase presented in Figure 14.

There is little impact of bottle weight on the transmission spectra. Figure 21, extracted from Figures 18 and 19, compares the transmission spectra for heavy and light weighted Antique Green glass. The implication is that the bottle thickness and hence 'absorbing power' is similar at the position of measurement. Transmission spectra for the other bottle types used are presented in Figures 22-24. In essence, the transmission spectra are essentially independent of bottle thickness for each glass type.

Figure 21. Comparison of % transmission versus wavelength for heavy ('Antique Green Glass') and light ('Lean Antique Green Glass') weighted bottles at 15 cm from bottle opening.

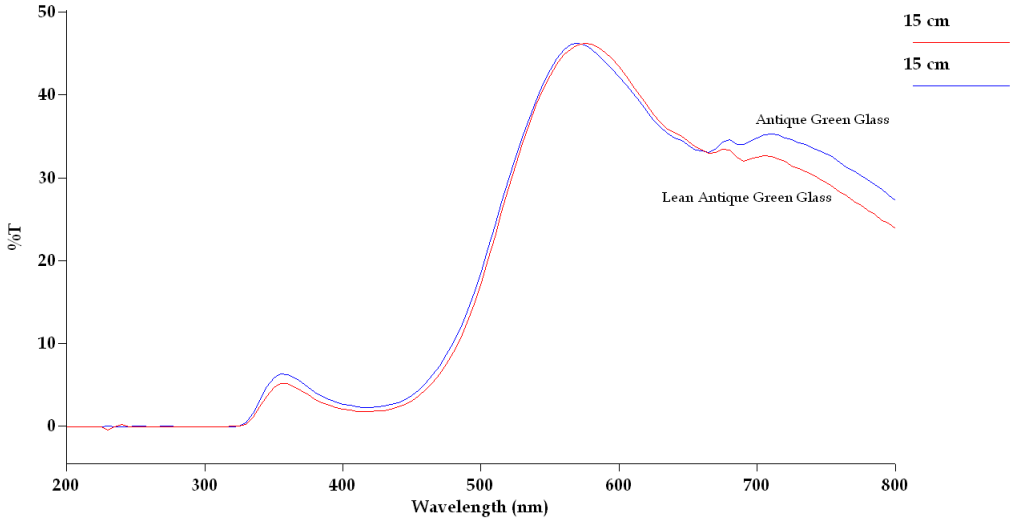


Figure 22. Comparison of % transmission versus wavelength for heavy (Flint Glass) and light (Lean Flint Glass) weighted bottles at 15 cm from bottle opening.

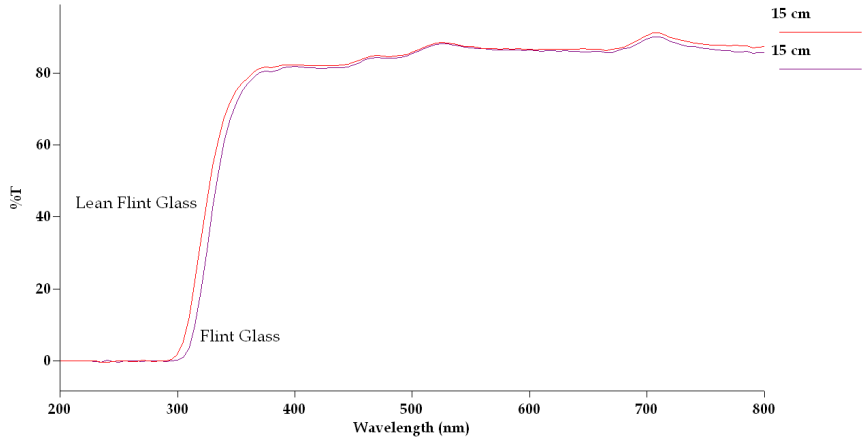


Figure 23. Comparison of % transmission versus wavelength for heavy (Arctic Blue Glass) and light (Lean Arctic Blue Glass) weighted bottles at 15 cm from bottle opening.

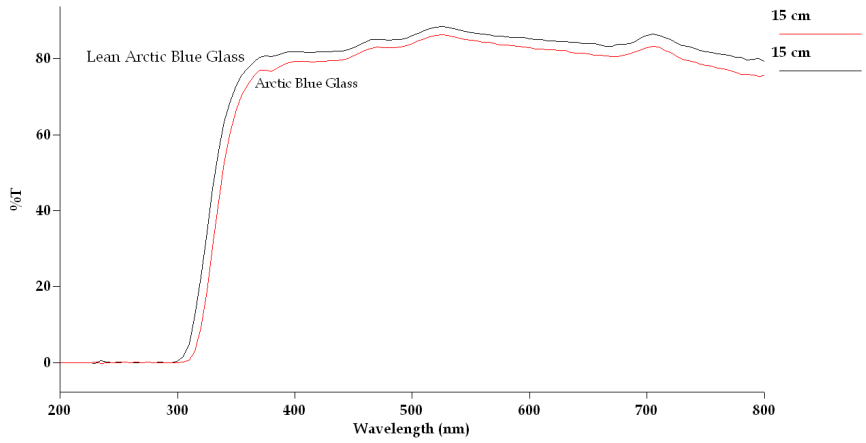
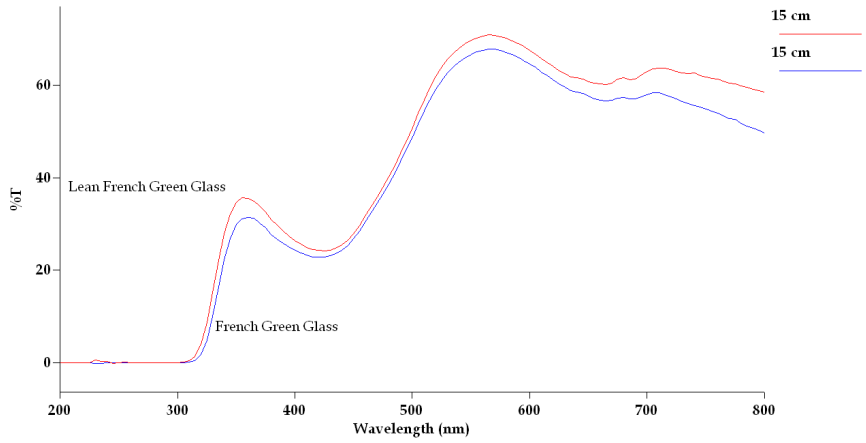


Figure 24. Comparison of % transmission versus wavelength for heavy (French Green Glass) and light (Lean French Green Glass) weighted bottles at 15 cm from bottle opening.



The second approach used to characterise the potential of the different bottles to transmit radiation was to record the transmission spectra over the entire length of the bottle in 1 cm increments using a Varian Cary 50 Bio UV-Vis Spectrophotometer. The percentage transmission was recorded at the wavelength of maximum transmission for each bottle type (eg: 570 nm for Antique Green, see Figures 21). Figures 25 and 26 show the results for heavy and light weighted Antique Green bottles respectively, while Figure 27 compares the spectra for the bottles types.

Figure 25. Percent transmission as a function of bottle length (heavy Antique Green)

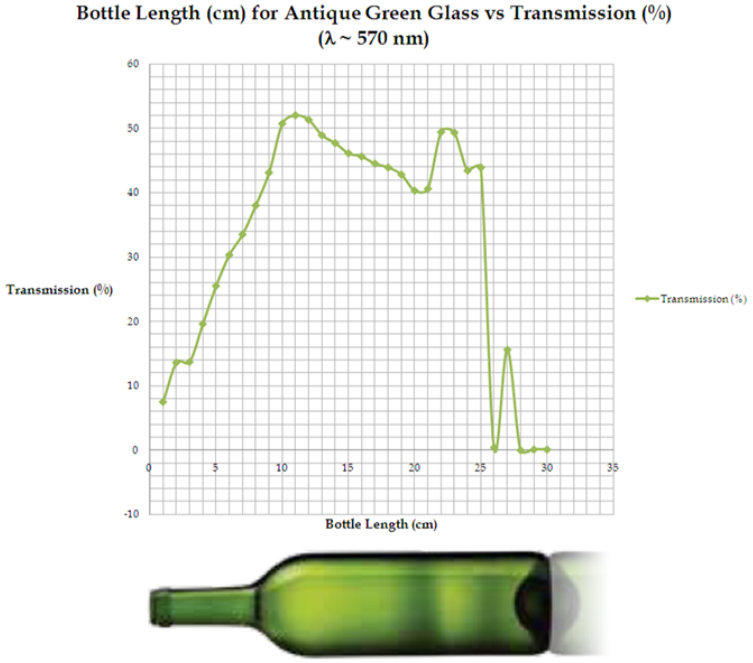


Figure 26. Percent transmission as a function of bottle length (lightweighted Antique Green)

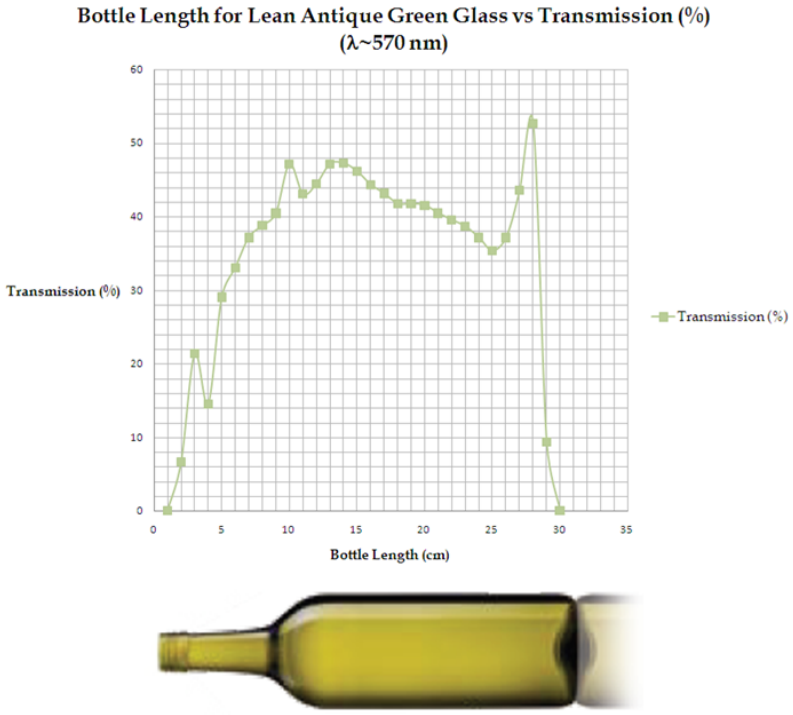
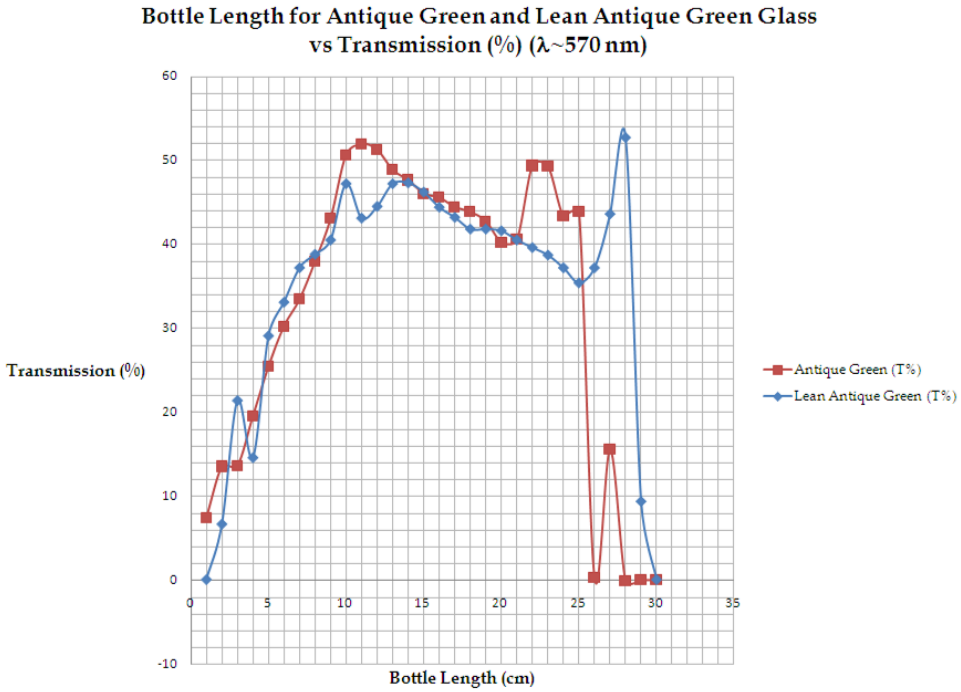


Figure 27. Comparison of percent transmission values as a function of bottle length for heavy and light (lean) weighted Antique Green bottles.



From the plots in Figures 25-27, the following points can be drawn:

- there is negligible transmission in the neck region of the bottle;
- both heavy and light weighted bottles show similar % transmission along the main body of the bottle;
- the drop to zero transmission at 26 cm from the bottle opening in the heavy weighted bottle (Figure 25) is a consequence of the 'settle wave', a thicker layer of glass that results from the production practice: this settle wave is absent in the light weighted bottle (Figure 26)
- the smaller punt in the light weighted bottle (Figure 26) allows transmission along a longer section of the bottle.

Similar comparisons between the heavy and light weighted bottles were observed for the other bottle types examined in this study. Figures 28-30 present the comparative plots with images of the bottle type inserted under the % transmission plot. The impact of the settle wave (heavy style) and shorter punt (lighter style) is apparent for each pair of bottles.

Figure 28. Comparison of percent transmission values as a function of bottle length for heavy and light (lean) weighted Flint bottles.

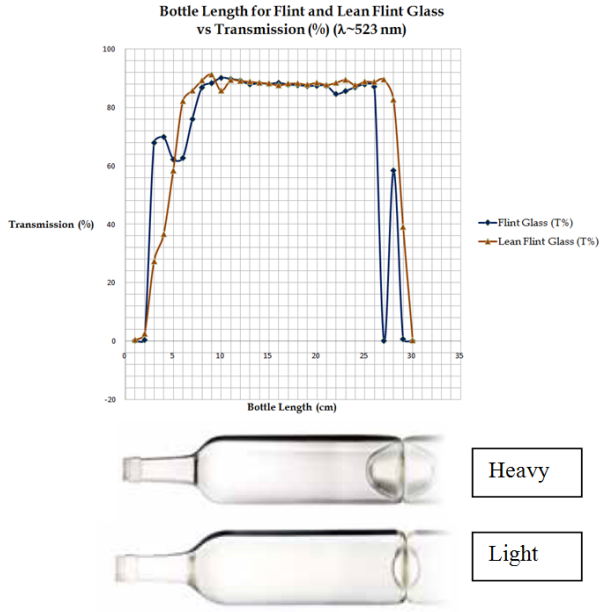


Figure 29. Comparison of percent transmission values as a function of bottle length for heavy and light (lean) weighted Arctic Blue bottles.

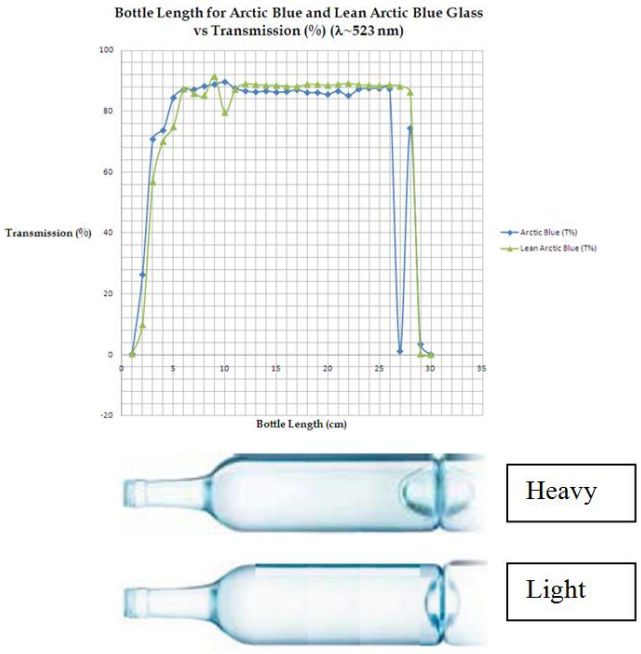
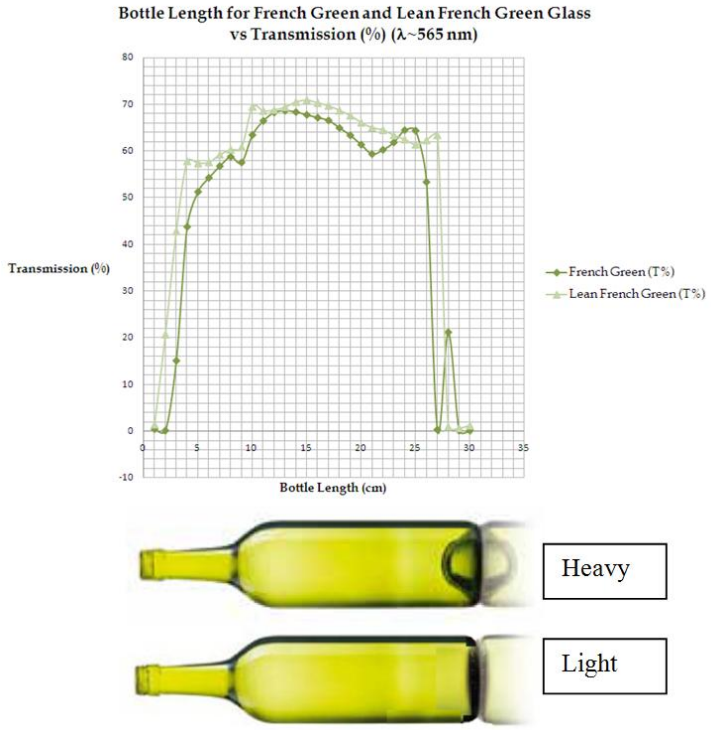


Figure 30. Comparison of percent transmission values as a function of bottle length for heavy and light (lean) weighted French Green bottles.



3.3 Impact of bottle type on the development of colour in wines exposed to radiation.

The impact of radiation less than 400 nm from the xenon lamp on the Chardonnay sample used in this experiment is presented in Figure 31. It is apparent from this difference spectrum that there are two major increases in absorbance over the 6 hr exposure period: one less than 400 nm and the other at 480 nm. With pigmentation as the focus of this experiment, the impact of the eight different bottle types described above (4 colours, both heavy and light weighted) on the absorbance at 480 nm. Figure 32 (A to D) illustrates these results.

Figure 31. Difference absorbance spectra illustrating the increase in pigment production (λ = 480 nm) over the 6 hr exposure period for a Chardonnay wine with added (-)-epicatechin (100 mg/L) at 45°C and irradiated with xenon lamp radiation less than 400 nm.

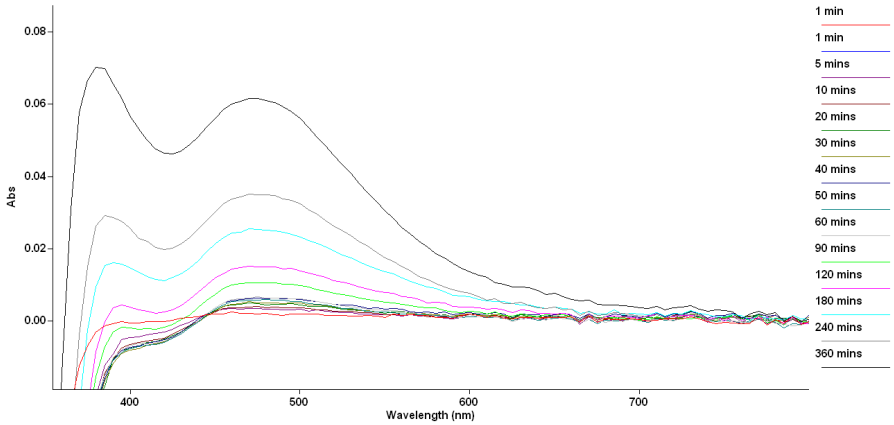
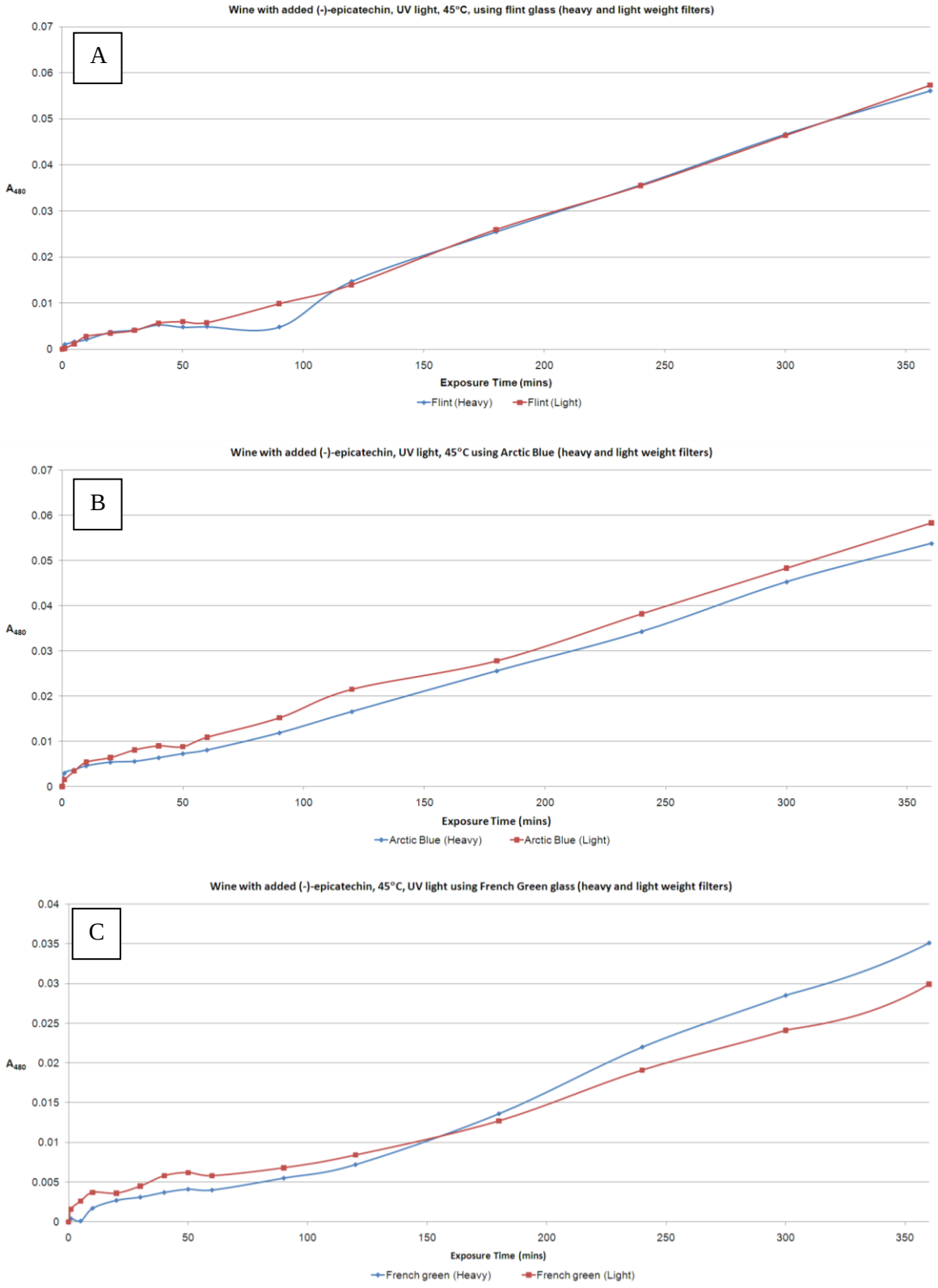
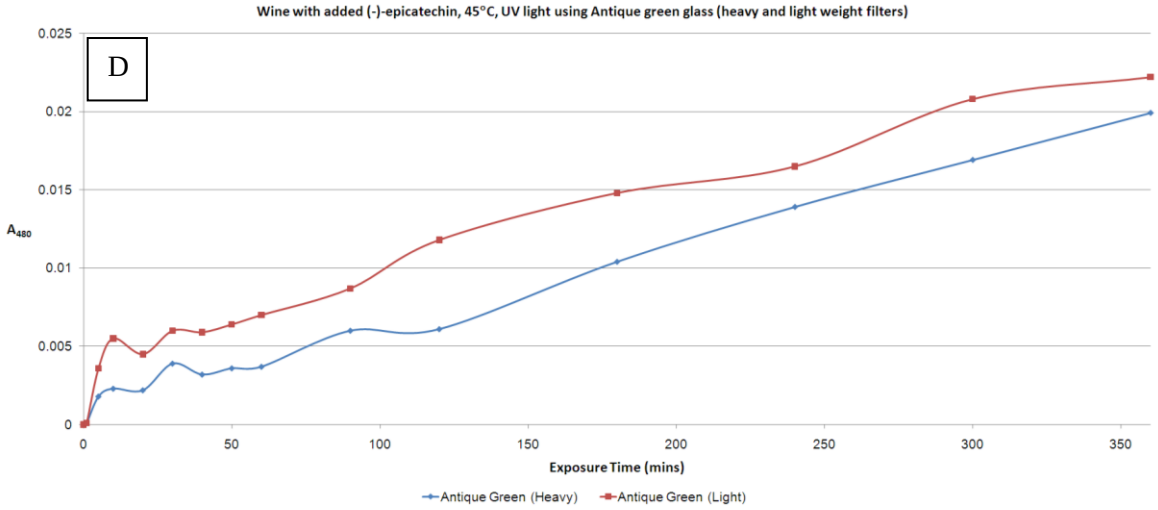


Figure 32. Changes in the absorbance at 480 nm as a function of bottle type. **A**: Flint. **B**: Arctic Blue; **C**: French Green; **D**: Antique Green



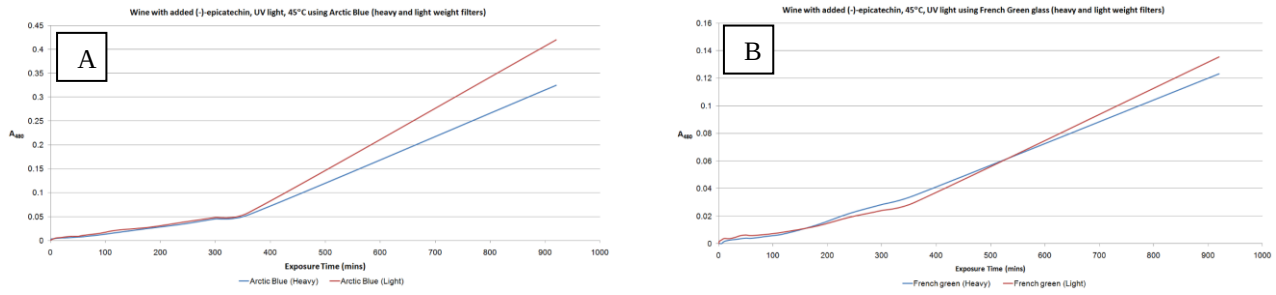


From these results in Figure 32, the following conclusions can be drawn:

- there is little impact of Flint glass on the development of pigmentation: after 6 hours, the absorbance reaches just under 0.06 units (Figure 32A) compared with 0.06 units in the absence of a glass filter (Figure 16);
- there is no difference in the increase of the A_{480} value between the heavy and light weighted bottles (Figure 32 A);

Exposure times used for the Arctic Blue and French Green studies: 15 hrs rather than 6 hrs as in Figure 32. The results (Figure 33) show a marked difference after 15 hours between the heavy and light weight Arctic Blue bottles, even though no difference was observed after 6 hrs exposure. The difference between the two types of French Green bottles is less apparent in comparison with the Arctic Blue bottles. Although these results are preliminary, they identify a further example of bottle colour and bottle weight influence on the development of pigmentation caused by exposure to light.

Figure 33. Changes in the absorbance at 480 nm as a function of bottle type. **A**: Arctic Blue; **B**: French Green.



4. Identification of pigments formed from wine in different glass bottles when exposed to radiation under controlled temperature conditions

Xanthylium pigments, formed from glyoxylic acid and catechin-type phenolic compounds, are now well-recognised as pigments formed under oxidative conditions (Es-Safi *et al.*, 2000; Clark *et al.*, 2002; Maury *et al.*, 2010). To determine whether xanthylium and/or other pigments are formed in the light exposure experiments described above in Section 3, large-scale exposure experiments using the set-up shown in Figure 6 were performed. The intention was to expose wine in bottles to generate sufficient volume of pigmented material for subsequent analysis.

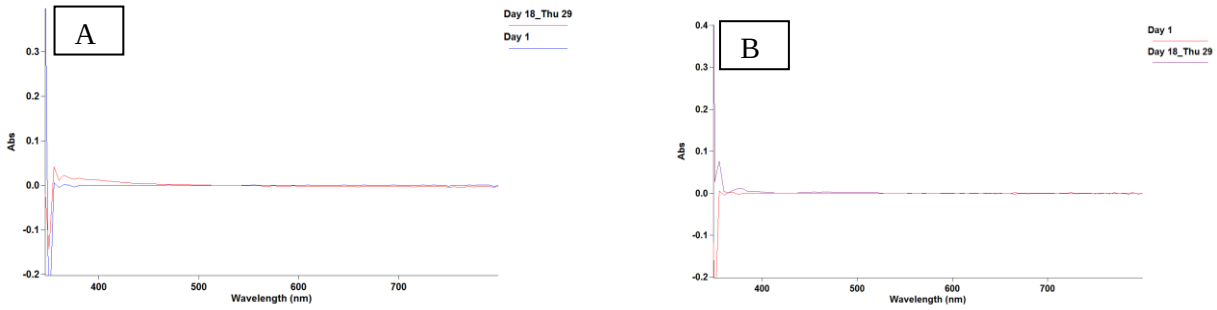
4.1 Bottle exposure experiments under controlled temperature conditions

All bottle types (AG, FRG, AB and FG), both heavy and light weighted bottles, were filled with Chardonnay to which (+)-catechin (100mg/L) had been added, headspace flushed with N_2 and sealed. All bottles were irradiated for 18 days (16 hrs light, 8 hrs darkness) at an ambient temperature of 30 ± 2°C. Bottle temperatures, however, were recorded to reach 38 ± 3°C through absorption of heat during the exposure to light. Eight bottles could be irradiated in any one experiment (see Figures 5 and 6). Bottles were aerated only during sampling for absorbance measurements. Difference absorption spectra were recorded periodically from Day 1 to Day 18.

Two controls were also established for each bottle type. One control was prepared with the Chardonnay wine without added (+)-catechin and was stored at room temperature (about 25°C) and in the dark for 18 days. The second control consisted of the Chardonnay with added (+)-catechin, also stored in the dark for 18 days at room temperature.

Figure 34 shows the results for the samples in Flint bottles that were stored in the dark. In essence, there was negligible pigmentation development for the two control samples, noting however, that these samples were stored at an ambient temperature of 25°C, rather than the light exposure study temperature of 30°C (bottles reaching 38 ± 3°C).

Figure 34. Difference absorbance spectra for wine in heavy weight Flint bottles and stored in the dark at 25°C for 18 days. **A:** neat wine; **B:** wine + 100 mg/L (+)-catechin.



The results of the 18-day exposure study for the Chardonnay with 100 mg/L (+)-catechin added in the four different bottle types (each type of two different weights) are presented in Figures 35 – 38. It is apparent from these difference spectra that there are some commonalities between bottle types and also some significant differences. Exposure of the wine resulted in the growth of two absorption peaks: one centred around 380 nm tails into the visible region and the other is around 450 nm (compared to 480 nm in small scale irradiation experiments). The relative absorbance of these two peaks is dependent on bottle colour. Visually, the colour of the wine after exposure decreased in the order: Flint > Arctic Blue > French Green > Antique Green.

Figure 35. Difference absorption spectra for the Chardonnay with 100 mg/L added (+)-catechin in Antique Green bottles over 18 days of exposure to light. **A:** heavy weighted bottle; **B:** light weighted bottle.

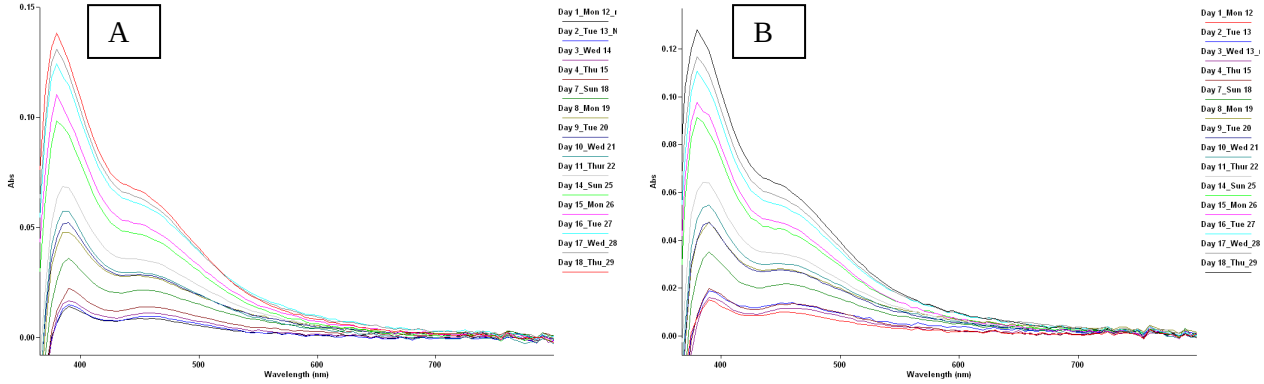
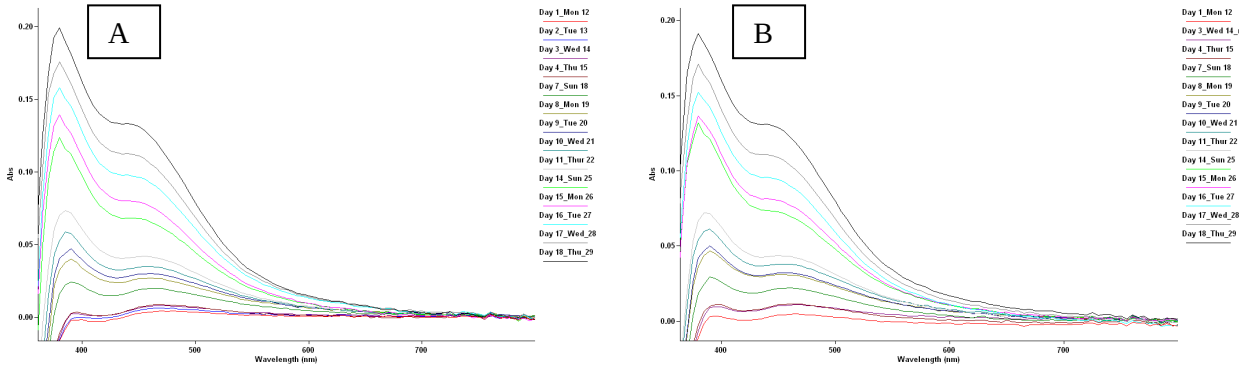


Figure 36. Difference absorption spectra for the Chardonnay sample with 100 mg/L added (+)-catechin in French Green bottles over 18 days of exposure to light. **A:** heavy weighted bottle; **B:** light weighted bottle.

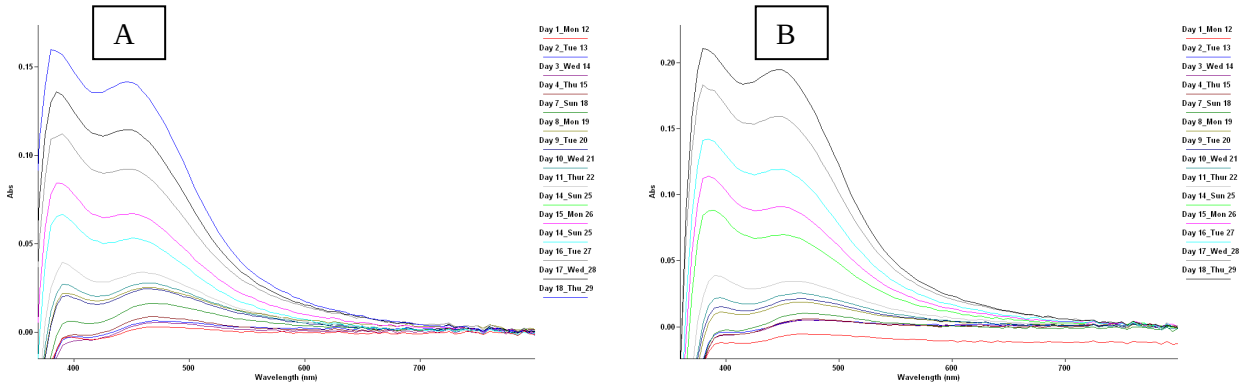


The wine stored in the Antique Green bottle showed a marked increase in the 380 nm absorbance, reaching 0.13 absorbance units after 18 days exposure. The absorbance at 450 nm only achieved a value of 0.063 absorbance units by the end of the experiment. The relative height of the two peaks was followed throughout the time course of the experiment (Figure 35). There is essentially no difference between the absorbance profiles for the heavy (Figure 35A) and light (Figure 35B) weighted bottles.

Two peaks were also observed in the difference spectra for the wine stored in French Green bottles (Figure 36) and again, there is essentially no difference between the bottles of different weight. There are, however, some major differences when compared with that observed for the wine in Antique Green bottles:

- the absorbance values are higher (A_{380} = 0.20 and A_{450} = 0.13) reflecting the higher observed colour of the exposed wine;
- the ratio of the absorbance values for the two peaks (A_{380}/A_{450}) is less with the French Green bottle than with Antique Green (compare Figures 35 and 36);
- there is a slight lag period in the growth of the peak at 380 nm, but after 4 days exposure, the increase in absorbance is faster than for the 450 nm peak.

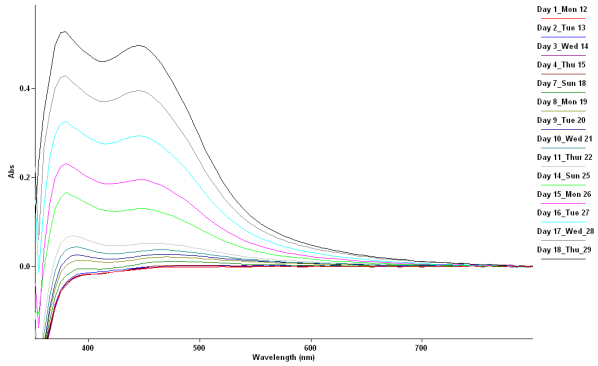
Figure 37. Difference absorption spectra for the Chardonnay sample with 100 mg/L added (+)-catechin in Arctic Blue bottles over 18 days of exposure to light. **A:** heavy weighted bottle; **B:** light weighted bottle.



The impact of bottle colour on the difference absorption spectra for the light exposed samples is obvious when the results for the Arctic Blue bottles are examined (Figure 37):

- the absorbance values for the two peaks are much closer in intensity than observed with Antique Green and French Green;
- the A_{450} values are higher than with Antique Green and French Green, reflecting the observed darker colour of the exposed wine after 18 days;
- noticeably higher absorbance values were found with the light weighted bottle compared with the heavy weighted bottle at 380 nm (heavy: 0.16; light: 0.21) and 450 nm (heavy: 0.14; light: 0.19).

Figure 38. Difference absorption spectra for the Chardonnay sample with 100 mg/L added (+)-catechin in heavy weighted Flint bottle over 18 days of exposure to light.



Only data for the heavy weighted Flint bottle is available and Figure 38 presents the spectral changes over the 18 day exposure period. The two peaks at 380 nm and 450 nm are similar in intensity and considerably higher in value in comparison with all other bottle types used as filters in this experiment.

In summary, the data in Figures 30 – 33 represent a unique set of results that has significant implications for the careful storage of wine. Clearly, and as perhaps expected, there was less colour development in the darker bottles than in the Arctic Blue and Flint. Significantly, however, pigmentation was observed to develop in all bottle types, implying that bottle colour does not provide total protection from light-induced changes, as may have been assumed previously.

The influence of bottle colour on the absorbance profiles in Figures 35–38 suggest that a series of complex and possibly competing reactions are occurring. Time did not allow a detailed investigation of the reactions that lead to initiation and continuation of pigment development. This investigation forms the basis of a longer-term study on the chemistry of the reaction sequences that are occurring.

Some preliminary work on sensory changes and on the identification of the pigments formed as a consequence of light exposure is described in the following sections. Again, this is an area of research that requires a more detailed investigation.

4.2 Sensory properties of wines irradiated with light at constant temperature

4.2.1 Aroma profiles[1]

The Chardonnay samples to which 100 mg/L (+)-catechin had been added and which had been exposed to light for 18 days were subjected to an aroma sensory analysis. Table 1 lists the descriptors per bottle type. The most common descriptor identified was acetaldehyde indicating that oxidative processes have been significant.

It must be stressed that this is only a preliminary study and a more rigorous trial design is required to allow quantification of the levels of oxidation aromas to determine if there is a relationship with bottle colour. This is an important area for future work.

Table 1. Aroma descriptors for Chardonnay samples after 18 days irradiation with light at controlled temperature conditions.

Sample Code	**Descriptors**
Antique Green – heavy	Acetaldehyde, caramel, malt, honey, almond/nutty
Antique Green – light	Apple cider, honey dew melon, fig, touch of ammonium, mousey taint
French Green – heavy	Acetaldehyde
French Green – light	Acetaldehyde, nutty/almond, acetic acid
Arctic Blue- heavy	Acetaldehyde, quince
Arctic blue – light	Acetaldehyde
Flint – heavy	Acetaldehyde

4.2.2 Colour profiles

On page 28, we noted that there was a bottle influence on colour development resulting from exposure to light. To obtain a better assessment (quantitative and descriptive) of the colour variation for the wine stored in different bottles, CIELab measurements were conducted on a Shimadzu UV-1700 UV-Visible spectrophotometer with UVPC Colour Analysis software (version 3.00; Shimadzu Scientific Instruments, Kyoto, Japan). The L^*, a^* and b^* CIELab values were calculated at the daylight illuminant D65 and with a 10-degree observer angle. The wine sample transmission was scanned over the range 380 to 780 nm with samples in 10 mm quartz cuvettes. A wine-like model solution was used as the blank. The L^* parameter gives an indication of the intensity of colour from essentially none (100) to maximum (0), a^* gives an indication of green ($-a^*$) to red ($+a^*$) colouration and b^* gives an indication of yellow ($+b^*$) to blue (-b*). Table 2 presents the data.

[1]We thank John Blackman of the NWGIC in Wagga Wagga for assistance with this aroma sensory study.

Table 2. CIELab values for the Chardonnay samples after 18 days irradiation with light at controlled temperature conditions,

Sample ID	***L****	***a****	***b****
Antique Green – heavy	97.05	-1.03	12.77
Antique Green – light	97.2	-1.07	12.11
French Green – heavy	95.23	-0.11	18.16
French Green – light	95.31	-0.11	17.80
Arctic Blue- heavy	94.48	0.47	19.65
Arctic blue – light	92.65	1.67	24.18
Flint – heavy	85.83	6.53	45.31

The data in Table 2 are in accordance with the general visual assessment of the wines. In summary, it can be concluded that:

- the greatest colour intensity was observed for Flint (heavy): L^*=85.83;
- the least colour intensity was observed for Antique Green (heavy): L^*= 97.05;
- there is a significant shift to the red colouration in the order: Antique Green < French Green < Arctic Blue < Flint;
- more red colouration occurs in the light weight Arctic Blue bottle than in the corresponding heavy weight bottle;
- the increase in yellow colouration follows the same bottle trend as the red colouration.

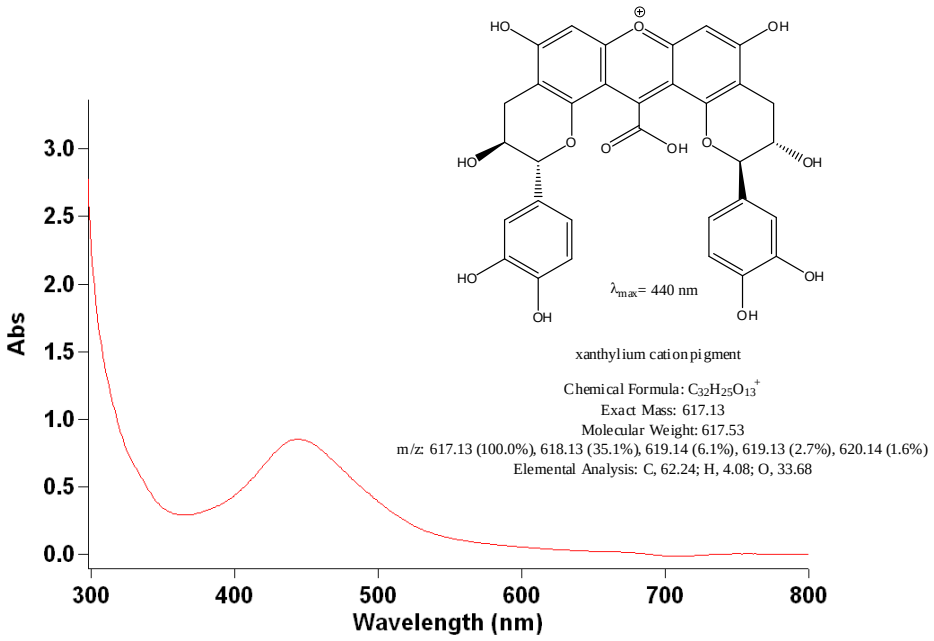
While it must be noted that this is an unreplicated study, the data in Table 2 underscores the importance of bottle colour influencing the development of colour as a consequence of exposure to light. Further work would allow more specific targeting of the important drivers of this colour development process including the effect of using lower temperatures, more specific radiation sources and intensity levels of these sources.

4.3 Identification of pigments formed as a consequence of exposure to light

Xanthylium pigments are well-established as one of the major contributors to white wine colour development, at least in model studies (Es-Safi, *et al.,* 2000; Clark *et al.,* 2002). Although not yet widely detected in white wines, one study by Maury *et al.* (2010) on the relationship between bottle colour and light exposure reported the presence of xanthylium cation pigments in a Sauvignon Blanc wine to which (+)-catechin had been added.

Xanthylium pigments are formed in a series of reactions that are initiated by the bridging of two catechin-type phenolic compounds by glyoxylic acid, the latter resulting from the light induced cleavage of tartaric acid (Clark *et al.,* 2003). Figure 39 shows the UV/Visible spectrum for a xanthylium pigment prepared by reacting (-)-epicatechin with glyoxylic acid and demonstrates the characteristic absorbance profile with a maximum at 440 nm. Clark *et al.* (2003) have developed this reaction to prepare a ‘standard marker’ for the detection of xanthylium pigments in diverse wine-like systems. The cationic character of the xanthylium pigment allows for ready detection by mass spectrometry (see below).

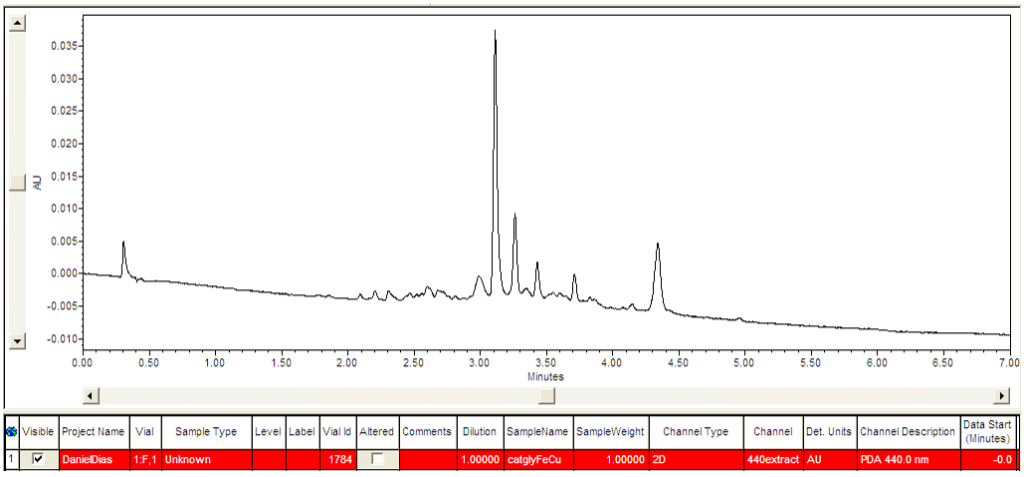
Figure 39. UV/Visible spectrum for an (+)-catechin-derived xanthylium pigment together with the structure and elemental analysis data.



4.3.1 Detection of xanthylium type pigments by UPLC

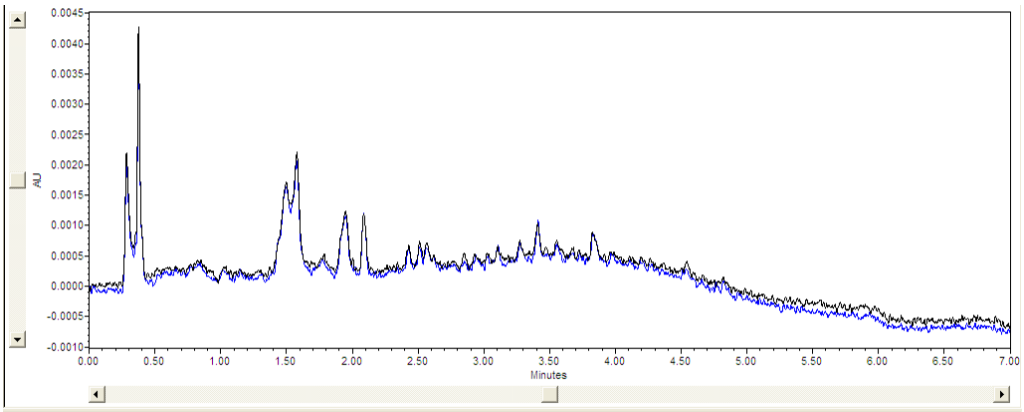
Figure 40 represents a chromatogram (UPLC) for a (+)-catechin-derived xanthylium pigment, prepared by the standard marker procedure referred to above. Three peaks in the chromatogram correspond to different isomeric forms of the xanthylium pigments (Clark *et al.*, 2007). The retention times for these peaks are at 3.11, 3.26 and 3.43 mins. Further, the peak at 4.34 mins corresponds to the ethyl ester of the carboxylic acid form of the xanthylium pigment shown in Figure 40. These peaks and retention times can be used as indicators of the presence of xanthylium pigments in unknown systems.

Figure 40. UPLC chromatogram for a standard xanthylium pigment formed from (+)-catechin and glyoxylic acid.



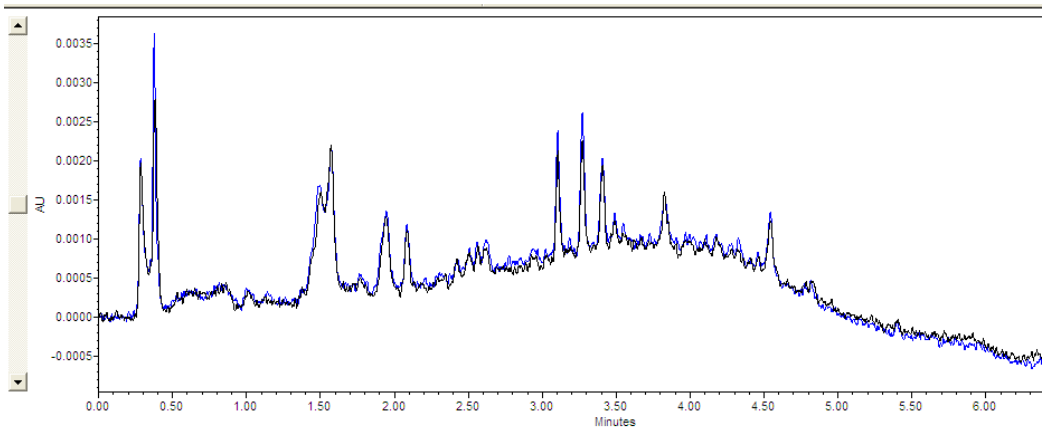
Figures 41 to 44 present chromatograms for the samples of Chardonnay to which 100 mg/L (+)-catechin had been added in the eight different bottles after 18 days exposure to light (16 hrs/day light on; 8 hrs/day light off).

Figure 41. UPLC chromatogram comparing the Chardonnay wine after 18 days exposure to light in heavyweight Antique Green (blue) and lightweight Antique Green (black) under controlled temperature conditions.



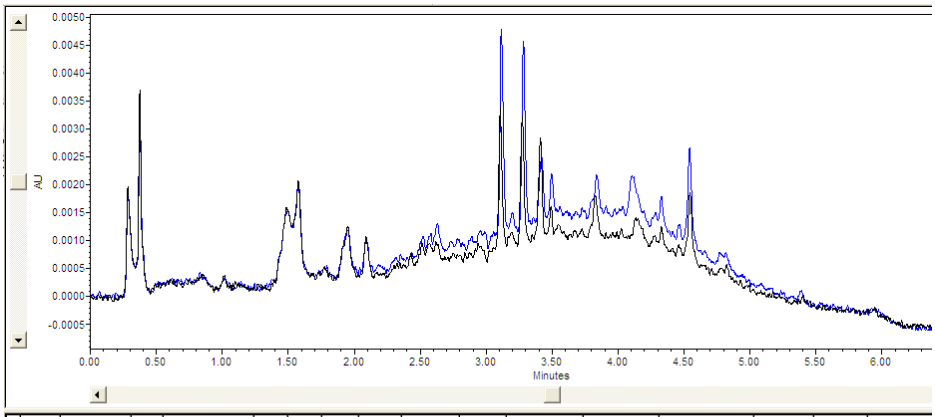
	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel Description	Data Start (Minutes)
1	☑	DanielDias	2:F,2	Unknown			1649	☐		1.00000	S1LAGDAY18	1.00000	2D	440extract	AU	PDA 440.0 nm	-0.0
2	☑	DanielDias	2:F,1	Unknown			1646	☐		1.00000	S1AGDAY18	1.00000	2D	440extract	AU	PDA 440.0 nm	-0.0

Figure 42. UPLC chromatogram comparing the Chardonnay wine after 18 days exposure to light in heavyweight French Green (blue) and lightweight French Green (black) under controlled temperature conditions.



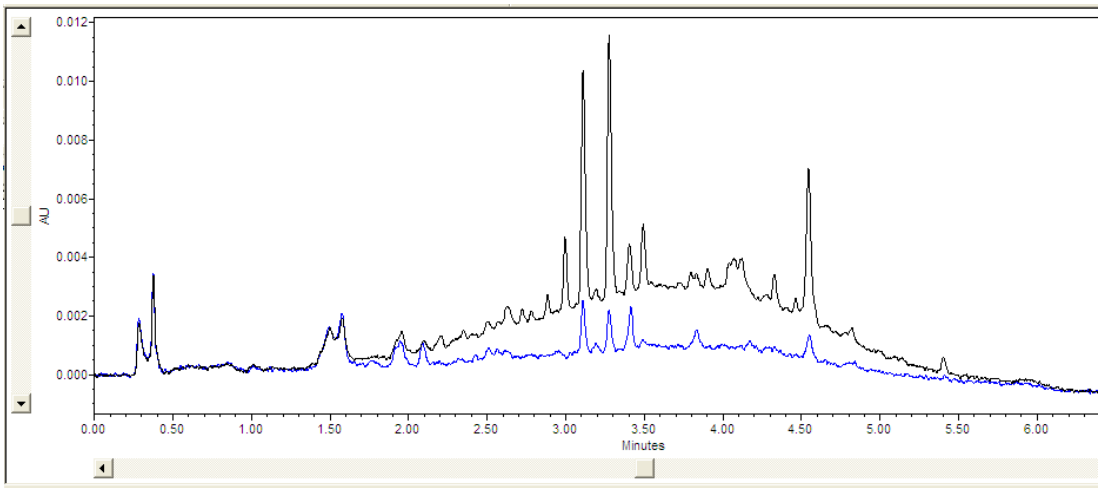
	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel De
1	☑	DanielDias	2:F,4	Unknown			1655	☐		1.00000	S1LFRGDAY18	1.00000	2D	440extract	AU	PDA 440.0
2	☑	DanielDias	2:F,3	Unknown			1652	☐		1.00000	S1FRGDAY18	1.00000	2D	440extract	AU	PDA 440.0

Figure 43. UPLC chromatogram comparing the Chardonnay wine after 18 days exposure to light in heavyweight Arctic Blue (black) and lightweight Arctic Blue (blue) under controlled temperature conditions.



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel Des
1	☑	DanielDias	2:F,5	Unknown			1658	☐		1.00000	S1ABDAY18	1.00000	2D	440extract	AU	PDA 440.0 n
2	☑	DanielDias	2:F,6	Unknown			1661	☐		1.00000	S1LABDAY18	1.00000	2D	440extract	AU	PDA 440.0 n

Figure 44. UPLC chromatogram comparing the Chardonnay wine after 18 days exposure to light in heavyweight Flint (black) and lightweight Flint (blue) under controlled temperature conditions.



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel Des
1	☑	DanielDias	2:F,7	Unknown			1664	☐		1.00000	S1FGDAY18	1.00000	2D	440extract	AU	PDA 440.0 n
2	☑	DanielDias	2:F,8	Unknown			1667	☐		1.00000	S1LFGDAY18	1.00000	2D	440extract	AU	PDA 440.0 n

It is apparent by matching retention times in the chromatograms for the exposed Chardonnay samples (Figures 41-44) with those for a xanthylium standard (Figure 40) that xanthylium pigments are formed in varying amounts in the treated samples. The highest production occurs in the Flint (heavy) bottle (Figure 44, black) and the lowest amount is formed in the Antique Green samples. This is in accord with the colour analysis described above (Table 2). Xanthylium pigment production is more extensive in the lightweight Arctic Blue bottle than in the heavy weight Arctic Blue bottle (Figure 43), again in accord with the colour data (Table 2).

4.3.2 Detection of xanthylium type pigments by LC-MS

Further confirmation of the presence of xanthylium pigments was obtained by LC-MS. The pigments are actually cationic species that makes their detection in the positive ion mode relatively easy. Further, there are several linkage isomers that can be formed and a characteristic pattern of

peaks is commonly observed. All exposure study samples were analysed by liquid-chromatography-mass spectrometry (LC-MS) with data consistent with the assignment of pigment peaks (reference to 440 nm chromatogram) as xanthylium cations in their acid and ethyl ester forms. The acid forms had parent ion signals at 617 *m/z* and the ethyl ester form was 645 *m/z*. Fragmentation ions at 465 and 152 *m/z* for the acid forms and 493 *m/z* for the ester forms were consistent with ion fragments reported previously for these compounds (Labrouche *et al.* 2005).

4.3.3 Approaches to the determination of other pigments

Chromatography with either photo-diode array or mass spectrometric detection confirmed the presence of xanthylium pigments in the wines exposed to light. To determine if other pigments were also formed as a consequence of exposure to light, a preparative chromatographic separation procedure was initiated in order to isolate pigmented fractions for subsequent analysis by mass spectrometry and nuclear magnetic resonance spectroscopy (NMR).

Preparative HPLC was carried out on an Agilent 1200 series equipped with a quaternary pump (G1311A), solvent degasser (11322A), fraction collector (G1314C) and PDA detector (G1314B VWD). A gradient method was adopted, time 0-5 (100% H_2O), 5-75 mins (70% H_2O:CH_3CN) and 76 mins (100% H_2O) on a Phenomenex Synergi Hydro-RP 80, 250 × 15 (10 µ) column at a flow rate of 5.0 mL/min.

After 18 days of exposure, the wine in the Flint bottles was freeze dried. The resulting brown gummy extract (~18 g) was filtered incrementally using 0.3 µm filters and the resulting extract was resuspended in 12 mL of deionised water and subjected to reverse phase preparative HPLC purification in which 26 fractions were collected. These fractions were then analysed by ^{1}H NMR on a Varian INOVA 400 MHz NMR spectrometer. Spectra were recorded in D_2O with referencing to TMS solvent signals (0 ppm).

Surprisingly, xanthylium type pigments, which were observed without the pre-concentration step, were not detected by NMR and UPLC in this experiment. Irrespective, this preliminary study on sample fractionation has opened up the possibility of a detailed investigation into the mechanism of pigment formation. Such a study was outside the bounds of this pilot study. However, the basis for a more intensive study has been established and there is every likelihood that considerable benefit will be derived from further examination of pigment production.

4.4 Bottle exposure experiments under uncontrolled temperature conditions

4.4.1 Experimental design and absorption spectra

The experiment described here was essentially identical to that in Section 4.1, except that temperature was not controlled and irradiation was carried out over three days only without aeration. Samples of Chardonnay with added (+)-catechin (100 mg/L) were added to the Flint, Arctic Blue, French Green and Antique Green light weight ('light weighted') bottles. These bottles were irradiated for 3 days (16 hrs light, 8 hrs darkness) for 3 days. During the periods of light exposure, the temperature reached at least 80^{o}C. The short time period (3 days compared with 18 days under controlled temperature conditions) was sufficient for visual perception of pigmentation.

Figure 45 presents the difference absorption spectra for samples from each bottle type after 3 days exposure. Growth of a peak around 380 nm tailing into the visible region is obvious, with the largest increase occurring for the Antique Green light weight bottle. This is exactly the reverse of the results obtained when the temperature was controlled. Figure 46 shows a close-up of the difference spectra in the visible region, confirming the significantly higher absorbance obtained for the wine in Antique Green. It is apparent that the colour development is a consequence of tailing into the visible

region. That is, there is no growth of a peak at 450 nm, as was observed when the same wine was exposed to light under controlled temperature conditions (see Figure 33, for example). This suggests that pigments, possibly in addition to xanthylium cation pigments, are being formed.

Figure 45. Comparison of the difference absorption spectra for the Chardonnay samples in light weighted ('lean') Antique Green (LAG), French Green (LFRG), Arctic Blue (LAB) and Flint (LFG) after 3 days irradiation without temperature control.

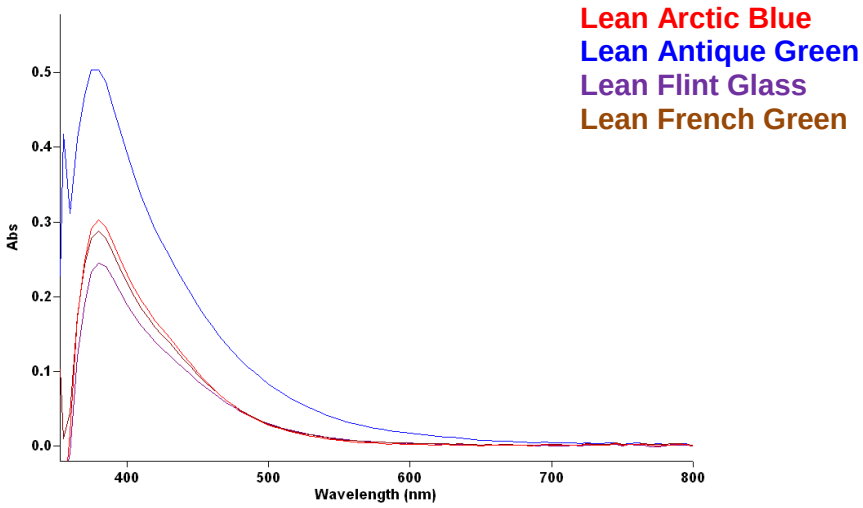
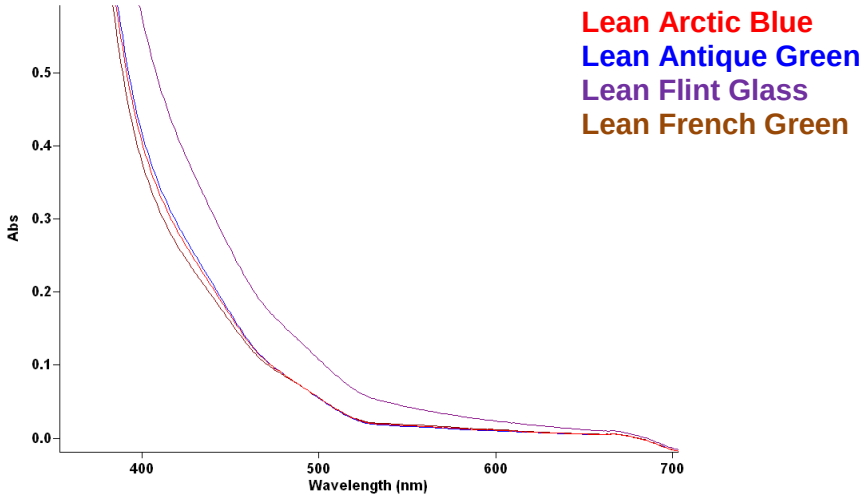


Figure 46. Comparison of the difference absorption spectra (zoom on visible region) for the Chardonnay samples in light weighted ('lean') Antique Green (LAG), French Green (LFRG), Arctic Blue (LAB) and Flint (LFG) after 3 days irradiation without temperature control.



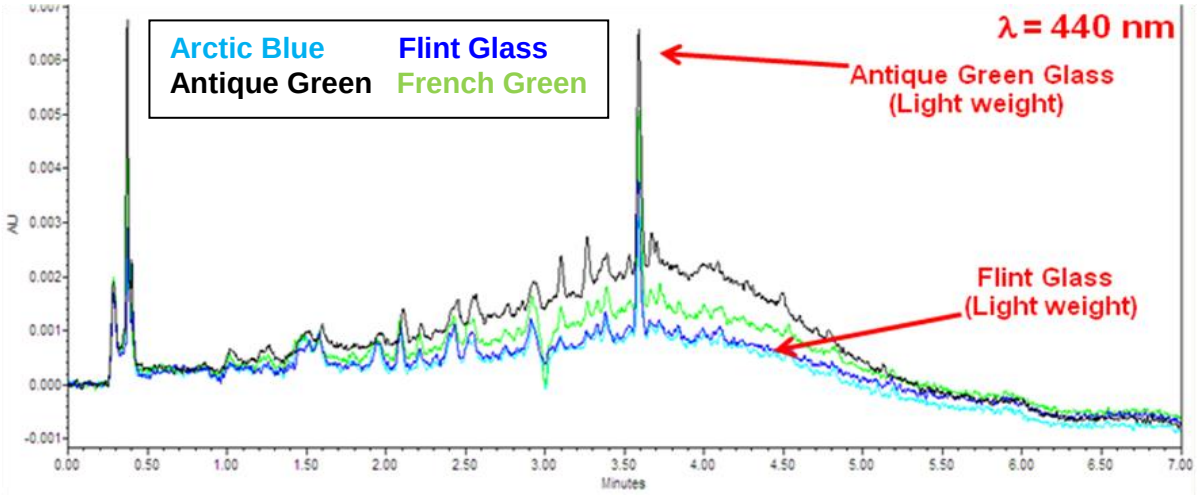
4.4.2. UPLC analyses of pigments formed under uncontrolled temperature conditions

UPLC chromatograms at 440 nm for the Chardonnay samples in the four light weighted bottles after 3 days exposure to light without temperature controlled are presented in Figure 47. These chromatograms are significantly different to those obtained for samples exposed to light under controlled temperature conditions (Figure 41-44). This confirms the observation made in Section 4.4.1 that higher pigmentation is occurring in the darker bottles (Antique Green). There is no evidence for the presence of xanthylium pigments in the uncontrolled temperature samples (compare

Figure 43 and Figure 47). Rather, there is a single peak at 3.6 min that is dominant and common to all samples. The extracted absorbance profile for this peak suggests that it a catechin-based phenolic compound. LC-MS indicated a *m/z* value of 562.7 (positive ion mode) corresponding to this peak at 3.6 mins in the DAD chromatogram. Its full structural identity was not determined.

The increase in the background of the chromatogram in Figure 47 suggests that polymeric pigments may have developed during this exposure experiment without temperature control. The chromatographic conditions used did not allow resolution of the components that contribute to the increase in baseline. Preparative purification and a full structural characterisation would be required to identify the major pigments formed during this experiment.

Figure 47. Overlay of UPLC chromatograms for Chardonnay samples after 3 days exposure to light with temperature control.



4.4.3. Sensory analysis of samples exposed to light without temperature control[2]

The Chardonnay samples that had been exposed to light for 3 days without temperature were subjected to a preliminary aroma profiling analysis. Table 3 presents the descriptors. Acetaldehyde, a descriptor for oxidation, was noted only in the Antique Green sample. This is different to the descriptors obtained for the samples exposed to light with temperature control (Table 1). In addition to acetaldehyde, the honey and kerosene descriptors in Table 3 may well reflect the formation of odorants such as sotolon and TDN, both of which require oxidative processes at elevated temperatures to be formed.

Although preliminary, the data in Table 3 suggest a way forward for assessing the impacts of both light and temperature on aroma profiles. The obvious oxidative aroma development in the Antique Green sample is in harmony with the highest level of pigmentation development in this dark bottle.

[2] We thank John Blackman of the NWGIC in Wagga Wagga for assistance with this aroma sensory study.

Table 3. Aroma descriptors for Chardonnay samples after 3 days exposure to light without temperature control.

Sample ID	Descriptors
Antique Green – light weight	Acetaldehyde, orange, guava
French Green – light weight	Stewed apricot, orange marmalade
Arctic Blue – light weight	Honey, apricot, orange marmalade
Flint – light weight	Orange marmalade, quince, honey, kerosene, apricot jam

CIELab measurements were also carried out on these samples (Table 4). In terms of colour intensity, the Antique Green sample is the most intense (*L**: 93.07), in line with the visual assessment of colour. The values for the *a** parameter show the shift to the red is greatest for the Antique Green samples. The *b** parameter indicates that all samples exhibit significant yellow colouration with again the Antique Green sample being the most yellow.

The CIELab measurements for these uncontrolled temperature samples are different to those for the temperature controlled samples (Table 2). Not only is the colour intensity the reverse, but the green to red and blue to yellow parameters also differ. The different colour parameters are further evidence that different pigmentation processes are occurring between the controlled and uncontrolled temperature samples. Further work is required to establish the basis for this difference with the potential to develop a marker for sample degradation as a consequence of light or temperature exposure.

Table 4. CIELab values for Chardonnay samples after 3 days exposure to light without temperature control.

Sample ID	*L**	*a**	*b**
Antique Green – light weight	93.07	-1.27	28.87
French Green – light weight	96.13	-2.57	19.59
Arctic Blue – light weight	95.67	-2.43	21.72
Flint – light weight	96.31	-2.23	19.01

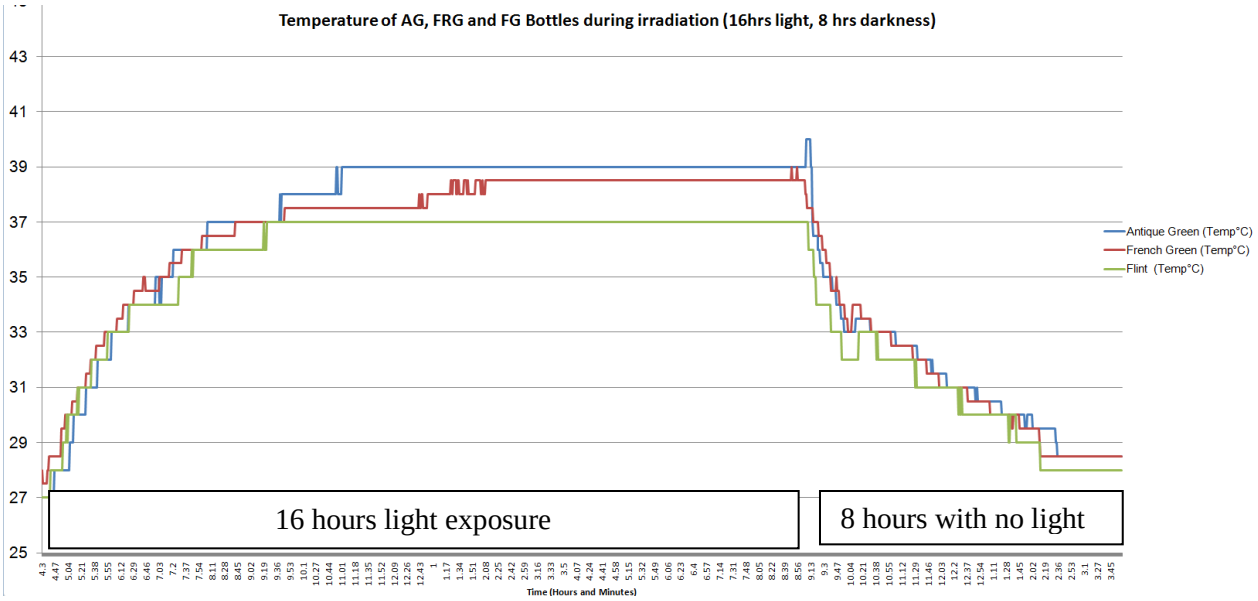
4.4.4. Differentiating between light and temperature effects that result in pigment production

Maury *et al.* (2010) exposed a Sauvignon Blanc wine in different coloured bottles (Antique Green, Classic Green, French Green and Flint) to sunlight for 70 days. The bottles were stored externally and subjected to a wide variation in temperature, both maximum and minimum values. While the conditions used may be perhaps extreme, they represent a 'worst-case' scenario. The results of the Maury *et al.* (2010) study showed that more colour development occurred in the dark green (Antique Green, Classic Green) bottle than in Flint or French Green. This trend parallels the results of the uncontrolled temperature study performed in this project.

The outcomes of the experiments performed in this project have allowed a separation of light and temperature effects. Under controlled temperature (30^{o}C, ambient, 38 ± 3^{o}C bottle temperature) and light conditions, the lighter coloured bottles (Flint and Arctic Blue) show the highest amount of colour development, while minimal colour development occurs in Antique Green bottles. This can be related to the higher levels of low wavelength light that can be transmitted by the Flint and Arctic Blue bottles. Exposure under the same light regime, but without temperature control, reversed the order of the bottle influence on colour. That is, when temperatures were allowed to rise to 80^{o}C or more simply using the light as the heat source, the Chardonnay sample stored in Antique Green bottles showed the most colour development.

Maury *et al.* (2010) proposed that dark coloured glass absorbs more heat and retains the heat longer and this becomes the driver of pigmentation development in darker bottles. Data to support this claim were obtained in this project. In the bottle exposure experiment under controlled temperature conditions, thermocouples were placed in the 'light box' (Figure 6), one assessing ambient temperature, the other attached to the surface of the bottle. Figure 48 illustrates the temperature changes recorded on the surface of the bottle over a 24 hr measurement period. When the light was turned on, the temperature of the bottles increased at much the same rate, but the plateau temperature for Antique Green was some 2^{o}C higher than for Flint. When the light was turned off, the temperature for the Flint bottle dropped slightly faster than for the Antique Green and reached a temperature about 1^{o}C lower by the end of the 'dark' (no light) period.

Figure 48. Temperature on surface of Antique Green (blue), French Green (red) and Flint (green) bottles during light and dark periods over a 24 hr day.



The controlled/uncontrolled temperature experiments in this project also showed differences in the type of pigments that are formed as a consequence of exposure to light. Xanthylium pigments, the type derived from glyoxylic acid, were identified as a major contributor to colour development in the controlled temperature experiments. On the other hand, the same pigments were not found in the uncontrolled temperature experiments. While the pigments that form under uncontrolled temperature conditions have not been identified, analysis of the UV absorbance spectra suggests that it may share a similar xanthylium type skeleton. Nevertheless, an optimised preparative HPLC method and full structure elucidation by NMR and MS would validate this.

Xanthylium pigments were found in the uncontrolled temperature light exposure experiments of Maury *et al.* (2010), but not under conditions different to those used in the experiments described here. The exposure periods are quite different, 70 days versus 3 days, and aeration conditions also differ. George *et al.* (2006) described several factors that can affect the stability of xanthylium pigments, one of which is light exposure. The possibility exists that xanthylium pigments may be precursors of other pigments, forming, breaking down and re-forming over time. This needs to be the subject of further research.

It is none-the-less clear that the exposure experiments performed in this project have opened up the possibility of identifying markers, both pigments and aroma compounds, which could be used to identify the occurrence of wine degradation in storage or transport.

5. Influence of wine components

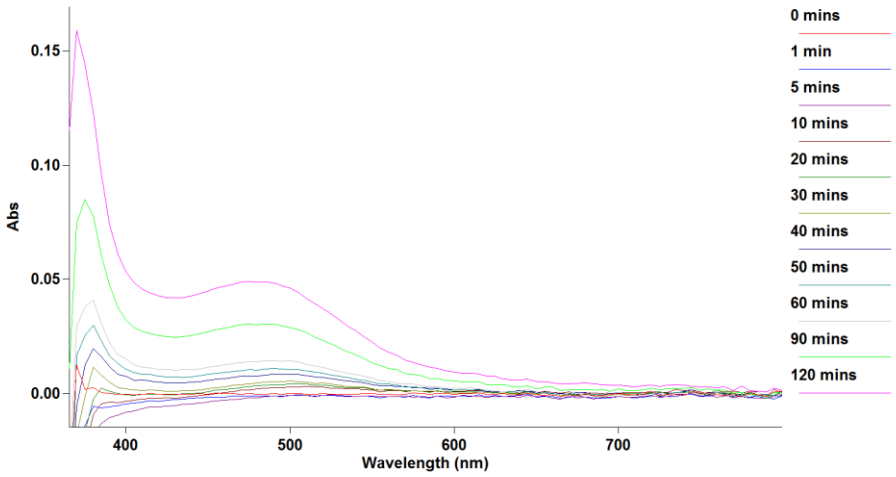
The wine components that were investigated for their influence on photodegradation were:

- class of phenolic compound (catechin-type and caffeic acid type)
- iron tartrate
- riboflavin
- molecular oxygen

5.1. Class of phenolic compounds

Preliminary experiments were initiated to compare the colour development in a Chardonnay wine with enhanced concentrations of (-)-epicatechin, caffeic acid and combinations of the two phenolic compounds. The data in Figure 49 illustrates a rapid increase in absorbance around 480 – 490 nm for a sample containing both (-)-epicatechin and caffeic acid. There is also better resolution between the changing absorbance at wavelengths less than 400 nm (due to phenolic re-arrangement) and the absorbance band in the visible region in this case compared to that for a Chardonnay containing added (-)-epicatechin only (see Figure 10). Additional work is required to determine the nature of the pigmentation that is occurring in this case.

Figure 49. Difference absorption spectra for a Chardonnay sample with added (-)-epicatechin (100 mg/L) and caffeic acid (200 mg/L) exposed at 45^{o}C to radiation from a xenon-arc lamp over 120 minutes.



5.2. Iron tartrate[3]

Clark *et al.* (2007) described the sunlight induced degradation of tartaric acid to give glyoxylic acid, a known pigment precursor. It was argued that trace contamination of iron was the basis for the initiation of the photodegradation process. The photoactivity of iron(III) tartrate is well-established, with its application to photography being recognised for more than 100 years (Ware, 1999). Abrahamson *et al.* (1994) examined the photochemistry of iron(III) carboxylate complexes in some detail. While focusing on iron(III) citrate, the authors reported that iron(III) tartrate showed marked photoactivity in the pH range 2.7 to 4.0. The work of Abrahamson *et al.* (1994) used a high

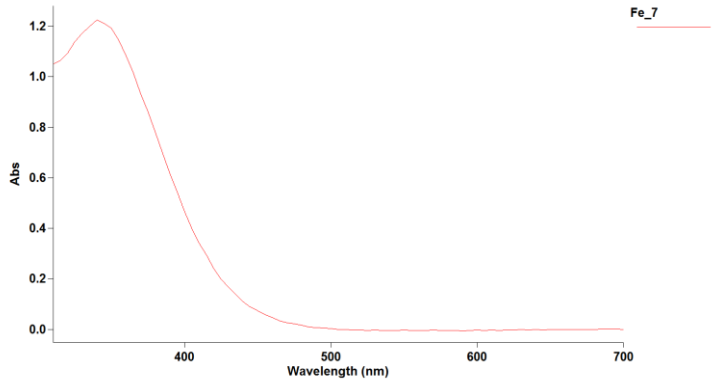
[3] These experiments were performed at the NWGIC in Wagga Wagga in conjunction with Dr Andrew Clark

Fe(III) to carboxylate ratio, the opposite to the situation in wine. However, the combination of the work of Clark *et al.* (2007) and Abrahamson *et al.* (1994) suggested that the photochemistry of iron(III) tartrate would benefit from further study to determine if it were a potential means of initiating light-induced pigment formation.

In our studies, we used iron (5 mg/L) in the model wine base. This concentration of iron was chosen as it is typical of concentrations in wine (Navarre, 1991). The ratio of iron(III) to tartaric acid was 0.005 (8.95×10^{-5}M Fe; 0.019M tartaric acid). Stock solutions were prepared by adding iron(II) sulfate to the model wine base. The solution immediately exhibited a pale yellow colour, indicative of iron(III) complexes with ligands containing oxygen donor atoms (Abrahamson *et al.*, 1994).

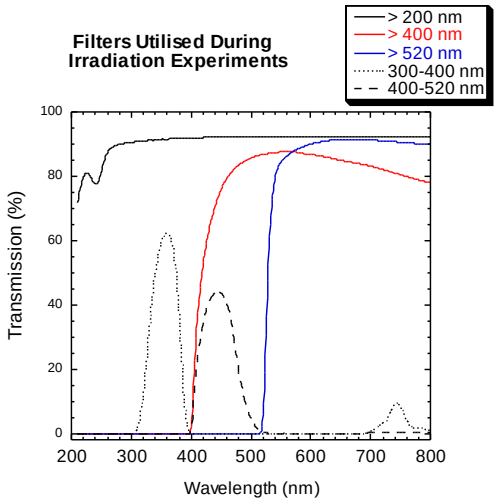
The absorption spectrum of an iron/wine base solution is shown in Figure 50. The spectrum shows a well defined absorbance peak centred around 350 nm, indicating the capacity to absorb radiation in the low visible/ultraviolet region, a region identified earlier in this project as critical for pigment development.

Figure 50. Absorbance spectrum of a wine base solution containing added iron.



To determine the critical impact of wavelength on iron(III) photoactivity, irradiation experiments were performed using the small scale set up shown in Figure 1. Various cut-off filters were placed between the xenon arc lamp and the spectrophotometer cell. The transmission spectra of the cut-off filters are shown in Figure 51.

Figure 51. Transmission spectra of cut-off filters.



The wine base samples containing iron (5 mg/L) were placed in a quartz cuvette (effectively transmissive to below 200 nm), sealed with a Teflon stopper and placed in the spectrophotometer cell holder at a distance of 14.5 cm from the xenon arc lamp. The cuvette was maintained at 45°C during a 30 minute irradiation time. The production of glyoxylic acid was monitored by UPLC, based on the mechanism for iron(III) tartrate photodegradation proposed by Clark *et al.* (2007).

Figure 52 presents the results of the exposure experiments for the different cut-off filters used while Figure 53 shows the UPLC chromatograms. Table 5 summarises the actual concentrations of glyoxylic acid produced.

Figure 52. Production of glyoxylic acid at different wavelengths of light.

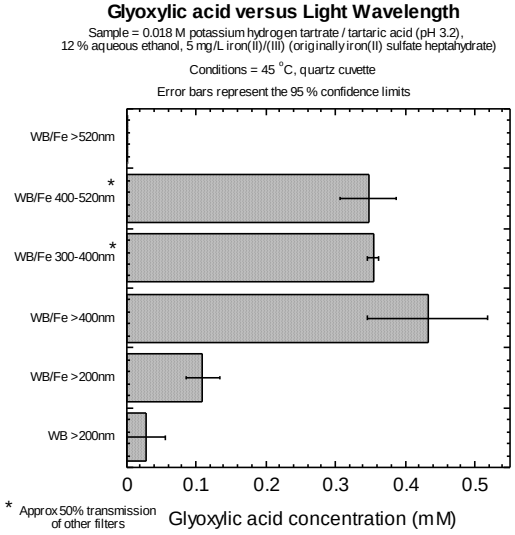


Figure 53. Chromatograms for exposed wine base solutions. **A**: hydrogen peroxide; **B**: tartaric acid; **C**: glyoxylic acid; **D**: unidentified organic acid.

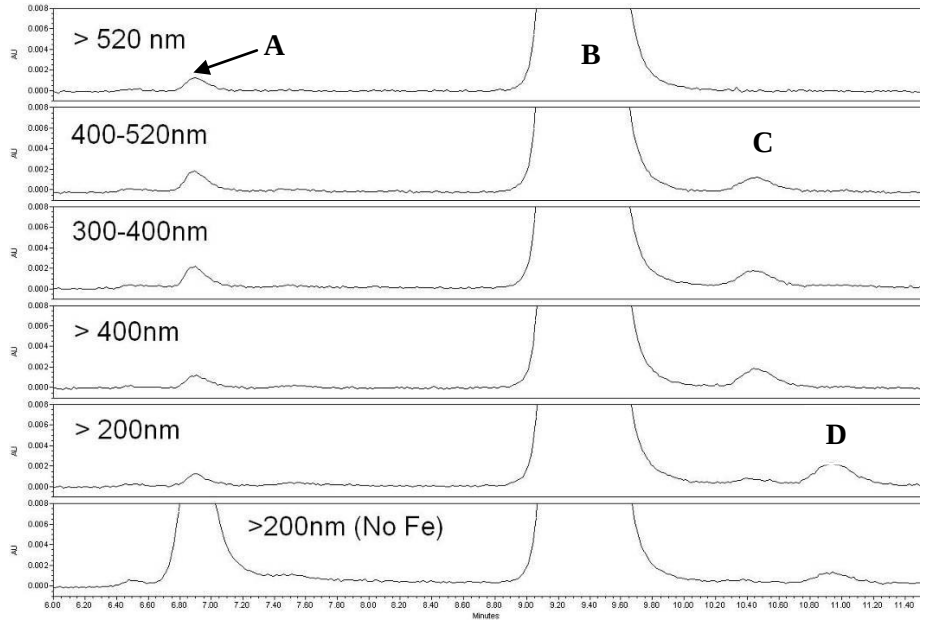


Table 5. Concentrations of glyoxylic acid generated as a function of wavelength. WB= wine base

Sample	Glyoxylic acid concentration (mM)
WB/Fe >520 nm	Not detected
WB/Fe 400-520 nm	0.35 ± 0.04
WB/Fe 300-400 nm	0.354 ± 0.008
WB/Fe >400 nm	0.43 ± 0.09
WB/Fe >200 nm	0.11 ± 0.02
WB >200 nm	0.03 ± 0.03

The following conclusions can be drawn from this experiment

- exposure to light above 520 nm does not produce any glyoxylic acid;
- wavelengths between 300 nm and 520 nm are critical for the formation of glyoxylic acid;
- comparison between the amount produced and the different wavelength ranges is difficult as the intensity of light reaching the sample varies from one cut-off filter to another.

The results for the exposure studies that allow radiation down to 200 nm to reach the sample (Table 5) are more of academic interest as all glass bottles eliminate wavelengths below 300 nm reaching the sample. Wavelengths below 300 nm can induce different photochemical processes such as the well documented production of hydrogen peroxide from water (Legrini *et al.*, 1993). This is evident in the sample irradiated at > 200 nm without iron whereby H_2O_2 accumulates (Figure 53, plot f). This hydrogen peroxide can lower the yield of glyoxylic acid by oxidising it to formic acid.

The influence of experimental conditions on the production of glyoxylic acid was also investigated. The variables examined were:

- Control: wine base containing Fe (5 mg/L)
- Wine base without added iron (No Fe);
- Lower oxygen concentration achieved by flushing the sample and headspace with nitrogen (low oxygen)
- No exposure to light, but held at the same temperature as all other samples (no light)
- Omission of ethanol and iron from the Control (No EtOH and Fe)
- Omission of ethanol only from the Control (No EtOH)

All exposure experiments were performed at 45^{o}C using a cut-off filter that allowed only light less than 400 nm to reach the sample. The results are presented graphically in Figure 54 and the actual concentrations of glyoxylic acid formed are given in Table 6.

Figure 54. Production of glyoxylic acid in relation to wine base composition and experimental conditions.

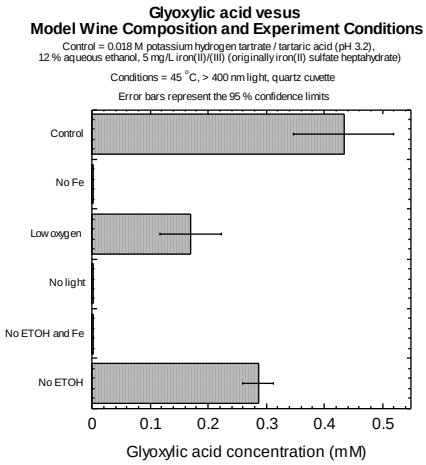


Table 6. Glyoxylic acid concentrations produced in relation to wine base composition and experimental conditions.

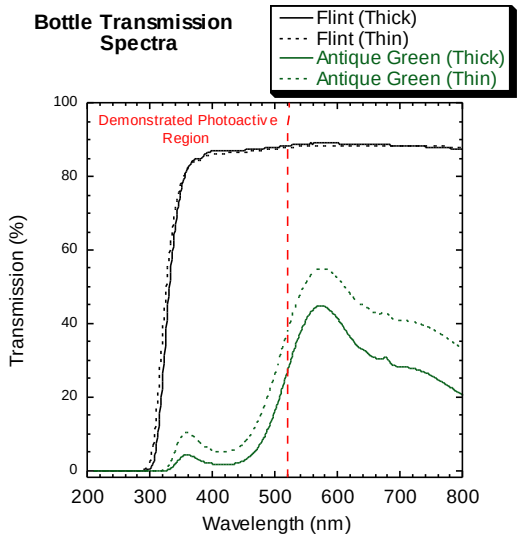
Sample	Glyoxylic acid concentration (mM)
Control	0.43 ± 0.09
No iron ions	Not detected
Low oxygen	0.17 ± 0.05
No light	Not detected
No ethanol and no iron ions	Not detected
No ethanol	0.29 ± 0.03

These results clearly demonstrate that

- the presence of iron is essential for the production of glyoxylic acid;
- the presence of light is essential for the production of glyoxylic acid;
- the absence of ethanol decreased the amount of glyoxylic acid produced. This observation is surprising, given that ethanol is a known radical scavenger; that is, a higher concentration of glyoxylic acid would be expected if a radical mechanism (i.e. Fenton chemistry) dominated the tartaric acid cleavage step. This observation is one that requires further study, but supports the importance of the photochemical step in the production of glyoxylic acid in these systems.

The relationship between colour development and bottle colour and thickness showed the highest pigmentation levels were found when wine samples were exposed to light with a Flint glass filter in place and the lowest levels when an Antique Green glass filter was used (see pages 36-38). Glyoxylic acid is a known precursor of xanthylium pigments and so the impact of glass bottle filters on the production of glyoxylic acid was examined. Sections of glass bottles were cut from Flint and Antique Green bottles, both the traditional heavyweight (thick) and newer lightweight (thin) bottles. Figure 55 shows the relationship between the transmission spectra of the different glass types in relation to the photoactive region (< 520 nm) for glyoxylic acid production.

Figure 55. Flint and Antique Green glass transmission spectra in relation to the wavelength of light required for glyoxylic acid production.



When the wine base sample containing iron (5 mg/L) was exposed at 45°C using the different glass filters, the results obtained for glyoxylic acid production are shown in Figure 56, with the supporting chromatograms in Figure 57. Table 7 lists the actual concentrations measured.

Figure 56. Comparison of Flint and Antique Green glass filters on the production of glyoxylic acid

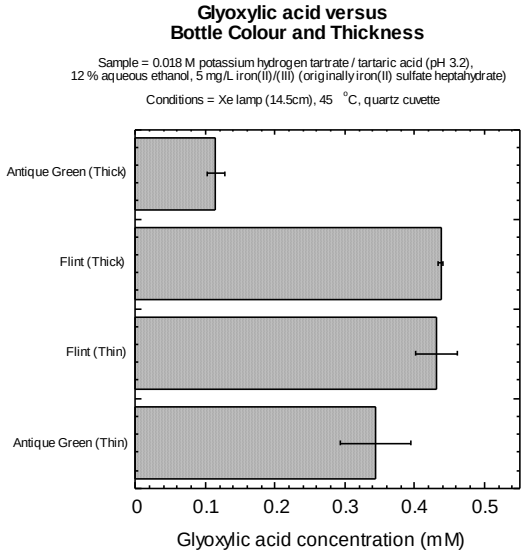


Figure 57. Chromatograms showing the amount of glyoxylic produced as a function of glass filter colour and thickness. **A:** hydrogen peroxide; **B:** tartaric acid; **C:** glyoxylic acid.

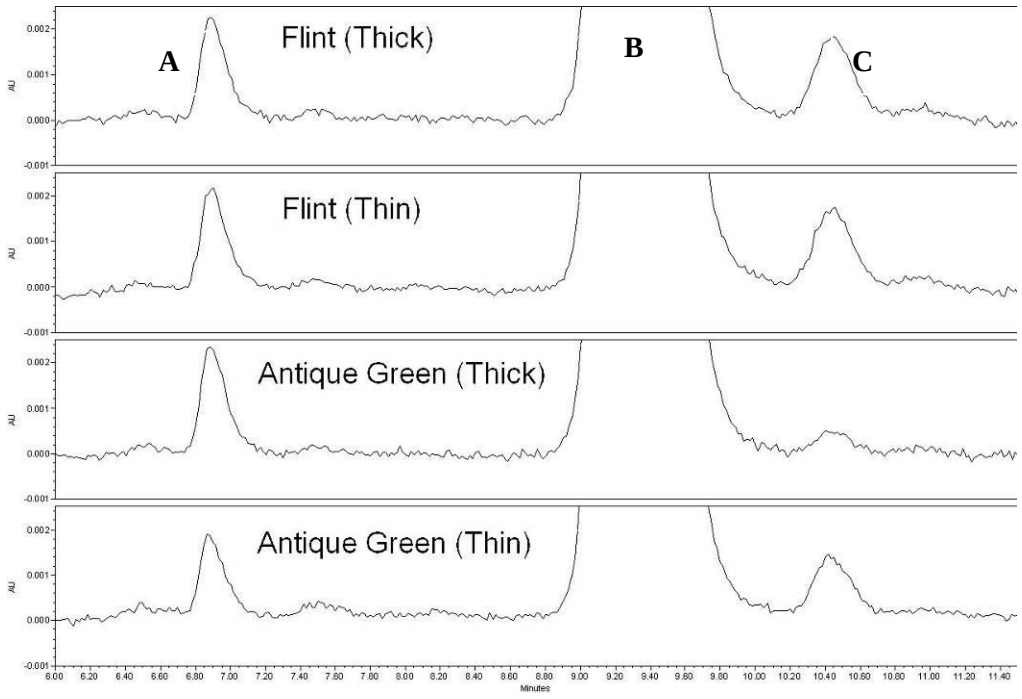
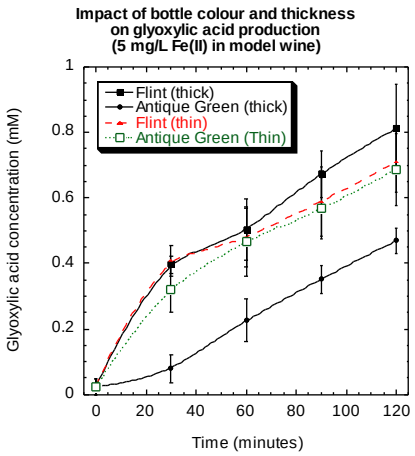


Table 7. Concentrations of glyoxylic acid produced as a function of glass filter colour and thickness.

Sample	Glyoxylic acid concentration (mM)
Flint (Thick)	0.437 ± 0.003
Flint (Thin)	0.43 ± 0.03
Antique Green (Thick)	0.11 ± 0.01
Antique Green (Thin)	0.34 ± 0.05

Figure 58 presents the time course for the production of glyoxylic acid when using Flint and Antique Green glass as filters in the irradiation experiment. There is a significant difference between the two weights for Antique Green in terms of the rate and amount of glyoxylic acid production that is not apparent with the Flint glass. While these results require further examination using all glass types used in this project, it is possible that there may be a threshold of light that is required to generate a high yield of glyoxylic acid. This threshold is exceeded with Flint (both weights), but only with the light weight Antique Green. This is in general agreement with the exposure studies described in Figure 28. The data in Figure 58 also show that the amount of glyoxylic acid increases throughout the exposure, in contrast to the fluctuating concentration reported for sunlight exposure by Clark *et al*. (2007). This study, however, involved a significant concentration of iron whereas Clark *et al.* (2007) had only trace levels of iron present (~10 µg/L). These data again underscore the critical role that iron, as iron(III), plays in this photoactivation process.

Figure 58. Time course for the production of glyoxylic acid in relation to bottle colour and bottle thickness; (thick = heavy weight; thin = light weight).



Clearly, Flint glass allows the higher production of glyoxylic acid and the amount produced is independent of bottle thickness. While the heavy weight Antique Green glass filter results in less glyoxylic acid being formed, it is important to note that it does not stop the production. This is in agreement with the transmission spectra/photoactive region shown in Figure 55. The results in this section also underscore the critical role of iron(III) tartrate as a potential photoactivator in the wine matrix. While this work was performed in a model wine matrix with only a minimum number of components, there are many potential binding agents for iron(III) in a white wine matrix. Knowledge of the distribution of the iron(III) species, that is its chemical speciation, is an essential adjunct to this preliminary photoactivation study.

5.3 Riboflavin

Riboflavin has been reported to be a photoactivator for the production of aroma changes in white wine. There has not been any work on the relationship between riboflavin and pigment production and the experiments described in this Section were designed to examine this relationship.

5.3.1 Background

Goldsmith *et al.* (2005) reported that the photo-physical event leading to the formation of light-struck flavour in beer, exposed to visible light, is the excitation of riboflavin to its triplet state followed by electron transfer from iso-α-acids. Beer components were shown to have the ability to quench the riboflavin triplet and provide protection against lightstruck character formation (Goldsmith *et al.*, 2005). There are naturally occurring quenchers, such as catechin and tryptophan, ascorbic acid (a known triplet state quencher and antioxidant) and other water-soluble plant polyphenols such as rutin and (-)-epigallocatechin gallate have been shown to be efficient quenchers of the triplet-excited state of riboflavin in aqueous solution (Becker, 2005). Riboflavin is present in a wide variety of products, where it accordingly may act as a photosensitiser inducing protein oxidation as in milk and beer or inducing lipid oxidation as in cheese (Skibsted, 2000; Li, 2000, Mortensen, 2002). In terms of its mechanism, riboflavin absorbs light which forms the very reactive triplet-excited state by intersystem crossing from the initially populated singlet-excited state (Skibsted, 2000 and Li, 2000). Riboflavin is a water-soluble vitamin and the long lived triplet-excited state reacts with other compounds present in the aqueous phase by electron transfer (Eqs. (3), (4)) or by hydrogen-atom transfer (Eqs. (5), (6)) following excitation and intersystem crossing (Eqs. (1), (2)) as shown in the following reaction sequence valid for aerobic conditions and which includes subsequent generation of the superoxide anion radical (Eq. (6)) (Becker, 2005). ROH represent a

phenolic compound which may reduce the triplet riboflavin, $^3Rib^*$, as shown in Eq. (3) or deactivate $^3Rib^*$ by transfer of a hydrogen atom to form $^2RibH^\bullet$, as shown in Eq. (5) (Becker, 2005).

$$Rib + h\nu \longrightarrow {}^1Rib^* \quad (1)$$

$${}^1Rib^* \longrightarrow {}^3Rib^* \quad (2)$$

$${}^3Rib^* + ROH \longrightarrow {}^2Rib^{\bullet -} + {}^2ROH^{\bullet +} \quad (3)$$

$${}^2ROH^{\bullet +} \longrightarrow {}^2RO^\bullet + H^+ \quad (4)$$

$${}^3Rib^* + ROH \longrightarrow {}^2RibH^\bullet + {}^2RO^\bullet \quad (5)$$

$${}^2RibH^\bullet + O_2 \longrightarrow {}^1Rib + H^+ + O_2^{\bullet -} \quad (6)$$

In a study by Pozdrik *et al.* (2006), the disappearance of riboflavin absorbance at 445 nm from beers or model beers on light exposure was found to be directly linked to light-struck character formation. The addition of (+)-catechin, (-)-epicatechin, tryptophol or ascorbic acid was able to reduce, but not stop, the absorbance loss or light-struck character formation in both model beer and lagers that were exposed to light (Pozdrik, 2006). This work by Pozdrik *et al.* (2006) opens up two possibilities for white wine. First, if riboflavin is involved in pigment production, the catechin-type phenolic compounds may mask this influence. Secondly, riboflavin may preferentially absorb radiation and thereby offer some form of protection towards pigment development of catechin-type phenolic compounds discussed in Section 4. This component of the report describes experiments designed to ascertain if one or the other of the above possibilities could be identified.

5.3.2 Exposure experiments

Varying concentrations of riboflavin and (-)-epicatechin were added to the Chardonnay wine. Exposure to UV light (<400nm) in the small scale irradiation set up (Figure 1) at 45^{o}C was performed in the same way described in Section 1. Oxygen would have been present in this sample. Only low wavelength radiation was used as this was shown in Section 4 to be critical for phenolic reactivity leading to pigment production. This approach differs from previous work on riboflavin/aroma changes described by Becker *et al.,* (2005) and Goldsmith *et al.,* (2005) where visible light was used, focussing on the riboflavin absorbance at 445 nm.

Figure 59 shows the difference absorption spectra measured over time. Two significant changes are apparent: there is a decrease in the absorbance at 450 nm, due to loss of riboflavin, and an increase in absorbance at 520 nm. Visual inspection of the cuvette during the irradiation shows the loss of the blue fluorescence of the excited riboflavin from the onset of the irradiation and an increase in colour after a protracted period of irradiation. The onset of the absorbance increase was much slower than in the absence of riboflavin and the wavelength for the increase has shifted to 520 nm from 480 nm (compare Figure 37 as an example). Figure 60 plots the time course of the absorbance change at 450 nm and 520 nm.

Figure 59. Difference absorbance spectra for the irradiation with light < 400 nm at 45°C of a Chardonnay sample containing (-)-epicatechin (10 mg/L) and riboflavin (10 mg/L).

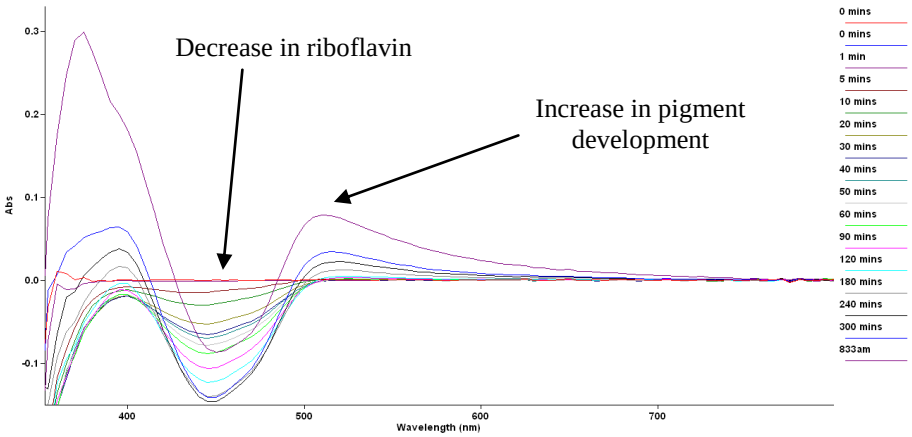
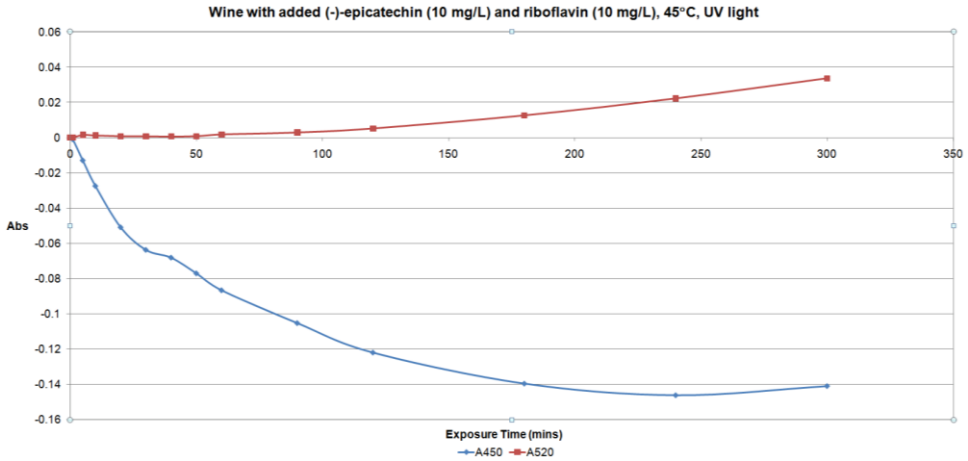
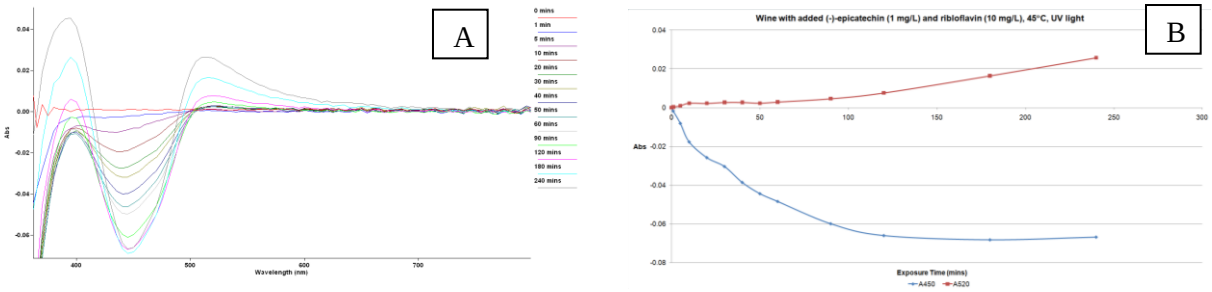


Figure 60. Time course of the change in absorbance at 450 nm (blue) and 520 nm (red) for the irradiation with light < 400 nm at 45°C of a Chardonnay sample containing (-)-epicatechin (10 mg/L) and riboflavin (10 mg/L).



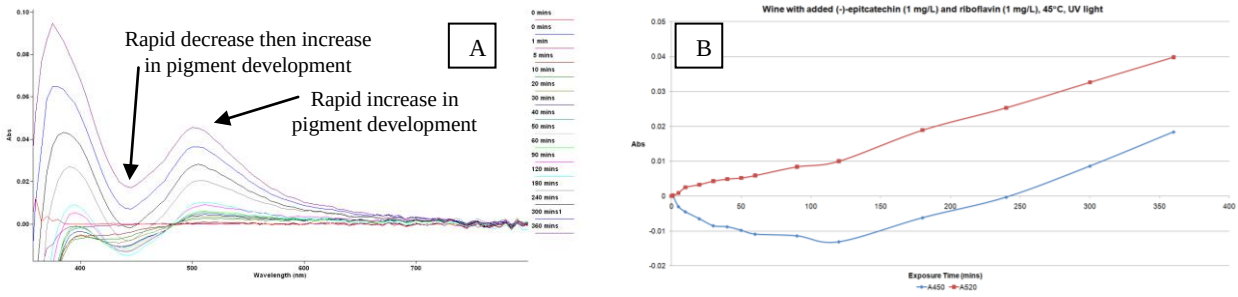
Decreasing the amount of added (-)-epicatechin generates the same type of profile with a loss of absorbance at 450 nm and an increase at 520 nm (Figure 61), the latter occurring only after the riboflavin has been depleted. The magnitude of the 520 nm increase is less in this case with a lower (-)-epicatechin concentration, but intriguingly not in proportion to the reduction in the concentration of (-)-epicatechin, suggesting a more complex mechansim for the production of pigments in the presence of decayed riboflavin.

Figure 61. Difference absorbance spectra (**A**) and time course of absorbance change at 450 nm (blue) and 520 nm (red) (**B**) for the irradiation with light < 400 nm at 45°C of a Chardonnay sample containing of (-)-epicatechin (1 mg/L) and riboflavin (10 mg/L).



The effect of riboflavin (10 mg/L) acting as a protector against pigment development is apparent from the two studies described above. However, the concentration of riboflavin used is considerably higher than would be found naturally in wine. Thus, the experiment was repeated, this time with 1 mg/L of each of (-)-epicatechin and riboflavin. The results in Figure 62 show the same initial loss of riboflavin and the delayed onset of pigment production, as reflected in the absorbance increase at 520 nm. In this experiment with the low riboflavin concentration, the loss of riboflavin occurred after about 100 mins, as was observed with riboflavin (10 mg/L) (Figure 61 B). However, the absorbance at 520 nm commenced to increased after about 30 mins, before the riboflavin had fully degraded. Further, the absorbance at 450 nm commenced to increase after 125 mins and continued to increase during the rest of the exposure period. The decrease at 450 nm was coupled with an observable loss of fluorescence that did not return when the increase in visible absorbance occurred. This indicates that the riboflavin is not re-forming. Rather the results suggest that a second pigment is forming or a different pigmentation mechanism is occurring. To add to the complexity of the system the A_{520} value in this case is higher than that for the experiment with (-)-epicatechin (1 mg/L) and riboflavin (10 mg/L) (Figure 61), and about the same as that for the experiments where both components were added at 10 mg/L (Figure 60). Similar spectral changes were also observed when the experiment was performed using (-)-epicatechin (1 mg/L) and riboflavin (5 mg/L).

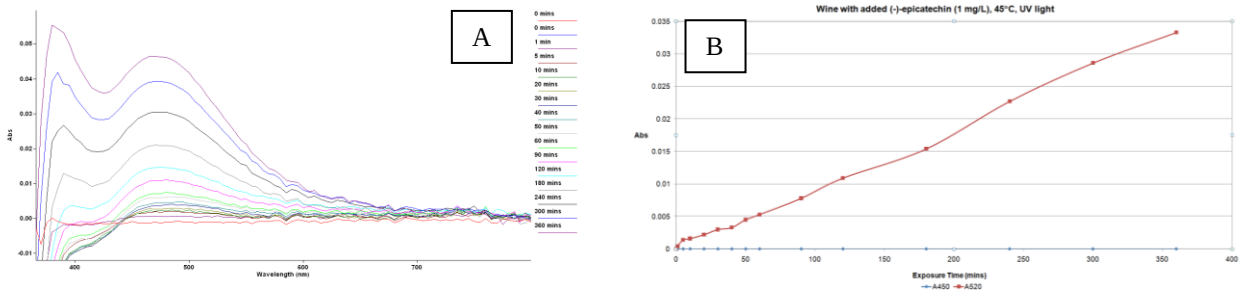
Figure 62. Difference absorbance spectra (**A**) and time course of absorbance change at 450 nm (blue) and 520 nm (red) (**B**) for the irradiation with light < 400 nm at 45°C of a Chardonnay sample containing 1 mg/Litre of (-)-epicatechin and 1 mg/Litre riboflavin.



To confirm the importance of riboflavin in these spectral changes, the impact of the <400 nm radiation of the Chardonnay sample to which only (-)-epicatechin (1 mg/L) had been added was examined. The result (Figure 63) shows no change in the 450 nm absorbance value while the two maxima (380 nm and 480 nm) were exactly as observed in earlier Sections of this report (see Figure 31). Clearly riboflavin plays a critical role in protecting the wine against pigment development while

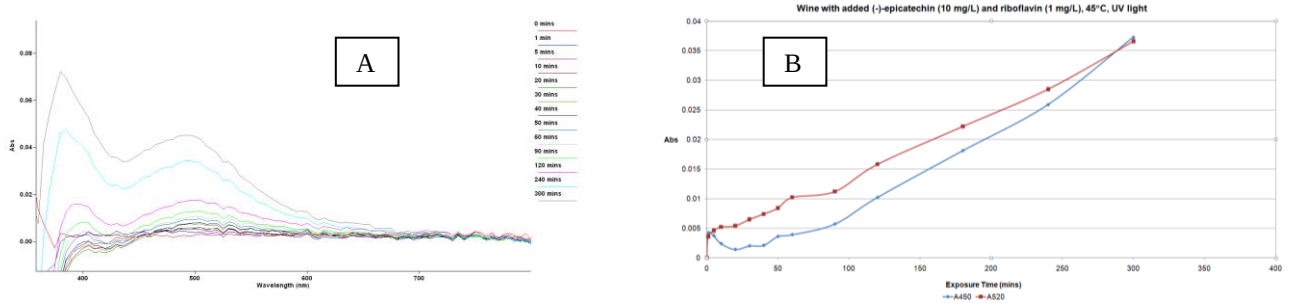
it remains in the wine. Pigmentation commences when the riboflavin has been broken down by the influence of the radiation.

Figure 63. Difference absorbance spectra (**A**) and time course of absorbance change at 450 nm (blue) and 520 nm (red) (**B**) for the irradiation with light < 400 nm at 45°C of a Chardonnay sample containing 1 mg/Litre of (-)-epicatechin.



Under conditions where the (-)-epicatechin concentration exceeded the amount of riboflavin added to the wine (10 mg/L and 1 mg/L respectively), i.e. in relative proportion that more closely resembles wine, a further set of spectral changes was observed. Figure 64 shows that the decrease in the A_{450} value appears to be much faster than in the experiments described above, but in reality, the increase in absorbance would appear to be commencing before the riboflavin is fully broken down. Further, there is a growth of a peak at 380 nm, similar to that observed with (-)-epicatechin alone (Figure 63), while the other increases in absorbance are more midway between the 520 nm (for mixed (-)-epicatechin/riboflavin systems; (Figure 60-63) and 480 nm (for (-)-epicatechin alone). The time course data in Figure 64 B) have been plotted for the A_{450} and A_{520} values for consistency with other data presented here.

Figure 64. Difference absorbance spectra (**A**) and time course of absorbance change at 450 nm (blue) and 520 nm (red) (**B**) for the irradiation with light < 400 nm at 45°C of a Chardonnay sample containing (-)-epicatechin (10 mg/L) and riboflavin (1 mg/L).

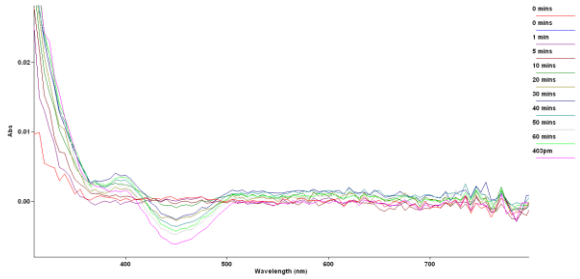


It is apparent from these preliminary experiments in this pilot study that a series of complex mechanisms are active in the light induced activity of riboflavin-containing wines. The concentrations of both riboflavin and (-)-epicatechin, both actual and relative, influence the spectral profiles during irradiation with light less than 400 nm. Further investigation of these effects is required for a full understanding of the different mechanistic processes at work. In addition, studies of the effect of riboflavin on pigment development should be linked to aroma profile studies. This would allow a complete picture as to whether riboflavin may have some advantage as a protector against wine colour development without any detrimental effect on wine aroma.

5.3.3 Irradiation studies using model systems.

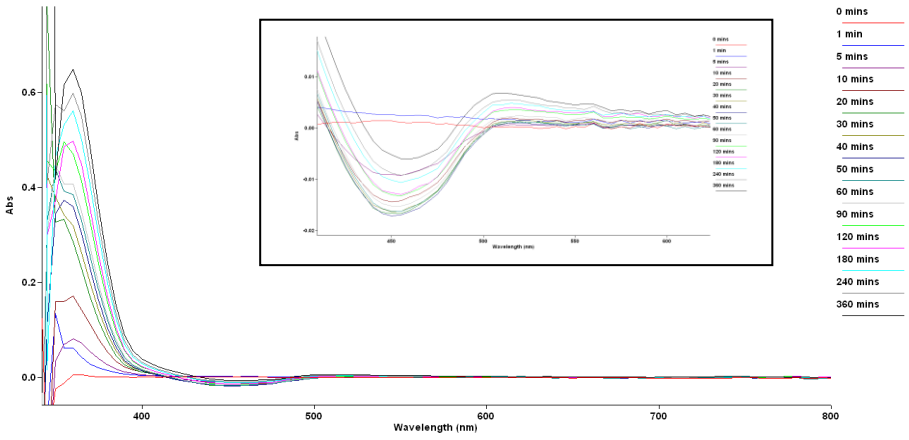
In an attempt to gain some insight of the other wine components that might be influencing the pigment development process, some trial experiments were initiated with the model systems used elsewhere in this project. Figure 65 shows the changes in the difference absorption spectra for a model system containing (-)-epicatechin (5 mg/L) and riboflavin (1 mg/L) during irradiation with light <400 nm at 45°C. The loss of riboflavin at 450 nm was observed, but an increase in pigmentation around 500 nm was not apparent. If, as described in Section 5.2, iron(III) tartrate is a photo-initiator, the low concentration of iron here from contamination only may have been insufficient for the production of glyoxylic acid, the precursor of the xanthylium cation pigments.

Figure 65. Difference absorbance spectra (A) for the irradiation with light < 400 nm at 45°C of a model wine system containing of (-)-epicatechin (5 mg/L) and riboflavin (1 mg/L).



Increasing the complexity of the model such that it contains caffeic acid (100 mg/L) in addition to the (-)-epicatechin and riboflavin shows the loss of ribflavin absorbance at 450 nm and a slight increase in absorbance in the 500 to 525 nm range. Figure 66 presents the results.

Figure 66. Difference absorbance spectra (A) for the irradiation with light < 400 nm at 45°C of a model wine system containing (-)-epicatechin (5 mg/L), riboflavin (1 mg/L) and caffeic acid (100 mg/L).



The delay in the production of absorbance in the visible region is observed in this case as well, but the spectral pattern tends to suggest that caffeic acid produces different pigments to those generated in the presence of catechin-type phenolic compounds.

Irradiation experiments with model systems showed the loss of riboflavin, but considerably lower production of pigments than found following the irradiation of a Chardonnay that had the same levels of (-)-epicatechin and riboflavin added. This implies that there may be other wine components

involved in the pigmentation process and more detailed studies are required to understand the complex processses that are occurring following the exposure of wine to low wavelength light.

5.3.4 UPLC analysis of the addition of riboflavin to white wine

Ultra performance liquid chromatography (UPLC) was used to monitor the degradation of riboflavin and potential formation of pigments during irradiation of solutions with varying concentrations of (-)-epicatechin and riboflavin. Figures 67 and 69 present respectively the 280 nm and 440 nm chromatograms for a standard aqueous solution of riboflavin and the Chardonnay sample used in these experiments, while Figure 68 shows the extracted absorbance profile for riboflavin. These wavelengths were selected as they are commonly used for monitoring pigment production in white wine Clark *et al.*, (2003). Riboflavin (Figure 67) is readily detected at 3.167 min and its extracted absorbance profile (Figure 68) indicates that it has the capacity to absorb radiation at several different wavelength ranges. The Chardonnay sample (Figure 69) does not show any absorbance at 440 nm, indicating that no pigments are present.

Figure 67. UPLC chromatogram for riboflavin in aqueous solution (blue: 280 nm; black: 440 nm).

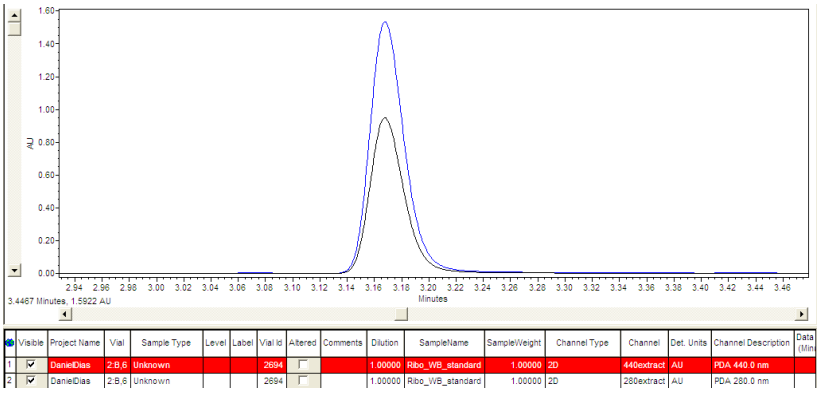


Figure 68. UPLC PDA contour plot and extracted UV profile of riboflavin in the chromatography solvent.

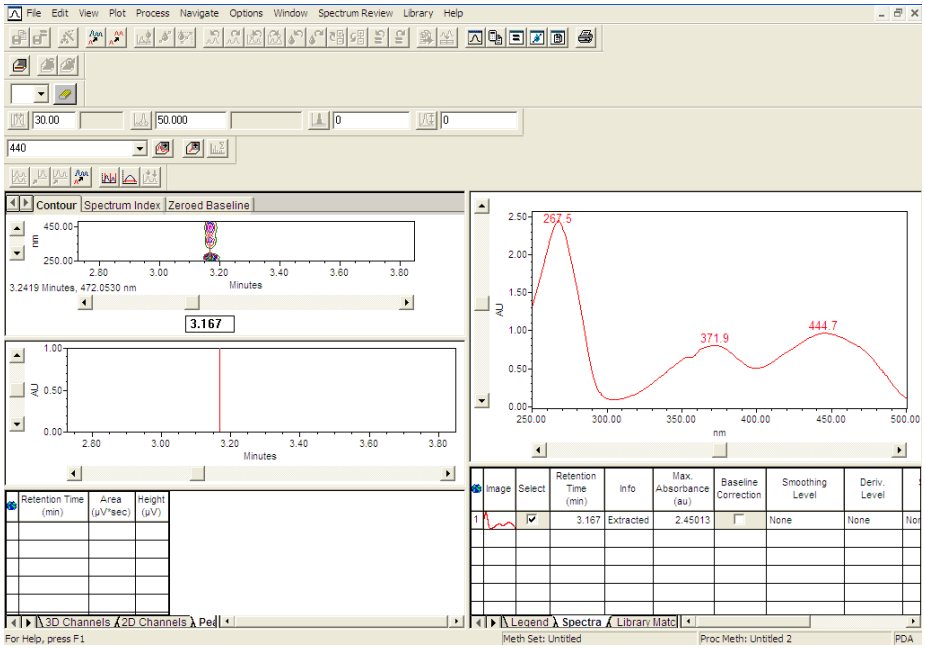
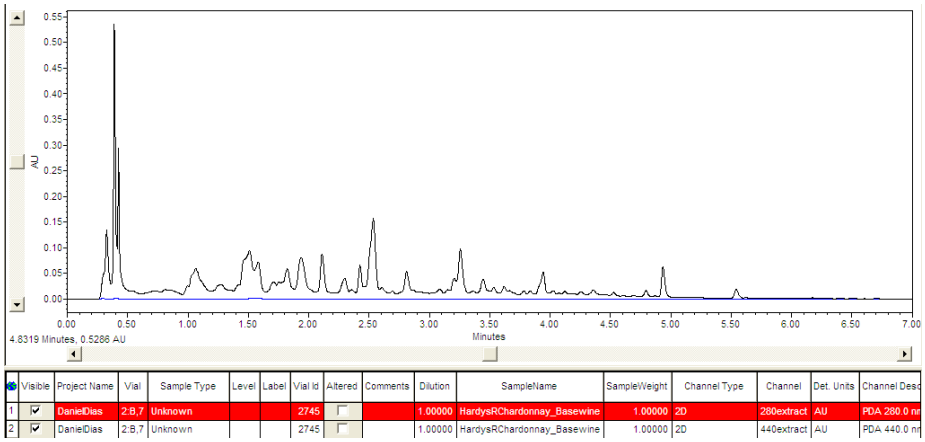


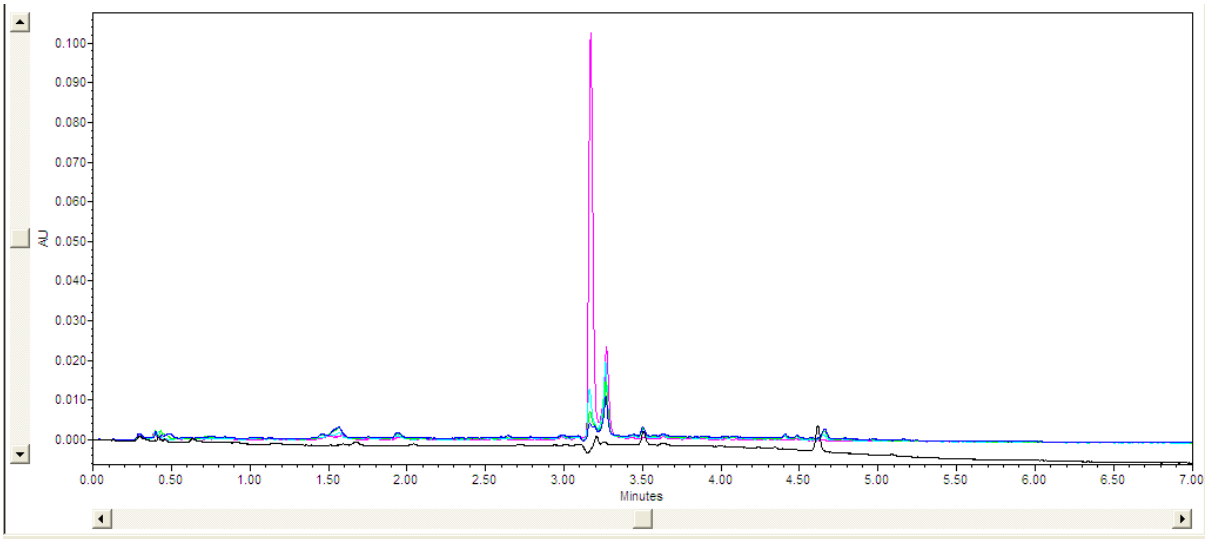
Figure 69. UPLC chromatogram for the Chardonnay wine (blue: 280 nm; black: 440 nm).



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel Desc
1	☑	DanielDias	2:B,7	Unknown			2745	☐		1.00000	HardysRChardonnay_Basewine	1.00000	2D	280extract	AU	PDA 280.0 nm
2	☑	DanielDias	2:B,7	Unknown			2745	☐		1.00000	HardysRChardonnay_Basewine	1.00000	2D	440extract	AU	PDA 440.0 nm

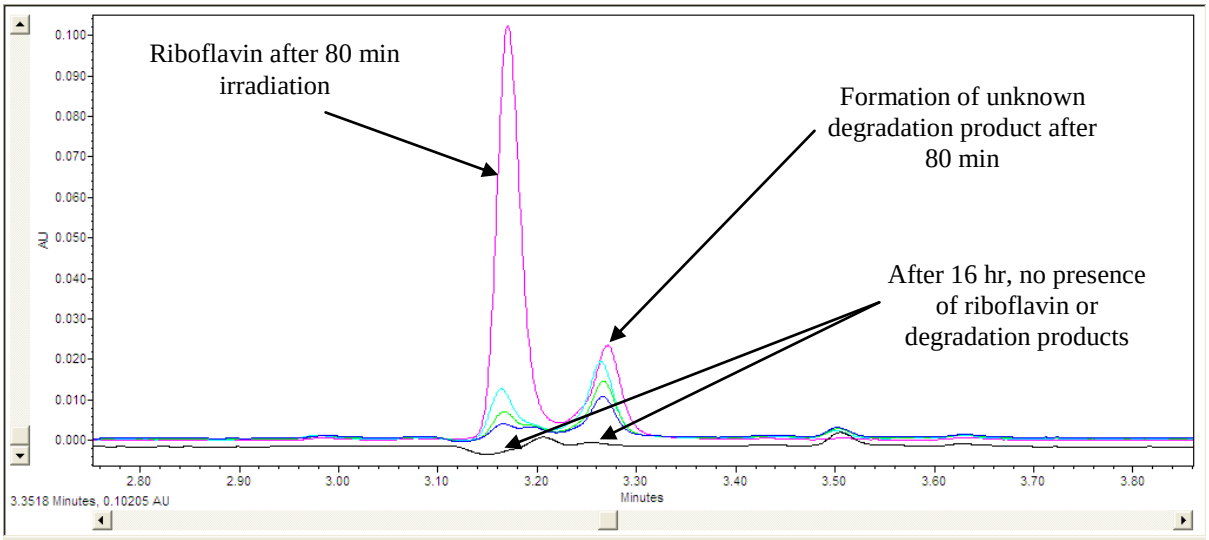
The Chardonnay containing added (+)-catechin (10 mg/L) and riboflavin (10 mg/L) was irradiated with light < 400 nm at 45°C without deoxygenating the solution before irradiation. Samples for UPLC analysis were taken at 80, 200, 260, 320, 380 mins and after 16 hrs. Figure 70 shows that riboflavin decreases rapidly as described in Section 5.3.2. An expansion of the chromatogram in the 2.8 to 3.8 minutes region (Figure 71) identifies the decrease in riboflavin absorbance more clearly and also illustrates that a product is being formed with a retention time of 3.28 mins. After 16 hrs irradiation, the peak at 3.28 minutes is absent suggesting that further molecular structural re-arrangement has occurred.

Figure 70. UPLC chromatogram (440 nm) for a Chardonnay sample containing (+)-catechin (10 mg/L) and riboflavin (10 mg/L). Chromatograms were recorded after 80 (pink), 260 (turquoise), 320 (green) and 380 (blue) minutes and after 16 hrs (black).



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel
1	☑	DanielDias	2:B,8	Unknown			2870	☐		1.00000	WineR10Cat10_UV_45C_843am_r1	1.00000	2D	440extract	AU	PDA 440
2	☑	DanielDias	2:B,8	Unknown			2856	☐		1.00000	WineR10Cat10_UV_45C_380mins_r1	1.00000	2D	440extract	AU	PDA 440
3	☑	DanielDias	2:B,8	Unknown			2847	☐		1.00000	WineR10Cat10_UV_45C_320mins_r1	1.00000	2D	440extract	AU	PDA 440
4	☑	DanielDias	2:B,8	Unknown			2750	☐		1.00000	WineR10Cat10_UV_45C_260mins_r1	1.00000	2D	440extract	AU	PDA 440
5	☑	DanielDias	2:A,7	Unknown			2682	☑		1.00000	WineR10Cat10_UV_45C80mins_r1	1.00000	2D	440extract	AU	PDA 440

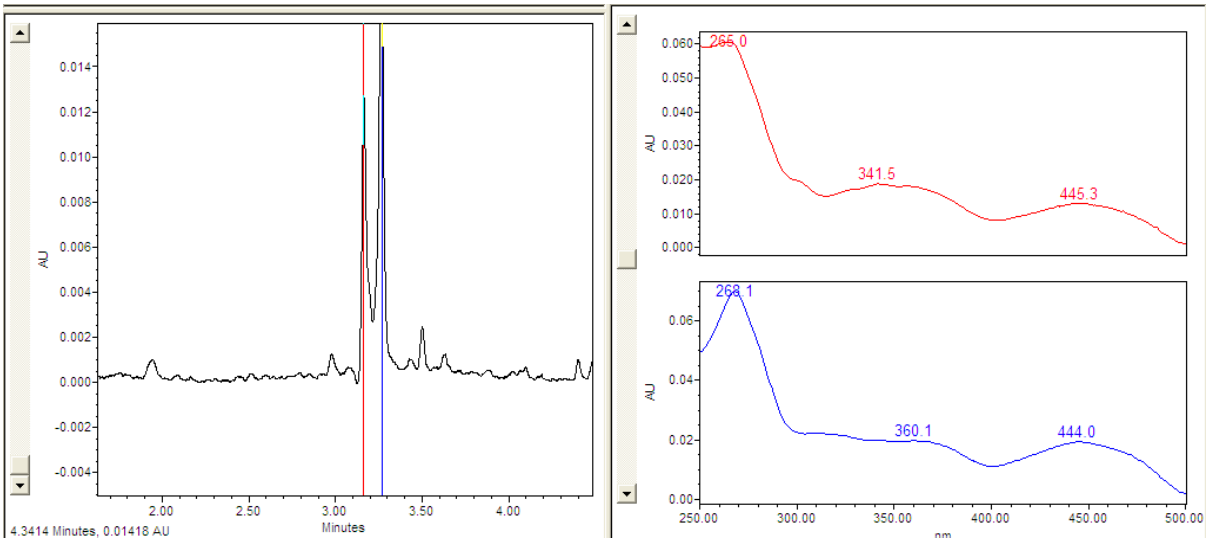
Figure 71. Expanded section (2.8 to 3.8 min) for the UPLC chromatogram shown in Figure 70. The same colour coding of chromatograms applies here.



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel
1	☑	DanielDias	2:B,8	Unknown			2870	☐		1.00000	WineR10Cat10_UV_45C_843am_r1	1.00000	2D	440extract	AU	PDA 440
2	☑	DanielDias	2:B,8	Unknown			2856	☐		1.00000	WineR10Cat10_UV_45C_380mins_r1	1.00000	2D	440extract	AU	PDA 440
3	☑	DanielDias	2:B,8	Unknown			2847	☐		1.00000	WineR10Cat10_UV_45C_320mins_r1	1.00000	2D	440extract	AU	PDA 440
4	☑	DanielDias	2:B,8	Unknown			2750	☐		1.00000	WineR10Cat10_UV_45C_260mins_r1	1.00000	2D	440extract	AU	PDA 440
5	☑	DanielDias	2:A,7	Unknown			2682	☑		1.00000	WineR10Cat10_UV_45C80mins_r1	1.00000	2D	440extract	AU	PDA 440

Figure 72 shows the UPLC chromatogram with corresponding extracted UV/visible spectra after 260 mins exposure. The sample contains a mixture of riboflavin and an unknown product. The unknown product does not seem to show the typical xanthylium type absorbance, but appears to be similar to that of riboflavin. After long exposure under UV light, the degradation products are not present, suggesting the degradation products formed by the depletion of riboflavin also break down. Other minor products (eg: at 3.5 mins retention time) seem to be formed after long exposure.

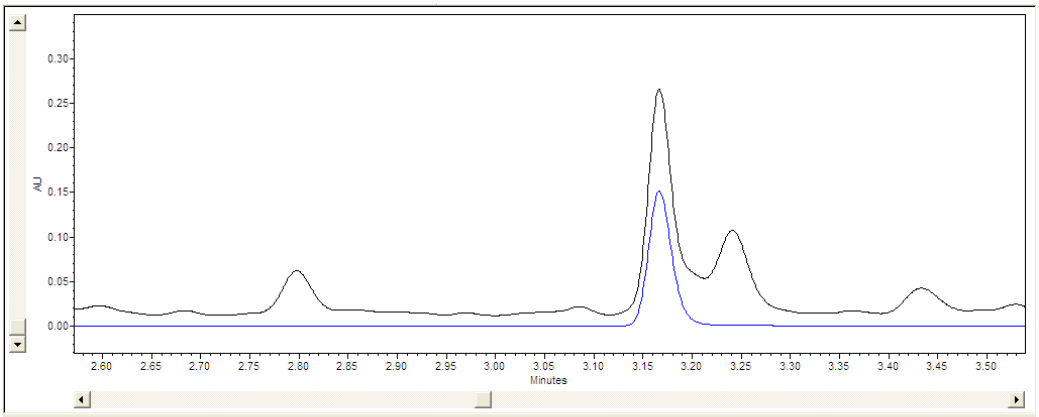
Figure 72. UPLC chromatogram showing extracted UV/visible spectra for riboflavin (red) and for the unknown degradation product (blue) at 260 minutes of irradiation.



Intriguingly, the UPLC chromatograms for the irradiated samples did not show the presence of any wine pigments containing the phenolic catechin moiety. The xanthylium cation pigments that were detected in irradiation studies without added riboflavin (Section 4.3) were not found in this experiment. The small absorbance increase observed in the irradiation experiments described in Section 5.3.2 suggests that pigment formation in the presence of riboflavin may be considerably less than in its absence. Whether any pigment formation is a consequence of riboflavin degradation products themselves or of oxidation of the phenolic compounds or a combination of phenolic compound and riboflavin degradation products remains a question requiring further research.

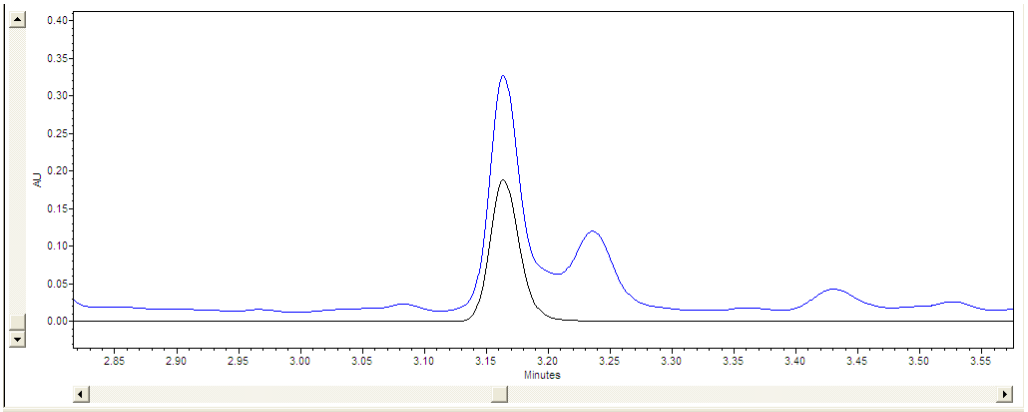
To confirm that light and not heat was causing the changes observed here, a sample of the Chardonnay with added (+)-catechin (10 mg/L) and riboflavin (10 mg/L) was heated in darkness at 45°C for about 1 hour. Figure 73 presents the UPLC chromatogram prior to heating while Figure 74 shows the result after 60 mins heating at 45°C in the absence of light. There is clearly no change in the chromatogram (compare Figure 67 in the presence of light).

Figure 73. UPLC chromatograms at 280 nm (black) and 440 nm (blue) for a Chardonnay sample containing (+)-catechin (10 mg/L) and riboflavin (10 mg/L) prior to any treatment.



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel
1	☑	DanielDias	2:A,7	Unknown			2658	☐		1.00000	WineRib10mgCat10mg45dark_time0r1	1.00000	2D	280extract	AU	PDA 28
2	☑	DanielDias	2:A,7	Unknown			2658	☐		1.00000	WineRib10mgCat10mg45dark_time0r1	1.00000	2D	440extract	AU	PDA 44

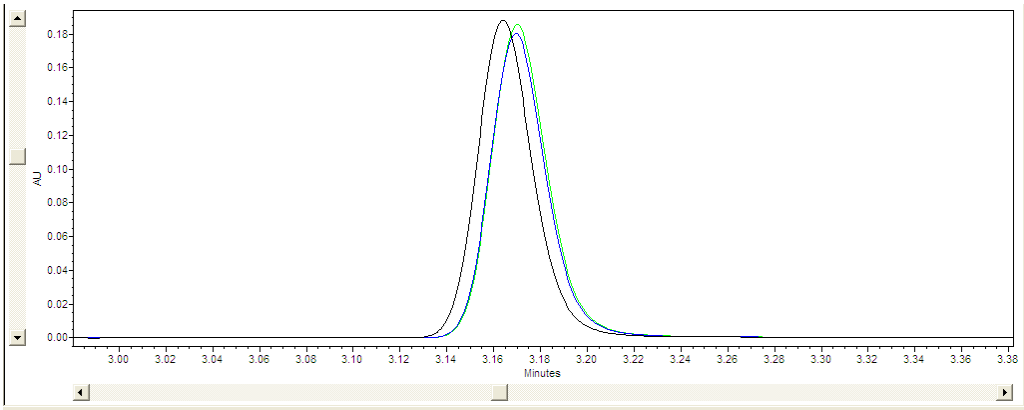
Figure 74. UPLC chromatograms at 280 nm (blue) and 440 nm (black) for a Chardonnay sample containing (+)-catechin (10 mg/L) and riboflavin (10 mg/L) after 60 min at 45°C in the absence of light.



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel
1	☑	DanielDias	2:B,2	Unknown			2673	☐		1.00000	WineRib10mgCat10mg45dark_60m_r1	1.00000	2D	440extract	AU	PDA 44
2	☑	DanielDias	2:B,2	Unknown			2673	☐		1.00000	WineRib10mgCat10mg45dark_60m_r1	1.00000	2D	280extract	AU	PDA 28

As further confirmation that light is required for the degradation of riboflavin under the experimental conditions used here, a sample of riboflavin (10 mg/L) in white wine was maintained at 45^{o}C in the absence of light for 60 mins. The 440 nm chromatograms (Figure 75), recorded for triplicate samples clearly show that no riboflavin degradation is occurring.

Figure 75. UPLC chromatograms (440 nm) for a riboflavin (10 mg/L in white wine) maintained at 45^{o}C for 60 mins.



	Visible	Project Name	Vial	Sample Type	Level	Label	Vial Id	Altered	Comments	Dilution	SampleName	SampleWeight	Channel Type	Channel	Det. Units	Channel
1	☑	DanielDias	2:B,2	Unknown			2673	☐		1.00000	WineRib10mgCat10mg45dark_60m_r1	1.00000	2D	440extract	AU	PDA 44
2	☑	DanielDias	2:B,3	Unknown			2676	☐		1.00000	WineRib10mgCat10mg45dark_60m_r2	1.00000	2D	440extract	AU	PDA 44
3	☑	DanielDias	2:B,4	Unknown			2679	☐		1.00000	WineRib10mgCat10mg45dark_60m_r3	1.00000	2D	440extract	AU	PDA 44

5.3.5 Summary

The preliminary experiments summarised in this Section open up several questions regarding the possibility of using riboflavin as a 'protecting agent' to prevent or minimise the development of phenolic-derived pigments that are formed during the exposure of white wine to short wavelength radiation. Riboflavin absorbs the <400 nm radiation and appears thereby to stop catechin-type phenolic pigment development. The nature of the pigmentation that was observed post degradation of the riboflavin has not yet been identified, but tentative evidence was obtained to suggest that it may be due more to a degradation product of riboflavin rather than a phenolic-based pigment.

A more detailed study is therefore required to determine the mechanism by which riboflavin induces phenolic protection. This potential advantage of riboflavin against light-induced pigmentation will need to be balanced against the reported role of light impact on riboflavin causing off-flavour development. A chemical and sensory analysis project would be required to examine the dual role that riboflavin may have in white wine.

5.4 Molecular oxygen[4]

Molecular oxygen, whether dissolved or in the bottle headspace, is likely to contribute to the oxidative processes involved in the formation of pigments based on catechin-type phenolic compounds. This section of the report describes experiments designed to determine the impact of molecular oxygen on pigment development in a Chardonnay wine. Measurement of dissolved and headspace oxygen was performed using a Presens analyser (Fibox 3 LCD v7). Only Flint glass bottles were used as the measurement of oxygen levels using the Presens analyser is more effective when the glass is not coloured. 'Low' and 'High' oxygen levels were generated artificially as described below.

[4] This experiment was carried out in conjunction with Dr Andrew Clark of the NWGIC in Wagga Wagga and used the NWGIC's Presens analyser.

5.4.1 Experimental approach

The general experimental approach adopted was:

- a Chardonnay sample was prepared by adding (+)-catechin (100 mg/L) and allowing the wine to equilibrate in darkness for 2 hours;
- 6 Flint glass bottles were prepared by placing one oxygen sensor just above the punt (for dissolved oxygen measurements) and another in the bottle neck (for headspace measurements);
- approximately 740 mL of the Chardonnay wine was added to each bottle;
- the wine in 3 bottles was flushed with carbon dioxide (CO_2) for 5 mins and sealed with a screw cap ('Low' oxygen): the bottles were stored for 7 days to equilibrate before irradiation experiments;
- the wine in the other 3 bottles was designated as 'High' oxygen: the bottles were stored for 7 days to equilibrate before irradiation experiments;
- during the irradiation process, the 'High' oxygen bottles were aerated each day for 1 hour, sealed and allowed to equilibrate for 30 mins prior to irradiation.

5.4.2 Preliminary wine analysis

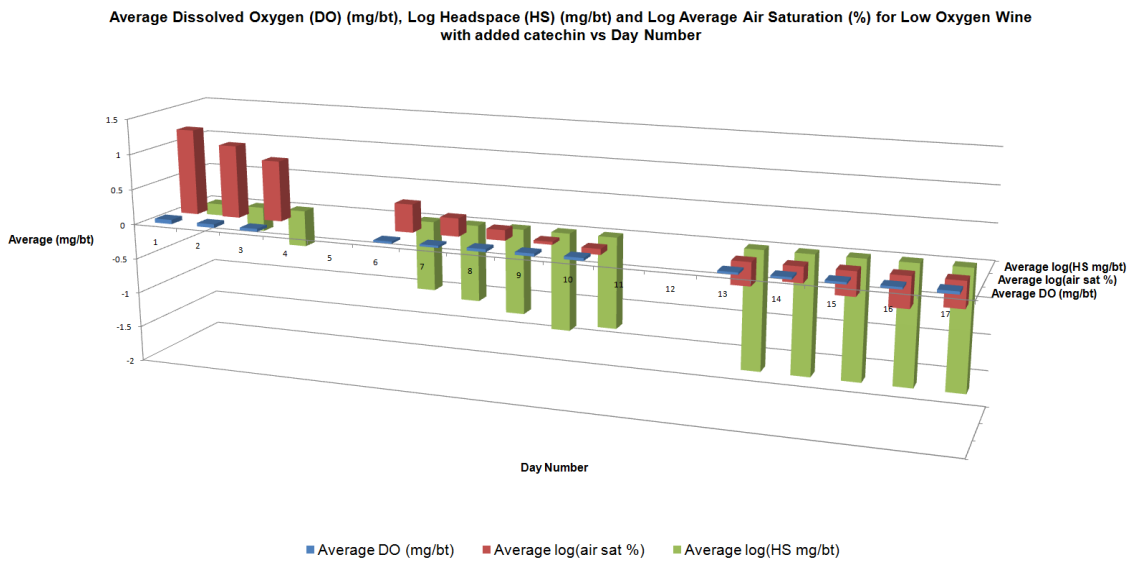
The wine was analysed for oxygen scavenging components, sulfur dioxide and ascorbic acid prior to bottling. The free and total sulfur dioxide concentrations measured (flow analysis method) were 30.0 mg/L (mean of 3; range 29.7 – 30.4) and 146 mg/L (mean of 3; range 143 – 150) respectively. The ascorbic acid concentration was measured by UPLC (calibration curve) and found to be 260 mg/L (n = 3).

5.4.3 Variation in oxygen levels during irradiation

Figures 76 ('Low' oxygen) and 77 ('High' oxygen) present data in graphical format, averaged over the 3 bottles in each category, of the changes in the measured parameters for headspace oxygen (as average log (HS mg/bottle); green bars), % air saturation (as average log(air sat%); red bars) and dissolved oxygen (average DO mg/bottle; blue bars). All experimental values are listed in Appendix 5.

The loss of headspace oxygen in the 'Low' oxygen case (Figure 76) is consistent with the consumption of oxygen by various wine components and then stabilisation as the oxygen was not being replenished.

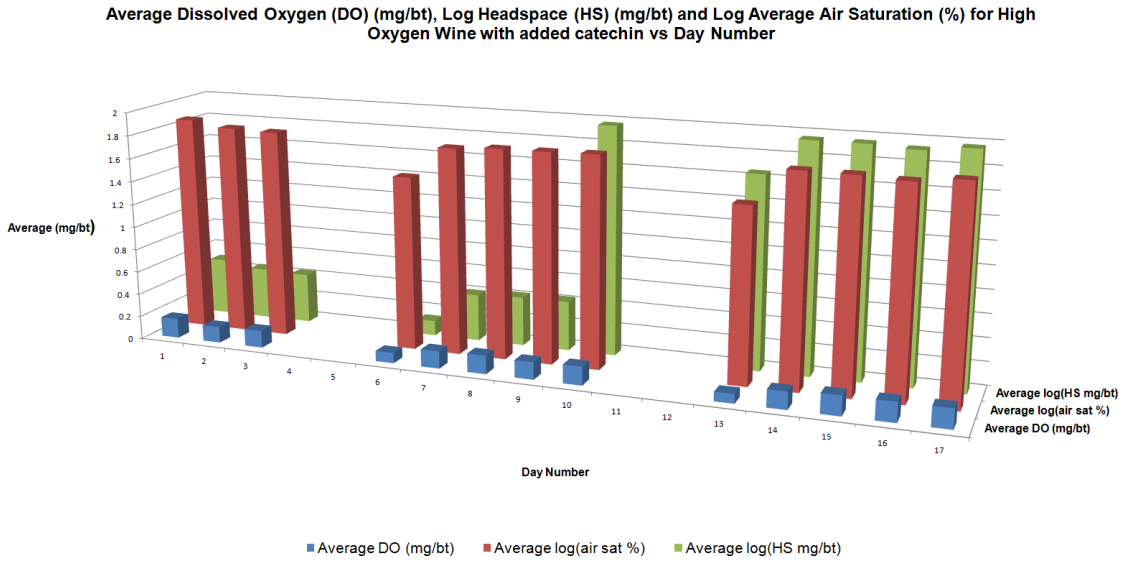
Figure 76. Plot showing average (DO), average log (HS) and average log (air saturation %) for 'Low' oxygen samples. See text for details.



In the 'High' oxygen case (Figure 77) shows that even with daily aeration, the headspace oxygen is consumed up to day 9 after which it remains at a high level. Visual inspection of the wines at day 10 indicated that they were 'browner' than the 'Low' oxygen wines. These 'High' oxygen wines became progressively darker from day 10 to day 17.

These observations are significant and are in accord with the comments of Bradshaw *et al.* (2004) that oxygen may only be required to initiate the reactions that lead to pigmentation. That is, there may be a critical point after which oxygen exposure is not needed for on-going pigmentation development. Further study of this point is vital.

Figure 77. Plot showing average (DO), average log (HS) and average log (air saturation %) for 'High' oxygen samples. See text for details.



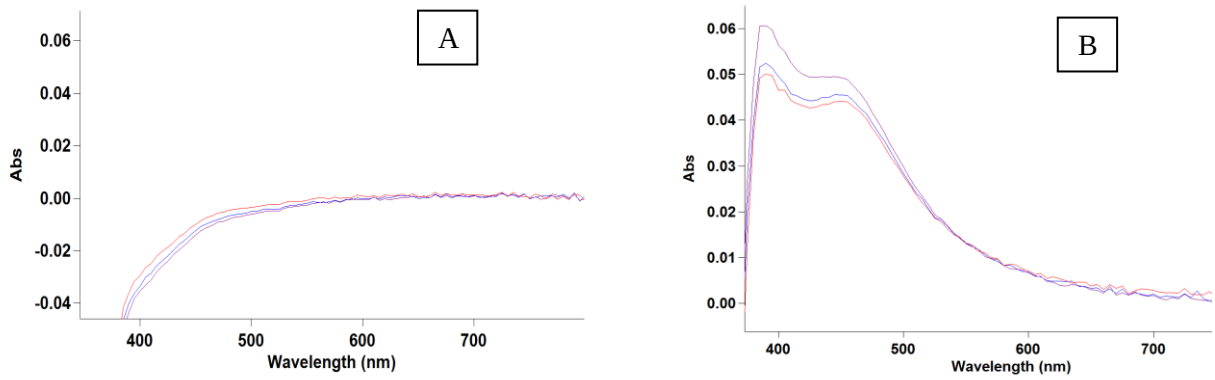
5.4.4 Pigmentation development

Images of the bottles taken after the irradiation period are shown in Figure 78. It is apparent that oxygen is important for the development of pigmentation (left hand set are 'High' oxygen). This requirement for oxygen is reinforced by the difference absorption spectra in Figure 79. Only the oxygenated samples showed an increase in pigmentation after the 17 days exposure to radiation.

Figure 78. Image showing 'Low' (right) and 'High' (left) oxygenated bottles after irradiation for 17 days.

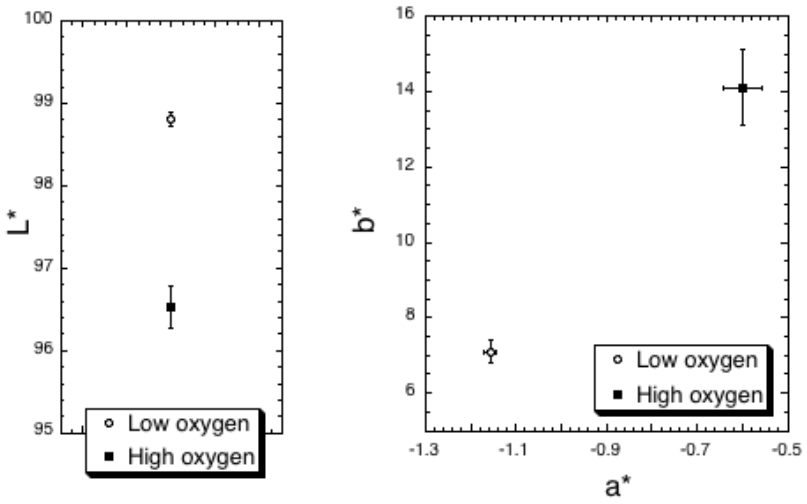


Figure 79. Difference absorption spectra for the 'Low' (A) and 'High' (B) samples after irradiation for 17 days.



CIELab data for the 'High' and 'Low' oxygen samples are plotted in Figure 80. The *L** parameter indicates 'less lightness' in the 'High' oxygen samples, in accord with the images in Figure 78. The relative positions of the 'High' and 'Low' oxygen samples on the *b*/a** plot indicate that there is more red and yellow in the 'High' oxygen samples.

Figure 80. CIELab data for the 'Low' and 'High' oxygen samples (triplicate samples).



5.4.5 Identification of pigments by UPLC

Xanthylium pigments were identified in Section 4.3 as one of the contributors to pigmentation development following exposure of a Chardonnay wine to light. UPLC chromatograms were recorded for the 'High' and 'Low' oxygen samples used in this experiment to determine the extent to which oxygen is important in their formation.

Figure 81 presents the 280 nm UPLC chromatogram for the 'High' and 'Low' oxygen samples. The peak labelled 'Catechin' refers to the phenolic compound that is the building block for the pigments. The 'High' oxygen sample (black trace) shows that less catechin remains unreacted after 17 days exposure than the 'Low' oxygen sample (red trace). The 440 nm UPLC chromatogram (Figure 82) shows the presence of xanthylium pigments in the 'High' oxygen sample only. Other pigments may also be present, but a full scale chromatographic separation could not be fitted in to the time scale of this pilot project.

Figure 81. UPLC chromatogram at 280 nm for the 'High' and 'Low' oxygen samples after 17 days irradiation.

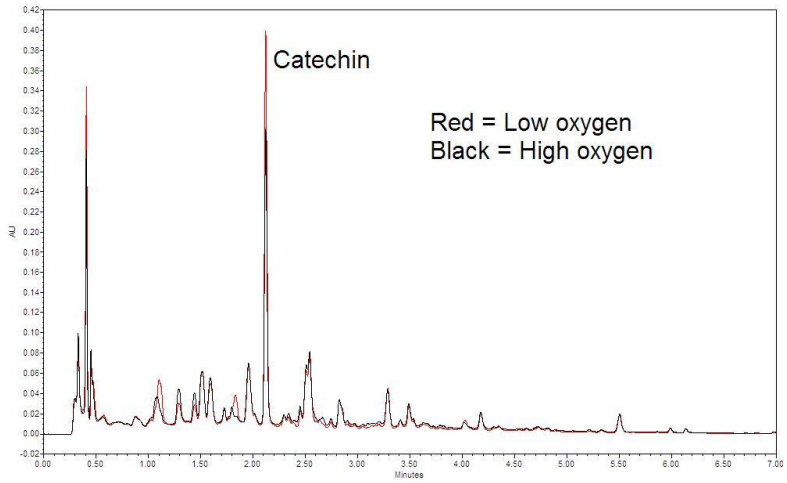
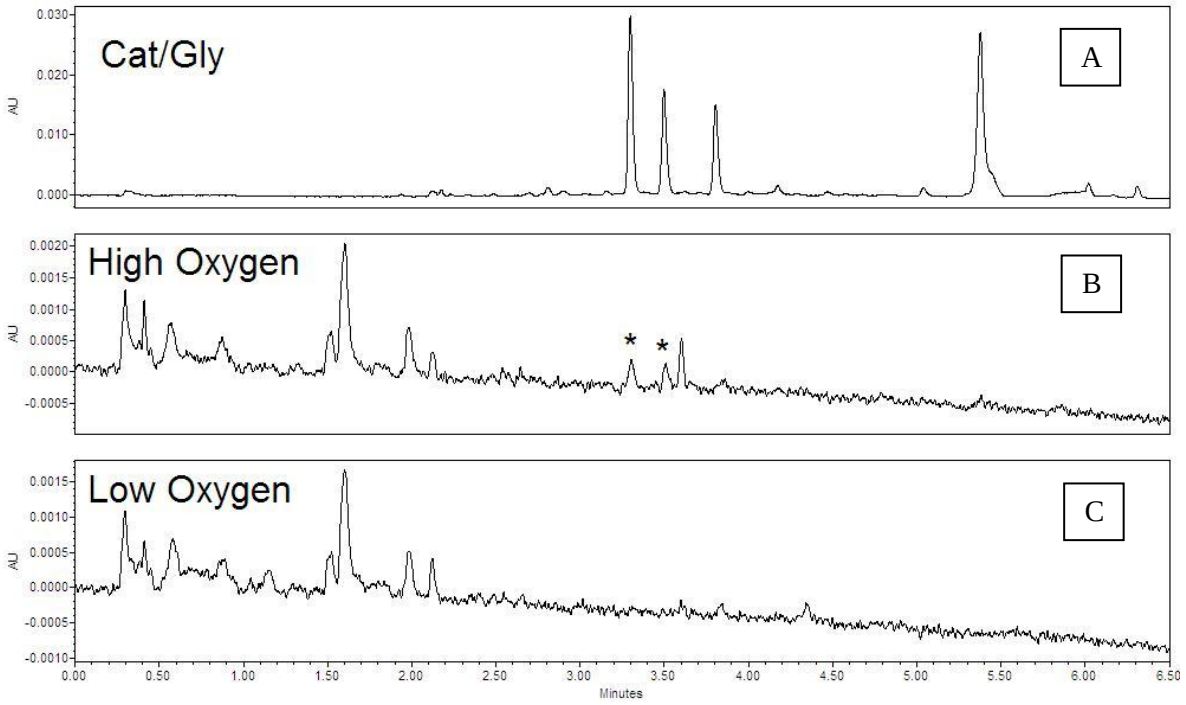


Figure 82. UPLC chromatogram at 440 nm for the 'High' and 'Low' oxygen samples after 17 days irradiation. **A**: xanthylium standard; **B**: 'High' oxygen sample with xanthylium pigments marked with *; **C**: 'Low' oxygen sample.



5.4.6 Summary

It is evident that molecular oxygen plays an important role in the production of pigments during irradiation with light. Pigmentation is greater in the bottles that were exposed to a high oxygen environment and xanthylium pigments could only detected in the high oxygen samples.

Although preliminary, the results in this Section of the project suggest a pathway for the phenomenon of 'random oxidation'. The results gained here demonstrate that both light and oxygen are critical for pigment production. Thus in situations where the oxygen concentration within a bottle varies, from (for example) poor seals, an environment is established that will allow pigmentation to develop if the wine is exposed to light.

Only one type of pigment was identified in these experiments and further work is required to determine if other pigments are also formed. The development of pigmentation under the conditions used here needs to be correlated with changes in the concentrations of the oxygen-scavenging components of the wine matrix, sulfur dioxide and ascorbic acid. Further, the role of variable oxygen concentrations, both dissolved and headspace, on pigmentation needs to be assessed, rather than just the two extreme situations used here. The link between changes in either the dissolved or headspace concentrations and pigment development would provide a much stronger basis for developing criteria to minimise the random oxidation phenomenon.

6. Potential activity of glass surface

The surface of glass is well-known to activate some chemical processes and, given the different compositions of glass used to make the bottles that were employed in this project, two experiments were performed to check for surface activity:

- measurement of the loss of dissolved oxygen on bottling

- decay of ascorbic acid in a model wine system.

These experiments were chosen as they both involve oxidative processes and the general chemistry that has been examined here is clearly oxidation chemistry.

6.1 Dissolved oxygen decay[5]

This experiment was carried out to compare the rate of loss of dissolved oxygen in wine as a function of bottle type. Light weighted Flint and Arctic Blue bottles were chosen as these colours are the only two of the four used in this project that are compatible with the oxygen measurement technique that was used. A Prensens analyser (Fibox 3 LCD v7) was used to measure the dissolved oxygen concentration. The response of the sensors to atmospheric oxygen is instantaneous, but sensors in solution require an equilibration or wetting period. Once 'wet', the response of the sensors to dissolved oxygen is rapid. The procedure described below contains this wetting period. The instrumentation also requires input of the sample temperature: this was measured in a separate bottle to those used for the dissolved oxygen measurements.

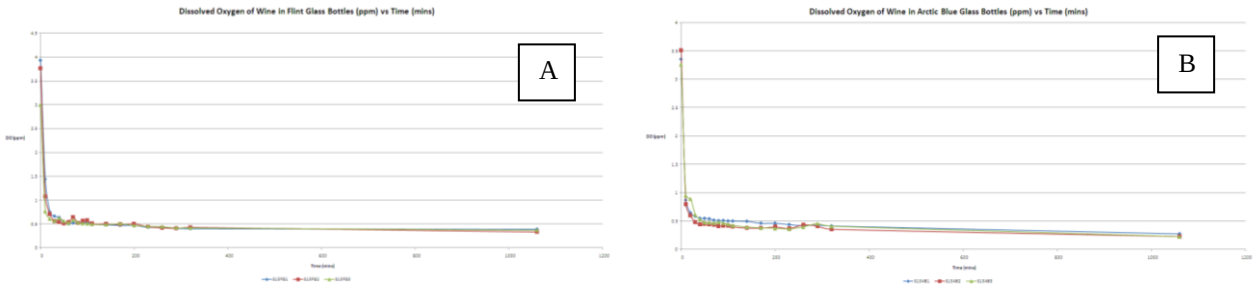
The experimental procedure that was adopted was:

- Flint and Arctic Blue bottles were prepared in triplicate
- a Chardonnay wine was used for the trial
- an oxygen sensor was placed in the lower section of each bottle and left overnight in air to equilibrate;
- the next morning, the Chardonnay wine was subsequently added to each bottle to just above the level of the sensor (wetting step);
- after 30 mins, this wine was removed and the bottles were filled to the top of the bottle (no headspace) with a fresh sample of the same wine
- dissolved oxygen measurements were made at time zero, immediately after filling, and at 10 mins intervals for the first 110 mins and then at 30 mins intervals up to 320 mins with the final measurement being made the next morning (16 hrs after the experiment began);
- one additional Flint and Arctic Blue bottle was filled with the Chardonnay and a temperature probe placed in the bottle to allow accurate recording of the temperature during the experiment.

Figure 83 presents a graphical representation of the data for the triplicate measurements in each bottle type. Actual dissolved oxygen measurements are given in Appendix 5. Although there is some difference in the starting value between the Flint and Arctic Blue bottles, the rapid loss of dissolved oxygen in the first measurement period is the same for both bottle types and the final value is the same in both cases. There is also no significant difference between the triplicate samples for the Flint and Arctic Blue bottles. In essence, the data from this experiment indicate that there is no influence of bottle colour, and hence surface chemical composition, on the decay of dissolved oxygen post-bottling.

[5] This experiment was carried out in conjunction with Dr Andrew Clark of the NWGIC in Wagga Wagga and used the NWGIC's Presens analyser.

Figure 83. Assessment of the decay of dissolved oxygen in wine stored in Flint (A) and Arctic Blue (B) bottles.



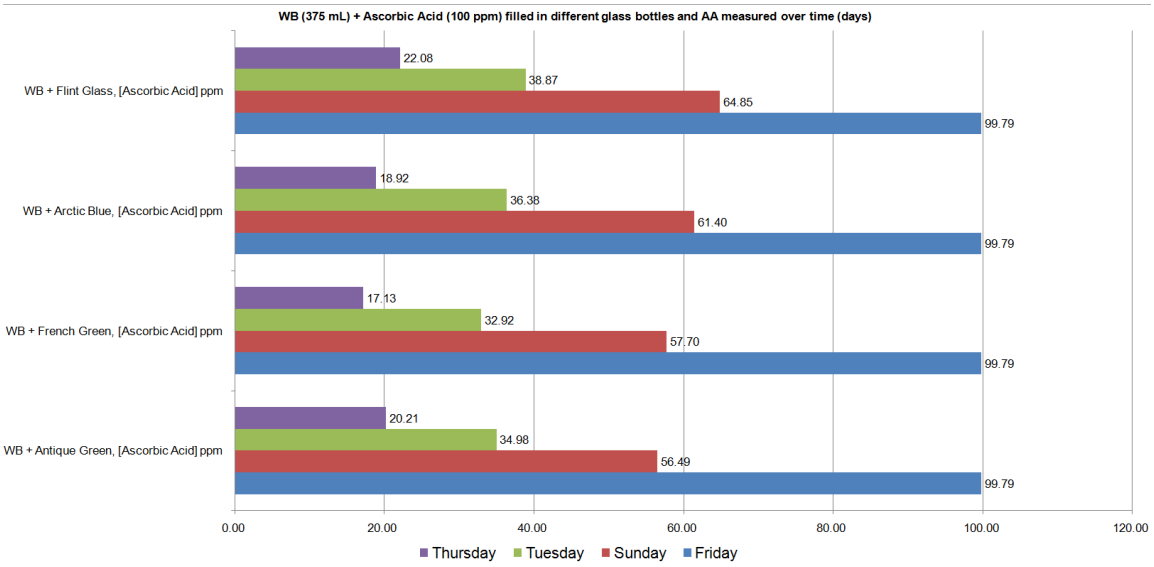
6.2 Decay of ascorbic acid in a model wine system

Ascorbic acid (100 mg/Litre) was added to a model wine system (see Method for details of preparation). The solution was then added to Flint, Arctic Blue, French Green and Antique Green bottles in triplicate (after flushing with nitrogen) and the bottles stored away from light at room temperature. The concentration of ascorbic acid was monitored on four occasions over a six day period by titration with 2,6-dichlorophenolindophenol. Table 8 sets out the details of the samples used and the results are presented in Figure 84.

Table 8. Details of samples used in the ascorbic acid decay experiment.

Code	**Bottle Colour**	
S14AG1	Antique Green	Model Wine + *100* mg/L ascorbic acid
S14AG2	Antique Green	Model Wine + *100* mg/L ascorbic acid
S14AG3	Antique Green	Model Wine + *100* mg/L ascorbic acid
S14FRG1	French Green	Model Wine + *100* mg/L ascorbic acid
S14FRG2	French Green	Model Wine + *100* mg/L ascorbic acid
S14FRG3	French Green	Model Wine + *100* mg/L ascorbic acid
S14AB1	Arctic Blue	Model Wine + *100* mg/L ascorbic acid
S14AB2	Arctic Blue	Model Wine + *100* mg/L ascorbic acid
S14AB3	Arctic Blue	Model Wine + *100* mg/L ascorbic acid
S14FG1	Flint Glass	Model Wine + *100* mg/L ascorbic acid
S14FG2	Flint Glass	Model Wine + *100* mg/L ascorbic acid
S14FG3	Flint Glass	Model Wine + *100* mg/L ascorbic acid

Figure 84. Comparison of the rates of decay of ascorbic acid in a model wine system stored in different bottle types over a six day period (first day = Friday; last day = Thursday).

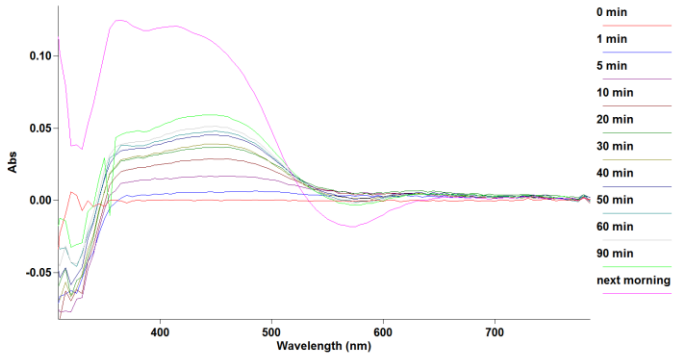


It is apparent from the data in Figure 84 that the decay rates are identical in all bottle types, again confirming (as with the dissolved oxygen decay rates) that there is no influence of the surface of the glass bottle on the solution chemistry oxidative processes.

7. Preliminary study of light impacts on red wine

A preliminary study on the effect of low wavelength radiation on red wine was carried out. The difference absorption spectra for a red wine to which (-)-epicatechin (100 mg/L) had been added and exposed to radiation < 400 nm are shown in Figure 85. There is a very clear increase in absorbance between about 380 nm to 480 nm without any obvious maximum absorbance, suggesting that multiple compounds may be forming. There is also a marked decrease in absorbance below about 350 nm, indicating that significant structural re-arrangement of phenolic compounds is occurring. The spectra in Figure 85 also show a decrease in absorbance centred around 550 nm, particularly after 16 hrs exposure (curve labelled 'next morning'). This corresponds to the region where red wine colour is measured (520 nm is the industry standard). The loss of absorbance suggests that bleaching of the pigmented species in red wine is occurring.

Figure 85. Difference absorption spectra for a red wine containing added (-)-epicatechin (100 mg/L) during exposure to radiation < 400 nm at 45^{o}C.



Further work is required to identify the processes and changes in phenolic composition that are occurring during exposure of a red wine to light.

OUTCOMES/CONCLUSIONS

This pilot project was designed to examine the influence of light, temperature, glass transmission (colour and bottle thickness) which impacts on the development of pigmentation or enhanced colour of white wine. Particular focus was made on the relationship between the wavelength of radiation necessary for the photo-activation process and glass transmissivity. In addition to these environmental variables, the link between various wine components (type of phenolic compound, iron(III) tartrate, riboflavin and molecular oxygen was also investigated).

To achieve these goals, two experimental strategies were used:

- a small scale wine irradiation setup which shows the impact of light (ultraviolet, low visible light wavelengths and specific wavelengths of light) on white wine under controlled/uncontrolled temperature conditions;
- a large scale wine irradiation setup allowed for whole wine bottles to be irradiated for a set-time under controlled/uncontrolled temperature conditions which shows the impact of bottle glass colour.

The main findings of this study were:

- Ultraviolet and, to a lesser extent, low wavelength visible light contributes to pigment production in white wine under controlled temperature conditions
- Ultraviolet light causes greater pigmentation in wine compared to red, orange and yellow light
- Temperature, that is heating wine in darkness at 25°C, 45°C, 50°C and 60°C does contribute to pigment development but not to the extent when UV light is present
- The transmission of ultraviolet light is greatest through Flint > Arctic Blue > French Green and Antique Green
- All glass types allow the transmission of low wavelength ultraviolet light to pass through to the wine, resulting in pigmentation development
- There is no significant difference in light induced wine pigmentation in the visible region between heavy and light weighted bottles of the same colour
- Under controlled temperature and light conditions xanthylium type compounds were identified by UPLC and LC-MS. Greater pigment production was observed in Flint> Arctic Blue > French Green > Antique Green bottles
- Under uncontrolled temperature and light conditions unknown pigments were identified, but not characterized by UPLC and LC-MS. Greater pigment production was observed in Antique Green > French Green > Arctic Blue and Flint bottles
- Aroma (descriptors) and colour profiles (CIELab) validated these findings
- Iron tartrate was found to be the photoactive species responsible for the degradation of tartaric acid to glyoxylic acid;
    - the presence of iron is essential for the production of glyoxylic acid;
    - the presence of light is essential for the production of glyoxylic acid;
    - the absence of ethanol decreased the amount of glyoxylic acid produced.
    - the relationship between wine colour development and bottle colour and thickness showed the highest pigmentation levels were found when wine samples were exposed to light with a Flint glass filter in place and the lowest levels when an Antique Green glass filter was used.
- Riboflavin was found to be a possible ‘protecting agent’ to prevent or minimise the development of phenolic-derived pigments that are formed during the exposure of white wine to short wavelength radiation.

- Molecular oxygen plays an important role in the production of pigments during irradiation with light. Pigmentation is greater in the bottles that were exposed to a high oxygen environment and xanthylium pigments could only detected in the high oxygen samples.
- The decay rates of ascorbic acid in Flint, Arctic Blue, French Green and Antique Green bottles were found to be identical in all bottle types; that is, there is no influence of the surface of the glass bottle on the solution chemistry oxidative processes.
- Pigment development was also identified in preliminary studies on red wine.

The communication strategies adopted for the dissemination of the research outputs to the wine industry and the scientific community are:

- One published industry technical paper, with a further two in preparation;
- One wine industry technical conference poster presentation and one chemistry conference poster presentation with a second chemistry conference presentation to occur in November 2010;
- Three scientific papers are in preparation;
- Two multiple day programs for Year 10 science students as part of the 'Growing Tall Poppies' initiative.

Collaboration with Dr Andrew Clark and John Blackman of the National Wine and Grape Industry Centre was established during this project, especially through the sharing of equipment and facilities. The outputs of the project will be fed into the oenology teaching program at CSU.

RECOMMENDATIONS

There are no formal recommendations arising from this one-year pilot project. The results obtained show a clear influence of light on the development of pigmentation and the research program has identified a series of issues that require further experimentation to generate a complete account of the impact of light on wine stability.

The results show that wine stored in lighter coloured bottles develops higher levels of pigmentation when exposed to low wavelength visible/UV radiation provided the temperature is controlled. In an uncontrolled temperature situation, wine in darker bottles develops more colour as a result of pigmentation reactions. These results have major implications for the proper storage and transport of white wine.

Appendix 1: Communication

Scientific (refereed) publications in preparation
Differentiation of light and temperatures effects on pigment development in white wine.

Iron(III) tartrate as a potential precursor of light induced oxidative degradation of white wine.

Influence of riboflavin and molecular oxygen on the light induced oxidative degradation of white wine.

Industry technical articles
Published
Daniel A Dias, Kenneth P Ghiggino, Trevor A Smith and Geoffrey R Scollary (2010). Ultraviolet light – a contributing factor to pigment development in white wine. Australian and New Zealand Wine Industry Journal, 25(3), 52-61.

In Preparation
Chemical and environmental factors that influence light induced oxidative degradation of white wine. For the Australian and New Zealand Wine Industry Journal

Caring for your wine – what type of glass bottle to use? For Practical Winery & Vineyard (by invitation)

Conference presentations (posters)

1. Ghiggino KP, Smith TA, Dias D and Scollary, GR. "Photodecomposition of wine and the effects of bottle glass colour" The 13th RACI National Convention. Chemistry for a Sustainable World, Melbourne, Australia, 4th – 8th July, **2010**.

2. Dias D, Smith TA, Ghiggino KP and Scollary, GR. "The photo- and thermo-oxidative spoilage of white wine and effect of bottle colour" The 14th Australian Wine Industry Technical Conference (AWITC), Adelaide, Australia, 3rd – 8th July, **2010**.

3. Dias D, Smith TA, Ghiggino KP and Scollary, GR. "Photodecomposition of wine and the effects of bottle glass colour", 6th Asian Photochemistry Conference, Wellington, New Zealand, 14th – 18th Nov, **2010**.

Presentation to school students

Presentation to Year 10 students from Santa Maria College, Northcote, February 15th-18th and March 1st-5th as part of the Growing Tall Poppies program.
See also http://coecxs.org/index.php?mod=Dynamic&id=96

Appendix 2: Intellectual Property

The project outputs are in the form of new scientific knowledge that is and will be published in scientific and wine industry technical journals. The project outputs have generated scientific 'know-how' for industry's benefit.

Appendix 3: References

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Appendix 4: Staff

The following table lists staff involved in the project. The role of each person and the funding source is also identified.

Name	Agency	Role in project	Funding
Dr Geoffrey Scollary	The University of Melbourne	Project Supervisor	University of Melbourne In-kind
Professor Ken Ghiggino	The University of Melbourne	Chief Investigator	University of Melbourne In-kind
Associate Professor Trevor Smith	The University of Melbourne	Chief Investigator	University of Melbourne In-kind
Dr Daniel Dias	The University of Melbourne	Research Fellow	GWRDC funds
Dr Andrew Clark	NWGIC, CSU	Collaborator	CSU in-kind
Mr John Blackman	NWGIC, CSU	Collaborator	CSU in-kind

Appendix 5: Raw Data

Dissolved oxygen data relevant to Section 6.1 are presented in the following two tables.

Dissolved oxygen measurements for a Chardonnay in Flint (light weighted) bottles.

Time (mins)	S13FG1	DO (ppm)	S13FG2	DO (ppm)	S13FG3	DO (ppm)
0	10.35	3.93	10.37	3.77	10.39	3
10	10.45	1.44	10.47	1.08	10.49	0.7645
20	10.55	0.7495	10.57	0.7052	10.59	0.6064
30	11.05	0.664	11.07	0.5639	11.09	0.5618
40	11.15	0.6375	11.17	0.5456	11.19	0.6219
50	11.25	0.5343	11.27	0.5151	11.29	0.5604
60	11.35	0.5044	11.37	0.5415	11.39	0.5186
70	11.45	0.5215	11.47	0.6475	11.49	0.5806
80	11.55	0.523	11.57	0.5202	11.59	0.529
90	12.05	0.5288	12.07	0.5667	12.09	0.5121
100	12.15	0.529	12.17	0.5788	12.19	0.5073
110	12.25	0.4972	12.27	0.5113	12.29	0.4951
140	12.55	0.4873	12.57	0.5064	12.59	0.4877
170	1.25	0.4728	1.27	0.4921	1.29	0.5075
200	1.55	0.4705	1.57	0.5077	1.59	0.469
230	2.25	0.4341	2.27	0.4436	2.29	0.4493
260	2.55	0.4357	2.57	0.4236	2.59	0.4445
290	3.25	0.4122	3.27	0.4121	3.29	0.4196
320	3.55	0.4044	3.57	0.4335	3.59	0.4181
1060	9.35	0.3912	9.35	0.3364	9.35	0.3703

Table X: Dissolved oxygen measurements for a Chardonnay in Arctic Blue (light weighted) bottles.

Time (mins)	S13AB1	DO (ppm)	S13AB2	DO (ppm)	S13AB3	DO (ppm)
0	10.41	3.36	10.43	3.51	10.44	3.26
10	10.51	0.8686	10.53	0.797	10.54	0.9533
20	11.01	0.6443	11.03	0.5971	11.04	0.8923
30	11.11	0.5868	11.13	0.4829	11.14	0.6153
40	11.21	0.5485	11.23	0.4421	11.24	0.5288
50	11.31	0.5469	11.33	0.4479	11.34	0.4776
60	11.41	0.5401	11.43	0.4427	11.44	0.4751
70	11.51	0.5149	11.53	0.4369	11.54	0.4677
80	12.01	0.5074	12.03	0.4126	12.04	0.4815
90	12.11	0.5091	12.13	0.4203	12.14	0.4657
100	12.21	0.5013	12.23	0.4192	12.24	0.4458
110	12.31	0.4997	12.33	0.4007	12.34	0.4219
140	1.01	0.4954	1.03	0.378	1.04	0.3947
170	1.31	0.4608	1.33	0.3789	1.34	0.3808
200	2.01	0.461	2.03	0.3994	2.04	0.3711
230	2.31	0.4379	2.33	0.3735	2.34	0.3616
260	3.01	0.4202	3.03	0.4375	3.04	0.3967
290	3.31	0.4308	3.33	0.4131	3.34	0.4562
320	4.01	0.4148	4.03	0.359	4.04	0.4084
1060	9.35	0.2745	9.35	0.2297	9.35	0.2242

Appendix 6: Budget Reconciliation

Appended as a separate document.