Modeling White Wine Changes Arising From Radiant Heat Exposure

Honours Thesis

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Table of Contents

Section #	Title	Page #
	Title Page	1
	Author's Declaration	2
	Acknowledgements	2
	Table of Contents	3
	Abstract	5
1.	Introduction	6
2.	Theory	10
2.1	Browning: Oxidative Browning	10
2.1.1	Enzymatic Browning	10
2.1.2	Non-Enzymatic Browning	11
2.1.2.1	Oxidation of Wine Phenols	11
2.2	Sulfur Dioxide	14
2.2.1	Chemistry of Sulfur Dioxide	15
2.2.1.1	Free Sulfur Dioxide	15
2.2.1.2	Bound Sulfur Dioxide	16
2.2.1.3	Free versus Bound SO_2	18
2.2.1.4	SO_2 and Non-enzymatic Browning	18
2.3	Colour and Colour Measurement	20
2.4	Other Quality Parameters	21
2.4.1	Aroma/Sensory	21
2.4.2	Haze and Precipitation Issues	22
3.	Material and Methods	23
3.1	Wine	23
3.1.1	Wine Production	23
3.1.2	Bottling	24
3.1.2.1	Pre-bottling Analysis	24
3.1.2.1.1	Stability Tests	24
3.1.2.1.1.1	Tartrate Stability	24
3.1.2.1.1.2	Protein Stability	25
3.1.2.2	Filtration	26
3.1.2.3	Bottling 100 mL and 750 mL Bottles	26
3.1.2.4	Post Bottling Analysis	27
3.2	Heat Treatments	27
3.2.1	Heat Summation	28
3.3	Analysis Timetable	28

3.4	Analysis Methods	29
3.4.1	FT-IR Method	29
3.4.2	UV-Vis Method	30
3.4.3	Colour Space – CIELab	30
3.4.4	FIASTAR 5000 with auto sampler	31
3.4.4.1	Free SO_2 Method	31
3.4.4.2	Total SO_2 Method	31
3.5	Sensory Assessment	32
3.6	Statistical Analysis	32
3.6.1	Initial Statistical Treatment	32
3.6.2	PARAFAC Analaysis	32
3.6.2.1	CIELab and Sulfur Dioxide	33
3.6.2.2	UV-Vis	33
3.6.2.3	FT-IR	33
4.	Results and Discussion	34
4.1	Wine Analysis	34
4.1.1	Pre and Post Bottling Analysis	34
4.1.2	Wine Analysis–Initial(Day0) and Final(Day57)	34
4.1.3	Sulfur Dioxide	36
4.1.3.1	Free Sulfur Dioxide Results	36
4.1.3.2	Total Sulfur Dioxide Results	39
4.1.4	Colour – CIELAb Results	43
4.1.4.1	‘L*’ Results	43
4.1.4.2	‘a*’ Results	45
4.1.4.3	‘b*’Results	47
4.1.4.4	‘a*’ vs ’b*’	49
4.1.4.5	Overall CIELab	50
4.2	PARAFAC Results	51
4.2.1	PARAFAC – Combined CIELab and SO_2	52
4.2.2	PARAFAC – UV-Vis	55
4.2.3	PARAFAC – FT-IR	58
4.3	Sensory Assessment	61
5.	Conclusion	63
6.	Bibliography	65

Abstract

Bottled white wine is subject to many environmental influences between production and consumption. Radiant heat exposure is probably the most likely to occur and has the potential to cause chemical and sensory changes to the wine, both of which can have consequences for the wine's perceived value.

This research project set out to identify whether the chemical changes could be modeled by subjecting a commercial white wine to varying degrees of heat exposure over an extended period of time with analysis of key components at regular intervals carried out using instrumental methods. The instruments used were ultra violet-visible (UV-Vis) and Fourier transform infra red (FT-IR) spectrophotometers and a specific sulfur dioxide auto-analyser used by wine industry. The instruments and methods were chosen as they were able to be replicated in winery laboratories. The UV-Vis was utilized to provide both spectral data and to produce standard colour space values using the CIELab scales. There was also limited sensory assessment carried out to determine if the treatments had produced significant sensory changes by the end of the storage period.

The results were then processed using chemometrics (specifically the PARAFAC methodology) to identify the key components and produce a model which explains the behavior of the components.

The project found that there were significant differences between the varying levels of radiant heat exposure with wine exposed to higher temperatures appearing markedly different in both chemical and sensory terms.

Using PARAFAC, it was found that valid models of compositional change could be developed using the UV-Vis scans and the CIELab and sulfur dioxide data. It was found that the FT-IR models were unable to produce valid models in this project.

1. Introduction

Wine is a complex mixture of organic and inorganic components. The interaction between these elements and the environment is complex and has been, traditionally, a difficult area to study. The adoption of modern, automated instruments has provided researchers with methods that allow a large number of replicates to be analysed quickly and cheaply. The use of chemometrics then permits researchers to look for underlying patterns that can be used to develop models of wine behavior.

In this research project, the interaction between wine and heat has been studied using these techniques, with the aim of developing a model of behavior for some key wine quality parameters when bottled wine is subjected to radiant heat.

Wine is a heavily traded product with markets often at great geographic distances from the production point, especially for Australian and New Zealand producers. During storage and shipment to these markets, wine can be subjected to environmental extremes and one of the most likely to occur is heat exposure.

Much time is spent in the winery minimizing heat exposure of bulk wine in storage. Bulk storage tanks are generally kept at low, stable temperatures. However, once the wine is in bottle and released to the market, the winery has much less control over the environmental conditions the wine will be subjected too.

While temperature controlled warehousing is becoming the norm within the industry, it is common practice for wine produced in Australia to be shipped in non-refrigerated containers to the end market. Such trips may be across continental Australia during the hot summer by road or rail; or by ship to overseas markets, of which, the most important ones require passage across the equator, with significant periods of potentially high to very high ambient temperatures.

In addition to this, the great majority of bottled wine at the point of sale and in storage with the actual consumer is not stored under controlled temperature conditions. In this situation, the actual temperatures may not be all that high (room temperature), however, there will be variation of temperature over the day, usually with a low at night and a maximum during the day.

This means that nearly all wine produced in bottle experiences some degree of radiant heat exposure between bottling and final consumption.

Radiant heat is generally considered to cause undesirable effects in wine, especially white wine. These effects include increased levels of oxidative browning of wine (this is most serious in young white wine), loss of fruit aromas, development of 'cooked' aromas and haze issues to do with protein denaturation (Boulton, et al. 1996; Reynolds, et al., 2010; Margalit, 2004)). A summary (Boulton et al. 1996) of research (Ribéreau-Gayon 1933, Ough 1985) has shown that the relative rates of oxygen uptake, browning, and total SO_2 decline in wine is 270, 20.7 and 1.7 times faster at 40°C as compared to 10°C. This can result in a potential reduction in the perceived quality and value of the wine, sometimes to the point of total loss.

Minimising oxygen levels in the wine post-ferment and sulfur dioxide (SO_2) addition are the most common methods used by winemakers to mediate the effects of oxidative browning and other oxidative processes. Sulfur dioxide is used because it is oxidised preferentially to the phenolic compounds which can polymerise to form brown pigments. However, the use of SO_2 has limits for a number of reasons; too much SO_2 in a wine causes quality issues in the bouquet and palate of the wine, rendering the wine unpleasant to drink. SO_2 can also be a contributor to the formation of unpleasant 'reductive' characters in wines bottled with low oxygen pickup using near oxygen impermeable screw cap closures. There are also possible allergenic reactions associated with higher levels of SO_2 (Yang & Purchase, 1985). For these reasons, there are legal limits to additions of SO_2 to wine in all developed markets.

It is important for winemakers to have knowledge about the effects on SO_2 levels on wines over time, especially those that may be subject to heat exposure. This would allow winemakers to make better informed decisions on adjustments that may need to be made to the levels of SO_2 at bottling to compensate for temperature exposure, so as to maximize the time the wine spends at the optimum organoleptic profile for the target market.

It is also important that any model produced could be developed for use in wineries. The need here is to use instrumentation and laboratory practices that could be carried out in a typical, reasonably well equipped winery laboratory.

In this project, the same wine was subjected to four different constant temperature regimes and one cyclic temperature regime. The stable

temperature regimes were chosen to cover a spread of temperatures that may be encountered for an extended length by wine in bottle in the period between bottling and consumption. The cyclic regime was chosen to simulate the daily variations evident in possible storage conditions such as in a retail store or a consumer's wine rack.

The wines were analysed instrumentally at regular intervals and the key parameters of colour and free and total SO_s levels tracked. As well, the mid infra-red spectra were recorded to ascertain whether changes in these spectra could be used to develop a model of wine behavior during heating.

Colour is an indication of the level of oxidative browning that has occurred. Many research projects that have concentrated on measuring oxidative browning have used absorbance at A_{420} (Simpson, 1978; Salacha, et al. 2007; Peng, 1998). In these projects, increasing absorbance A_{420} has been used to indicate increased browning in the wine (Peng, 1998). In this project, a different methodology using tristimulus colorimetry is used to look at the overall changes in the colour space. The colour values were obtained by using a scanning ultra violet-visible light (UV-Vis) spectrophotometer. Such instruments equip most moderately sized winery laboratories and are easy and familiar to use for winery personnel.

The SO_2 levels were monitored using an auto-analyser that had been specifically setup for the measurement of SO_2 levels in wine. Although this instrument is not as common as UV-Vis spectrophotometers, the ability to monitor SO_2 levels is an absolute necessity for wineries and the equipment and knowledge to monitor this is universally available.

The spectrophotometer used for the mid infra-red analysis was chosen as it is currently used to monitor wine composition in wineries. It is relatively common in large wineries and laboratories that perform contract analysis for wineries. It had the advantages of being relatively easy to use, while also allowing a large number of analyses to be performed quickly.

A secondary facet of the project was to ascertain whether the use of 100 mL medicine flats would give similar results compared to wine industry standard 750 mL bottles. To evaluate this, a small number of 750 mL bottles were bottled and subjected to the same treatments and analysis as the 100 mL bottles and the results compared. This has the implication that the results obtained within this project using the 100 mL bottles (where there were large numbers of replicates) are able to be applied to 750 mL bottles satisfactorily.

The data generated from the methods employed was subject to multidimensional chemometric analysis to construct models of compositional change in the wine arising from radiant heat exposure.

2. Theory

2.1 Browning: Oxidative Browning

As one of the principal sources of quality degradation in bottled wine over time (especially white wine), it is worth elucidating the mechanism of oxidative browning further. It is also a process where temperature has been identified as factor in the rate of reaction (Robinson, et al. 2010).

There are a number of substrates in wine that are susceptible to oxidation, such as metal ions, SO_2, organic acids, ethanol and phenolic compounds. The major substrate associated with browning in wine are the phenolic compounds (Boulton et al., 1996).

Oxidative browning is generally separated into two categories – enzymatic browning and non-enzymatic browning.

2.1.1 Enzymatic Browning

Enzymatic browning of phenols generally takes place in the presence of atmospheric oxygen and polyphenol oxidase (PPO) (Li, et al., 2008). It is a commonly accepted source of browning in food (figure 1.)

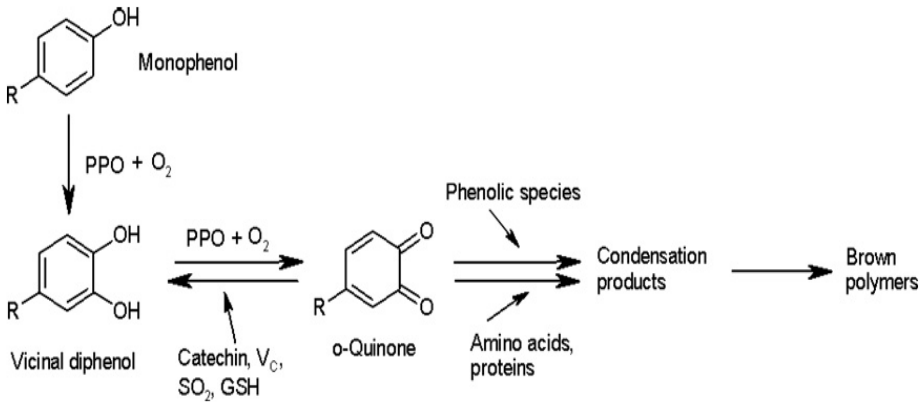


Figure 2.1: Enzymatic Browning in Grape Must. H. Li, et al. ,2008)

In the case of wine, it is a process that is most likely to occur early in the production process, when the wine is at the stages of must (crushed grapes) and unfermented juice. At this stage, SO_2 levels may be low and other treatments that may remove or inhibit the enzymatic reactions have not occurred.

Once a wine has been finished and bottled, and provided that laccase is not present, enzymatic browning is essentially eliminated (Boulton, et al., 1996).

As the grapes used in the project wine were of high quality and good winemaking practices were used to produce the wine, enzymatic browning can be deemed to have had little influence on any colour changes found.

2.1.2 Non-enzymatic Browning

Non-enzymatic browning (also referred to as chemical browning) in wine is mainly considered to be associated with oxidation of phenols (Waterhouse and Laurie, 2006). Specifically, the most susceptible groups are the *o*-Diphenols, which includes caffeic acid and related esters, catechin, epicatechin, anthocyanins and derivatives, and gallic acid, as well as the levels of flavan-3-ols (Waterhouse and Laurie, 2005).

2.1.2.1 Oxidation of wine phenols

There are several pathways that lead to browning involving phenols.

These include oxidation of phenols, followed by polymerization of the oxidized products.

o-Diphenols are oxidized to *o*-quinones and semi-quinones may be produced. Oxygen is reduced to hydrogen peroxide (H_2O_2) (Figure 2.2).

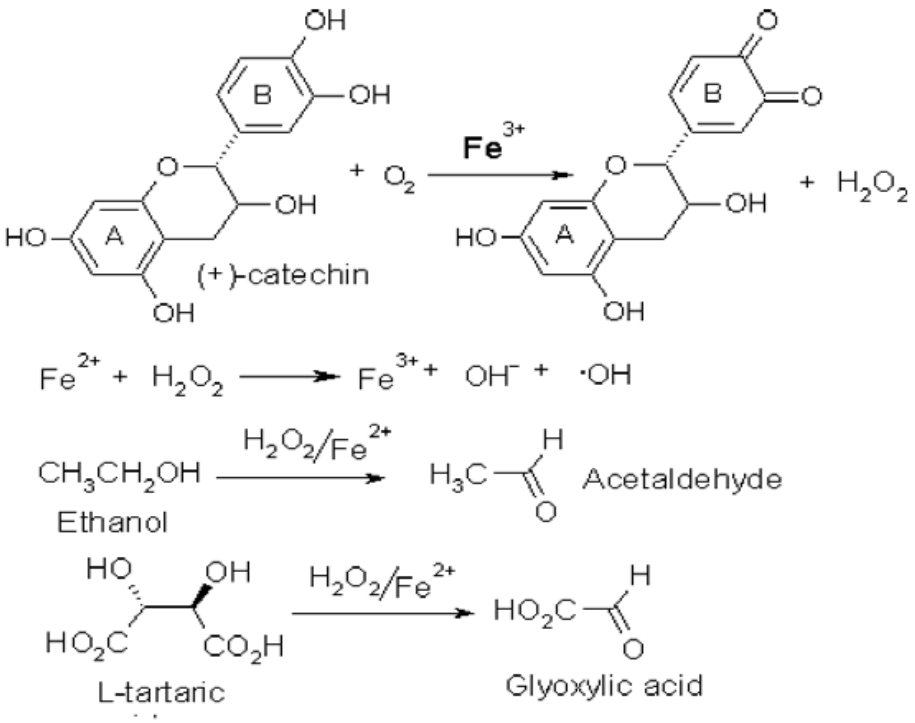


Figure 2.2: Oxidation of (+)-catechin, ethanol and L-tartaric acid (Danilewicz,2003)

The quinones formed are not stable and may continue to react, ultimately to cause pigment formation. This process is similar to that which occurs in enzymatic browning (Robards, et al, 1999; Singleton, 1987).

Figure 2.3 shows a suggested pathway, where condensation reactions occur to form coloured, high molecular weight products.

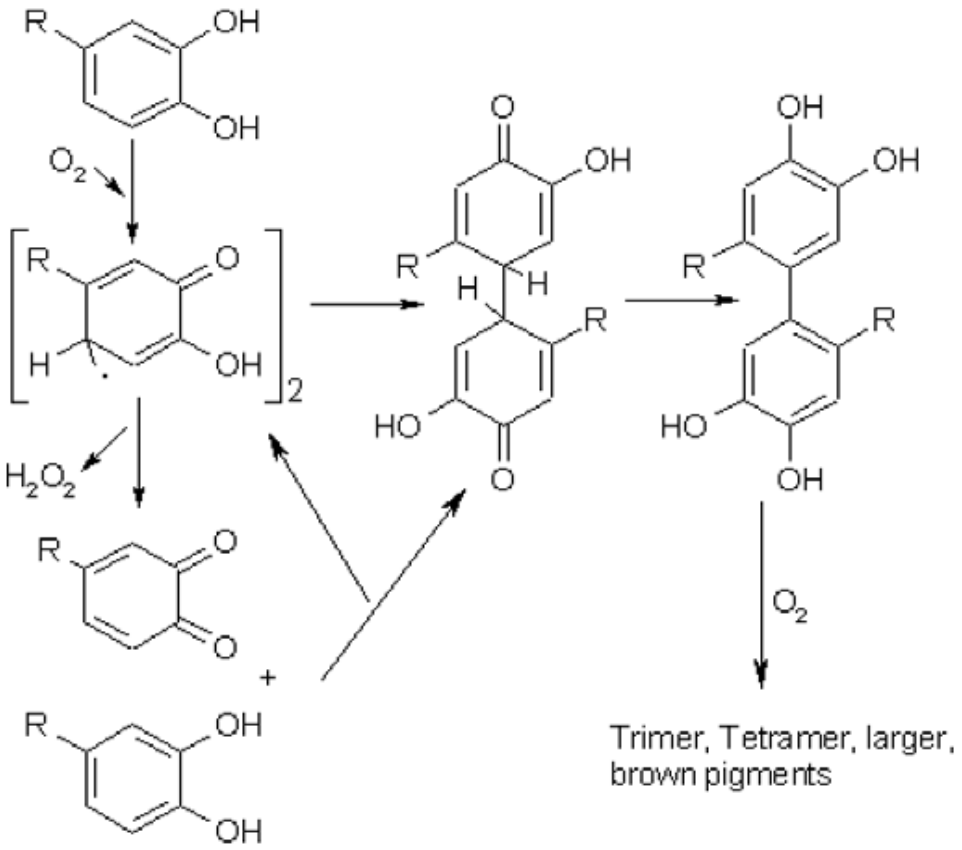


Figure 2.3: Regeneration of oligomers by reaction between twosemiquinones or a quinone and a phenol (Singleton, 1987)

High electronic affinity can cause quinones to combine with nucleophilic compounds such as phenols, sulphydryl compounds and amines. The dimers or polymers produced can undergo a structural rearrangement through enol-like conversion reactions to form new diphenols (Li, 2008,).

Figure 2.3 shows such a reaction, with a quinone-phenol dimer being converted to a new diphenol dimer.

The resultant dimers and polymers in coupled oxidation have lower redox potentials than the parent phenols, and are therefore much more susceptible to further oxidation (Boulton et al.,1996; Li et al., 2001). The regenerated *o*-diphenols can be renewably oxidized, which will lead to an acceleration of the polymerization reactions of the phenols (Boulton et al., 1996).

As this proceeds, more H_2O_2 is produced and, in the presence of transition metals, more ethanol oxidized to acetaldehyde. This leads to some phenols, which had not been originally easily oxidised, becoming susceptible through the structure rearrangement depicted in Figure 2.3 (Boulton et al., 1996).

These reactions are very fast under alkaline conditions but are very slow in acidic media such as wine (Boulton et al., 1996). Here the presence of metal ions allows the process to proceed, albeit at a slow rate.

In what, at first, seems counterintuitive, the total oxygen uptake is actually greater under acidic conditions; where the reaction speeds are slow, than in alkaline conditions, where the reaction speeds are at their greatest. Boulton (1996) attributes this to there being more polymerization regenerating oxidisable units under the slower, acidic conditions.

In the case of the fast reaction, if oxidisable phenols are being rapidly converted to quinones, the slower polymerization reactions are minimized. Once the phenol pool is exhausted, there can be no further reaction with quinones and oxidation ceases (Boulton et al., 1996).

In the media with slow reaction times, fewer quinones may be formed, however each quinone has a better chance to undergo substitution by another phenolic molecule or other nucleophile regenerating a hydroquinone. Continued slow oxidation allows each cycle to extend the polymeric substitution-regeneration reaction, while leaving more of the original phenol available to continue the process (Boulton et al., 1996).

The net result is that, although the process may be slow in wine, the magnitude of the oxidation will be greater than under alkaline conditions.

As these reactions are considered to be subject to Arrhenius's equation, that is that the rate constant of a chemical reaction is exponentially related to the temperature of the system, an increase in temperature should lead to an increase in the reaction rate. (Marias and Pool, 1980; Robinson, et al. 2010).

2.2 Sulfur Dioxide

Sulfur Dioxide has been used for many years in winemaking due to the many useful properties that make it an almost indispensable aid in winemaking (Margalit, 2004).

These beneficial properties are as follows:

1. Antimicrobial: It inhibits the development of microorganisms. It has a greater activity on bacteria than on yeasts. At low concentrations, the inhibition is transitory. High concentrations can destroy a percentage of the microbial population. The effectiveness of a given concentration is increased by lowering the initial population, by filtration for example. During storage, SO_2 hinders the development of all microorganisms (yeasts, lactic acid bacteria, acetic acid bacteria). In this way, haze formation by yeasts, the refermentation of sweet wines, the development of mycodermic yeasts (flor yeasts) and various bacterial spoilages are avoided.

2. Antioxidant: in the presence of catalyst, it binds with dissolved oxygen according to the following reaction:

$$2\ SO_2 + O_2 \rightarrow 2\ SO_3$$

   This reaction is relatively slow. It serves to protects wines from chemical oxidations, but it has no effect on enzymatic oxidations, which are very quick. SO_2 protects wine from an excessively intense oxidation of its phenolic compounds and certain elements of its aroma. It prevents madeirization; an oxidation process producing a sweeter, lower-acidity wine. It also contributes to the establishment of a sufficiently low oxidation-reduction potential, favoring wine aroma and taste development during storage and ageing.

3. Enzymatic Oxidation Inhibition: it instantaneously inhibits the functioning of oxidation enzymes (tyrosinase, laccase) and can ensure their destruction over time. Before fermentation, SO_2 protects musts from oxidation by this mechanism. As we are working with finished wine form good quality grapes, this property is of little import for this project.

4. Binding ethanal and other similar products, it protects wine aromas and makes the ‘flat’ character disappear.

Of these, it is the anti-oxidant abilities that are most relevant to this project.

Management of SO_2 levels in wine can be problematic for winemakers as maintaining precise SO_2 levels is complicated due to the complex chemical equilibriums involved.

First of all, excessive concentrations should be avoided for health reasons (Yang & Purchase, 1985), and there are strict rules on the level of SO_2 permitted in wine that is ready for market. Generally, these rules specify maximum total SO_2 levels, although some markets also have free SO_2 specifications.

Excessive concentrations can also interfere with wine bouquet. Initially, the aroma can become dull. At higher concentrations, it gives wines characteristic defects: a metallic character, a pungent aroma, a sharpness in the nose and a 'soapy' smell. (Zoecklin, et al,1999).

However, in insufficient concentrations, it does not ensure stability of the wine. Excessive oxidation or microbial development can compromise the wine's quality and ultimately, reduce the wines value – both commercially and in terms of customer satisfaction.

Yeast also produce small quantities of SO_2 during fermentation. In general, the amount formed is rarely more than 10 mg/L, but in certain cases it can exceed 30 mg/L. Consequently, the total absence of sulfur dioxide in wine is rare; even when SO_2 is not added by the winemaker.

It is general practice in the wine industry to minimize oxidation and inihibit microbial populations by using a low concentration of SO_2 that is added to the must immediately after the grapes are crushed. Additional additions occur after fermentation is finished, with the objective of maintaining an effective level throughout the remainder of winemaking process, storage and bottling.

2.2.1. Chemistry of Sulfur Dioxide

2.2.1.1. Free Sulfur Dioxide

As with carbon dioxide, sulfur dioxide is a weak diprotic acid.

$$SO_2 + H_2O \leftrightarrows HSO_3^- + H^+ \qquad pKa1 = 1.81 \text{ in water at } 25°C$$

$$HSO_3^- \leftrightarrows SO_3^- + H^+ \qquad pKa2 = 7.20 \text{ in water at } 25°C$$

Figure 2.4. shows the species distribution diagram for SO in water at 25°*C.*

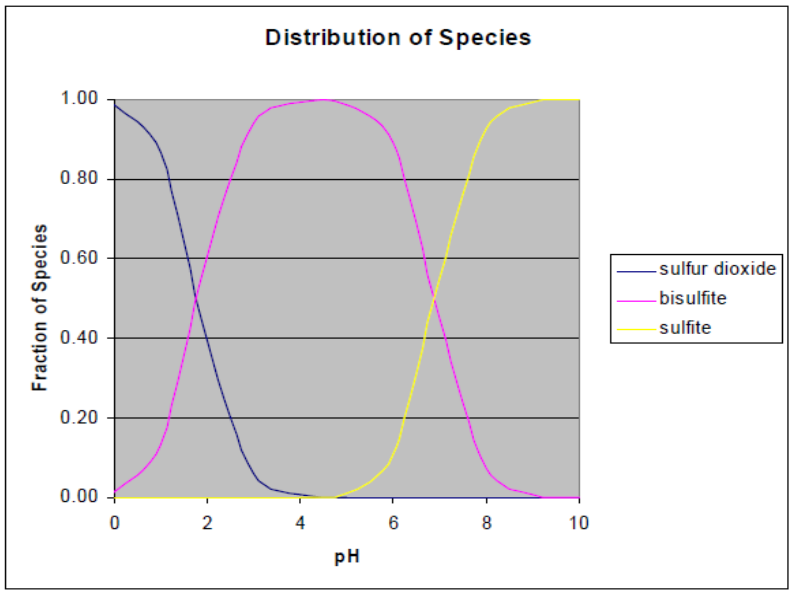


Figure 2.4: Distribution of Sulfur Dioxide forms: pH 0 – pH 10

At the pH of wine (~3.0-3.4) the major species present is bisulfite (HSO_3^-).

As ΔH° = −60 kJ for pKa1, the Ka decreases with temperature. The concentration of free sulfur dioxide is larger at higher temperatures.

As temperature increases, free SO_2 increases and bound SO_2 decreases. (SO_2 bound to acetaldehyde remains constant.) This is because increased temperatures cause partial dissociation of the bound SO_2 form, resulting in increased free SO2 and hence increased molecular SO_2 concentrations.

For example, a wine containing 68 mg/L of free SO_2 at 0°C (30°F) will contain 85 mg /L at 15°C (57°F) and 100 mg/L at 30°C (84°F) (Peynaud, 1984). .

The Ka's above are measured in water and in wine (about 10% alcohol), the values again decrease, which in turn increases the free SO_2. Thus the higher the alcohol concentration that is in wine, the larger the amount of free SO_2.

2.2.1.2. Bound Sulfur Dioxide

Bisulfite addition compounds form with aldehydes and ketones:

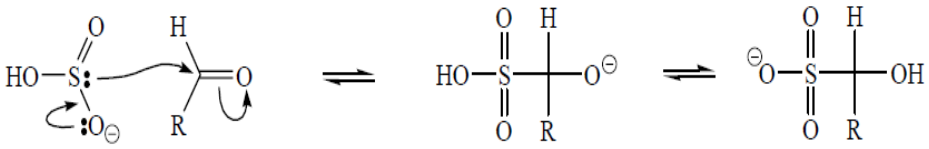


Figure 2.5: Bisulfite Addition Compounds (xxxx,200X)

The equilibrium constant for the formation of these addition products is large and consequently most aldehydes and ketones in wine will be present in the bound form.

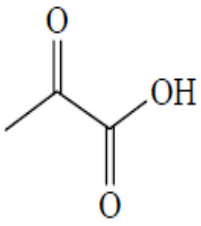
These addition compounds represent the bound SO_2 and the sum of the free SO_2 ($SO_2 + HSO3^-$) plus bound SO_2 is equal to the total SO_2.

Ethanal

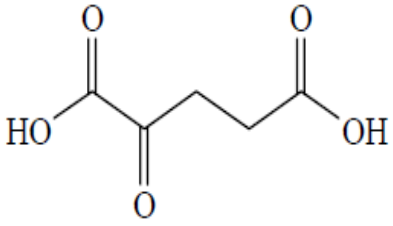
The equilibrium constant for the formation of the addition product of ethanol is large, $K = 6.7 \times 10^5$ and consequently the presence of free ethanal is negligible in wine containing free SO_2. In wine no longer containing free SO_2, a weak dissociation of the ethanal addition product liberates a trace of ethanal. This ethanal is said to be responsible for the flat character in wine.

Ketonic acids

Pyruvic and α-ketoglutaric acid are present in wine from alcoholic fermentation.



pyruvic acid



α-ketoglutaric acid

These acids are formed at the beginning of fermentation. After initially increasing, their concentration decreases. Elevated temperatures and pH, along with aeration, favor their synthesis and accumulation.

Sugars and Sugar derivatives

Sugars are either aldoses (aldehydes) or ketoses (ketones) and will form addition compounds. Fructose and sucrose are virtually unreactive. Glucose

has some reactivity, however, the equilibrium constant for the formation of the addition product with glucose is small.

2.2.1.3. Free vs Bound SO_2

The plot of bound SO_2 versus free is not necessarily a straight line. This is because, in a multi-component system containing different concentrations of carbonyls with various equilibrium constants, the SO_2 is preferentially bound to those carbonyls with the largest values of K. Thus not until nearly all the acetaldehyde is bound to SO_2 will the remaining carbonyls begin to be significantly bound. The shape of the curve therefore depends on the relative concentrations of the different binding compounds and their K values. In practice, free acetaldehyde and free SO_2 cannot co-exist and more than 99% of the acetaldehyde will be bound before any free SO_2 can be determined. Conversely, less than 0.1% of the total glucose is generally sulphite bound, despite its relatively high concentration, because its K value is relatively small.

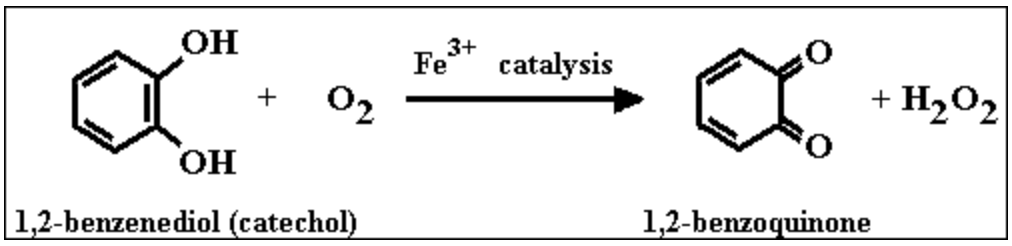
SO_2 and Non-Enzymatic Browning

In term of this project, the most important characteristic is the relationship between SO_2 and non-enzymatic oxidation.

If we refer back to the mechanisms of non-enzymatic oxidation, the points at which SO_2 can inhibit the reaction appear quite plain. However, the precise mechanisms by which SO_2 protects wine from oxidation are not fully understood, nor widely agreed upon. Nevertheless, SO_2 is deemed to possess anti-oxidant activity because it is preferentially oxidised over other compounds which, when oxidised, would lead to undesirable aromatic/flavour changes (Margalit, 2004).

The direct reaction of SO_2 with oxygen appears to be insignificant in wine, as the reaction is relatively slow (Margalit, 2004). Oxygen reacts with polyphenols before it can be removed by SO_2. Instead, the main antioxidant role of SO_2 appears to be its reaction with hydrogen peroxide (Danilewicz, 2003). The most reactive polyphenol grouping in wine is the catechols (in this explanation catechols are used as a simple example to show the reaction of the most reactive wine polyphenol grouping). The following equation repres*ents their reaction with O_2 in wine:*

1,2-benzenediol (catechol), through Fe^{3+} catalysis, reacts with O_2 to form 1,2-benzoquinone and hydrogen peroxide (Wildenradt and Singleton, 1974).



Essentially, transition metals oxidise catechol (1,2-benzenediol) and these reduced metals are then re-oxidised by oxygen. Indeed, work by Poulton (1970) suggests that an intermediate is formed in the presence of oxygen which then reacts rapidly with SO_2 since, under model wine conditions, a half time of approximately 30 days was required for SO_2 to consume the oxygen in a saturated solution. In a white wine, the half time was 1.2 days.

Hydrogen peroxide is a strong oxidising agent and will oxidise other wine compounds, potentially leading to an undesirable aroma. For example, ethanol may be oxidised to form acetaldehyde, which possesses a stale aroma, as shown in figure 2.2.

However, when SO_2 is present, the bisulphite ion (HSO_3^-) is deemed to undergo nucleophilic attack by hydrogen peroxide (H_2O_2) with displacement of water and the formation of a peroxymonosulphite ion:

$$HSO_3^- + H_2O_2 \text{ ===> } HOOSO_2^- + H_2O$$

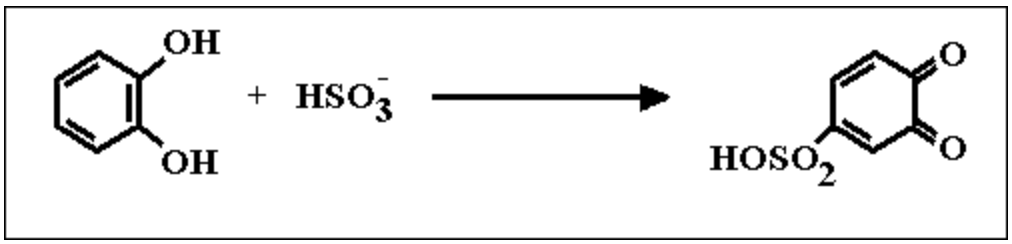
Acid catalyzed rearrangement subsequently results in the formation of a sulphate dianion:

$$HOOSO_2^- + H^+ \text{ ===> } SO_4^{2-} + 2H^+$$

Thus, SO_2 removes the strong oxidising agent H_2O_2, preventing it from oxidising other wine compounds which would potentially lead to undesirable oxidised aromas. (This action, however, is competitive and the formation of acetaldehyde has been shown to occur even in model wine solutions with high (177 mg/l) concentrations of SO_2 (Wildenradt and Singleton, 1974).)

From the equations above it can be seen that, for every mole of oxygen uptaken, one mole of SO_2 and one mole of a quinone will form.

Quinones are highly reactive with bisulphite and the reaction of 1,2-benzoquinone with bisulphite proceeds as follows:



However, some of the quinone will not react with bisulfite due to the fact that they condense with other compounds. If all the quinone reacted with bisulfite then a further mole of SO_2 would be taken up, resulting in the uptake of two moles of SO_2 for every mole of oxygen. However, since not all quinones will react in this way, the overall SO_2 loss for every mole of oxygen should be between one and two moles of SO_2. The antioxidant activity of bisulphite is primarily restricted to its reaction with hydrogen peroxide. The direct reaction of SO_2 with oxygen seems to be a chain process, prevented by the reaction of scavenging phenols with SO_2 (Danilewicz, 2003).

The end result though, is that SO_2 slows the rate of non-enzymatic oxidation markedly. As SO_2 levels decrease with ageing, the expectation is that the level of non-enzymatic browning will increase.

2.2. Colour and Colour Measurement

CIE L*a*b* (CIELab) is the color space specified by the International Commission on Illumination (French Commission internationale de l'éclairage or CIE). It describes all the colors visible to the human eye and was created to serve as a device independent model to be used as a reference.

The three coordinates of CIELab represent the lightness of the color (**L*** = 0 yields black and **L*** = 100 indicates diffuse white; specular white may be higher), its position between red/magenta and green (**a***, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (**b***, negative values indicate blue and positive values indicate yellow). The asterisk (*) after L, a and b are part of the full name, since they represent L*, a* and b*, to distinguish them from Hunter's L, a and b, a previous colour space definition (Hunterlab, 2006). Figure 2.6 shows a three dimensional representation of the colour space.

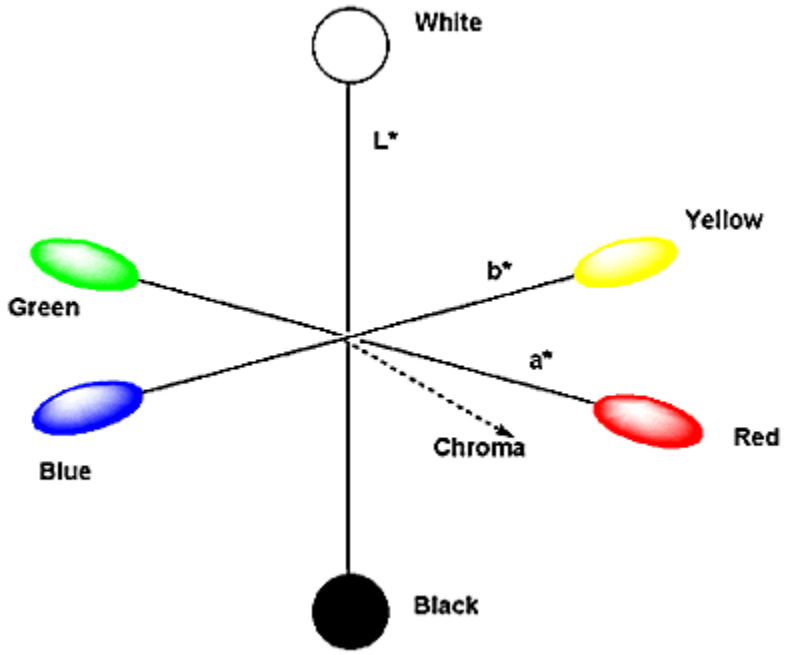


Figure 2.6: 3 Dimensional Representation of CIELab Colour Space (Gordon, 2001)

Since the L*a*b* model is a three-dimensional model, it can only be represented properly in a three-dimensional space. Two-dimensional depictions are chromaticity diagrams: sections of the color solid with a fixed lightness. It is crucial to realize that the visual representations of the full gamut of colors in this model are never accurate; they are there just to help in understanding the concept.

Because the red/green and yellow/blue opponent channels are computed as differences of lightness transformations of (putative) cone responses, CIELAB is a chromatic value color space.

2.3. Other Quality Parameters

2.3.1 Aroma/Sensory

The typical white wine ageing profile proceeds from a beginning where the wine exhibits maximum 'fresh fruit' aromas, such as citrus or floral tones, which gradually gives way over time to more developed aromas such as honey or toast. Finally, in the last stages the wine exhibits aromas of burnt rubber and oxidised tones.

It has long been known that heating wine causes changes that mimic the effects of normal ageing (Rossi and Singleton, 1966; Singleton, et al, 1979; Singleton, 2001)

2.3.2 Haze and Precipitation Issues

Wine that has finished fermentation contains protein from both the grape and from the yeast and some of this protein is susceptible to denaturation by heat.

When exposed to such temperatures, the protein denatures and can form a haze and eventually precipitate out of solution (Boulton, et al, 1996).

This precipitate is harmless and does not affect the wine's organoleptic qualities; however, it is a severe fault in the eye of the consumer, as the expectation is for a crystal clear beverage.

To prevent this, winemaker's conduct stability tests, subjecting the wine to high temperatures in an effort to estimate the degree to which the wine is susceptible to forming this haze. If a wine is found to be susceptible, the generally accepted method is to use bentonite (a montmorillonite earth) to remove the proteins in question (Boulton, et al. 1996).

The wine in this project was treated in such a manner, as detailed in the Materials and Methods section.

3. Material and Methods

3.1 Wine

3.1.1 Wine Production

Approximately 100 litres of 2010 vintage Semillon from a commercial winery in the Hunter Valley were obtained for use as the test wine.

The wine was made using conventional winemaking techniques – the grapes were harvested, then processed through a destemmer/crusher (the destemmer removes stalks and material other than grapes, the crusher breaks the berry open), the crushed grapes (must) were then chilled by pumping through a heat exchanger, before the juice was extracted by being pressed using a tank press. Sulfur dioxide was added at the must stage and to the juice from the press to slow oxidation and to prevent unwanted fermentation occurring.

The juice was kept chilled while the solids were allowed to settle to the bottom of the storage tank. Once the settling was complete, the clear juice was pumped off the solids (racked).

The juice was then inoculated with yeast and fermented to dryness (nearly all fermentable sugars consumed). During this time, the temperature of the ferment was maintained by cooling the fermenting tank as required.

Once fermentation was complete, SO_2 was added to minimize oxidation and to prevent any further microbial action. The yeast was allowed to settle out and the clear wine racked to a new tank where the wine was chilled to -2^{o}C to minimize oxidation and to promote precipitation of potassium bitartrate (see Tartrate Stability).

During this stage, the 100 litres required for the project were racked from the storage tank into 25 litre stainless steel carboys, using dry ice to provide an inert atmosphere within the carboy, to minimize oxygen pickup whilst filling. The carboys were filled to capacity to remove any ullage for a similar reason.

The carboys were then immediately transported to the Charles Sturt University Experimental Winery, where they were then transferred to a 200 litre, variable capacity tank, again under CO_2 cover to minimize oxygen pickup.

The wine was then prepared for bottling.

Before bottling can occur, there are a number of quality control processes carried out in normal wine production to preserve the quality of the wine and to minimize the formation of undesirable precipitates during storage.

3.1.2 Bottling

3.1.2.1 Pre- Bottling Analysis

Prior to bottling, wine is usually analysed for pH, titratable acidity, Free SO_2, Total SO_2 and ethanol.

The methods detailed in Section 3.4.1 below were used to perform the pre and post bottling wine analysis (Day 0 results) as well as the Day 57 analysis results.

Samples were run in duplicate, with each duplicate analysed twice and the results averaged.

The Free and Total SO_2 were measured using the Foss FIASTAR 5000 Autoanalyser with Foss Autosampler, see 3.4.4 for details of the method used.

It is common to adjust the pH, TA and SO_2 levels prior to bottling, if necessary. In this case, all the levels were judged to be within industry standards for this style of wine, so no further adjustments were carried out.

3.1.2.1.1 Stability Tests

3.1.2.1.1.1 Tartrate Stability

Wine after fermentation is usually super-saturated with potassium bitartrate (KHT). As the actual solubility of KHT varies with changing temperature (decreasing with lower temperature and vice versa), it is possible that the solubility product of KHT will be exceeded and crystal formation will commence. The resultant crystals are harmless and do not impact the wines organoleptic qualities, however, the end consumer generally consider them to be unacceptable.

Hence the wine is 'cold' stabilized to prevent the formation of potassium bitartrate crystals. This is usually carried out by chilling the bulk wine to sub-zero temperatures for an extended period to allow the KHT to precipitate out. This was carried out in the commercial winery before the wine was transported to the Experimental Winery at CSU Wagga Wagga.

Using the test method described in Iland et al. (2004) , where a filtered sample of wine is held for 72 hours at -4^{o}C and then checked for the presence of crystals, it was determined that the test wine was cold stable (no crystals had formed) and did not require further stabilisation before bottling.

3.1.2.1.2 Protein Stability

In addition to potassium bitartrate crystal formation, wine may experience haze issues caused by the denaturation and precipitation of proteins from the grape and yeasts that were involved in the fermentation, especially where a finished wine is subjected to heating – this is referred to as 'protein' or 'heat' stability. The most common treatment to prevent this is to carry out a bentonite fining prior to bottling. The added bentonite exerts an electrostatic attraction towards protein, binding it and allowing it to precipitate out and clear, stable wine decanted from the bentonite/protein deposit.

The wine provided had not been protein stabilized and as the project involved holding at elevated temperatures for an extended period, it was necessary to carry out a bentonite fining prior to bottling.

To determine the dose of bentonite required, the trial protocol detailed in Iland et al (2004) was used. Here a stock suspension of bentonite (Iland et al. 2004) uses a 5% bentonite concentration, in this case a 10% bentonite suspension was used) is used to dose a series of 100 mL containers containing the wine. Each dose corresponds to a dose rate of bentonite to be used in the bulk wine. The solids in the treated wine were then allowed to settle overnight and the clear supernatant liquid decanted off. The wine was then filtered through a 0.45 micron membrane filter into test tubes. These test tubes were incubated in an 80^{o}C water bath for 6 hours and then removed. Once they had cooled to room temperature, they were checked for the presence of a haze by passing a narrow beam of light through the test tube. The lowest dose rate of bentonite which produced no haze was considered to produce a protein stable wine.

From this analysis, it was determined that a dose of 300 mg/L of 10% bentonite solution would be sufficient to minimize the likelihood of protein instability occurring within the testing period.

This was carried out by making a 10% bentonite slurry in hot water that was left overnight to allow the bentonite to rehydrate. As there were 100 Litres of wine, 300 g of this solution was added to the wine and stirred in under carbon dioxide (CO_2) cover to prevent unnecessary oxygen ingress. The wine was left

for 48 hours to allow adequate settling of the protein/bentonite mixture (lees) from the wine. The clear supernatant was then siphoned off the lees, again under CO_2 cover.

The wine was then retested for protein stability using the method described in Iland and found to be stable and suitable for bottling.

3.1.2.2 Filtration

As is standard industry practice for white wines, the wine was filtered pre-bottling. This is done to remove solids, providing bright, clear wine and to lower microbial populations to levels that minimize any chance of spoilage.

In this case, the wine was pumped through a 20 cm filter press using Cuno 60S filter pads into a 200 litre variable capacity tank which had been dosed with dry ice to provide CO_2 cover so as to minimize oxidation through oxygen pickup. This removed any remaining solids and lessened the microbial load in the juice.

Cuno 60S filter pads are capable of only producing 'nominally' sterile product, however, as the wine had a low pH, very low residual sugar levels and adequate Free SO_2, the risk of microbial contamination was considered very low and the level of filtration deemed adequate for the trial purposes.

3.1.2.3 Bottling 100ml and 750 mL Bottles

As the wine was within industry standard quality control parameters, bottling could proceed.

For this project, 100 mL medicine flats and wine industry standard Bordeaux style 750 mL wine bottles were sanitized by rinsing with water containing 1000 mg/L of SO_2 and left upside down to drain dry on a draining rack.

A pea sized drop of dry ice was used in each bottle prior to filling to minimize oxygen uptake by providing an inert gas cover while bottling occurred.

The wine was then siphoned into the bottles. The 100 mL medicine flats were sealed with screw caps with cellulose wadding, while the 750 mL bottles were sealed using industry standard roll on, tamper evident aluminium enclosures (ROTE caps).

3.1.2.4 Post Bottling Analysis

The wine was analysed post bottling for the same parameters (pH, titratable acidity, % alcohol and free and total SO_2) using the same methodology as detailed in the pre-bottling analysis section. This also forms the basis of the Day 0 results.

3.2 Heat Treatments

The radiant heat treatments consisted of constant heat exposure at 16°C, 25°C, 35°C and 45°C. There was also a cyclic treatment which cycled between 16°C and 25°C over a 24 hour period.

Before the samples were committed to the various incubators, the temperatures of the incubators were logged for 7 days to check the accuracy of their thermostats. The temperatures were found to be within 1°C of the set points for all locations, apart from times when the incubator doors were opened. After the doors were closed, the air temperature quickly returned to the set point. As the samples were placed within cartons where air circulation was limited, along with their own thermal inertia, it was considered that the limited opening of incubator doors would not affect the internal sample temperatures during the course of the project.

The wines were stored in cardboard cartons to exclude light and were placed into the following:

Temp – Cyclic: 16°C – 25°C incubator, where the temperature was cycled from 16°C – 25°C on a 24 hour basis, with the set point at 12 hours for each temperature.

Temp 16: 16°C storage room, where the temperature was kept at a constant 16°C.

Temp 25: 25°C incubator, where the temperature was kept at a constant 25°C.

Temp 35: 35°C incubator, where the temperature was kept at a constant 35°C.

Temp 45: 45°C Incubator, where the temperature was kept at a constant 45°C.

3.2.1 Heat Summation

To provide a measure that related the results to the amount of heat exposure, a heat summation was calculated to provide a figure for Heat Degree Hours (HDH). This was to be derived from data logger recordings in the original experimental design; however, problems with the data loggers meant that the HDH had to be calculated.

For the constant temperature treatments, the HDH was calculated as follows:

$$\text{HDH } (^{o}\text{C Hrs}) = \text{Incubator Set Temperature } (^{o}\text{C}) \times \text{Time (Hrs)}$$

For the cyclic incubator,

$$\text{HDH } (^{o}\text{CHrs}) = 20.8\ (^{o}\text{C}) \times \text{Time (Hrs)}$$

The figure of 20.8 ^{o}CHrs was obtained through integrating data logger data from the cyclic incubator over a 5 day period.

3.3 Analysis Timetable

The wine was analysed according to the schedule in table 3.1:

Table 3.1: Analysis Schedule

Days	0	4	8	12	16	23	30	37	44	51	57
Hours	0	96	192	288	384	552	720	888	1056	1224	1368

At each analysis point, 3 100 mL sample bottles and 1 750 mL bottle were taken as quickly as possible from the cartons in the incubators and taken to the instrument room at CSU Experimental Winery.

Samples were scanned in random order on a single day to eliminate any variables such as instrument drift.

They were then scanned using a Winescan FT120 Fourier Transform Infra Red spectrometer (FTIR) for the infra red region (see section 3.4.1 for method) and with a Shimadzu UV-1700 Ultra Violet/Visible spectrometer (UV/Vis) in the ultra violet-visible spectrum (section 3.4.2 for details on the method).

A FIASTAR 5000 autoanalyser was used to determine the Free SO_2 and Total SO_2 (see 3.4.4 for method used).

All replicates were analysed in duplicate, with multiple measurements by the instruments, as detailed in their respective methods.

3.4 Analysis Methods

3.4.1 FTIR Method

FT-MIR spectroscopy is an indirect analytical method whereby the concentrations of analytes of interest in a sample are predicted on the basis of a predetermined calibration algorithm developed for each respective analyte (Skoog *et al*., 1997). The technology is based on the measurement of the frequencies of fundamental vibrations of covalent bonds in functional groups such as C-C, C-H, O-H, C=O and N-H in the mid-infrared region of the electromagnetic spectrum. This region is usually defined as ranging from 400 to 4000 cm^{-1} or, in terms of nanometres, from 25000 to 2500 nm (Smith, 1999). The characteristic wave numbers at which molecules absorb infrared light depend on the bond itself and its immediate molecular environment. Absorbance is directly proportional to the concentration of a particular component (Skoog *et al*., 1997; Smith, 1999). This relationship is established during a calibration process through the application of chemometric techniques that include partial least squares (PLS) regression (Eriksson *et al*., 1999; Esbensen, 2002). In a matrix with the chemical complexity of grape juice or wine, the calibration process is extensive and large numbers of samples typically are needed to meet the important requirement that all possible sources of physical and chemical variation to be expected in future unknown samples are accounted for in the calibration model (Nieuwoudt *et al*., 2004).

The FT-IR spectrum of each sample replicate was obtained using a WineScan FT120 spectrometer equipped with a Michelson interferometer and CaF2-lined cuvette with a path length of 37 µm (FOSS Analytical, Denmark). As the WineScan is a specialised instrument designed especially for quantification in wine- and grape-derived matrices, some instrument settings are pre-set by the manufacturer and cannot be changed by the user. These include the sample temperature, which is set at 40°C, the scanning interval set from 930 to 5011 cm^{-1} at 4 cm^{-1} intervals, and the number of repeated scans of each sample, which is set at 20 (Bevin, et al. 2007).

Zero liquid S-6060 (Foss Analytical, Denmark) was scanned prior to the addition of the samples to obtain a background scan. Repeated scans of each sample were averaged and processed to a single beam transmittance

spectrum through a series of mathematical procedures, including Fourier transformation (Foss Analytical, Denmark, 2001).

Background absorbance was corrected for by division of the sample spectrum by the zero liquid spectrum, at each recorded wave number. Finally, the corrected transmittance spectrum was converted to a linearised absorbance spectrum (Foss Analytical, Denmark, 2001).

For the wine analysis requirements (pH, titratable acidity and % alcohol), the absorbance spectra are loaded into the FOSS Winescan software, where they are compared to a calibration matrix to produce the analysis results.

For PARAFAC analysis, raw data that made up the spectral information was saved as comma delimited text files from the Winescan program and uploaded into Matlab (R2010a) for further treatment as described in Statistical Analysis.

3.4.2 UV/Vis Method

A Shimadzu UV-1700 UV/Vis spectrophotometer, under the control of Shimadzu UV Probe Version 2.21 software package (Shimadzu Corporation, 2005), was used in transmittance mode using medium speed scans through a 10 mm Quartz cell recording at 1.0 wave number intervals over a range of 250 - 800 nm.

A water baseline was first established before samples were scanned, with a water blank used for each scan.

Each replicate was scanned in duplicate with two scan per duplicate.

Each day's scans were saved as comma delimited text files to allow uploading into Matlab for PARAFAC analysis described in Statistical analysis

Scan data was also saved as UVPC Spectrum (.spc) files for use in the Shimadzu UVPC Color Analysis Personal Spectroscopy Software (Shimadzu Corporation, Version 3.0) for conversion to colour values.

3.4.3 Colour Space - CIELab

The scans from the UV/Vis spectrophotometer were uploaded into Shimadzu UVPC Color Analysis Personal Spectroscopy Software (Shimadzu Corporation, Version 3.0) where

The uniform colour space CIE1976-(L*a*b*) (CIELab) was used. As is required by the weighted-ordinate method, the visible spectra of the samples were weighted according to the characteristic factor of the selected visual reference conditions: 10^{o} visual field observer and standard illuminant D65, which corresponds to natural daylight (Wyszecki and Stiles, 1982).

The CIELab values were uploaded into Matlab (R2010a) for further treatment detailed in Statistical Analysis.

3.4.4 FIASTAR 5000 with auto sampler

Free and total SO_2 were measured using a Foss FIASTAR 5000 auto-analyser with FOSS auto sampler and processed by FOSS FIASTAR software on the attached computer.

On each analysis day, a calibration run was performed using freshly made sulfur dioxide standards as per the method detailed in the Foss FIASTAR 5000 manual (Foss Analytical, 2006).

3.4.4.1 Free SO_2 Method

The sample was injected by the auto-analyser into a water carrier and is then acidified with hydrochloric acid to liberate sulphur dioxide gas from the sample. This free SO_2 diffuses through a gas permeable membrane into a pH 8.4 Phosphate buffer solution. 5,5´-Dithio – bis (2-nitrobenzoic acid) (DTNB) reagent is then added and reacts with the free SO_2. The color reaction produces a strong yellow color, which is measured at 420 nm internally (Foss, 2006). This is compared to the calibration standard results to produce a final measurement.

3.4.4.2 Total SO_2 Method

The sample is injected into a pH 8.4 phosphate buffer solution by the auto-analyser. DTNB reagent is then added and the stream is heated to 50^{o}C. The DTNB reacts with all forms of SO_2 and produces a strong yellow color which is dialysed into a suitable absorbance range. The final color is measured at 420 nm internally (Foss, 2006). This is compared to the calibration standard results to produce a final measurement.

Each replicate was analysed in duplicate, with each duplicate analysed twice.

3.5 Sensory Assessment

Limited sensory analysis was carried out at the conclusion of the analysis program. Here the purpose was to determine whether any treatments had impacted the organoleptic qualities of the wines compared with the wine stored under ideal conditions (the Temp 16 treatment).

Difference testing of wines exposed to radiant heat treatments in 750 mL bottles was assessed using balanced duo trio tests (Anonymous, 2008) with panelists asked to determine the same sample as the reference during comparisons. In all, 15 panellists ranging in age from 23 to 48, participated in the sensory testing and were chosen on the basis of availability and interest. All comparisons were conducted in purposefully designed sensory booths with red light illumination to mask colour differences between samples. Approximately 30 mL of each wine was poured into standard XL5 tasting glasses and presented to panellists. Compusense 5.0 ™ software (Compusense Inc, Guleph) was used to determine blinding codes for samples, design of sample comparisons such that all samples were compared to each other, and statistical analysis of results.

3.6 Statistical Analysis

3.6.1 Initial Statistical Treament

The free and total SO_2 and CIELab results were averaged across treatments for the initial review of results, with the 100 mL and 750 mL bottles averaged separately and the respective standard error measurements calculated.

3.6.2 PARAFAC Analysis

PARAFAC enables the data decomposition of three (or higher) dimensions of data matrices to determine linear interactions of attributes treatments and experimental duration (Bro, 1997).

Data cubes consisting of samples x attributes x sampling days were constructed. Where multiple scans per replicate were recorded for a specific instrumental procedures, these were averaged for each replicate and each data cube consisted of 20 samples x number of attributes x 11 sampling days. For these analyses, the 100 mL and 750 mL bottles were not treated separately.

PARAFAC was performed using PLS Toolbox (Eigenvector Research Inc., Wenatchee, Version 5.8.2, Release 9277) and MATLAB (Version 2010a). The

number of factors extracted for each PARAFAC model was sequentially reduced until a stable core consistency diagnostic value was achieved (core consistency=100) (Bro & Kiers, 2003).

3.6.2.1 CIELAB and Sulfur Dioxide

Data in cubes was centred across mode 1 (samples) for sulfur dioxide and CIELab attributes. For the purposes of this project, the CIELab and sulfur dioxide values were folded into a cube for PARAFAC analysis. In this case the data cube consisted of 20 samples x 100 attributes (20 'L*', 20 'a*'. 20 'b*', 20 free SO_2 and 20 total SO_2) x 11 sample days.

3.6.2.2 UV-Vis

Raw data for UV-VIS spectra was not preprocessed for PARAFAC analysis. Before the PARAFAC was performed, the wave numbers used were cut to the region 341-739 nm (i.e. spanning the visual region). Means for data points within replicates were calculated and the data cubed. The resultant cube consisted of 20 samples x 398 wavelengths x 11 sampling days.

3.6.2.3 FT-IR

Prior to constructing data cubes with FT-IR spectra, FOSS pin numbers were converted to wave numbers (Wave number (cm^{-1}) = 3.858×pinnumber) and data was normalised using standard normal variate scaling (Zeaiter, et al., 2009). In this case the data cube consisted of 20 samples x 1060 wave numbers x 11 sample days. As the instrument manual (Foss Analytical, 2005) recommends that the water absorption peaks in the regions between wave numbers 1543-1717 and 2971-3627 cm^{-1} as well as carbon dioxide, which has a fundamental absorption peak between 2276-2431 cm^{-1} be excluded. A PARAFAC analysis was performed with these included but it was not possible to reach a stable core consistency, as a result, these wave numbers were not included in the PARAFAC analysis presented in this report.

4. Results and Discussion

Table 4.1 below summarises the analysis days, temperature treatments and the HDH.

Table 4.1: Heat Degree Hours-Sum of Temperatures (^{o}C Hrs)

Days	16-25 Cyclic	16 C	25 C	35 C	45 C
0	0	0	0	0	0
4	1928	1536	2400	3360	4320
8	3856	3072	4800	6720	8640
12	5784	4608	7200	10080	12960
16	7712	6144	9600	13440	17280
23	11086	8832	13800	19320	24840
30	14460	11520	18000	25200	32400
37	17834	14208	22200	31080	39960
44	21208	16896	26400	36960	47520
51	24582	19584	30600	42840	55080
57	27474	21888	34200	47880	61560

4.1 Wine Analysis

4.1.1 Pre and Post Bottling Analysis

The analysis results for pre and post bottling analysis are below in Table 4.2.

Table 4.2: Pre and Post Bottling Analysis

Treatment	pH	Titratable Acidity (g/l Tartaric)	Alcohol (%w/v)	Free SO_2 (mg/l)	Total SO_2 (mg/l)
Pre-bottling	3.06+/-0.01	6.84+/-0.07	9.72+/-.05	34.2+/-0.2	148+/-1
Post-bottling	3.06+/-0.01	6.82+/-0.07	9.69+/-.05	32.1+/-0.2	143+/-.1

The results tabled are the average of 100 mL bottles in each treatment +/- the standard error measurement (S.E.M.).

All these parameters were judged to be within normal acceptable limits for pre and post bottling quality control of white wines in commercial wineries.

4.1.2 Wine Analysis – Initial (Day 0) and Final (Day 57) Analysis: Typical Wine Quality Control Parameters

The analysis results for typical wine quality control parameters at Day 0 and Day 57 for each treatment are detailed in Table 4.3.

Table 4.3: Initial and Final Wine Analysis by Treatment

Time (days)	Treatment	pH	Titratable Acidity (g/l Tartaric)	Alcohol (%w/v)	Free SO_2 (mg/l)	Total SO_2 (mg/l)
0	Initial	3.06+/-0.02	6.82+/-0.01	9.7+/-0.0	32.1+/-0.0	142+/-1
57	Temp 16	3.07+/-0.00	6.79+/-0.01	9.6+/-0.0	14.7+/-0.2	106+/-2
57	Temp 25	3.07+/-0.00	6.80+/-0.01	9.7+/-0.0	9.8+/-0.3	96+/-1
57	Temp 35	3.08+/-0.00	6.79+/-0.01	9.6+/-0.0	8.9+/-1.7	93+/-4
57	Temp 45	3.08+/-0.00	6.79+/-0.01	9.5+/-0.0	1.6+/-0.2	75+/-3
57	Temp Cyclic	3.08+/-0.00	6.75+/-0.01	9.5+/-0.0	11.6+/-0.2	1001+/-1

The results tabled are the average of 100 mL bottles in each treatment +/- the standard error measurement (S.E.M.).

The results showed little change for pH and titratable acidity between any treatment and the initial analysis.

Alcohol levels dropped across the treatments with the 45^{o}C treatment and the Cyclic (16^{o}C-25^{o}C) exhibiting the largest fall. The alcohol loss may be due to the permeability of the wadding in the seals of the medicine flats.

As expected, free and total SO_2 levels showed marked declines across all treatment, with the rate of decline increasing according to the degree of exposure to heat. As would be expected, the rate of decline of the cyclic treatment of 16^{o}C-25^{o}C sits roughly halfway between the 16^{o}C and the 25^{o}C treatments.

4.1.3 Sulfur Dioxide

4.1.3.1 Free SO_2 Results

The results of the Free SO_2 analyses are contained in table 4.4 for 100 mL bottles and table 4.5 for 750 mL bottles

Table 4.4: Days v Free SO_2 (mg/l) Results – 100 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	32.1 +/- 0.2	32.1 +/- 0.2	32.1 +/- 0.2	32.1 +/- 0.2	32.1 +/- 0.2
4	28.9 +/- 0.1	28.1 +/- 0.5	28.4 +/- 0.5	25.2 +/- 0.4	24.0 +/- 0.6
8	27.4 +/- 0.3	26.5 +/- 0.1	22.4 +/- 0.2	23.0 +/- 0.5	17.9 +/- 0.2
12	25.3 +/- 0.2	27.0 +/- 0.4	24.0 +/- 0.8	24.0 +/- 0.4	16.3 +/- 1.0
16	24.9 +/- 0.2	26.4 +/- 0.4	23.3 +/- 0.7	23.5 +/- 0.4	15.6 +/- 7.0
23	22.7 +/- 0.3	24.9 +/- 0.2	20.8 +/- 0.3	22.2 +/- 0.5	15.3 +/- 0.6
30	22.1 +/- 0.2	24.3 +/- 0.2	20.2 +/- 0.3	21.5 +/- 0.5	14.8 +/- 0.5
37	18.6 +/- 0.3	19.7 +/- 0.8	15.7 +/- 0.9	15.2 +/- 1.2	10.0 +/- 0.3
44	17.5 +/- 0.3	21.1 +/- 0.6	16.2 +/- 0.2	16.8 +/- 0.2	8.7 +/- 0.5
51	15.4 +/- 0.1	18.5 +/- 0.1	12.6 +/- 0.2	12.4 +/- 0.	4.3 +/- 0.3
57	11.6 +/- 0.2	14.7 +/- 0.2	9.8 +/- 0.3	8.9 +/- 1.7	1.6 +/- 0.2

The results tabled are the average of all 100 mL bottles analysis +/- the SEM.

Table 4.5: Days v Free SO_2 (mg/l) Results – 750 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	32.0 +/- 0.2	32.0 +/- 0.2	32.0 +/- 0.2	32.0 +/- 0.20	32.0 +/- 0.2
4	27.3 +/- 0.0	27.9 +/- 0.1	28.6 +/- 0.2	28.5 +/- 0.1	26.0 +/- 0.1
8	26.8 +/- 0.0	27.8 +/- 0.1	28.0 +/- 0.0	24.5 +/- 0.1	19.3 +/- 0.2
12	25.2 +/- 0.1	27.8 +/- 0.2	27.5 +/- 0.1	24.8 +/- 0.3	20.7 +/- 0.1
16	24.4 +/- 0.1	26.9 +/- 0.1	27.2 +/- 0.2	24.4 +/- 0.2	19.9 +/- 0.1
23	24.2 +/- 0.4	25.2 +/- 0.7	19.9 +/- 0.1	21.3 +/- 0.0	15.8 +/- 0.0
30	23.5 +/- 0.4	24.6 +/- 0.6	19.2 +/- 0.2	21.1 +/- 0.1	15.5 +/- 0.1
37	18.6 +/- 0.1	20.3 +/- 0.4	23.6 +/- 0.1	18.0 +/- 0.1	14.2 +/- 0.0
44	20.1 +/- 0.1	21.7 +/- 0.1	21.1 +/- 0.0	18.0 +/- 0.1	13.3 +/- 0.2
51	18.4 +/- 0.2	19.6 +/- 0.1	19.2 +/- 0.0	15.9 +/- 0.0	10.2 +/- 0.0
57	16.7 +/- 0.3	17.8 +/- 0.1	17.4 +/- 0.4	13.9 +/- 0.2	8.9 +/- 0.1

The results tabled are the average of all 750 mL bottles analysis +/- the SEM.

Figures 4.1 and 4.2 plot free SO_2 vs time for 100 mL and 750 mL bottles respectively.

Figure 4.1: Free SO_2 (mg/l) vs Time (Days) - 100 mL Bottles

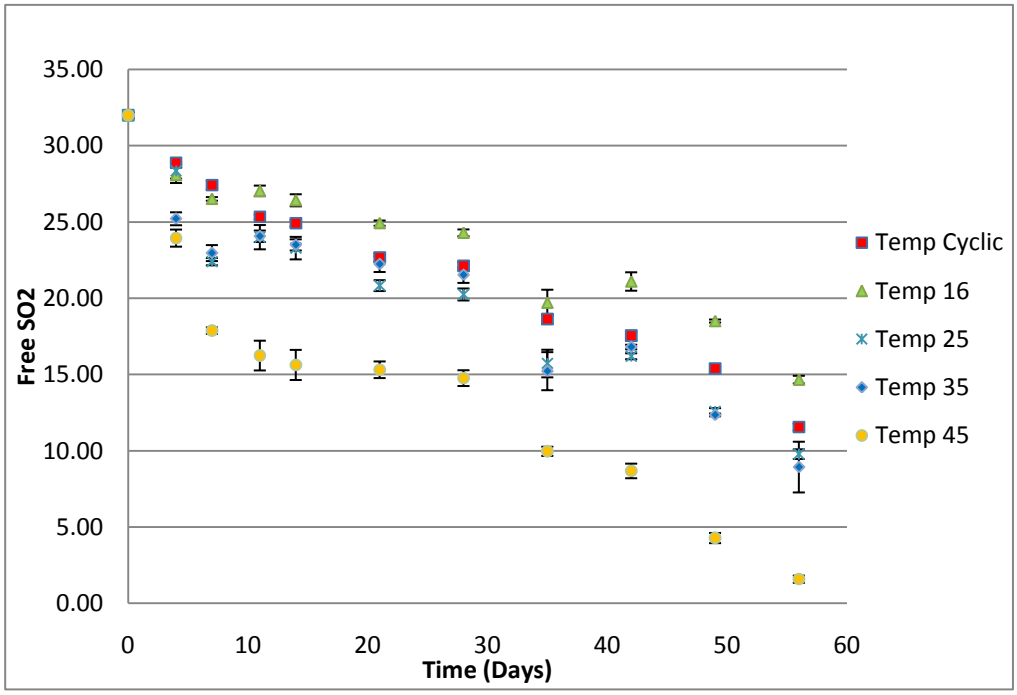
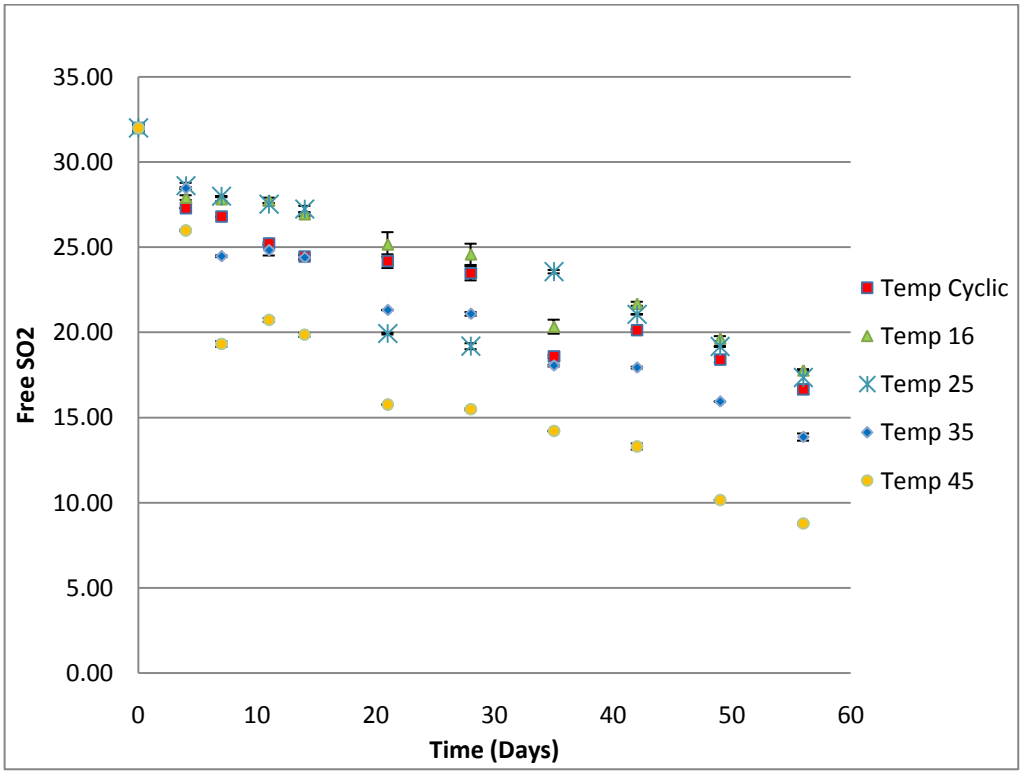


Figure 4.2: Free SO_2 (mg/l) vs Time (Days) - 750 mL Bottles



As expected free SO_2 declines over time, with the highest temperature treatments declining at the fastest rate. This trend is exhibited in both the 100 ml and 750 mL bottles, with the 750 mL bottles decreasing at a slower rate.

Figure 4.3 and 4.4 plot free SO_2 against the heat summation (HDH) for 100 mL and 750 mL bottles respectively.

Figure 4.3: Free SO_2 (mg/l) vs HDH (^oC Hrs) – 100 mL Bottles

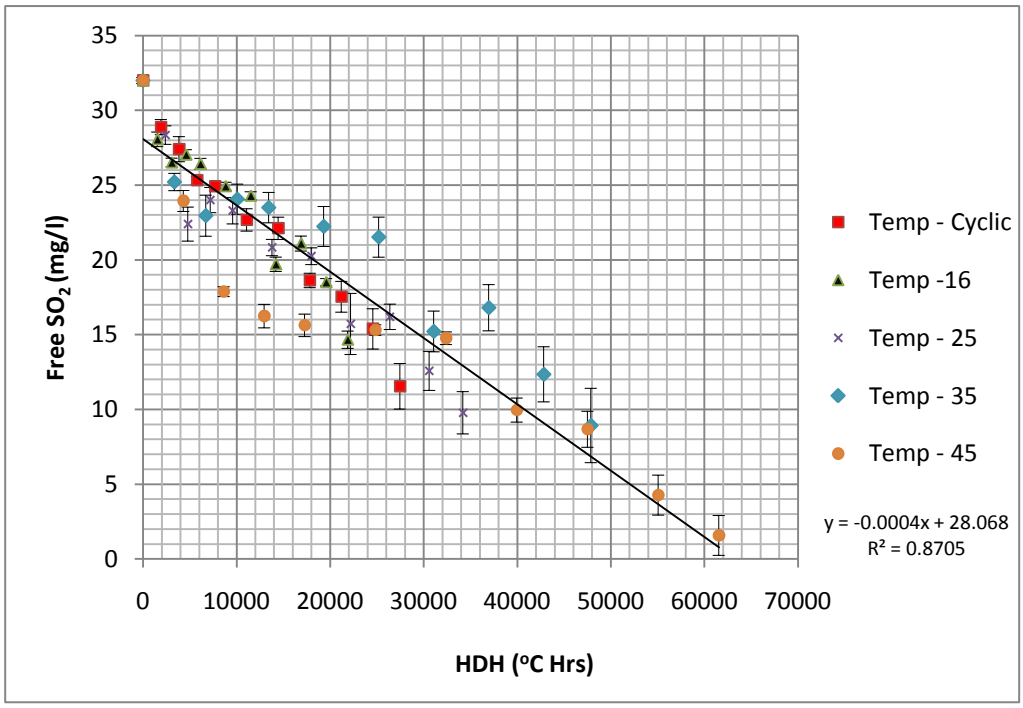
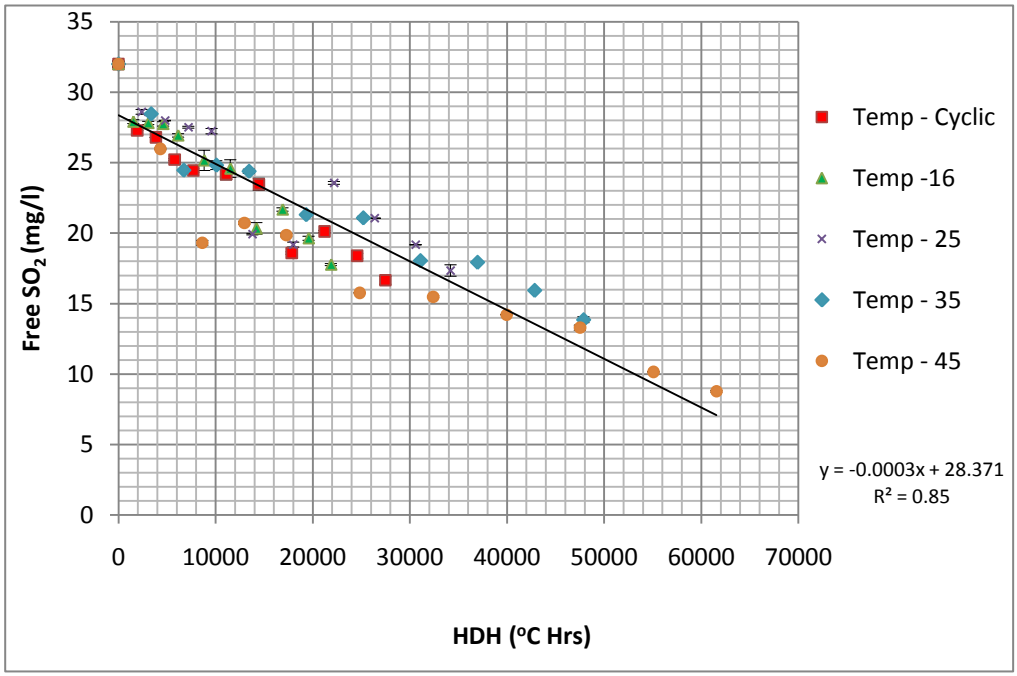


Figure 4.4: Free SO_2 (mg/l) vs HDH (^oC Hrs) – 750 mL Bottles



Plotting free SO_2 against the heat summation shows that there is good correlation between the heat load experienced and the decline in free SO_2, (R^2=0.87 for the 100 mL bottles and R^2=0.85 for the 750 mL bottles) for the linear regression.

Comparing the rate of decay of free SO_2 between the 100 mL and 750 mL bottles, there appears to be little difference between the two, with the 100 mL bottles possibly exhibiting a slightly higher rate of decay.

4.1.3.2 Total SO_2 Results

The results of the total SO_2 analyses are contained in table 4.6 for 100 mL bottles and table 4.7 for 750 mL bottles.

Table 4.6 Total SO_2 (mg/l) Results over Time – 100 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	143 +/- 1	143 +/- 1	143 +/- 1	143 +/- 1	143 +/- 1
4	134 +/- 3	141 +/- 1	127 +/- 3	128 +/- 3	11 +/- 1
8	133 +/- 2	139 +/- 0	126 +/- 3	126 +/- 3	111 +/- 1
12	119 +/- 1	124 +/- 1	120 +/- 1	118 +/- 1	107 +/- 1
16	119 +/- 1	124 +/- 1	120 +/- 1	118 +/- 1	106 +/- 7
23	118 +/- 1	123 +/- 1	119 +/- 1	117 +/- 1	106 +/- 1
30	117 +/- 1	123 +/- 1	119 +/- 1	117 +/- 1	106 +/- 1
37	116 +/- 3	121 +/- 2	108 +/- 2	107 +/- 4	93 +/- 2
44	115 +/- 3	120 +/- 2	107 +/- 2	110 +/- 2	92 +/- 2
51	106 +/- 1	111 +/- 0	105 +/- 0	102 +/- 1	81 +/- 2
57	1001 +/- 1	106 +/- 2	96 +/- 1	93 +/- 4	75 +/- 3

The results tabled are the average of all 100 mL bottles analysis +/- SEM.

Table 4.7: Total SO_2 (mg/l) Results over Time (750 mL Medicine Flats)

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	143 +/- 1	143 +/- 1	143 +/- 1	143 +/- 1	143 +/- 1
4	129 +/- 0.	133 +/- 0	135 +/- 0	132 +/- 0	119 +/- 0
8	127 +/- 0	133 +/- 0	134 +/- 0	131 +/- 0	117 +/- 0
12	118 +/- 0	127 +/- 0	124 +/- 0	124 +/- 0	108 +/- 1
16	117 +/- 0	127 +/- 0	123 +/- 0	124 +/- 0	107 +/- 1
23	116 +/- 1	126 +/- 1	122 +/- 0	123 +/- 0	107 +/- 1
30	116 +/- 1	126 +/- 1	122 +/- 0	123 +/- 0	107 +/- 2
37	114 +/- 0	119 +/- 1	105 +/- 0	117 +/- 0.	104 +/- 0
44	113 +/- 0	117 +/- 1	104 +/- 1	117 +/- 0	103 +/- 0
51	106 +/- 0	110 +/- 0	108 +/- 1	113 +/- 0.	103 +/- 0
57	108 +/- 0	113 +/- 0	105 +/- 0	106 +/- 0	101 +/- 0

The results tabled are the average of all 750 mL bottles analysis +/- SEM.

Figures 4.5 and 4.6 show plots of Total SO_2 vs Time for 100 mL and 750 mL bottles respectively.

Figure 4.5: Total SO_2 (mg/l) vs Time (Days) – 100 mL Bottles

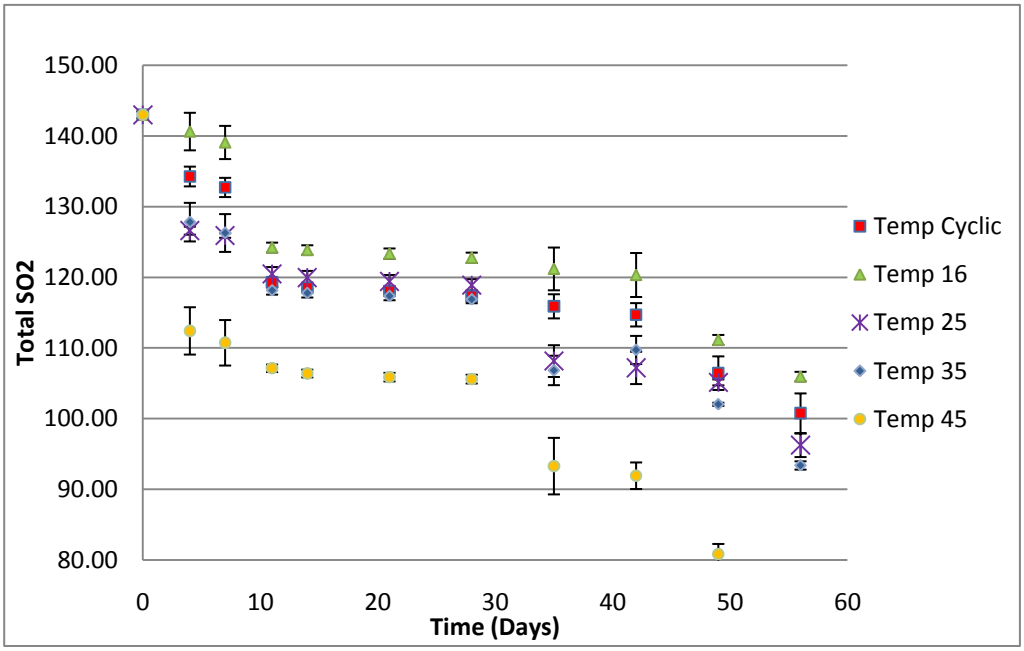
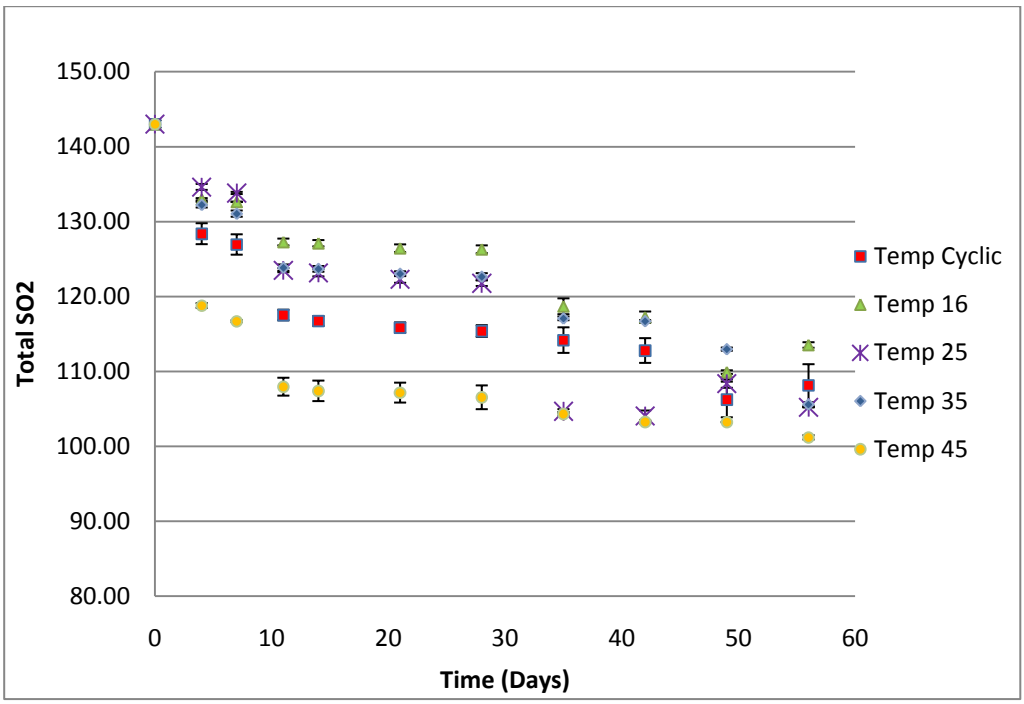


Figure 4.6: Total SO_2 (mg/l) vs Time (Days) – 750 mL Bottles



Figures 4.7 and 4.8 show plots of total SO_2 vs HDH for 100 mL and 750 mL bottles respectively.

Figure 4.7: Total SO_2 (mg/l) vs HDH (^oC Hrs) -100 mL Bottles

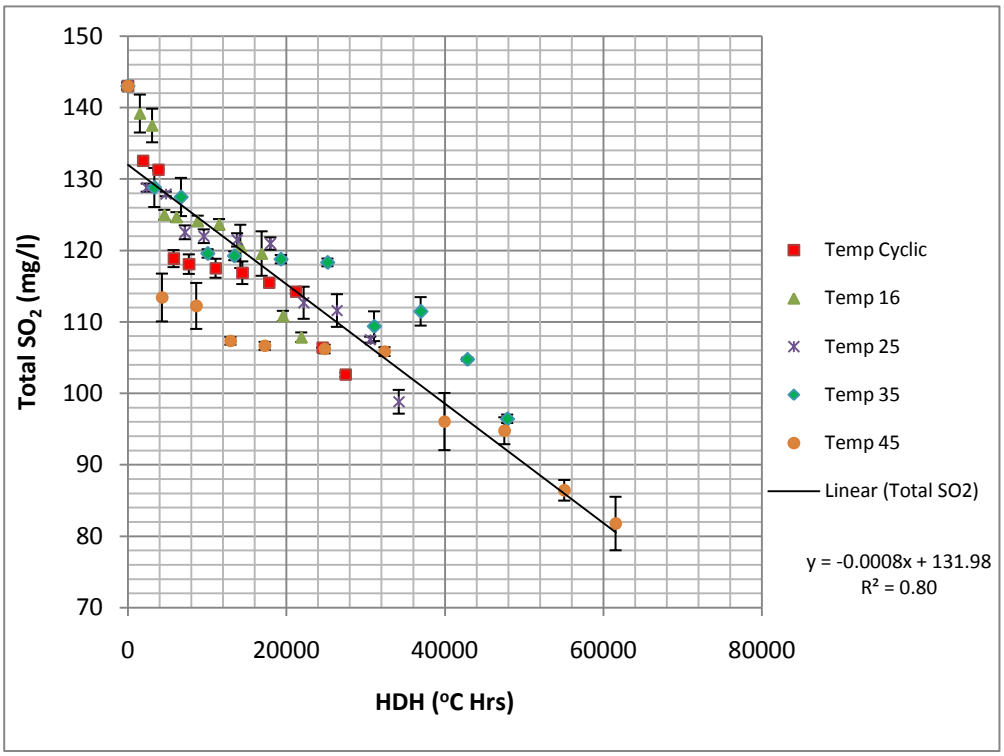
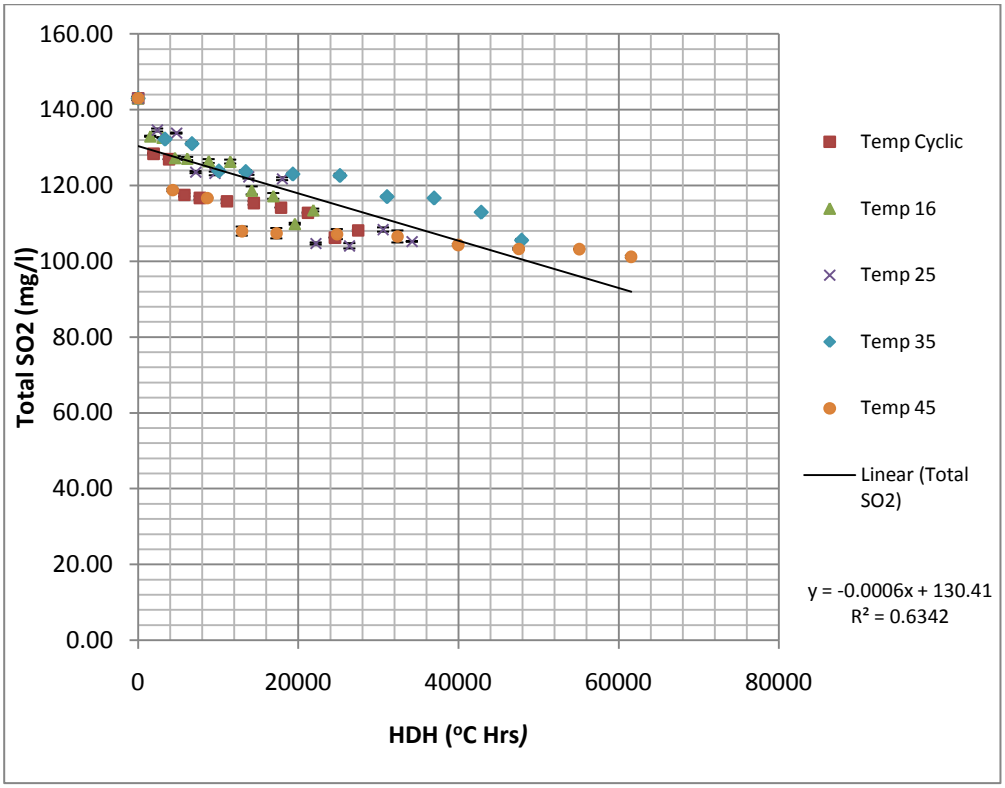
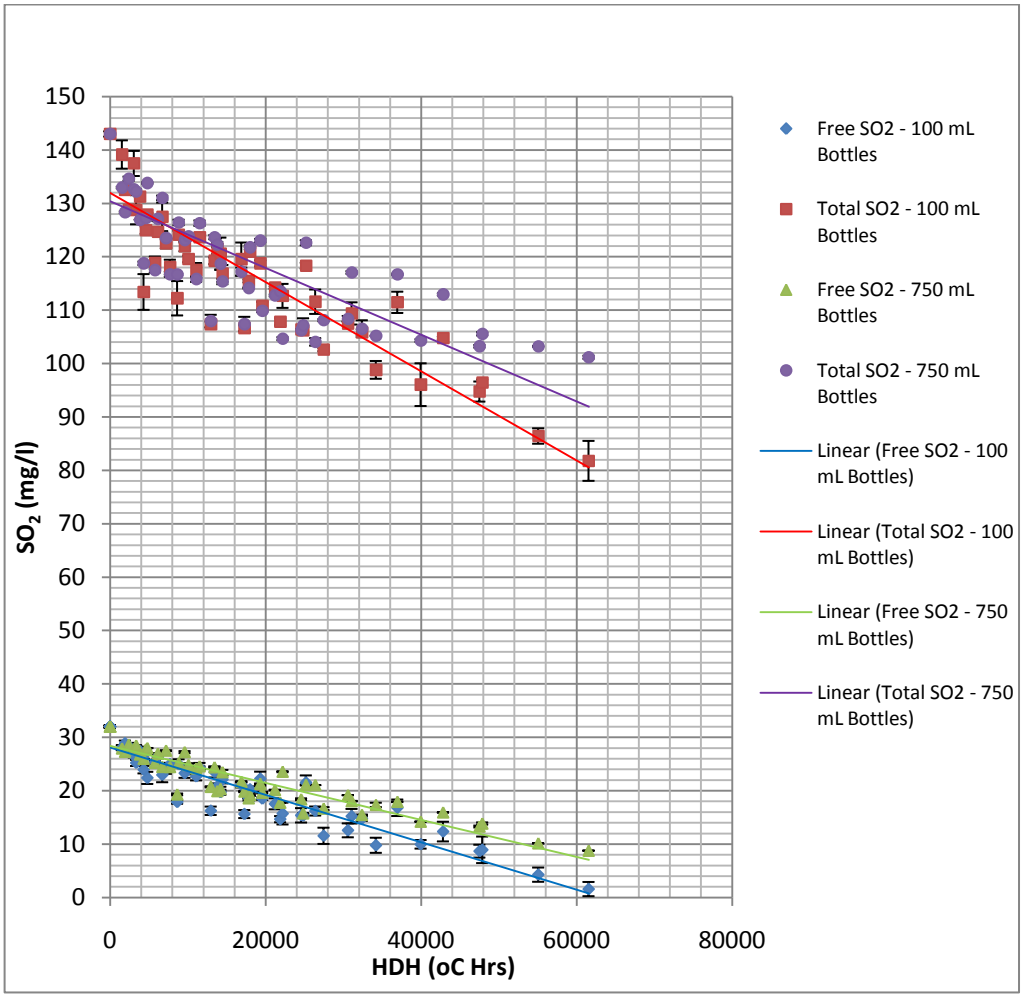


Figure 4.8: Total SO_2 (mg/l) vs HDH (^oC Hrs) -750 mL Bottles



Figures 4.7 and 4.8 show that total SO_2 levels, similarly to free SO_2 levels, decline when exposed to temperature over time and that this decline is at a faster rate than that of free SO_2 - if the slopes of the linear regression are compared, the rate of decline appears to be double that of free SO_2 (for 100 mL bottles, the slope is -0.0004 for free SO_2 and -0.0008 for total SO_2; while for 750 mL bottles, the slope is -0.0003 for free SO_2 and -0.0006 for total SO_2). Figure 4.9, where the free SO_2 and total SO_2 for both 100 mL and 750 mL bottles are plotted on the same chart, illustrates this.

Figure 4.9: Free and Total SO_2 (mg/L) v Heat Degree Hours (^oC Hrs) – 100 mL & 750 mL Bottles



The regression's also show that there is more variability in the total SO_2 results compared with the free SO_2 results and between the bottle types (R^2 of 0.87:0.80 for 100 mL bottles and 0.85:0.63 for 750 mL bottles).

The differences between the rates of decay of both free SO_2 and total SO_2 in the 100 mL and 750 mL bottles are not large, with the 750 mL bottles consistently lagging the 100 mL bottles. This means that the behavior of the 100 mL bottles is quite comparable with those obtained in 750 mL bottles.

Some caution has to be exercised in evaluating all the SO_2 vs HDH results, as there are a low number of results in the high HDH regions (from around 35000 ^{o}C Hrs) which could influence the regression lines.

4.1.4 Color – CIELab Results

4.1.4.1 'L*' Results

Tables 4.8 and 4.9 summarises the 'L*' results for 100 mL and 750 mL bottles respectively.

Table 4.8:'L*' Results – 100 mL

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	99.42 +/- 0.10	99.42 +/- 0.10	99.42 +/- 0.10	99.42 +/- 0.10	99.42 +/- 0.10
4	99.42 +/- 0.02	99.47 +/- 0.02	98.99 +/- 0.03	98.93 +/- 0.07	98.86 +/- 0.01
8	98.79 +/- 0.20	99.29 +/- 0.05	98.84 +/- 0.18	98.81 +/- 0.14	96.99 +/- 0.25
12	99.05 +/- 0.09	99.02 +/- 0.19	99.10 +/- 0.06	99.17 +/- 0.03	97.95 +/- 0.28
16	99.12 +/- 0.13	99.28 +/- 0.02	99.32 +/- 0.12	99.29 +/- 0.06	98.87 +/- 0.12
23	99.16 +/- 0.10	99.12 +/- 0.03	99.19 +/- 0.02	98.77 +/- 0.17	98.03 +/- 0.45
30	98.73 +/- 0.13	98.88 +/- 0.01	98.75 +/- 0.08	98.34 +/- 0.06	98.53 +/- 0.02
37	98.41 +/- 0.17	98.42 +/- 0.22	98.43 +/- 0.03	98.33 +/- 0.20	97.53 +/- 0.60
44	99.13 +/- 0.03	98.91 +/- 0.01	98.41 +/- 0.04	98.20 +/- 0.06	96.82 +/- 0.25
51	95.31 +/- 0.39	98.67 +/- 0.04	98.75 +/- 0.05	97.84 +/- 0.17	95.23 +/- 0.60
57	100.23 +/- 0.10	100.84 +/- 0.08	99.53 +/- 0.46	92.05 +/- 3.25	85.21 +/- 1.90

The results tabled are the average of all 100 mL bottles analysis +/- SEM.

Table 4.9: 'L*' Results – 750 mL

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	99.42 +/- 0.00	99.42 +/- 0.00	99.42 +/- 0.00	99.42 +/- 0.00	99.42 +/- 0.00
4	99.62 +/- 0.00	99.46 +/- 0.00	98.81 +/- 0.00	98.94 +/- 0.00	99.34 +/- 0.00
8	99.30 +/- 0.00	98.55 +/- 0.00	99.36 +/- 0.00	99.22 +/- 0.00	99.06 +/- 0.00
12	99.07 +/- 0.00	99.36 +/- 0.00	99.29 +/- 0.00	99.31 +/- 0.00	99.21 +/- 0.00
16	99.19 +/- 0.00	99.41 +/- 0.00	99.20 +/- 0.00	99.31 +/- 0.00	99.16 +/- 0.00
23	97.79 +/- 0.00	99.43 +/- 0.00	99.40 +/- 0.00	99.46 +/- 0.00	99.10 +/- 0.00
30	98.50 +/- 0.00	98.88 +/- 0.00	98.97 +/- 0.00	98.69 +/- 0.00	98.67 +/- 0.00
37	98.94 +/- 0.00	98.88 +/- 0.00	99.80 +/- 0.00	98.99 +/- 0.00	99.12 +/- 0.00
44	99.14 +/- 0.00	99.00 +/- 0.00	99.43 +/- 0.00	98.94 +/- 0.00	98.96 +/- 0.00
51	99.00 +/- 0.00	99.04 +/- 0.00	98.59 +/- 0.00	99.06 +/- 0.00	98.89 +/- 0.00
57	101.50 +/- 0.00	103.05 +/- 0.00	102.47 +/- 0.00	98.90 +/- 0.00	96.87 +/- 0.00

The results tabled are the average of all 750 mL bottles analysis +/- SEM.

Figures 4.10 and 4.11 plot 'L*' vs Time for the 100 mL and 750 mL bottles respectively.

Figure 4.10: 'L*' vs Time – 100 mL Bottles

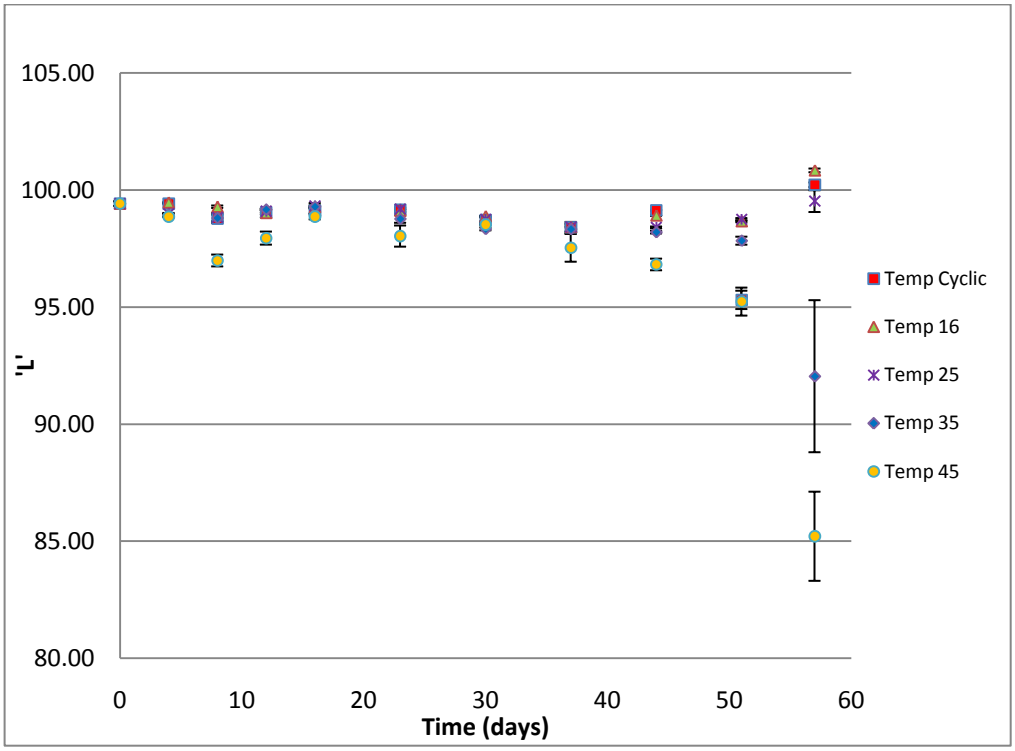
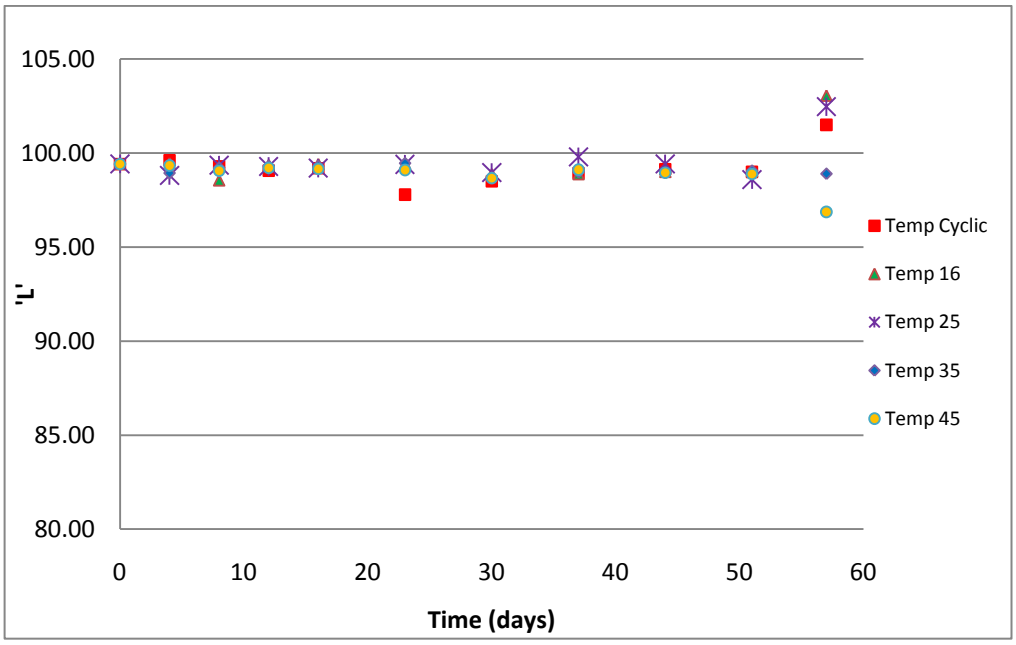


Figure 4.11: 'L*' vs Time – 750 mL Bottles



The results show that for both 100 mL and 750 mL bottles that the 'L*' value remains reasonably stable for a length of time (around 37 days for the 100 mL and 57 days for the 750 mL), that is there is no real change in the luminosity until then. At that point, however, the lower temperature treatments (Temp Cyclic, Temp 16 and Temp25)

start to rise above 100, separating from the Temp 35 and Temp 45 treatments. In the 100 mL bottles, Temp 35 and Temp 45 begin to decline markedly, indicating increasing 'darkness', in the 750 mL bottles, this process looks to have just started as the project finished with Temp 35 at a similar value to its' initial value and Temp 45 at a lower value than in previous measurements .

4.1.4.2 'a*' Results

Tables 4.10 and 4.11 summarise the 'a*' results for 100 mL and 750 mL bottles respectively.

Table 4.10: 'a*' Results – 100 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	-0.49 +/- 0.02	-0.49 +/- 0.20	-0.49 +/- 0.20	-0.49 +/- 0.02	-0.49 +/- 0.02
4	-0.49 +/- 0.01	-0.53 +/- 0.03	-0.39 +/- 0.02	-0.35 +/- 0.02	-0.48 +/- 0.03
8	-0.23 +/- 0.02	-0.38 +/- 0.02	-0.27 +/- 0.03	-0.51 +/- 0.18	-0.53 +/- 0.04
12	-0.26 +/- 0.01	-0.48 +/- 0.08	-0.48 +/- 0.05	-0.42 +/- 0.01	-0.63 +/- 0.01
16	-0.41 +/- 0.02	-0.31 +/- 0.02	-0.23 +/- 0.02	-0.39 +/- 0.01	-0.64 +/- 0.01
23	-0.20 +/- 0.04	-0.28 +/- 0.04	-0.35 +/- 0.04	-0.51 +/- 0.00	-0.46 +/- 0.11
30	-0.26 +/- 0.02	-0.25 +/- 0.08	-0.23 +/- 0.03	-0.43 +/- 0.01	-0.75 +/- 0.01
37	0.03 +/- 0.06	0.01 +/- 0.04	-0.30 +/- 0.05	-0.44 +/- 0.05	-0.68 +/- 0.08
44	-0.28 +/- 0.03	-0.12 +/- 0.01	-0.37 +/- 0.04	-0.55 +/- 0.01	-0.80 +/- 0.06
51	0.11 +/- 0.04	-0.06 +/- 0.02	-0.23 +/- 0.04	-0.45 +/- 0.03	-0.64 +/- 0.13
57	-0.17 +/- 0.01	-0.02 +/- 0.01	-0.63 +/- 0.04	-0.93 +/- 0.06	-1.18 +/- 0.03

The results tabled are the average of all 100 mL bottles analysis +/- SEM.

Table 4.11: 'a*' Results – 750 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	-0.50 +/- 0.00	-0.49 +/- 0.00	-0.49 +/- 0.00	-0.49 +/- 0.00	-0.49 +/- 0.00
4	-0.55 +/- 0.00	-0.62 +/- 0.00	-0.43 +/- 0.00	-0.43 +/- 0.00	-0.57 +/- 0.00
8	-0.36 +/- 0.00	-0.42 +/- 0.00	-0.39 +/- 0.00	-0.35 +/- 0.00	-0.67 +/- 0.00
12	-0.21 +/- 0.00	-0.40 +/- 0.00	-0.37 +/- 0.00	-0.46 +/- 0.00	-0.68 +/- 0.00
16	-0.35 +/- 0.00	-0.29 +/- 0.00	-0.39 +/- 0.00	-0.47 +/- 0.00	-0.65 +/- 0.00
23	-0.27 +/- 0.00	-0.25 +/- 0.00	-0.28 +/- 0.00	-0.58 +/- 0.00	-0.68 +/- 0.00
30	-0.26 +/- 0.00	-0.13 +/- 0.00	-0.42 +/- 0.00	-0.52 +/- 0.00	-0.80 +/- 0.00
37	-0.12 +/- 0.00	-0.04 +/- 0.00	-0.29 +/- 0.00	-0.51 +/- 0.00	-0.84 +/- 0.00
44	-0.26 +/- 0.00	-0.03 +/- 0.00	-0.38 +/- 0.00	-0.58 +/- 0.00	-1.01 +/- 0.00
51	-0.27 +/- 0.00	-0.18 +/- 0.00	-0.44 +/- 0.00	-0.66 +/- 0.00	-1.15 +/- 0.00
57	-0.31 +/- 0.00	-0.42 +/- 0.00	-0.72 +/- 0.00	-0.93 +/- 0.00	-1.68 +/- 0.00

The results tabled are the average of all 750 mL bottles analysis +/- SEM.

Figures 4.12 and 4.13 Plot ‘a*’ vs Time for the 100 mL and 750 mL bottles respectively.

Figure 4.12: ‘a*’ value v Time (days) -100 mL Bottles

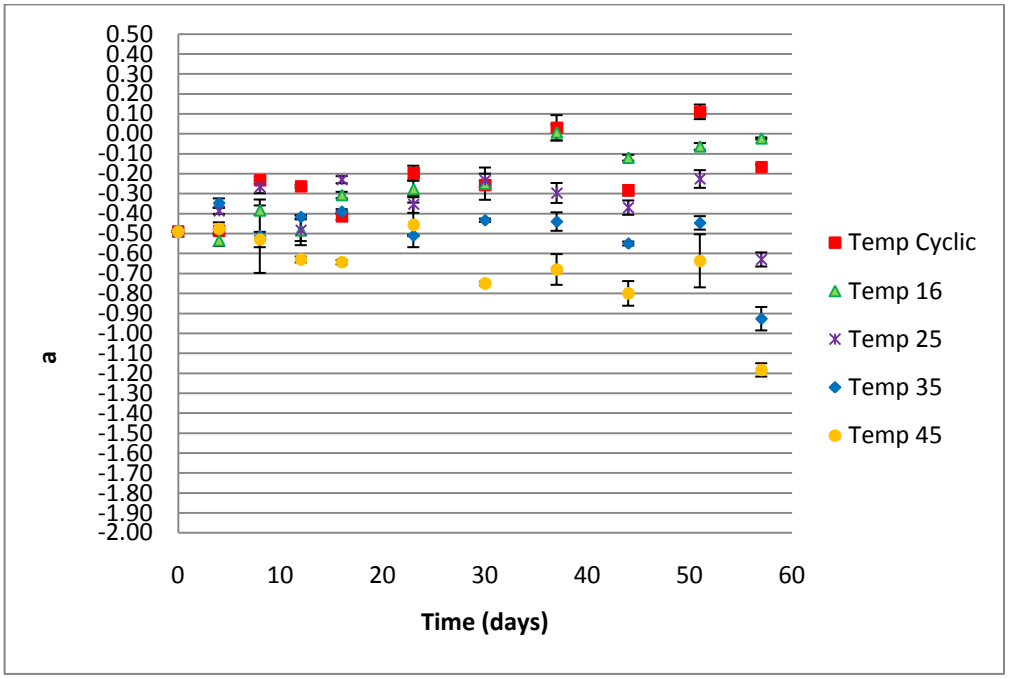
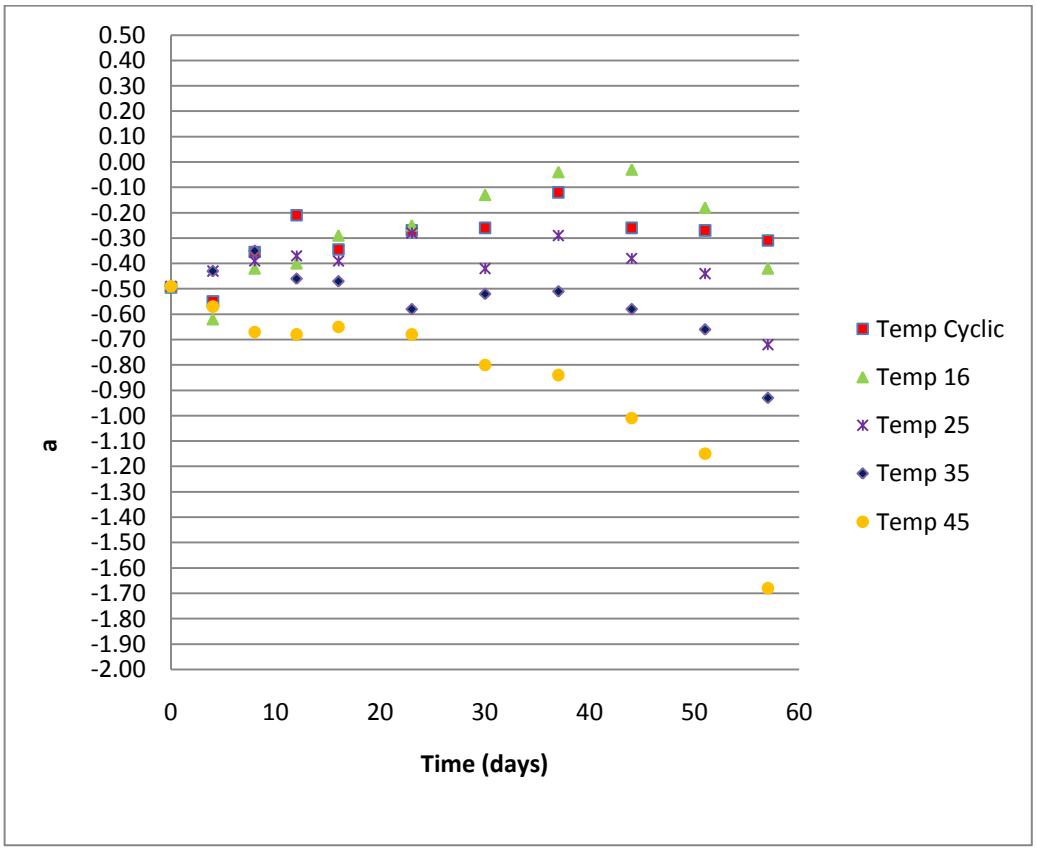


Figure 4.13: ‘a*’ value v Time (days) – 750 mL Bottles



The values for 'a*' for the lower temperature (cyclic, 16^{o}C) treatments and 100 mL bottles show a slight tendency to head towards the positive region (tending to reddish) over time, while the warmer treatments trend towards more negative (or more greenish) over time, especially the 35^{o}C and 45^{o}C results. The 750 mL bottles follow the same trend, except the decline for Temp 45 treatment appears to be greater.

4.1.4.3 'b*' Results

Tables 4.12 and 4.13 summarise the 'b*' results for 100 mL and 750 mL bottles respectively.

Table 4.12: 'b*' Results – 100 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	2.84 +/- 0.02	2.84 +/- 0.02	2.84 +/- 0.02	2.84 +/- 0.02	2.84 +/- 0.02
4	2.83 +/- 0.01	2.83 +/- 0.03	2.94 +/- 0.05	3.17 +/- 0.01	3.61 +/- 0.01
8	2.85 +/- 0.07	2.90 +/- 0.07	2.97 +/- 0.04	4.01 +/- 0.51	6.79 +/- 0.69
12	2.97 +/- 0.04	3.93 +/- 0.68	3.75 +/- 0.37	3.37 +/- 0.02	5.95 +/- 0.28
16	3.29 +/- 0.28	2.70 +/- 0.04	2.83 +/- 0.03	3.00 +/- 0.10	4.72 +/- 0.11
23	3.08 +/- 0.11	2.94 +/- 0.11	3.15 +/- 0.11	3.78 +/- 0.07	5.68 +/- 0.80
30	2.91 +/- 0.04	3.00 +/- 0.30	3.11 +/- 0.06	3.89 +/- 0.02	6.03 +/- 0.14
37	2.99 +/- 0.09	2.77 +/- 0.03	4.12 +/- 0.26	5.33 +/- 0.55	7.92 +/- 1.08
44	2.98 +/- 0.07	2.48 +/- 0.03	3.07 +/- 0.10	5.76 +/- 0.09	10.94 +/- 0.87
51	3.55 +/- 0.30	2.33 +/- 0.08	3.18 +/- 0.12	4.64 +/- 0.15	11.15 +/- 1.01
57	3.07 +/- 0.03	2.47 +/- 0.02	3.44 +/- 0.23	7.26 +/- 1.48	10.68 +/- 0.73

The results tabled are the average of all 100 mL bottles analysis +/- SEM.

Table 4.13: 'b*' Results – 750 mL Bottles

Day	Temp Cyclic	Temp 16	Temp 25	Temp 35	Temp 45
0	2.83 +/- 0.00	2.83 +/- 0.00	2.83 +/- 0.00	2.83 +/- 0.00	2.83 +/- 0.00
4	2.80 +/- 0.00	2.86 +/- 0.00	2.80 +/- 0.00	2.89 +/- 0.00	3.40 +/- 0.00
8	2.85 +/- 0.00	3.23 +/- 0.00	2.80 +/- 0.00	2.97 +/- 0.00	3.97 +/- 0.00
12	2.76 +/- 0.00	2.78 +/- 0.00	2.88 +/- 0.00	3.44 +/- 0.00	4.38 +/- 0.00
16	2.70 +/- 0.00	2.60 +/- 0.00	2.89 +/- 0.00	3.29 +/- 0.00	4.49 +/- 0.00
23	3.87 +/- 0.00	2.56 +/- 0.00	2.81 +/- 0.00	3.45 +/- 0.00	6.05 +/- 0.00
30	3.25 +/- 0.00	2.48 +/- 0.00	3.02 +/- 0.00	3.97 +/- 0.00	5.37 +/- 0.00
37	2.79 +/- 0.00	2.78 +/- 0.00	3.60 +/- 0.00	4.01 +/- 0.00	6.24 +/- 0.00
44	2.84 +/- 0.00	2.27 +/- 0.00	3.06 +/- 0.00	4.41 +/- 0.00	5.87 +/- 0.00
51	2.68 +/- 0.00	2.31 +/- 0.00	3.16 +/- 0.00	3.76 +/- 0.00	5.62 +/- 0.00
57	2.78 +/- 0.00	1.91 +/- 0.00	2.62 +/- 0.00	4.36 +/- 0.00	6.39 +/- 0.00

The results tabled are the average of all 750 mL bottles analysis +/- SEM.

Figures 4.14 and 4.15 Plot 'b*' vs Time for the 100 mL and 750 mL bottles respectively.

Figure 4.14: 'b*' value v Time (days) – 100 mL Bottles

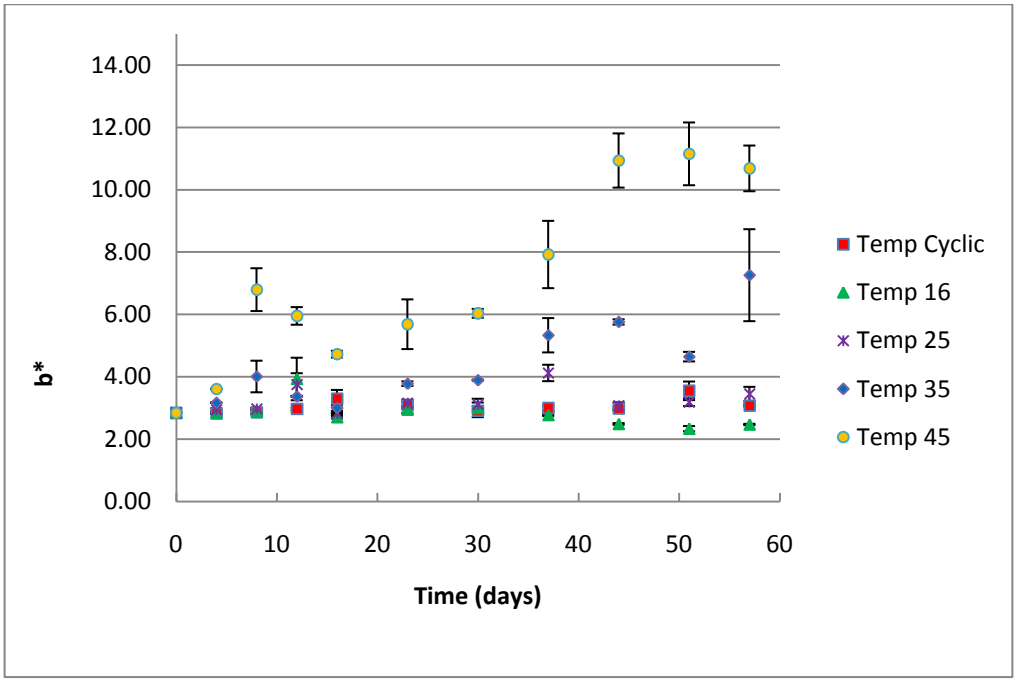
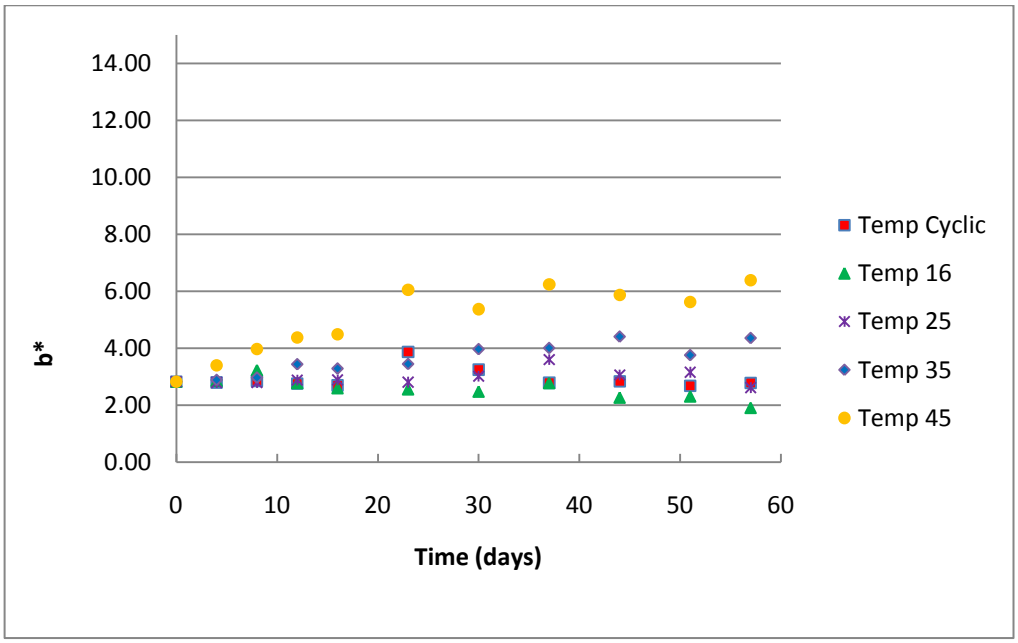


Figure 4.15: 'b*' value v Time (days) – 750 mL Bottles



The 'b*' results are consistent with the trends noted for 'L*' and 'a*', in that the lower temperature (cyclic, 16°C and 25°C) treatments show only slight

movement while the two higher temperature treatments diverge away from the others. In this case, the movement in their 'b*' values is taking them towards the yellow/orange regions of the scale. With the decreasing 'L*' values (i.e. darkening of the wine), this would be producing more brownish tones. Again the 750 mL trends are similar to the 100 mL results except that they appear to be slower to occur.

4.1.4.4 'a*' vs 'b*'

By plotting 'a*' vs 'b*' for the different treatments (figures 4.15 and 4.16 for 100 mL and 750 mL bottles respectively), it is possible to see the pattern of the shift in colour of the treatments.

Figure 4.15: 'a*' value vs 'b*' value by treament – 100 mL Bottles

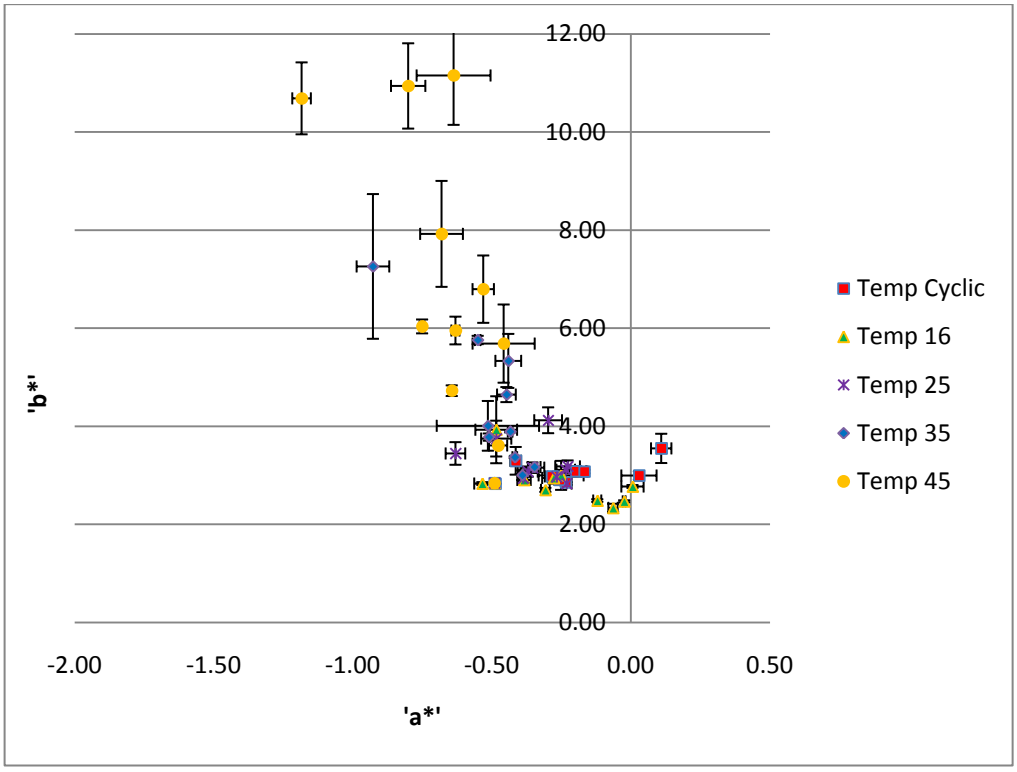
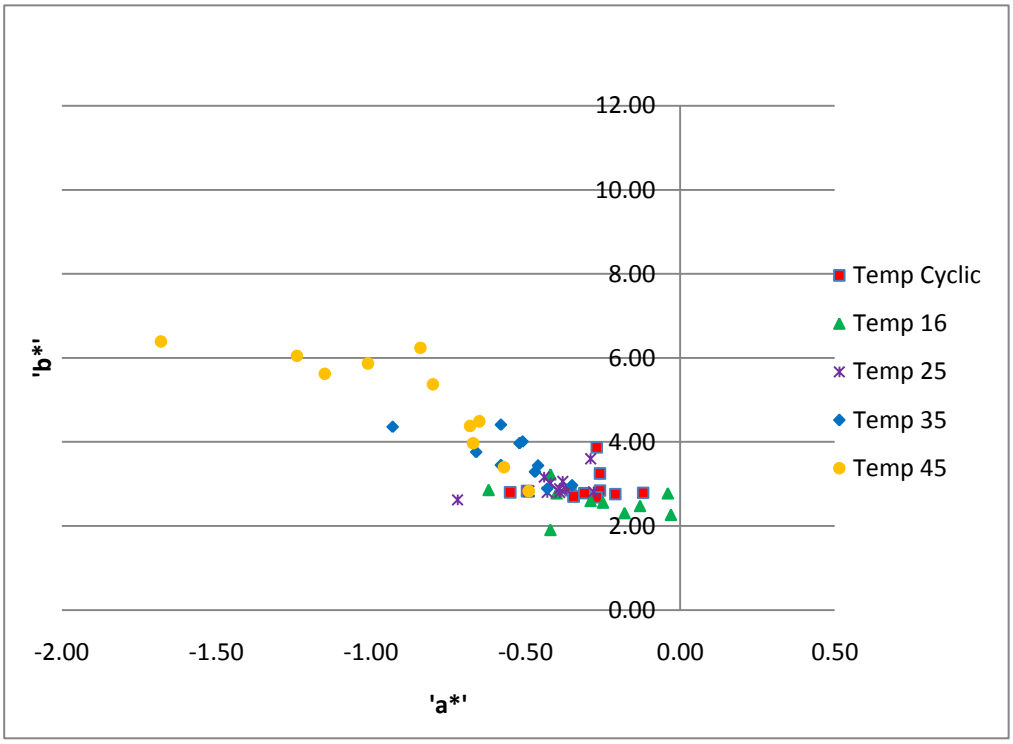


Figure 4.16: 'a*' value vs 'b*' value by treament – 750 mL Bottles



As was seen in the results for 'a*' and 'b*' previously, the lower temperature treatments for both the 100 mL and 750 mL bottles show a small movement in the 'b*' axis with some slight movement in the 'a*' axis. The Temp 35 and Temp 45 treatments show more movement in both axes, with the 100 mL bottles showing a large movement in 'b*' and a smaller movement in 'a*', interestingly, the 750 mL bottles show the reverse, with a large movement in 'a*' and a plateauing of movement in 'b*'.

4.1.4.5 Overall CIELab

The CIELab results, when evaluated in totality, make it is possible to draw some conclusions about the colour changes experienced.

The colour shift is towards the yellow-orange region of the colour space, with the higher temperature treatments, especially Temp 45 shifting much further in this direction. With the decrease in luminosity in Temp 35 and Temp 45 treatments, which makes the wine appear 'duller', the perceived effect on viewing is a definite shift towards a brown colour for the higher temperature treatments.

4.2 PARAFAC Results

Table 4.14 below describes the sample terminology for the PARAFACs performed.

Treatment	Replicate #	Sample Code	Type
Temp Cyclic	1	TCY1	100 mL bottle
	2	TCY2	100 mL bottle
	3	TCY3	100 mL bottle
	4	TCYBt	750 mL bottle
Temp 16	5	T16R1	100 mL bottle
	6	T16R2	100 mL bottle
	7	T16R3	100 mL bottle
	8	T16Bt	750 mL bottle
Temp 25	9	T25R1	100 mL bottle
	10	T25R2	100 mL bottle
	11	T25R3	100 mL bottle
	12	T25Bt	750 mL bottle
Temp 35	13	T35R1	100 mL bottle
	14	T35R2	100 mL bottle
	15	T35R3	100 mL bottle
	16	T35Bt	750 mL bottle
Temp 45	17	T45R1	100 mL bottle
	18	T45R2	100 mL bottle
	19	T45R3	100 mL bottle
	20	T45Bt	750 mL bottle

Table 4.14 Sample Codes

4.2.1 PARAFAC - Combined CIELab and SO_2

The CIELab (L*,a* and b*) values and the free and total SO_2 results for all treatments and bottle types were analysed using the PLS toolbox's PARAFAC module.

This resulted in a 2 factor model being derived which fitted 83.3% of the data variability and had a core consistency of 100.

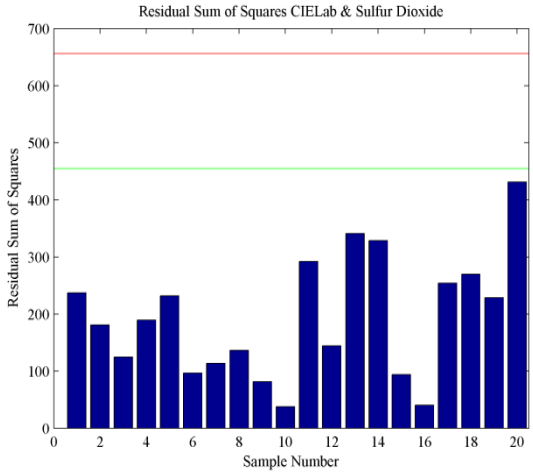


Figure 4.17: Residual Sum Of Squares

The residual sum of squares (figure 4.17) returned no samples that were outside the 95% confidence limit (green line =-95% confidence limit, red line = 99% confidence limit). All samples were retained for modeling.

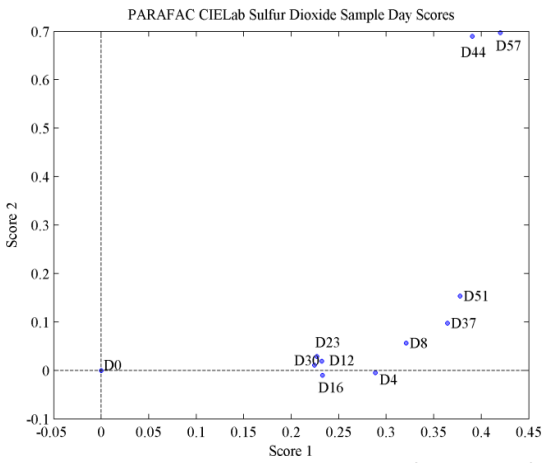


Figure 4.18:PARAFAC CIELab SO_2 Sample Day Scores

Figure 4.18 plots Score 1 vs Score 2 for the Sample Days. Here it can be seen that the time of exposure had some effects. Projected across Score 1 are sample days D0 to D57 in a more or less linear progression, indicating that treatment duration is cumulative i.e. the longer exposure of samples to radiant heat the greater the impact upon the measured attributes (as seen in the raw data for CIELab values and SO_2 levels).

D0 separates out with D4,D12,D16,D23 and D30 in a grouping in the centre. D8,D37 ,D44, D51 and D57 form another group. Score 2 results show little

change until around D37, where the samples exposed to the longest times separate out from bottom to top. For both scores, the results indicate that there is a time relationship to the model, with only moderate changes until around D37, when the rate of change appears to be .

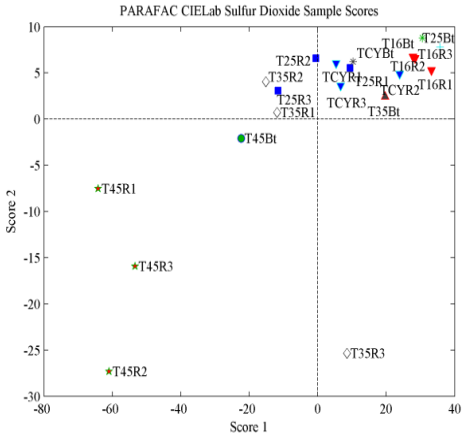


Figure 4.19:PARAFAC CIELab SO2 Sample Scores

The PARAFAC on the CIELab Sulfur Dioxide Sample scores (Figure 4.19), shows that there is a definite treatment effect, with the lowest temperature treatments in the top right corner and the highest temperature treatments in the lower left corner. The treatments are roughly placed in their order of temperature in this direction.

Interestingly, for both scores, it is the higher temperature samples which have separated out the most (apart from T35R3, which appears to be anomalous for score 2, this variation may be due to the variability in the 100 mL bottles). This may indicate that there are some reactions occuring at higher temperatures, that either do not occur at the lower temperatures or are much slower.

Another conclusion that may be drawn is that the 750 mL bottles exhibit a very similar pattern to the 100 mL bottles, but lag in absolute terms. This is an important finding, as it provides validation that the results obtained using the 100 mL bottles can be extrapolated to the behaviour of 750 mL bottles.

In figure 4.20, it is possible to see that two attributes exert a large effect arising from the experimental treatments, namely 'L*' (relatively highly positive in score 2, zero in score 1) and total SO_2 (relatively highly positive in score 1, slightly negative in score 2) with 'b*' (slightly negative for both scores 1 and 2) and free SO_2 (slightly positivie for score 1, zero for score 2) showing weak effects while 'a*' had a neglible effect (near zero for both scores). In essence, this means that Factor 1 is mostly due to

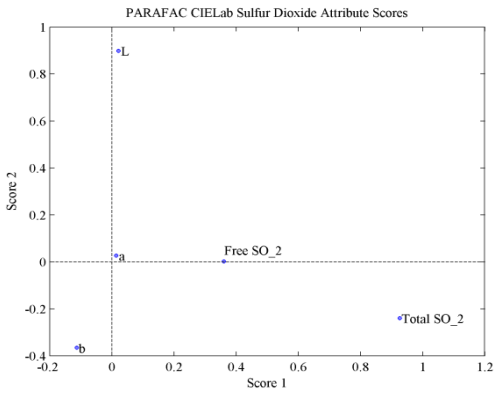


Figure 4.20:PARAFAC CIELab SO2 Attribute Scores

sulfur dioxide and, in particular, total SO_2, with almost no contribution from the CIELab values (a slight negative interaction with 'b*'). Factor 2 is predominantly influenced by 'L*', with a slight negative influence from 'b*' and total SO_2.

If figure 4.19 is re-examined, it is possible to see the effects of the experimental attributes in the factors. In the case of the Temp 45 treatments, the treatments are all highly negative for scores 1 and 2. The score 1 results would indicate that these treatments are undergoing more marked decline in sulfur dioxide (particularly total SO_2) than the other treatments (as factor 1 is highly positive for total SO_2 and reasonably positive for free SO_2, a negative score for the sample is associated with a decline in that factor), while 'b*' should be rising slightly compared with the other treatments (as 'b*' is negatively correlated with factor 1). 'a*' will be not be changing significantly, as it has a zero loading in factor 1.

With the score 2 results for all Temp 45 treatments, it would be expected that, as the scores are highly negative, the Temp 45 treatments would be experiencing a larger relative decline in 'L*' (as 'L*' is positively correlated to factor 2, a negative score would indicate a decline in value relative to the other treatments), with 'b*' rising (as 'b*' is negatively correlated with factor 2) and no influence on free SO_2 and 'a*' (zero loadings). There is a slight effect on total SO_2 from factor 2, in the opposite direction to factor 1. This is reflected in the raw data, with Temp 45 total SO_2 levels declining to a greater extent than the lower temperature treatments, accompanied by a marked decline in 'L*' values, while 'b*' values are higher than the lower temperature treatments. The 'a*' values are changing, but the changes are of a much smaller magnitude than the other values.

The sulfur dioxide results are also worth further examination. In the discussion on the sulfur dioxide and heat summation, it was noted that the rate of decline of total SO_2 was twice that of free SO_2. If the factor loadings are examined in figure 4.20, it can be seen that if the factor 2 negative effect is added to factor 1 (in essence reducing the total SO_2 in factor 1), that the relationship between free and total SO_2 would be somewhere near 2:1.

As the model appears to fit behavior exhibited in the raw data and has an excellent level of fitting and an acceptable core consistency, it can be concluded that this model is valid for explaining the relationship between CIELab values, sulfur dioxide, time and radiant heat exposure (treatments.

4.2.2 PARAFAC – UV-Vis

The UV-Vis spectral data was analysed using the PLS toolbox's PARAFAC module.

This resulted in a 3 factor model being derived which fitted 99.9% of the data variability and had a core consistency of 100.

The plots of the means of the scan replicates are plotted in figure 4.21. Here we can see that there has been some changes in the spectral data recorded.

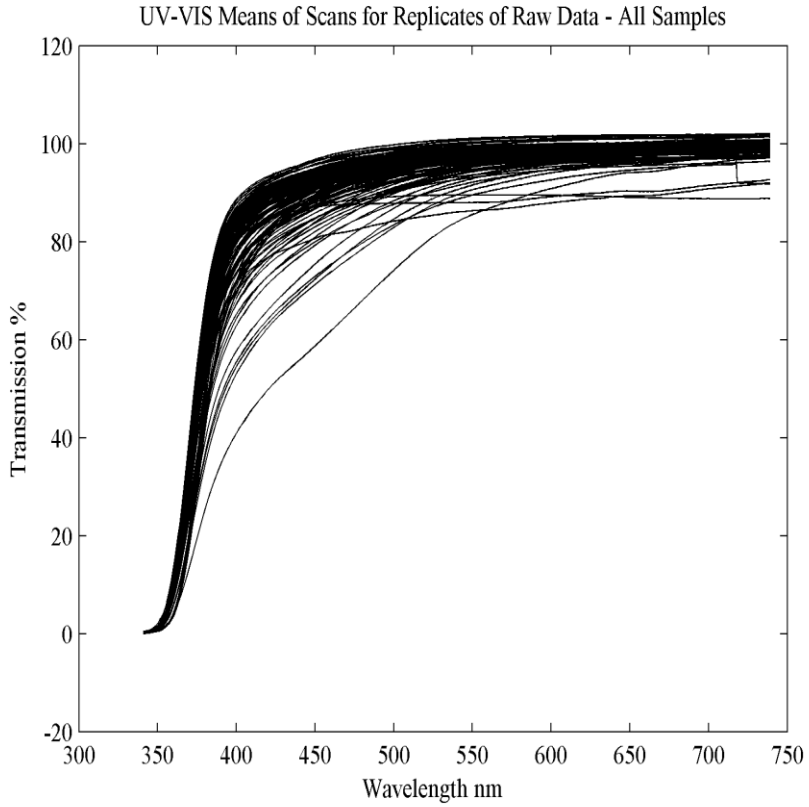
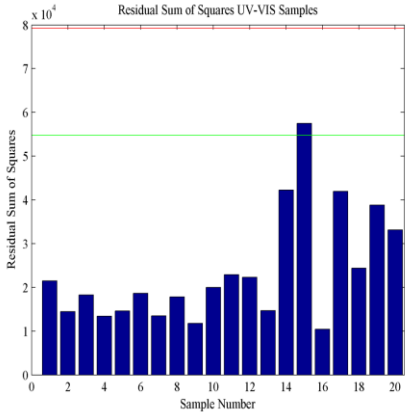
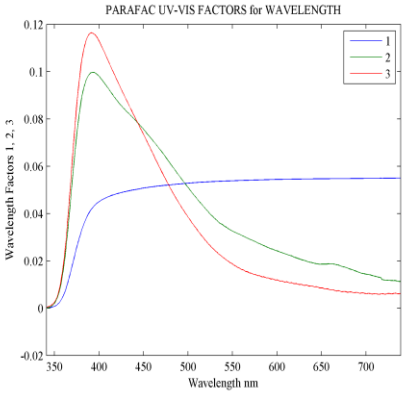


Figure 4.21: UV-Vis Means of Scans for Replicates of Raw Data - All Samples



The residual sum of squares (figure 4.24) returned one sample that was outside the 95% confidence limit, sample 15 (green line = 95% confidence limit, red line = 99% confidence limit). As sample 15 only marginally excluded the 95% confidence level, it was retained for modeling.

Figure 4.22: Residual Sum of Squares UV-Vis Samples



When the three factor loadings are plotted versus the wavelength (figure 4.23) (i.e. looking at their relationship to transmission), it is apparent that factor 1, which corresponds to the largest influence, reflects the raw data. Knowing this, factors 2 and 3 should become the focus, as they offer the best opportunity

Figure 4.23: PARAFAC UV-Vis Factors for Wavelength

to identify causes of the variability. Factors 2 and 3 appear to be largely similar, with a similar peak and curve shape indicating that they are modeling the same chemical processes with the factor 2 attributes processes occurring at a slower rate.

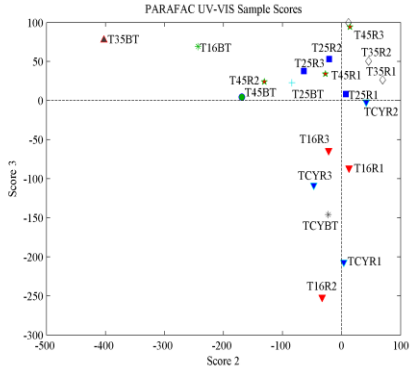


Figure 4.24: PARAFAC UV-Vis Sample Scores

In figure 4.24, it can be seen that there is a definite grouping in score 2, with the 750 mL bottles separated out to the left of the 100 mL bottles. This indicates that there is some form of vessel or volume effect being exhibited. The negative loadings show that the 750 mL bottles appear to be changing more slowly than the smaller 100 mL bottles. Referring back to the sulfur dioxide analysis and the CIELab values, this is quite evident, with the 750 mL bottles always showing a lagging tendency compared with the 100 mL bottles.

This is important, as this confirms the earlier tentative conclusions drawn about the ability of 100 mL bottles to reflect the changes in the 750 mL bottles. Here we have the trends identified in that analysis being observable in the model – that is, that the 100 mL bottles attributes change at a faster rate than the 750 mL bottles, but that the 750 mL bottles appear as if they will follow the same pattern of behavior.

For score 3, there is a group below the axis that consists of nearly all the two lowest heat loaded treatments (Temp16 and Temp Cyclic), apart from T16BT. The other treatments are clustered above that axis, with little ordering apparent.

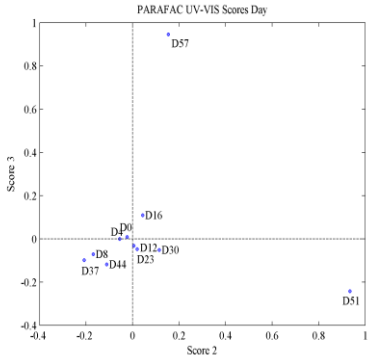


Figure 4.27 plots scores 2 and 3 for days. Here it appears that little is happening until Day 51 and Day 57 which diverge markedly from the rest of the days. Again, this is seen in the raw data, where the L*,a* and b* values move the most at these late points.

Figure 4.27: PARAFAC UV-Vis Scores Days

Taking all the above into consideration, it appears that this PARAFAC satisfactorily models the wine's behavior, with the raw data trends being captured in the model, while the model itself had an excellent fit and a satisfactory core consistency.

4.2.3 PARAFAC - FTIR

The figure below (figure 4.28) shows the plotted results for the FTIR scans. The areas between the green lines are the excluded zones as detailed in materials and methods.

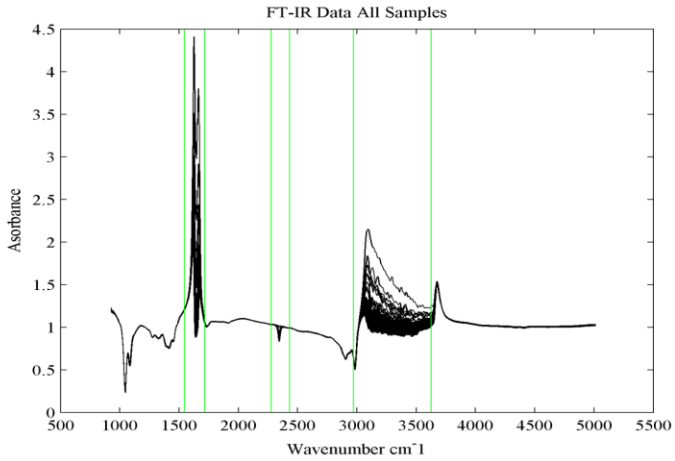


Figure 4.28: FT-IR Data All Samples - Absorbance v Wavenumber

Figure 4.29 shows the scans after SNV processing.

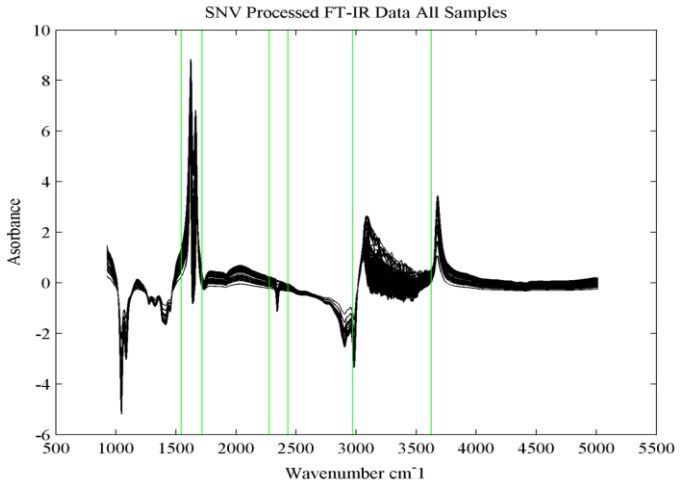


Figure4.29: SNV Processed FT-IR Data All Samples - Absorbance v Wavenumber

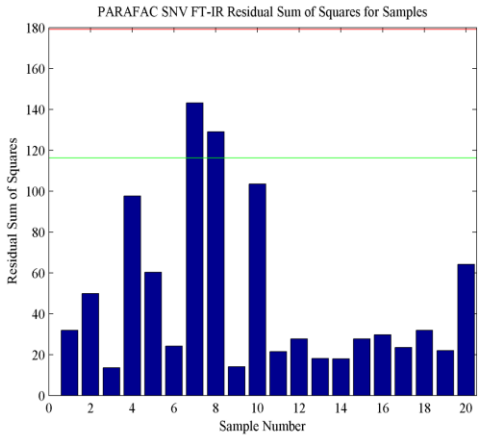


Figure 4.30: PARAFAC SNV FT-IR Residual Sum of Squares for Samples

The residual sum of squares (figure 4.30) indicated that samples 7 and 8 should be excluded. As they were significantly outside the 95% confidence limit (green line = 95% confidence limit, red line = 99% confidence limit), they were excluded from further analysis.

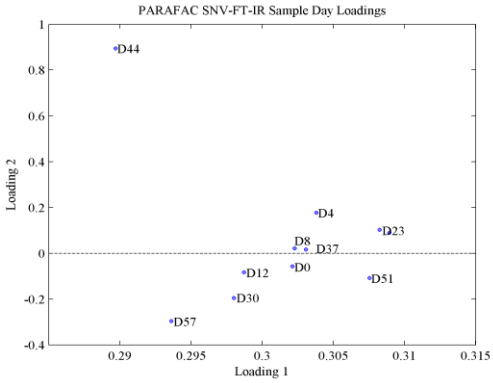


Figure 4.31: PARAFAC SNV FT-IR Sample Day Loadings

The loadings for Sample Days (figure 4.31) showed no real pattern for either loading 1 or loading 2, with the Days scattered in both directions.

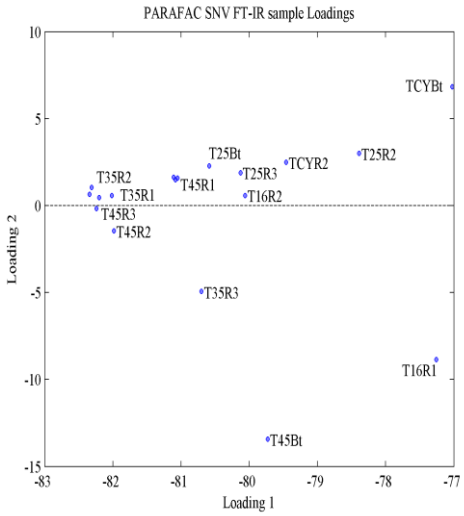


Figure 4.32: PARAFAC SNV FT-IR Sample Loadings

For treatment loadings (figure 4.32), loading 1 showed a weak trend of higher heat treatments to the left (more highly negative) with the lower temperature treatments to the right. These were roughly grouped; with the lower temperature treatments (TCY, T16 T25) in a wide spread grouping and T35 and T45 treatments mostly grouped together around -82, with T35R3 and T45Bt slightly separated

from the main group.

Loading 2 produced no conclusive trend, with most treatments clustered around zero, with a few outliers that were not from the same treatments.

As there were no consistent trends evident in the PARAFAC analysis apart from a weak treatment effect for loading 2, the construction of a model of compositional changes using the FTIR data was not considered to be viable.

It is possible that the analysis carried out was not in a suitable spectral range to develop a satisfactory model. A change in the region may produce a valid model, for example, a shift to measuring in the mid infra-red might result in better separation of the variables.

4.3 Sensory Assessment

Table 4.15 details the results of balanced duo-trio testing.

Table 4.15 – Results for Balanced Duo-Trio Tests

Treatment Comparison	n	Number correct	P	Significant
Temp 16 v's Temp CyclicC	15	10	0.151	No
Temp 16° v's Temp 25	15	9	0.304	No
Temp 16° v's Temp 35	15	15	0.000	Yes
Temp 16 v's Temp 45	14	12	0.006	Yes
Temp Cyclic v's Temp 25	14	6	0.788	No
Temp Cyclic v's Temp 35	14	11	0.029	Yes
Temp Cyclic v's Temp 45	15	12	0.018	Yes
Temp 25 v's Temp 35	15	13	0.004	Yes
Temp 25 v's Temp 45	15	15	0.000	Yes
Temp 35 v's Temp 45	15	12	0.018	Yes

There was no significant difference detected by the panelists between ideal storage at 16^{o}C (Temp 16) and storage at 25^{o}C (Temp 25) or with the cyclic treatment (Temp Cyclic). The cyclic results are within expectations, as the heat summation for Temp Cyclic is lower than the Temp 25 summation.

Storage at 35^{o}C (Temp 35) and 45^{o}C (Temp 45) did result in significant sensory differences compared with the lower temperature treatments. As well, the panel was able to distinguish between Temp 35 and Temp 45. This is consistent with the earlier CIELab analyses, where the Temp 45 treatment at Day 57 had separated markedly from the lower temperature treatments. This confirms that the extra heat load in Temp 35 and, particularly, Temp 45 has had effects not only on colour but on the organoleptic profile of the wine.

The sulfur dioxide results indicated that there is a relationship between compositional changes in the wine and the heat summation. It would be a worthwhile extension of this research to investigate whether the lower temperature treatments (and most particularly the 25^{o}C and cyclic treatments) exhibit similar sensory qualities when the heat summations are similar to those that the Temp 35 and Temp 45 treatments were exposed to by the end of the storage time. It would also be worth a more thorough examination of the changes in the sensory perception of the wine throughout the heat exposure. This could entail employing difference testing at each sampling point, followed by descriptive analysis once significant differences were found.

These results could then be used in conjunction with the compositional analysis to develop a fuller model of the wine behavior due to radiant heat exposure.

5. Conclusion

The major aim of this project was to establish whether suitable models of the behavior of white wine behavior when exposed to radiant heat could be developed.

During the course of the project and the ensuing data analysis it was clearly shown that radiant heat had profound effects on white wine. From changes in colour (to the yellow/orange/brown region of the colour space), to accelerated loss of sulfur dioxide (both free and total SO_2) and altered sensory characteristics all driven by the amount of radiant heat that the wine experienced. The greater the heat summation (the product of temperature and length of exposure to that temperature), the greater were the changes measured.

Moreover, within the limits of the project, it was found that the changes could be modeled using PARAFAC to analyse the UV-Vis spectral data and the CIELab and sulfur dioxide data with reasonable certainty. It was found that the FT-IR data obtained could not produce a valid model of behavior.

The project demonstrated that there was definite potential in the methodologies adopted and that further work should be carried out to further refine and validate the models. Such work should include a number of different wines to ascertain the validity of the results and models against a cross section of wines and wine styles. This would enable the effects of a range of different compositions (i.e. a mixture of different alcohols and pH's, oaked and un-oaked, different ages, etc.) on the models to be determined.

It would also be of use to provide more thorough sensory assessment of the wines throughout such a project. It would be interesting to know whether the sensory assessments were in line with the compositional changes measured over time. For instance, in much of the raw data, reinforced by the factors identified in the modeling, there is a noticeable change in the composition in the last few time points (commencement varying from Day 37 to Day 51, depending on bottle type and measured attribute), it would be of use to know if sensory assessment also captured these changes at or around the same time. Such sensory assessments could also be used as a further variable in the modeling to provide a more robust and useful tool.

Additionally, a longer time frame would enable further analysis and indicate whether the changes noted continued or whether there was some form of

plateau in the changes measured. It would also allow evaluation of the heat summation effects in the higher HDH regions.

A secondary objective was to evaluate the use of 100 mL medicine flat bottles against wine industry standard 750 mL bottles. If the 100 mL bottles were found to satisfactorily simulate the effects of radiant heat exposure in the 750 mL bottles, the results of the project could be directly related to industry standard bottles. It was found that although the 100 mL bottles tended to be more 'advanced' than the 750 mL bottles for all measured attributes, that similar results were obtained from the 750 mL analyses, just at a later time. This means that the results from the 100 mL bottles can be extrapolated to the 750 ml bottles with some certainty, however, it would be advantageous to have a longer time set to evaluate whether the 750 mL bottles actually continue to follow the same pathway, or whether they deviate at some stage. However, the use of 100 mL bottles for simulating compositional changes in white wine, at least in terms of radiant heat exposure, as a substitute for wine industry standard 750 mL bottles would seem to be valid, especially where there are limits on the amount of wine or the amount of temperature control storage available to the researcher.

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