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Investigating the phenolic composition of orange wines: studying the effects of oxidation and skin contact on white wine tannin

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A Dissertation

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of the requirements for the Degree of

Bachelor of Viticulture and Oenology with Honours

at

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by

Emily Rose Townshend

Lincoln University

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Viticulture and Oenology with Honours

Investigating the phenolic composition of orange wines: studying the effects of oxidation and skin contact on white wine tannin

by

Emily Rose Townshend

An in depth analysis of orange wines was conducted to assess factors affecting the phenolic composition. Orange wines were produced from Pinot Gris juice and table grape skins from September 2017 to March 2018. Factors included in this experiment were different ratios of skin contact to juice, additions of sulfur dioxide and aging. Basic wine data was analysed along with methylcellulose precipitable tannin, fractionation by solid-phase extraction and mean degree of polymerisation via acid catalysis in the presence of excess phloroglucinol. Increasing rates of skin addition resulted in a higher tannin composition in wine, along with an increase in pH and a decrease in TA and alcohol percentage. The use of SO2 limited tannin development, mean degree of polymerisation and affected the proportions observed in fractions two and three, but protected total phenolics from oxidation over time, especially those present in fraction one. The use of SO2 also resulted in orange wines with a lesser degree of browning. Orange wine phenolics develop in a similar way to red wine phenolics and are affected by similar winemaking conditions, however further study is required to confirm results.

Keywords: orange wine, phenolics, tannin, Pinot Gris, fractionation, mDP, colour, oxidation, skin contact, extended maceration.

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Chapter 1

Introduction

1.1 Background

"Orange wine" was a term first coined by David Harvey (UK wine-trade professional) in 2004 *[29, 38]* while tasting these wines in Italy. This method of white wine production has seen a recent re-emergence and rising popularity, although it has been in production for many years, and could be considered to be an ancient method of making wine *[29]*.

Orange wine has many diverse definitions and expectations from winemakers around the world, such as Italian winemaker Saša Radikon, who interprets it as meaning the wine "has to be macerated on natural yeasts and with no temperature control" *[29]*. However, one agreement is that it is essentially white wine made using the methods generally employed for red winemaking. This involves the use of skins, seeds and sometimes stems during the fermentation process, which may extend for weeks, months or even years *[21, 29, 38]*. This allows extraction of tannin into white wines and differentiates orange wines from commercially produced white wines from a sensory perspective, due to a stronger perception of astringency.

Tannin is an important component of orange wine structure from a sensory perspective, as it is a major contributor to mouthfeel and texture through the perception of astringency. Tannins are characterised by their ability to bind to proteins in saliva, which causes this astringency, or drying sensation of the mouth and tongue *[31]*. They are primarily found in the skins, seeds and stems of the bunch. Seeds and skins generally have the highest proportion, followed by stems *[46]*. The degree of tannin extraction from this grape matter may be manipulated through winemaking techniques, such as yeast selection, extended maceration, and thermal treatments *[2]*. Ultimately, it depends fundamentally on extended skin and seed contact with ethanol, such as during fermentation and extended maceration *[5]*. This helps to explain why wines made with an extended maceration time following fermentation often contain appreciably higher concentrations of tannin than those which are pressed off immediately following fermentation *[23]*. This is partially why commercially produced white wines have little-to-no astringent mouthfeel, as the skins and seeds usually do not remain in contact with the juice during fermentation. As mentioned above, the skins and seeds of orange wines remain in contact with the juice during fermentation and generally for a period at the end (extended maceration). This generally results in a greater amount of tannin extraction into the final wine, along with a more structured and astringent mouthfeel *[1, 4, 12, 30, 44]*.

Tannin content in white and red grapes can be, in fact, quite similar *[41]*. Therefore, it could be assumed that if a white wine was produced in the style of a red wine, with a fermentation involving skin contact, similar results could be observed in terms of tannin perception and chemical composition. However when we these wines are produced, described as “orange”, they have nearly less potential for tannin retention. This is largely due to one main compositional difference in the skin of red grapes; in that they contain anthocyanins, whereas white grapes do not. Tannins may precipitate out of white wines amongst grape and yeast solids, with no anthocyanins to bind to *[42]*. Otherwise, studies have presented that the skin and seed composition of red and white grapes is relatively similar *[43]*. This lack of anthocyanins means that the binding reaction between tannins and anthocyanins cannot take place. The solubility of tannin is therefore not increased and as a result, is more rapidly precipitated out of wine following fermentation and aging. For this reason, orange wines are generally less astringent than their red wine counterparts.

1.2 Aims and objectives

This study was focused on the ratio of skins to juice in fermentation trials of *Vitis vinifera* L. cv. Pinot Gris, as well as the effects of oxidation and accelerated aging on the phenolic composition and colour of orange wines. It includes a review of the current literature on the subject, experimental results, a discussion, conclusion and references. Observations include how tannin concentration and composition was altered when the skin: juice ratio is manipulated prior to fermentation, how additions of SO_2 maintained during fermentation and aging affect tannin, and how these wines could be expected to change over time using an accelerated aging period. Wines produced with a higher ratios of skins were expected to provide a greater extraction of tannin than those produced with a “normal” proportion of skins or solely with juice. Wines matured in an accelerated environment at 35° C were expected to show greater development of tannin and perhaps a greater mean degree of polymerisation (mDP). Wines produced without SO_2 were expected to demonstrate oxidation of phenolics and a higher absorbance reading for colour.

Chapter 2

Literature review

2.1 Production of orange wines

The Oxford Companion to Wine defines orange wines as wines produced "by fermenting the juice of ripe white grapes on their skins and pips, usually for between a week and a year" which results in wines which are amber/orange/gold/yellow in colour and possess a higher level of tannin than many commercial white wines *[38]*. Commercial styles of white wine are generally produced from grape juice only, following pressing and discarding of the grape skins. It is also noted that there are currently no regulatory frameworks for orange wines, and that they are all officially classed as white wines. This definition indicates that any white wine produced with skin contact during at least the duration of fermentation could be classed as an "orange" wine.

2.2 Current findings and observations

Proanthocyanadins, or condensed tannins, are polyphenolic flavonoid compounds consisting of oligomers and polymers of flavan-3-ols. They are widely found in the plant kingdom, including in *Vitis vinifera* grapes *[24, 40]*. They are an important component of red wine mouthfeel, providing structure and astringency. Their extraction depends largely on skin contact with juice during alcoholic fermentation, as the production of ethanol facilitates extraction *[5]*.

Although studies have been conducted in relation to white wines produced with skin contact, many of these studies have involved pre-fermentative maceration for a set time period prior to inoculation with yeast *[12, 15, 19, 33, 35, 48]*. Of these, some were focussed on the effect of this maceration on volatile aromatic compounds rather than the effect on the phenolic composition of the wines *[33, 48]*. Pre-fermentative maceration was found to be beneficial for the production of varietal aroma compounds such as varietal thiols, terpenes and norisoprenoids, as well as displaying an increase in fermentative aroma precursors. This can be attributed to the location of these compounds in the grape berry, being primarily in the skin.

However, pre-fermentative maceration has also been demonstrated to have an effect on the phenolic composition of white wines across different *Vitis vinifera* varieties, including Chardonnay *[34]*, Chenin Blanc *[1]*, Gewürztraminer *[44]*, Listán Blanco *[12]*, Riesling *[44]*, Sauvignon Blanc *[35]* and Zalema *[15]*. A general trend observed was that the total phenolic content of musts and wines was higher than that of control wines *[1, 12]* and was found to increase with the length of

maceration *[15, 19, 44]*. Studies involving different varietals have found significant differences in phenolic compositions. For example, the total phenolic content of Gewürztraminer with 35 hours of pre-fermentative maceration (220 mg/L) was found to be somewhat less than the total phenolics of Riesling after only 8 hours of pre-fermentative maceration (228 mg/L) *[44]*. This demonstrates that initial compositional differences can influence final phenolic content in white wines produced with the same degree of skin contact. This was also found to vary from year to year, with Gewürztraminer produced in 2009 presenting a total phenolics of 213 mg/L after 8 hours of pre-fermentative maceration, compared with 149 mg/L after 8 hours in 2010. This was attributed to 2010 being a cooler vintage with a cold ripening period and was harvested at a substantially lower 21 °brix than the 25 °brix achieved in 2009. This may have allowed further development and polymerisation of tannins in 2009 during berry maturation *[21]* which is indicated in the higher phenolic content of the wine subjected to the same maceration treatments as in 2010.

Some pre-fermentative studies have found that cooler temperatures facilitate higher extraction of some flavonoids and benzoic acids, particularly catechin, epicatechin, gallic acid, caffeic acid and caftaric acid, *[15, 19]*. It was found that wine produced from musts macerated for 6 hours on skins at 10 °C had roughly three times higher concentrations of catechin than wines produced without skin contact, 14.71 mg/L compared with 4.26 mg/L, respectively. Wines produced from the same maceration time at 20 °C contained half this amount, 7.09 mg/L. Other studies used a higher temperature of 16 °C and macerated for 24 hours, resulting in a catechin concentration of 8.38 mg/L compared with the control wine, 2.11 mg/L.

The effects of skin contact during fermentation of white wine on the phenolic composition have been studied, albeit not in great depth. Similar trends were observed to pre-fermentative maceration but showed more developed extraction of phenolic compounds. Where fermentation was studied on a daily basis, days 6-8 were demonstrated to present the highest concentrations of phenolic compounds. Most significant were the flavonols, which showed an approximately 40 fold increase when compared with a control fermentation without skins *[30]*. An initial lag phase was observed at the beginning of fermentation where the phenolic content did not significantly increase. This may be due to the time required for the seed lipid layer to become disrupted, which has been shown to be increased by the production of ethanol during fermentation *[18]*. These studies did not measure tannin, however, which is analysed in this experiment and is a new contribution.

Some studies explored different methods of including skin contact during fermentation, such as Chardonnay wines produced from an 8 day carbonic maceration (CM) compared with fermentation on skins *[34]*. It was found that wines that underwent carbonic maceration showed no significant differences from the wines produced without skin contact, apart from caffeic acid and quercetin

glycoside which showed significant increases. Wines produced with an on-skin fermentation showed significant increases in most phenolic compounds assessed, especially catechin and epicatechin which were 32.9 mg/L and 55.8 mg/L, respectively. Carbonic maceration wines presented 1.89 mg/L catechin and 0.90 mg/L epicatechin, while control wines with no skins presented 1.77 mg/L catechin and 1.42 mg/L epicatechin. Maceration with skins after crushing (pomace maceration) compared with whole berry maceration during fermentation was also explored *[4]*. It was found that pomace maceration resulted in a much higher total phenolic content in the final wines across two different varieties. Ribolla Gialla pomace macerated wines had a total phenolic concentration of 539 mg/L, compared with whole berry macerated wines and control wines, which had concentrations of 166 mg/L and 173 mg/L, respectively. The whole berry macerated wines actually had a lower concentration than that of control wines. This was likely due to the lack of phenolics being released from skins in the absence of crushing and pressing, the lack of contact with grape seeds, as well as a lowered surface area to volume ratio than the pomace macerated wines. Malvasia Istriana wines presented similar patterns, with pomace macerated wines having total phenolics concentrations of 134 mg/L compared with berry macerated wines and control wines, which contained 69 mg/L and 73 mg/L, respectively. This again demonstrated the lowered total phenolics concentration of the whole berry macerated wines while also illustrating the significant differences in phenolic composition that can occur between different grape varieties, as also found in pre-fermentative maceration. This could also be observed in the total phenolic content presented by fermentations of Gewürztraminer on skins, for which studies have found concentrations as high as 751 mg/L *[44]*.

One study looked at the effect of different skin contact percentages during fermentation on skins of Verdicchio wines *[13]* at 11-15 °C using reductive conditions in an industrial winery setting. These were 0 (C), 150 (15M) and 600 g/L (60M). Total phenolics was highest in the treatment with 600 g/L of pomace at the end of fermentation, 233 mg/L, compared with the 150 g/L and the control treatments, which were 136 mg/L and 85 mg/L, respectively. However, these differences became less evident after 6 months aging in stainless steel. 60M had decreased to 160 mg/L, 15M had increased to 163 mg/L and C had increase to 127 mg/L. Higher levels of skin maceration resulted in greater increases in the phenolic fraction during fermentation, with the most significant being gallic acid, caftaric acid and *cis*- and *trans*-coutaric acid. 60M also showed significant increases in monomeric flavonols catechin and epicatechin compared with 15M and the control. As flavan-3-ols are located in vacuoles within the skins of grapes, fermentation with skins allows these to pass into the fermenting must.

Where phenolics were determined by absorbance values, fermentations on skins of Chenin Blanc were found to present the highest absorbance values at 280 nm, 1.35 absorbance units (AU) compared with 0.64 AU for a pre-fermentative skin treatment and 0.63 AU for the control. These

wines also displayed the highest concentrations of catechin, gallic acid, polymeric phenols, coutaric acid and quercetin-3-glycoside when compared with pre-fermentative and control treatments. Polymeric phenols (not further specified) were found to be greatly increased at 39.24 mg/L compared with 8.86 mg/L for the pre-fermentative treatment and 8.58 mg/L for the control. Catechin was the next most significant at 18.32 mg/L compared with 8.45 mg/L for the pre-fermentative treatment and 7.87 mg/L for the control.

2.3 The effects of oxidation and accelerated aging on phenolic composition

Increased phenolic extraction has been demonstrated to result in additional browning of white wines during aging periods *[45]*. As browning of wine is mainly due to the either the enzymatic activity of polyphenol oxidase in juice prior to fermentation of oxidation of phenolic compounds during aging, it could be expected that higher concentrations of phenolics lead to a greater capacity for oxidative browning in orange wines. The oxidation of phenolic compounds such as catechin, epicatechin and hydroxycinnamic acids has been found to result in yellow and/or brown coloured compounds. These form from the polymerisation of *ortho*-quinones *[16]*. Where sulfur dioxide has been used in wine, a reduction in *ortho*-quinones in wine has been observed and therefore, a reduction in subsequent browning *[39]*. This indicates the capacity of sulfur dioxide as an antioxidant and therefore, the potential for protection of wine colour and oxidation of phenolics.

The temperature at which wine is stored directly influences the rate of physiochemical reactions that take place during wine aging *[21]*. As heat accelerates and activates reactions in wine, storage at higher temperatures tends to result in more browning *[21]* and has been demonstrated at short periods (6 weeks) to show accelerated changes in the concentrations of volatile aromatic compounds, such as esters and fatty acids *[6]*. Other studies have found that the use of higher temperature storage conditions (35 – 37° C) for longer periods of time (2 – 3 months) have not had significant negative effects on wine phenolics *[27, 47]* and therefore could be used to measure changes in wine composition during aging where time constraints are an issue.

2.4 The use of fractionation and mean degree of polymerisation (mDP) for further analysis of orange wine phenolics

Fractionation and mDP are methods commonly used for a more in-depth assessment of phenolic composition in red wines. However, there appears to be no known use of these types of analyses for orange wines at this stage. The results presented in this research will therefore be a new

contribution to the available knowledge on orange wine phenolic composition. The use of these methods for phenolic analysis are outlined below.

Fractionation helps researchers to understand the proportions of different phenolic compounds that exist in wine and how they may be affected by different winemaking treatments. The use of a specialized cartridge and different solvents allows separation of the wine into different fractions. The method developed by Jeffery, et al. [*22]* states that Fraction 1 contains phenolic acids, nonpolymeric flavanols, flavonols, anthocyanins, and pigmented monomers. In red wines, these pigments range from bright pink to orange in colour depending on the age of the wine. Fraction 2 contains polymeric polyphenols of a deep red colour. Fraction 3 also contains polymeric polyphenols, deep red to deep red/brown in colour. These fractions are also indicative of physiochemical properties, in that F2 is more hydrophilic, while F3 is predominantly hydrophobic. Higher proportions of F2 than F3 may indicate a greater solubility of phenolic compounds in wine and therefore a less astringent mouthfeel due to complexing reactions with other compounds. Hypothetically, applying this analysis to orange wines should give similar results, minus anthocyanins, with different hues visible than that of red wines, more those in the amber/yellow/orange/gold spectrum.

Mean degree of polymerisation (mDP) helps researchers to understand what affects tannin chain length and polymerisation over time. This analysis uses acid catalysis in the presence of excess phloroglucinol to cleave tannin chains in order to identify terminal and extension units of tannin chains *[25, 26]*. Through this analysis, it is possible to identify the proportion of terminal and extension units, the compounds present and the average length of the tannin chains (mean degree of polymerisation). As shorter chain lengths and flavan-3-ol monomers have been found to contribute to bitterness, while longer chain lengths have been found to contribute to astringency *[8, 10, 50]*, the sensory implications for wine production can be inferred. Therefore, this analysis could be used as a method of understanding the tannin composition in not only red wines, but orange wines also.

2.5 Scope of knowledge

The literature presented has mainly been concerned with the pre-fermentative skin contact, effects of skin contact on white wine sensory properties and volatile aromatic compounds. Data present on phenolic compounds is inconsistent in its depth of information, types of analysis used and grape varieties analysed. After extensive research, no studies on Pinot Gris have been found, although this is a common white grape used for the production of orange wines in new world wine countries such as New Zealand, due to its aromatic qualities. The data presented in this research will therefore be useful and applicable for modern white winemaking styles.

Chapter 3

Materials and methodology

3.1 Grape material

Due to the timing of the practical experiment (September - February 2017), fresh wine grape samples were unavailable. The material used for the production of orange wines was a combination of wine grape juice samples and table grape skins.

Vitis vinifera Pinot Gris juice samples were used from the 2015 and 2016 harvest from Pernod Ricard in Marlborough, New Zealand. The grapes had been sampled from the weighbridge, frozen in individual 200 mL plastic jars and later transported in September 2017 to Lincoln University in Christchurch, New Zealand. Skins were sourced from Lincoln New World in the form of green "Thompson seedless" (*Vitus vinifera*) table grapes, packed and shipped by Jasmine Vineyards, Delano, California, U.S.A. These were put through a crusher/de-stemmer (Marchisio, supplied by Viniquip International, Australia) then pressed twice to a maximum pressure of 300 kPA using a water bag press (Speidel, 40 L capacity, supplied by Vitis, NZ) until mostly dry by visual assessment. Juice was tasted regularly to ensure pressing could be stopped before high levels of phenolics were extracted from skins. Table grape juice was discarded and the skins collected and weighed. 4.055 kg of skins were gained and 15.5 L juice from 21 kg of fruit. A total fruit weight of 20 kg was estimated to cover all treatments. The fresh skin weight of grape berries is accepted as around 13-15% of the total berry weight. (Personal communication, Senior Lecturer Glen Creasy, 2017). The skin requirement for this experiment was calculated based on 1.1 kg of fruit per pot. Assuming 13% fresh skin weight, the requirement for each pot was 143 g. A total of 2.6 kg of skins at fresh weight was estimated to cover all four treatments requiring skins. One of these skin treatments was applied to each batch, with the aim of producing different tannin concentrations in the wine (see Table 3-1).

3.2 Fermentation and aging

Grapes were vinified as per a modified "French press" method *[7]*. Juice contained full solids and small macerated amounts of skins/seeds. Samples were kept frozen until needed and thawed gradually overnight in a warm water bath. Sample jars were then combined into a 25 L glass demijohn, the headspace gassed with CO_2, and stored in a 27° C warm room until the juice had reached 16° C. Juice was then distributed to provide a uniform 950 mL juice addition for each pot.

Juice was analysed for ° Brix, pH, titratable acidity (TA), free (FSO_2) and bound SO_2 (BSO_2). Different amounts of skin were added for each batch, and each batch was produced in triplicate for a total sample number of 15. Samples (50 mL) were taken from pots following skin additions and the juice was analysed again for ° Brix, pH, titratable acidity (TA), FSO_2 and BSO_2. This was to determine if the addition of table grape skins had affected basic chemical composition of the juice.

Table 3-1: Description of treatments and volumes.

Treatment	*Description*	*Replicates*	*Juice (ml)*	*Skin weight (g)*	*Total skin weight*	*Total pot volume (ml)*	*SO_2*
A	Pinot Gris juice only. No skins present. No SO_2 added.	3	950	0	0	950	N
B	Pinot Gris juice, "regular" skins. No SO_2 added. 5 day post-ferment maceration.	3	950	143	429	1093	N
C	Pinot Gris juice, "double" skins. No SO_2 added. 5 day post-ferment maceration.	3	950	286	858	1236	N
D	Pinot Gris juice, "regular" skins. SO_2 added. 5 day post-ferment maceration.	3	950	143	429	1093	25 ppm
E	Pinot Gris juice, "double" skins. SO_2 added. 5 day post-ferment maceration.	3	950	286	858	1236	25 ppm

Treatment A was considered the control wine and had no additions of skins or SO_2. Treatments B and D had what was considered to be a "normal" amount of skins added (13%) and treatments C and E had what was considered to be a "double" amount of skins added. Treatments D and E also had an addition of SO_2 in the form of a 5% potassium metabisulfite solution to provide an initial concentration of 25 ppm free sulfur dioxide (SO_2). All treatments were inoculated with the same wine yeast: Maurivin PDM *(Saccharomyces cerevisiae var. bayanus*), selected for a steady, reliable fermentation. Yeast was commercially available in dry form and was rehydrated according to manufacturer's instructions. Pots were then moved to a warm room maintained at 27-30° C for the duration of fermentation. Fermentation progression was monitored by checking temperature and weighing pots daily to measure weight loss via CO_2 (see Appendix A). An addition of diammonium

phosphate in a 20% solution was made to all pots on the third day after inoculation to add 60 mg/L of yeast assimilable nitrogen (YAN). Residual sugar (RS) was measured using Clinitest® tablets. Fermentation was assessed as completed and wines as dry when RS was measured at <2 g/L. Following the end of fermentation, treatments with skin additions had a 5 day extended maceration on skins. Pots were then pressed off skins by pushing down the plunger by hand, with a hold at full pressure for 10 seconds, then decanted to separate bottles. Pots were individually analysed for pH, TA, FSO_2, BSO_2 and alcohol content. Wines were cold settled for 14 days at 4° C in 1 L glass bottles and were not inoculated for malolactic fermentation. Following racking after cold settling, Treatments D and E were again analysed for SO_2 and adjusted to a final concentration of 25 ppm. Wines were then settled for a further 1 month, followed by a second racking. Treatments D and E had a final SO_2 analysis and top up to 25 ppm. For final bottling, each pot was separated into two 250 mL amber glass bottles and stored at two different temperatures during bottle maturation to estimate future compositional differences through accelerated aging.

3.3 Wine aging

At bottling, each replicate was split into two 250 mL bottles for a total of 30 bottles, 2 of each replicate. Any headspace was removed by adding sterilised marbles to bottles with low amounts of wine. 15 bottles were placed in a temperature controlled oven (240V, 50Hz, 'Clayson' Laboratory Apparatus LTD, NZ) at 35° C for 3 months. The remaining 15 bottles were placed in the Lincoln University Winery for the same time period. Ambient temperature in the winery was monitored with the use of two data loggers (TinyTalk topoclimate temperature data logger, Gemini Data Loggers UK), one placed next to the wine samples and one placed on the opposite side of the room to observe any differences between locations. Temperature data was recovered in 2 week intervals over the 3 month period to ensure data recovery in case of failure, using Tinytag Explorer 5.0 software (Gemini Data Loggers UK). Temperature graphs can be found in Appendix B.

3.4 Standard analysis of must and wine

Juice samples were analysed for ° Brix, pH, TA, FSO_2 and BSO_2 following the addition of skins, prior to fermentation. Following fermentation, samples were analysed for pH, TA, FSO_2, BSO_2, RS and alcohol. All wine samples were clarified for 20 minutes at 10000 rpm prior to analysis. Standard analysis was repeated following aging experiments, excluding RS as significant differences were not observed following fermentation. All analysis were as per standard methods *[20]*.

3.5 Spectral scans

Spectral scans were performed on all wines. Research wines had scans after fermentation and after aging. Commercial wines were analysed alongside. Scans were performed from 240 nm – 610 nm on a Shimadzu UV-1800 UV-Vis spectrophotometer (Shimadzu USA Manufacturing Inc., Canby, OR, U.S.A.).

3.6 Methylcellulose precipitable tannin assay

Total tannin was determined in all wines by the AWRI Standard Method "Determination of tannins in grapes and red wine using the MCP (methyl cellulose precipitable) tannin assay" *[3]*. This assay is based on tannin-polymer interactions, which produce insoluble tannin polymer complexes which then precipitate. The polymer in this case is methyl cellulose (0.04%). The reagents methyl cellulose (0.04%) and saturated ammonium sulfate required for this assay were made up as per the AWRI standard method instructions. The procedure followed was for the 1 mL assay. Treatment absorbance values were subtracted from control absorbance values at 280 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu USA Manufacturing Inc., Canby, OR, U.S.A). The difference was converted to mg/L epicatechin equivalents using a calibration curve produced from a 1000 mg/L epicatechin solution diluted to concentrations of 0, 25, 50, 75, 100 and 125 mg/L. The absorbance of each concentration at 280 nm was recorded and used to plot the calibration curve.

3.7 Fractionation

Samples from Treatments A, C and E only were selected to undergo fractionation due to the limited time frame of this study. In addition, all commercial wines were analysed. Solid-phase extraction was used to separate the wine into three fractions, each containing different concentrations of phenolic acids, non-polymeric flavanols, flavonols, anthocyanins, pigmented monomers and polymeric polyphenols. This was carried out according to the method as listed under the heading "Solid-Phase Extraction: Optimized Method" in Jeffery et al. *[22]*. High performance liquid chromatography (HPLC) analysis was carried out on the first fraction to determine the phenolic compounds present. Fractions two and three were measured at 280 nm on a UV-VIS spectrophotometer (Shimadzu UV-1800, Shimadzu USA Manufacturing Inc., Canby, OR, U.S.A) with scans performed from wavelengths of 240 – 610 nm.

3.8 Mean degree of polymerization (mDP)

Mean degree of polymerization (mDP) was carried out on all treatments and commercial wines. This was performed via the acid catalysis in the presence of excess phloroglucinol method outlined in Kemp, et al. *[25]*. This process seeks to identify the average amount and composition of terminal and extension units in a given sample containing proanthocyanidins, namely catechin, epicatechin, epicatechin gallate and epigallocatechin. Peaks were identified and calculated according to the method in Kennedy and Jones [*26]* to give the mDP. Peak areas under 5000 were excluded due to the arguably low concentrations, as well as replicate outliers in order to give the most accurate results.

3.9 Commercial orange wines

Five commercial orange wines were purchased and analysed alongside wine samples to provide a basis of commercial data for comparison of results from this study. Wines came from a variety of regions around the world and were mostly selected for their varietal composition and to have a range of data representative of several different styles within the "orange" category. Wines with a large percentage of Pinot Gris were selected where possible in order to relate more closely to the results from this study. Winemaking information is provided on each wine below, where available. All wines were clarified prior to analysis using a centrifuge for 20 minutes at 10000 rpm.

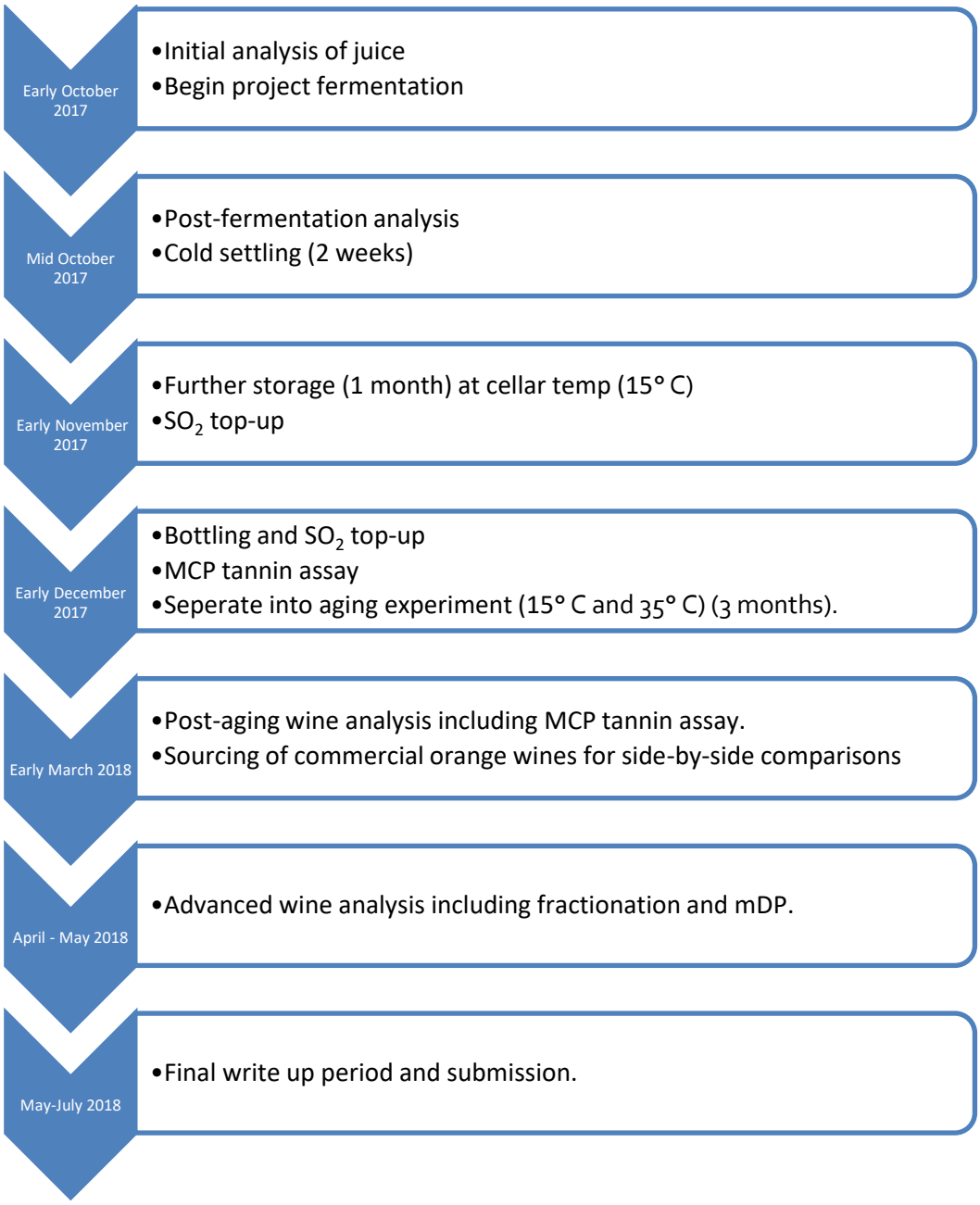
1. Muddy Water Skin Ferment 2016 (Waipara, NZ). 50:50 Chardonnay and Pinot Gris. 10% whole bunch fermented. 90% on skins for 30 days, 10% on skins for 120 days. Wild fermentation. MLF. No fining or filtration.
2. Valli "The Real McCoy" 2016 (Central Otago, NZ). 100% Pinot Gris. No fining or filtration. Made like Pinot Noir. 21 days on skins.
3. Vandal Gonzo White 2016 (Marlborough, NZ). Pinot Gris, Viognier & Riesling. Wild fermentation. Pressed off skins when ferment finished. 9 months barrel aging, 70% MLF. Sulfites added. Unfined and unfiltered.
4. Pheasant's Tears Rkatsiteli 2016 (Kakheti, Georgia). 100% Rkatsiteli, endemic Georgian variety. Skin fermented and aged in amphora for 6 months.
5. Denavolo Dinavolo Orange Wine 2010 (Emila-Romagna, Italy). Field-blend of Malvasia di Candia, Aromatica, Ortrugo, Marsanne, Sauvignon Blanc, Santa Maria and some unknown local varieties. Co-fermented with 4-7 months skin contact. Aged in botti and then bottled for 3 years before release.

Chapter 4

Results

4.1 Research wines

4.1.1 Timeline



4.1.2 Fermentation kinetics and wine production

Treatments C and E began fermentation first, abruptly at 3 days following inoculation with yeast. Treatments B and D began fermentation gradually at 3-4 days following inoculation. Treatment A began fermentation the latest, 5 days after inoculation. This may have been due to a lack of yeast assimilable nitrogen (YAN) and other nutrients in the juice which were supplemented by the addition of skins and soluble solids. It was also observed that the beginning of fermentation for treatment D occurred the day after the addition of DAP on day 3 following inoculation, which may indicate both a lack of nutrients and an impact of SO_2 on yeast population growth. Treatments C and E had completed fermentation by day 5 at which time the 5 day extended maceration period was determined to have begun. Treatments B and D had completed fermentation by day 6, at which time their extended maceration was determined to have begun. Treatment A completed fermentation by day 6, in one day. Treatments with double skins (C and E) completed fermentation in two days. Treatment B began fermentation a day earlier than its skins counterpart with SO_2 added (D), indicating that the presence of SO_2 had an impact on initial yeast population growth, although it had a slower fermentation (3 days). All treatments had completed fermentation and extended macerations by day 10. See Appendix A for individual ferment graphs.

4.1.3 Juice data

The basic juice composition of all treatments was analysed to determine whether the different skin treatments resulted in a change in juice chemical composition. Significant differences were observed between treatments with regards to the pH, free, bound and total SO_2. Significant differences were not observed between treatments in regards to ° Brix or TA (see Table 4-1). Juice pH was significantly different among treatments. The addition of skins resulted in increases in pH, with Treatments C and E having the highest pH. The presence of free sulfur dioxide in the juice prior to additions was noted. The probable cause may be field additions at the time of harvest, prior to juice sampling. This sampling took place at the winery weighbridge as fruit arrived. All treatments containing skins had higher bound and total SO_2 than treatment A. This is most likely due to the presence of a preservative sheet found in the boxes of purchased table grapes. Treatments C and E had significantly higher free, bound and total SO_2. As these treatments both contained an amount of skins double the amount considered to be “normal”, greater amounts of skins could then be expected to contribute to a higher FSO_2 in the juice. Treatment A contained the lowest TSO_2 which is to be expected as it did not have any skin additions.

Table 4-1: Basic chemical data of juice prior to inoculation

Treatment	*° Brix*	*pH*	*TA (g/L)*	*Free SO_2 (mg/L)*	*Bound SO_2 (mg/L)*	*Total SO_2 (mg/L)*
A	22.0	3.28	6.6	12	37	48
B	21.9	3.34	6.7	12	41	53
C	21.9	3.44	6.6	16	43	57
D	22.0	3.33	6.6	12	41	53
E	21.9	3.42	6.5	15	44	59
P-value	0.205	0.000*	0.755	0.012*	0.004*	0.001*

4.1.4 Basic wine data following fermentation (T1)

Following fermentation, the effects of both skin addition and SO_2 addition on wine chemical composition were assessed (see Table 4-2). The addition of skins and the rate of skin addition was found to significantly affect the wine pH, TA, alcohol percentage and tannin concentration. The pH stayed relatively stable during the fermentation process and retained the same patterns as previously noted, with increasing amounts of skin addition resulting in an increase in pH. Residual sugar was not significantly different across all treatments with all wines resulting in a final RS of <1 g/L, which can be classed as “dry”. The tannin concentration followed patterns of skin addition, with the lowest concentration observed in treatment A, medium concentrations observed in treatments B and D, and highest concentrations observed in both treatments with double skins (C and E). SO_2 was found to have a significant effect on tannin, with treatments D and E presenting lower tannin concentrations than their skin rate counterparts (B and C). Treatments D and E had top ups of SO_2 to maintain 25 mg/L prior to aging to continue observations of these effects on the development of wine composition. Tables of SO_2 additions and analysis of treatments D and E can be found in Appendix C.

Table 4-2: Basic wine data at T1

Treatment	*pH*	*TA (g/L)*	*RS (g/L)*	*Alcohol %*	*Tannin (mg/L)*
A	3.25	9.6	0.8	12.9	10
B	3.34	8.8	0.8	11.9	47
C	3.43	8.1	0.7	12.1	64
D	3.33	8.8	0.7	11.9	35
E	3.43	8.2	0.7	12.1	55
P-value skins	0.000*	0.000*	0.803	0.000*	0.005*
P-value SO_2	0.032*	0.715	0.615	0.959	0.313

T1 is immediately following fermentation. Tannin measurements are in epicatechin equivalents. SO_2 data displayed in Appendix C.

4.1.5 Basic wine data following aging (T2)

Following aging of wines, either at ambient cellar temperature or under accelerated conditions, the basic chemical composition of the wines were again analysed to observe how they had changed (see Table 4-3). All compositional parameters analysed were significantly affected by the addition and rate of grape skins in the initial fermentation. The pH of the aged wines follows similar patterns to those of the juice and newly finished wine, however all have reduced slightly and become more acidic. The TA of wines was reduced by the addition of skins, with treatment A producing the highest TA and treatment C presenting the lowest. Tannin concentration was significantly affected by all three factors; skin addition, SO_2 addition and the aging conditions. In the wines without SO_2, distinct differences were noted. Treatment A had the least amount of tannin but still showed an increase in the accelerated aging conditions. Treatments with skin contact were found to increase during accelerated conditions. Treatments with SO_2 also displayed increases in tannin during aging but had lower concentrations overall. Wines with SO_2 additions displayed similar patterns of increases in tannin under accelerated conditions but were diminished. This indicates that while the addition of SO_2 may not have a significant effect on tannin development initially following fermentation, it can impact the tannin development during the course of wine aging. Significant effects of SO_2 addition were observed only in the tannin concentration as the effect on pH following aging was found to be no longer significant. The same was found for aging conditions, with only tannin concentration being significantly affected. Treatments D & E were still found to have a proportion of FSO_2 at this time. Tables of SO_2 data and additions can be found in Appendix C.

Table 4-3: Basic wine data at T2

Treatment	*pH*	*TA (g/L)*	*Alcohol %*	*Tannin (mg/L)*
A	2.94	8.0	12.8	7
A - A	2.96	7.5	12.7	21
B	3.10	7.4	12.0	29
B - A	3.10	7.2	11.8	66
C	3.33	5.4	11.6	36
C - A	3.21	6.6	11.4	51
D	3.12	7.2	11.9	20
D - A	3.12	7.1	11.9	21
E	3.18	6.8	11.7	33
E - A	3.20	6.7	11.7	37
P-value skins	0.000*	0.000*	0.000*	0.000*
P-value SO_2	0.055	0.066	0.124	0.001*
P-value acceleration	0.248	0.802	0.287	0.004*

T2 is following 3 months of aging. Treatments with a single letter have been aged in ambient cellar conditions. Treatments with – A have been aged under accelerated conditions in a 35 ° C oven. SO_2 data displayed in Appendix C.

4.1.6 Spectral scans following fermentation (T1) and aging (T2)

Spectral scans at T1 and T2 showed interesting results. At T1, treatments B, D and E were indistinguishable from each other and displayed the highest phenolic peaks at 280nm (see Figure 4-1). Treatment A was clearly distinguishable from the other peaks as having the lowest absorbance at 280 nm. Treatment C was slightly lower than B, D and E but higher than A. All treatments were relatively similar except for A.

T2 at ambient cellar temperature presents similar patterns but the peaks are more distinguishable (see Figure (4-2). Treatment A presents the lowest phenolic peak, which is consistent with T1 and its role as a control. The phenolics in treatment A have decreased over the aging period. Treatments B and C are now more closely located, with both treatments decreasing in phenolic peaks slightly. Treatment B presents a surprisingly higher phenolic peak, given that Treatment C has “double” skins so should therefore present a higher amount of phenolics. This suggests then that most phenolics originate from juice, which is supported by smaller but not insignificant peak of treatment A. This helps to explain why measurements of total phenolics are of little use when investigating orange wine and why a stronger focus should be on tannin measurements. This pattern is also observed in

treatments D and E which presented the highest phenolic peaks, but which treatment D is actually higher than treatment E. This may indicate a capped effect, in which the addition of skins only facilitates extraction to a point. Treatments with SO_2 additions showed an increased phenolic peak following the aging period.

T2 at accelerated conditions demonstrates similar patterns to T2 at ambient cellar aging, yet all treatment phenolic peaks have increased (see Figure 4-3). Treatment A still possesses the lowest phenolic peak, while the treatments with SO_2 (D & E) display the highest phenolic peaks. This indicates the ability of SO_2 to protect phenolics from oxidation during bottle aging. The unusual trend of the treatments with "double" skins presenting lower phenolic peaks than the treatments with "normal" skins is still observed under accelerated conditions and indicates that wines will continue to develop in this way over time.

In all scans there is a "shoulder" peak present from approximately 300-380 nm. This is able to be explained through the spectral properties of different phenolic compounds. Nonflavonoid hydroxycinnamic acids and stilbenes have been found to absorb most strongly between 300 and 330 nm, while flavonols such as quercetin and kaempferol have been found to absorb at 365 nm. Overlapping of chemical properties presents difficulties in differentiating peaks at these wavelengths *[17]*.

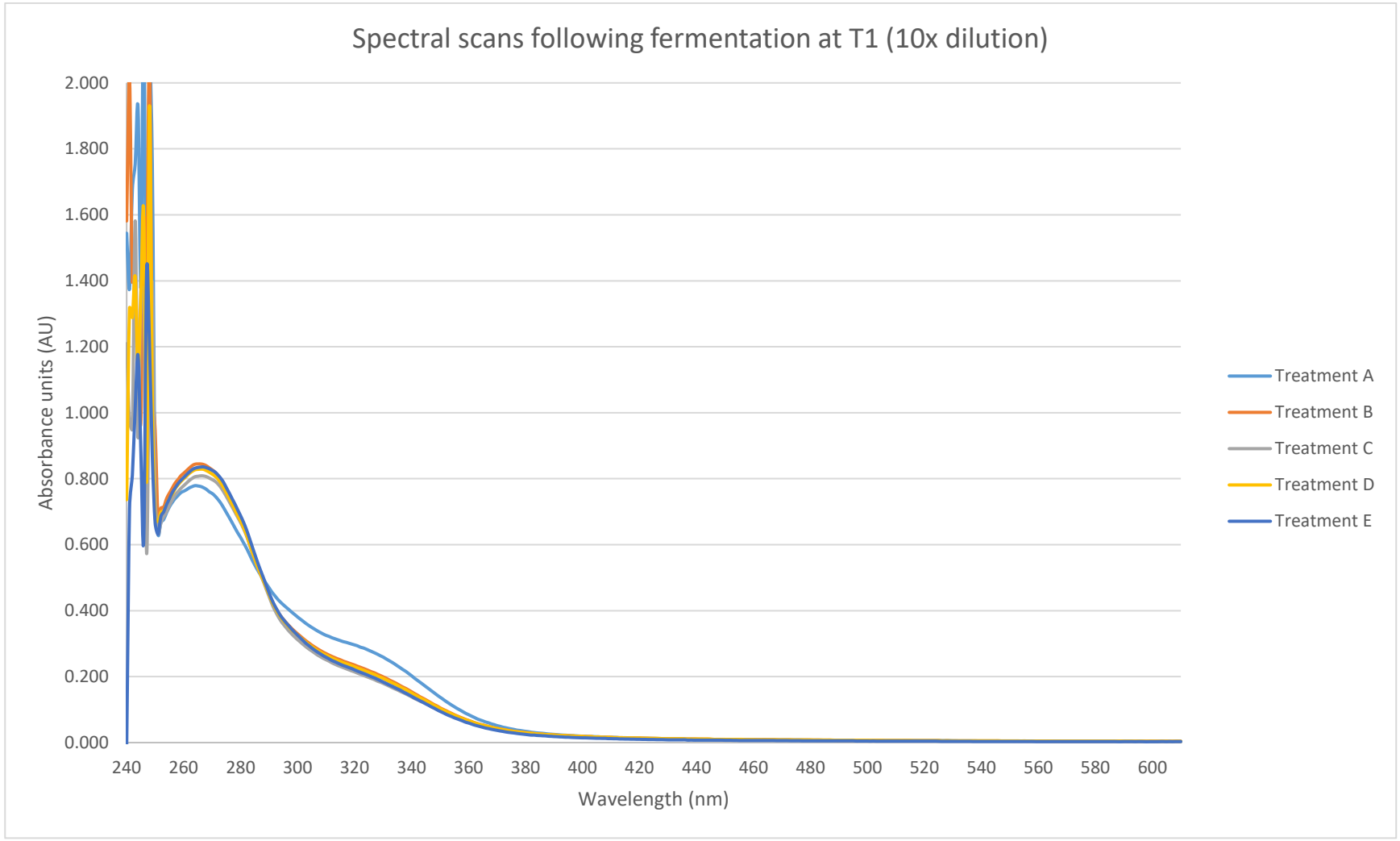


Figure 4-1: Spectral scans of all treatments at 10x dilution following fermentation (T1)

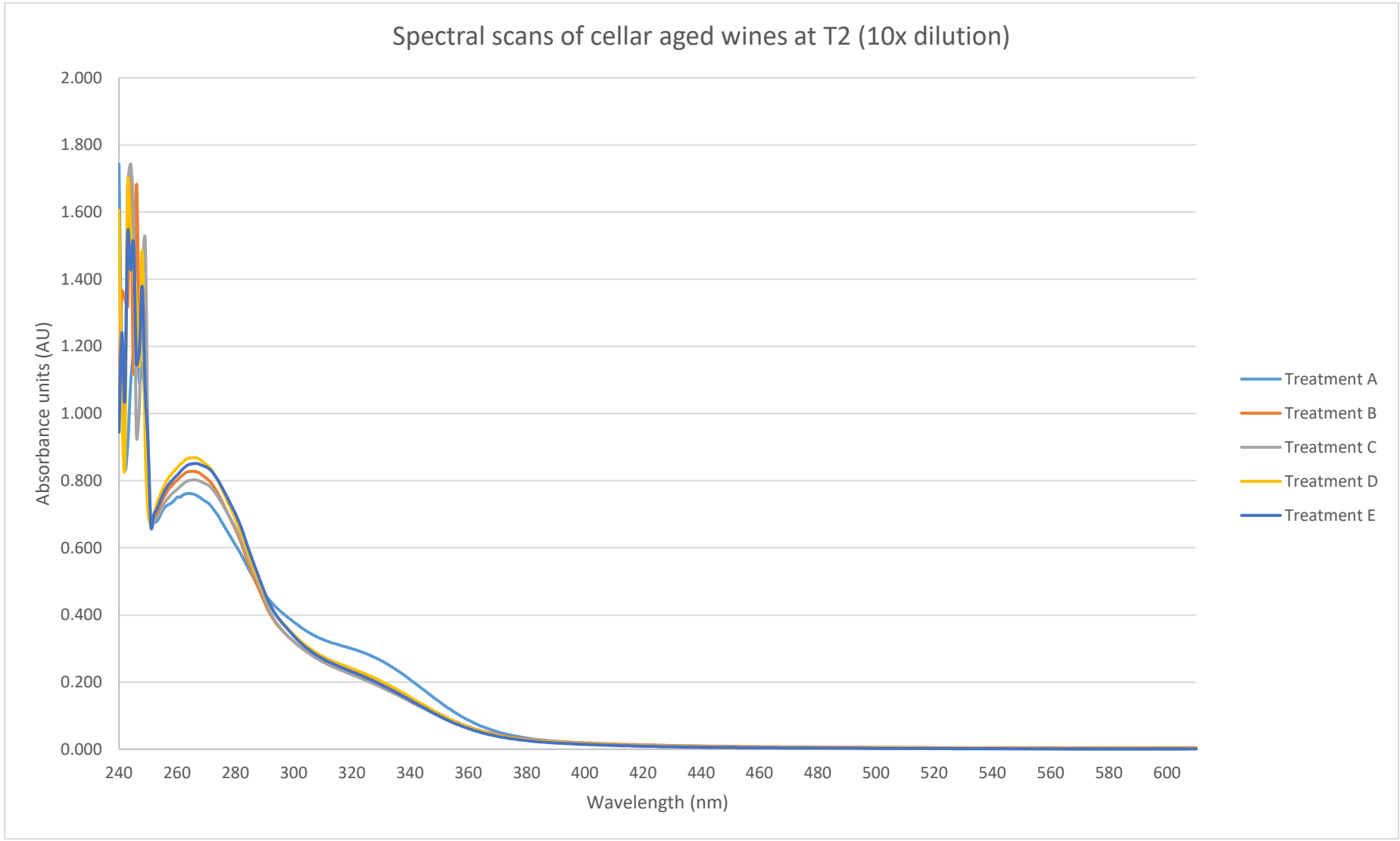


Figure 4-2: Spectral scans of all treatments at 10x dilution aged for 3 months at ambient cellar temperature (T2).

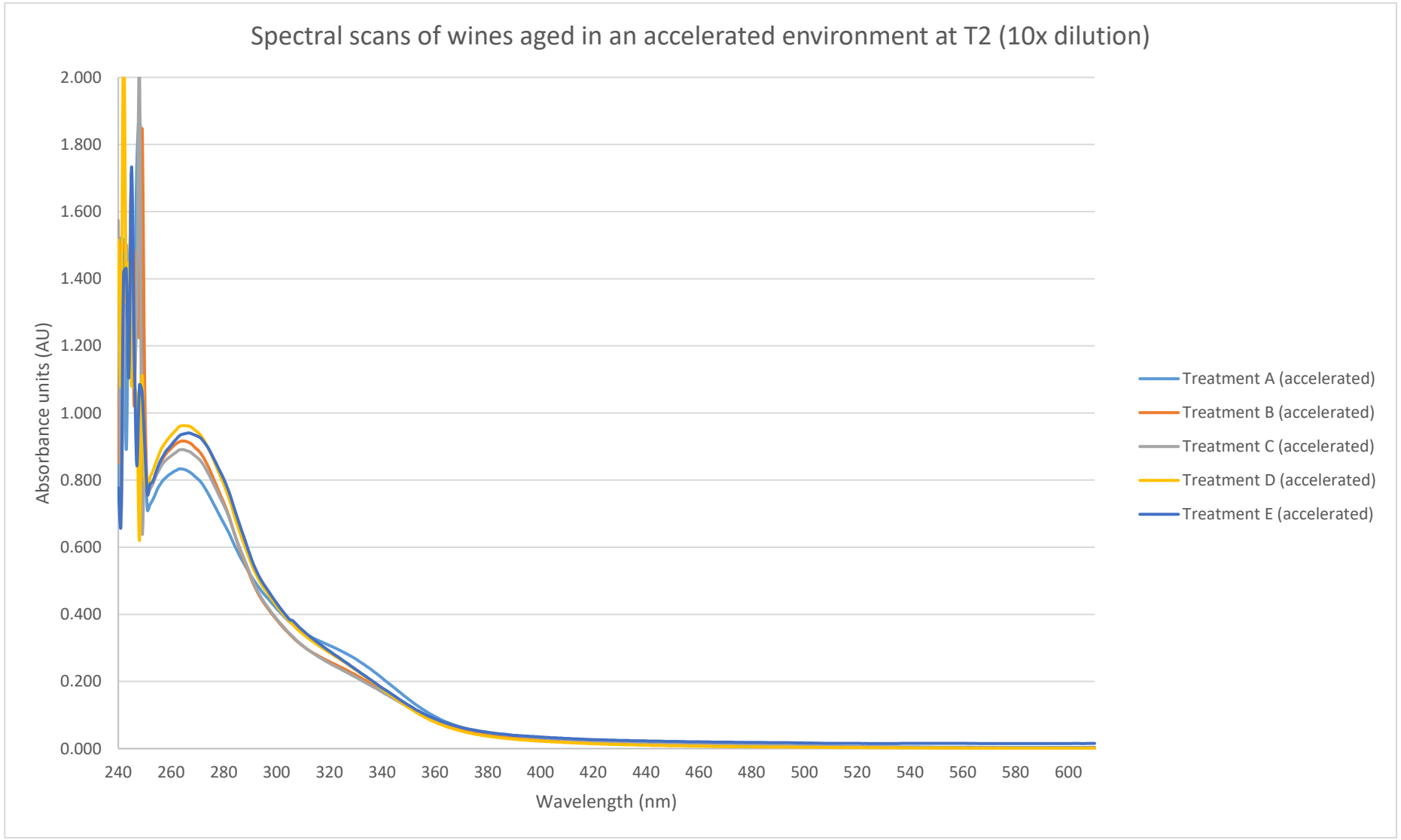


Figure 4-3: Spectral scans of all treatments at 10x dilution aged for 3 months in accelerated conditions (T2)

4.1.7 Colour following fermentation (T1) and aging (T2)

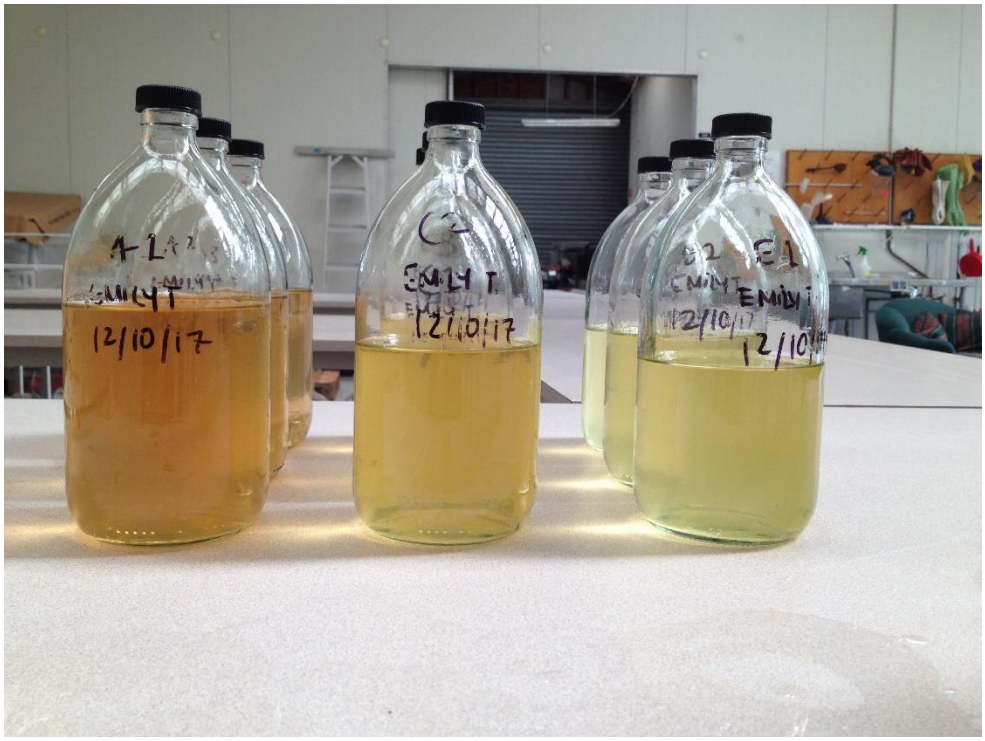
Visible colour differences were observed between all treatments at T1 (see Figure 4-4). Treatments D and E were the most visibly different from other treatments and almost identical to each other in hue. Treatments A, B and C showed distinct browning/orange characteristics while D & E remained a light greenish yellow. Browning of treatments A, B and C is most likely due to oxidation. The addition of SO_2 has preserved the colour of treatments D and E. Treatment A shows the most browning. This is potentially due to all replicates of treatment A beginning fermentation 5 days after inoculation, which may have allowed further opportunity for oxidation due to the lack of CO_2 produced by yeast. Interesting, minimal colour differences were observed during spectral measurements (see Figures 4-1, 4-2, 4-3). This suggests that the human eye is more sensitive to changes in hue than a spectrophotometer.

Observations made at T2 found that treatments aged under accelerated conditions presented deeper hues than those aged at ambient cellar temperature and were more brown/orange in colour (see figures 4-6, 4-7, 4-8). However, this effect was diminished in the presence of SO_2 (see Figure 4-8) and the wines of both aging treatments were much closer in hue. This effect was also visible in the spectral scans of wine aged under accelerated conditions (see Figure 4-3) in which a slight curve can be observed at 420 nm.

Figure 4-4: All treatments colours at T1

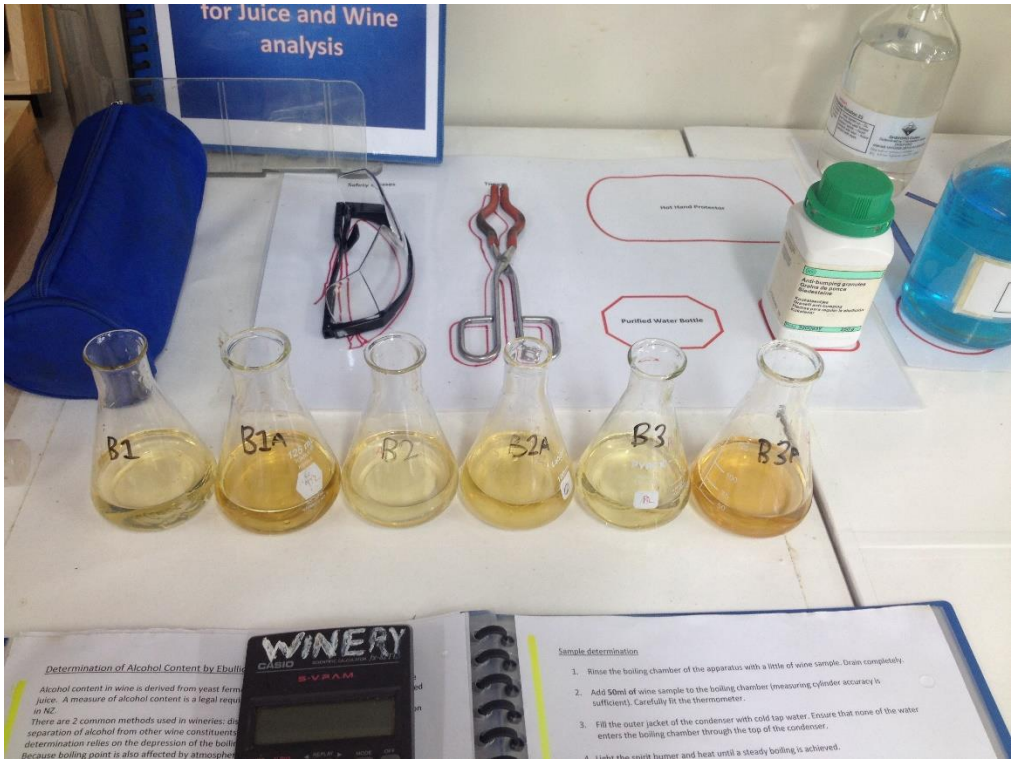
From left to right: Treatment A, B, C, D, E.

Figure 4-5: Treatments A, C and E colours at T1



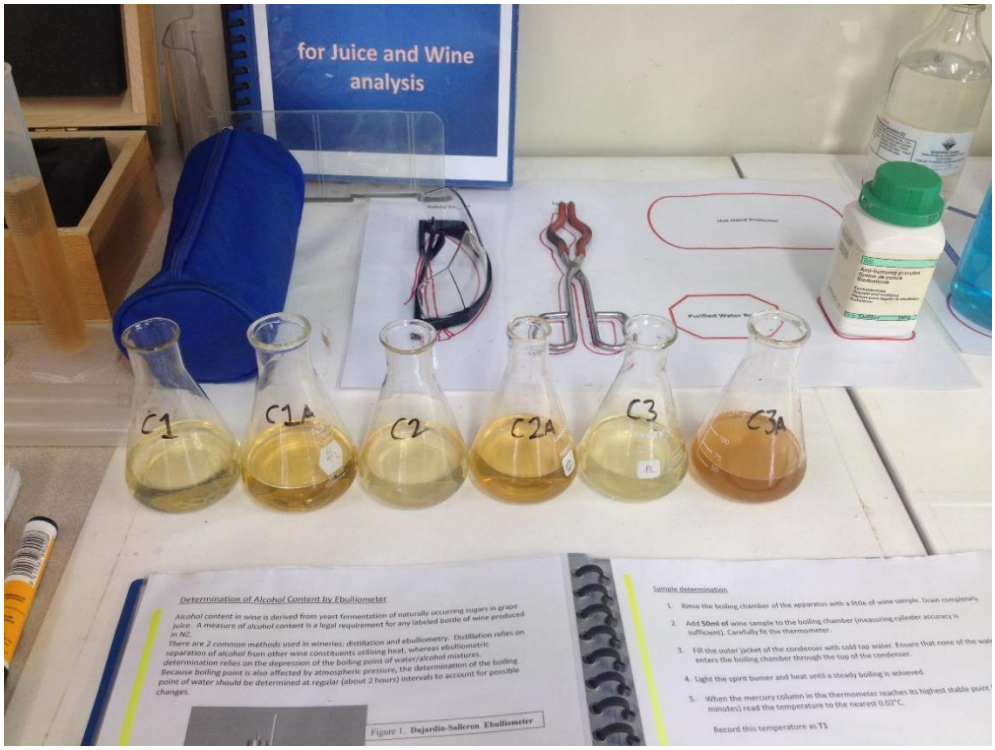
From left to right: Treatment A, C, E.

Figure 4-6: Treatment B and BA replicate colours at T2



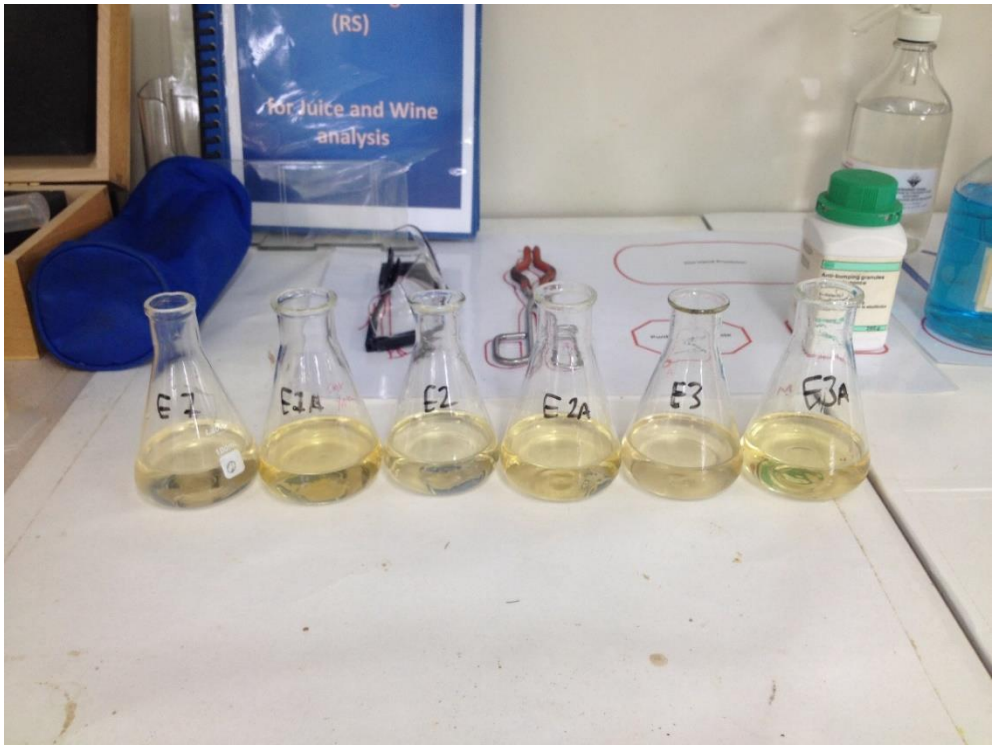
First, third and fifth wines have been aged at ambient cellar temperature. Second, fourth and sixth wines have been aged under accelerated conditions.

Figure 4-7: Treatment C and CA replicate colours at T2



First, third and fifth wines have been aged at ambient cellar temperature. Second, fourth and sixth wines have been aged under accelerated conditions.

Figure 4-8: Treatment E and EA replicate colours at T2



First, third and fifth wines have been aged at ambient cellar temperature. Second, fourth and sixth wines have been aged under accelerated conditions.

4.1.8 Fractionation following aging (T2)

Only treatments A, C and E along with their accelerated counterparts were measured due to research time constraints. This allowed measurement of the control, “double” skins, and “double” skins with SO_2 addition. Following separation of wine into fractions 1, 2 and 3, fraction 1 was sent for analysis by HPLC. The chemical compounds identified for each wine can be found in Table 4-4.

Treatment E when subjected to aging (EA) shows the highest concentration of catechin at 7.36 mg/L. Treatments A and AA show unusual results with concentrations of gallocatechin, epigallocatechin and epicatechin gallate among the highest out of all treatments. As the juice provided by Pernod Ricard contained some skins and seeds, this may have contributed to the concentrations of these compounds. The juice was stirred and uniformly distributed amongst treatments prior to the addition of skins, but it is plausible that differing proportions of skins or seeds were present in the treatments. This could be avoided by the use of fresh wine grape material. Alternatively, as fraction 1 contains only monomers, the comparatively low concentrations observed in treatments other than A may be due to electrophilic aromatic substitution (EAS), in which under acidic conditions proanthocyanidin chains can be cleaved and reformed *[28]*. Gallic acid is formed from the hydrolysis of hydrolysable oak tannins *[21]* and as these wines had no aging in oak, the minimal concentration of gallic acid is to be expected.

Table 4-4: Analysis of compounds in Fraction 1 of research wines by HPLC

Compounds	***A***	***AA***	***C***	***CA***	***E***	***EA***
	(mg/L)	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**
Gallic acid	0.44	0.59	0.45	0.39	0.44	0.62
Protocatechuic acid	4.51	5.41	3.16	3.28	3.62	2.68
Gallocatechin	23.66	26.19	29.24	23.54	21.62	21.27
Hydrobenzoic acid	1.01	1.39	1.06	0.88	0.92	1.06
Caftaric acid	7.10	6.33	2.57	3.05	4.86	5.25
Epigallocatechin	27.73	25.12	3.07	11.28	26.92	24.09
Catechin	0.66	0.43	4.42	1.36	6.60	7.36
Vanilic acid	1.62	0.58	2.98	1.27	3.32	2.78
Caffeic acid	0.49	0.68	0.35	0.46	0.06	1.33
Syringic acid	2.64	2.44	1.51	1.04	1.33	1.01
Epicatechin	0.33	0.56	1.45	2.31	1.76	2.19
Malvidin-3-O-glucoside	ND	ND	ND	ND	ND	ND
p-Coumaric acid	0.47	0.46	0.48	0.47	0.52	1.15
Ferulic acid	0.26	0.24	0.53	ND	ND	ND
Rutin	1.98	1.40	4.72	2.24	1.97	1.18
Epicatechin gallate	3.25	0.88	2.54	0.53	1.85	0.61
Quercetin	ND	ND	ND	ND	ND	ND

Single letter denotes aging at ambient cellar conditions. "A" following the treatment letter denotes aging under accelerated conditions. ND = not detected.

Fractions 1, 2 and 3 of these wines each had a spectral scan performed from 610 nm – 240 nm and were measured separately at 280 nm to determine the approximate tannin concentration in epicatechin equivalents using a calibration curve (see table 4-5). In fraction 1, treatment EA was observed to have the highest peak at 280 nm, distinctly separate from all other treatments which were grouped together (see Figure 4-9). All treatments were shown to have higher peaks when aged under accelerated conditions. In fractions 2 and 3, significant effects were observed for both the effect of skin addition and SO_2 for all treatments. Treatment A contained the lowest concentration of tannin in fraction 3 but a similar amount of tannin in fraction 2 as treatment E. Treatment C contained the highest concentrations of tannin in both fractions, significantly more than E, which has the same skin addition but also the addition of SO_2. Accelerated aging conditions had a significant effect on the concentration of tannin in fraction 2, demonstrating increases in the treatments which were aged under accelerated conditions. Fraction 3 was not significantly affected by aging conditions.

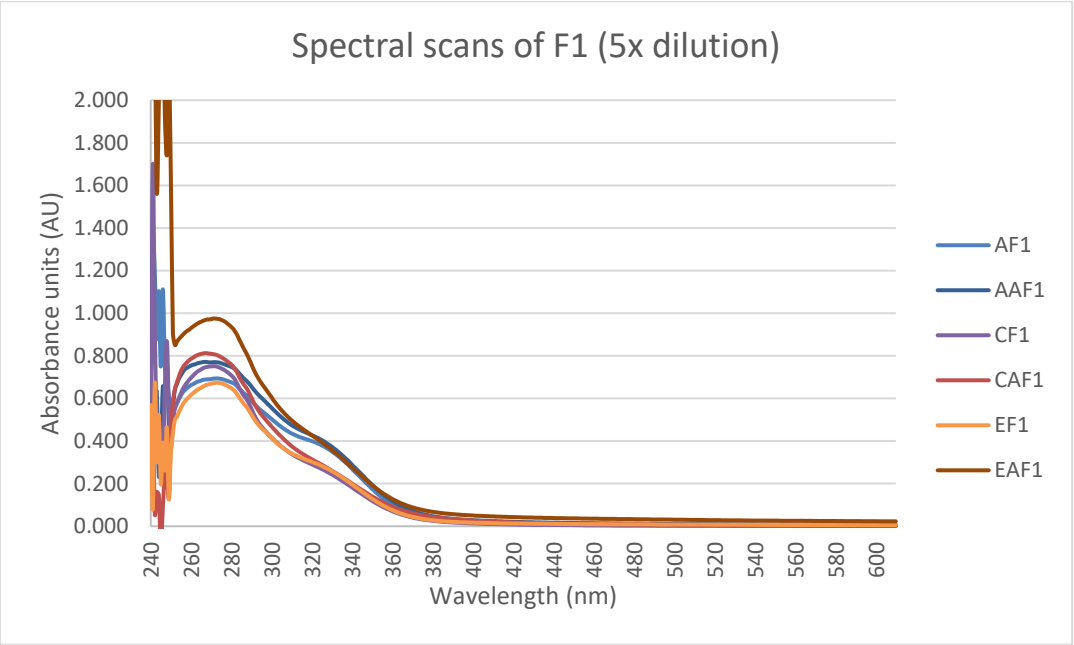
In all spectral scans, fraction 2 presented a higher phenolic peak than fraction 3 (see Figures 4-10 to 4-15). Treatment C shows a slightly higher peak than treatment A at 280 nm on fraction 2 and a much higher peak on fraction 3, double that of treatment A (see Figure 4-12). Treatment C also showed the most substantial increase in fraction 2 during accelerated aging,

Table 4-5: Fraction 2 and 3 tannin measurements of all treatments

Treatment	*Skins*	*SO_2 addition*	*Fraction 2 (mg/L)*	*Fraction 3 (mg/L)*
A	-	-	45	23
AA	-	-	46	21
C	2x	-	50	45
CA	2x	-	68	50
E	2x	Y	47	37
EA	2x	Y	49	35
P-value skins	-	-	0.003*	0.000*
P-value SO_2	-	-	0.009*	0.005*
P-value acceleration	-	-	0.028*	0.978

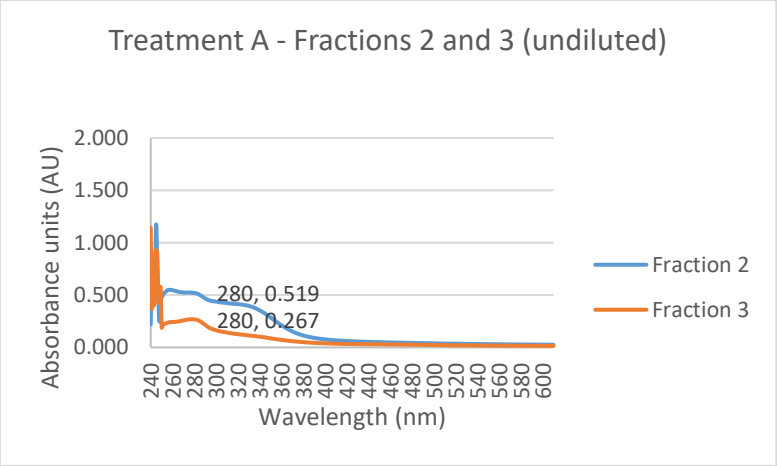
Tannin calculated from absorbance values plotted against a calibration curve. Measured in mg/L epicatechin equivalents. Single letter denotes aging in ambient cellar conditions. 'A' after the first letter denotes aging under accelerated conditions.

Figure 4-9: Spectral scans of research wines fraction 1



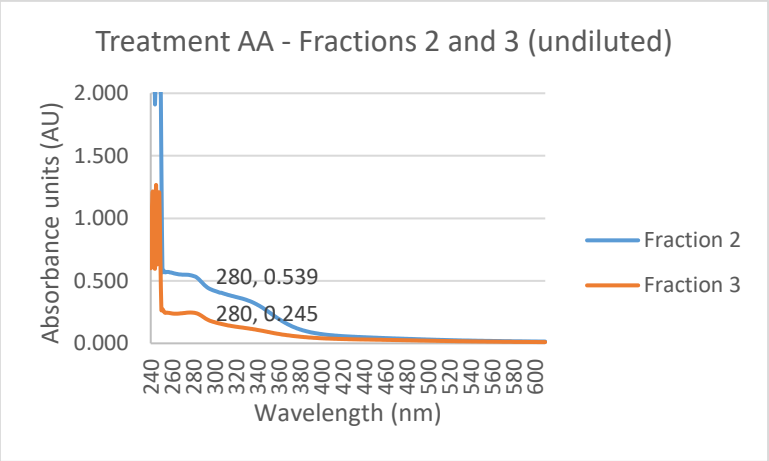
All research wines F1 had a 5x dilution.

Figure 4-10: Spectral scans of Treatment A, fractions 2 &3



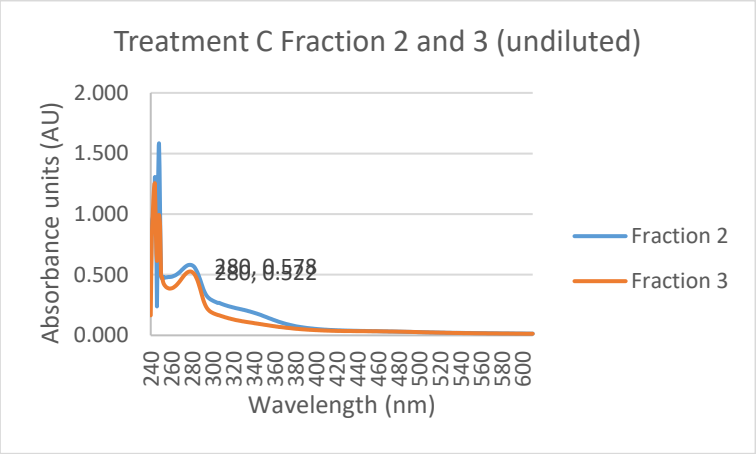
Aged in ambient cellar temperature. Fractions were undiluted.

Figure 4-11: Spectral scans of Treatment AA fractions 2 & 3



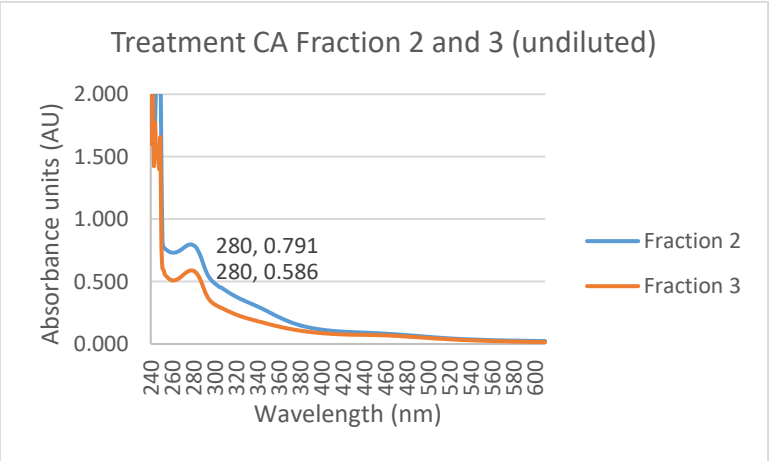
Aged in an accelerated environment. Fractions were undiluted.

Figure 4-12: Spectral scans of Treatment C fractions 2 & 3



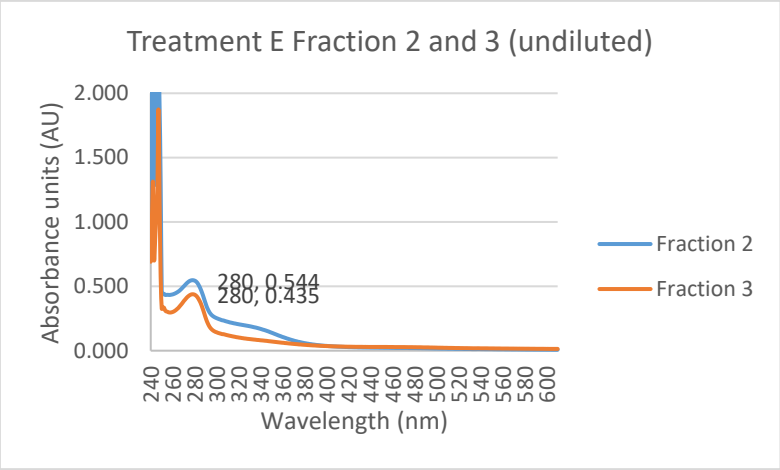
Aged in ambient cellar temperature. Fractions were undiluted.

Figure 4-13: Spectral scans of Treatment CA fractions 2 & 3



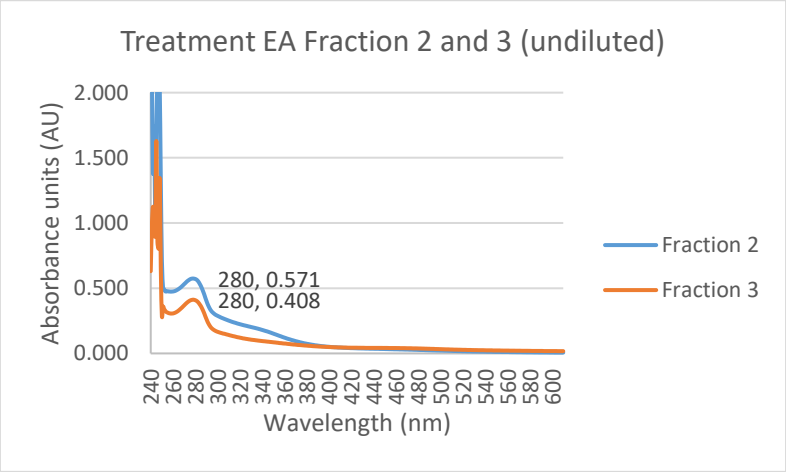
Aged in an accelerated environment. Fractions were undiluted.

Figure 4-14: Spectral scans of Treatment E fractions 2 & 3



Aged in ambient cellar temperature. Fractions were undiluted.

Figure 4-15: Spectral scans of Treatment EA fractions 2 & 3



Aged in an accelerated environment. Fractions were undiluted.

4.1.9 Mean degree of polymerisation (mDP) following aging (T2)

Mean degree of polymerisation showed high variation between treatments and across replicates. The averages are presented below (see Table 4-6). Both treatments A and AA's results for mDP are a result of both negative and triple digit numbers across replicates, indicating that the quantities of terminal and extension units are likely too low to be accurately detected and therefore reliably calculated. As this is the control treatment with no skins, this result appeared appropriate and was therefore not displayed. Treatments D & E presented the lowest mDP results initially, which

increased in the accelerated aging treatments. This indicates that the addition of SO_2 has an initial effect on tannin polymerisation which is diminished over time. However, the accelerated results of treatments D and E were still the lowest out of all skin treatments, but showed a pattern consistent with their skin treatments, 12.3 for the treatment with 1x skins and 15.9 for the treatment with 2x skins. Treatment C had an initially lower mDP than that of B, but increased over time to have the highest accurate mDP (Treatment CA). Treatment B presented the highest mDP of all treatments, however aging under accelerated conditions resulted in a decrease in the mDP. Statistical analysis was unable to be performed due to the wide variation in data, so the standard deviation is noted.

Table 4-6: Research wines mDP

Aging conditions	***B***	***C***	***D***	***E***
Ambient	16.4	11.1	5.7	4.4
Accelerated	14.0	17.6	12.3	15.9

Standard deviation = 5.23.

4.2 Commercial wines

4.2.1 Basic wine data

All commercial wines showed significant differences from each other in all basic chemical aspects (see Table 4-6). This is most likely due to the different grape varieties used in each wine, the chemical composition of berries at harvest and the unmistakably different winemaking methods used (see 3.9, above). Notably, the presence of free and/or bound SO_2 was observed in some of the wines; Valli, Vandal and Pheasant's Tears. This indicates that SO_2 was used during the production process of these wines or (most likely) at bottling.

Table 4-7: Basic chemical data of commercial wines

Wine	*pH*	*TA*	*FSO_2*	*BSO_2*	*TSO_2*	*RS*	*Alcohol %*	*Tannin (g/L)*
Muddy Water	3.4	4.8	0	0	0	2	11.8	0.83
Valli	3.2	4.6	5	70	75	2	12.4	0.25
Vandal	3.3	5.0	0	35	35	4	13.7	0.57
Denavolo	3.3	5.6	0	0	0	2	13.1	2.18
Pheasant's Tears	3.5	5.6	0	10	10	4	13.1	1.67
P-value	0.000*	0.000*	0.003*	0.000*	0.000*	0.006*	0.000*	0.000*

Tannin is measured in epicatechin equivalents and expressed in g/L due to the high concentrations observed.

4.2.2 Spectral scans

Spectral scans of all commercial wines showed distinguishable differences between each wine (see Figure 4-16). Denavolo presented the highest phenolic peak, followed by Pheasant's Tears. Vandal has the lowest, with Muddy Water and Valli having moderate absorbance readings. This data is reflected in Table 4-6, in which Dinavolo and Pheasant's tears presented the highest concentration of tannin, however, the results of Vandal and Valli are reversed. Total phenolics, although strongly related to tannin concentration, has been demonstrated to be not entirely representative of the tannin concentration in red wine *[32]*. Some colour is evident in the spectral scans, Muddy Water showing the strongest hue, noticeably different from the other wines even at 20 times dilution. Other commercial wines show slight absorbance readings in the visible spectrum (380-610 nm) for colour (420 nm) but are not visibly distinguishable. The "shoulder" peak present from approximately 300-380 nm is as explained earlier in this chapter (see Chapter 4, 4.1.5).

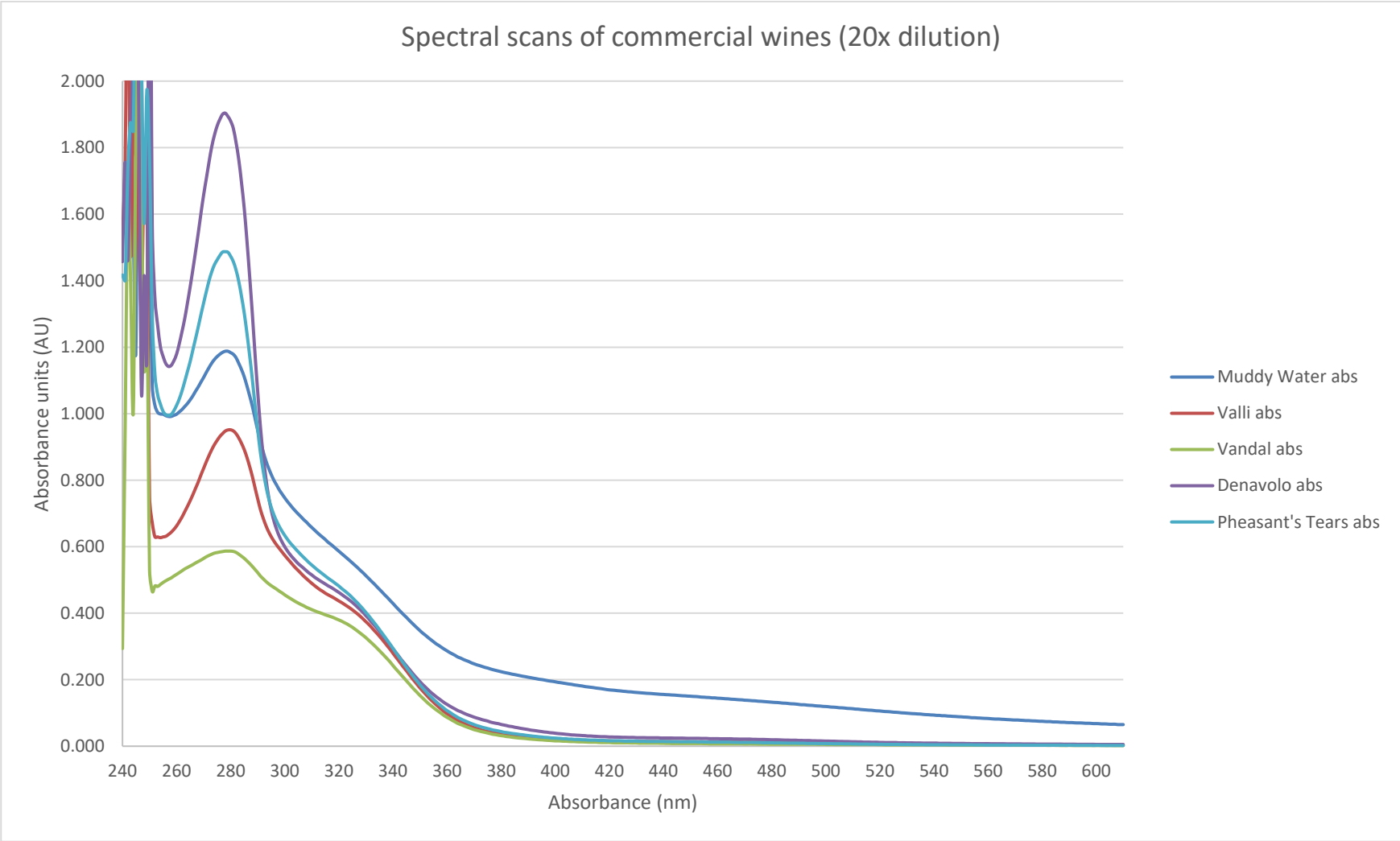
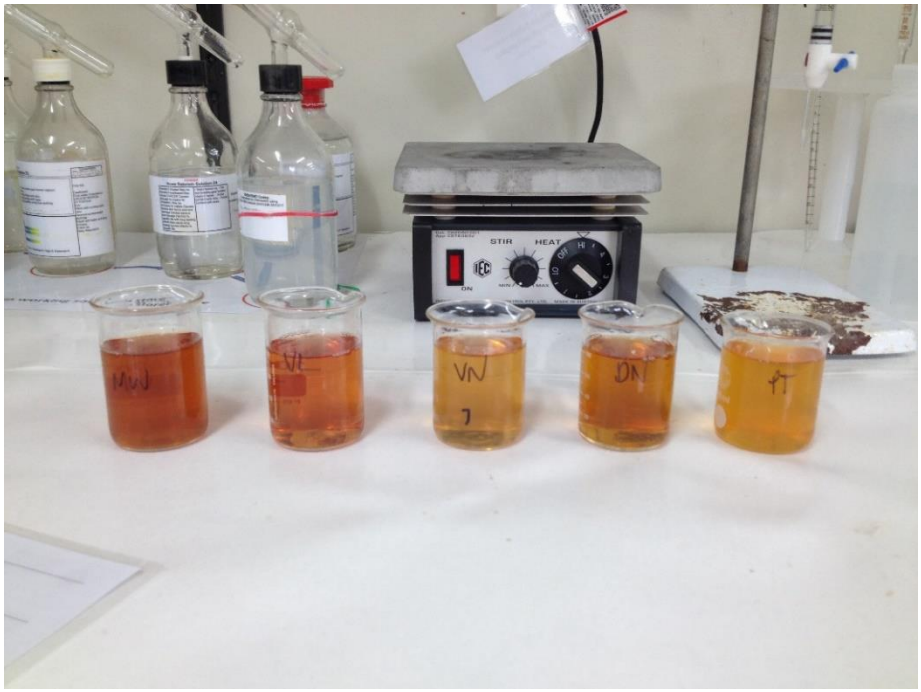


Figure 4-16: Spectral scans of commercial wines at 20x dilution

4.2.3 Colour

Visual observations show that Valli had the most orange hue, with a pink tinge. Vandal was the lightest wine, a light gold. Pheasant's Tears was a deep, bright amber. Muddy Water was the most strongly coloured, a dark brownish orange and was the cloudiest of all commercial wines. This persisted after it was passed through a centrifuge (see Figure 4-17). Valli, Vandal and Pheasant's Tears were determined the lightest coloured wines by visual estimate, which is consistent with the data presented in Table 4-7, in that these wines were found to have residual SO_2 present in the free and/or bound forms. The mechanism of action of SO_2 on wine colour is explained in the discussion (Chapter 5, 5.2).

Figure 4-17: Colour comparisons between commercial wines



From left to right: Muddy Water, Valli, Vandal, Denavolo, and Pheasant's Tears.

4.2.4 Fractionation

Fraction 1 of the commercial wines presented differences also (see Table 4-8). Muddy Water was comparatively low in gallic acid, protocatechuic acid and epigallocatechin but contained the second highest concentrations of catechin and epicatechin gallate, and the third highest concentration of epicatechin. It also contained the highest concentration of ferulic acid. Valli had the third highest

concentration of gallic acid, the second highest of rutin and protocatechuic and caffeic acids, the highest of catechin, epicatechin gallate, hydrobenzoic and caftaric acids. Valli was also the only wine of both research and commercial wines in which malvidin-3-O-glucoside was detected. Vandal had the second highest caftaric acid, second highest, vanilic and ferulic acids, the highest concentration of rutin and was one of three wines that contained quercetin. Denavolo contained the second highest concentration of gallic acid and p-coumaric acids, the highest concentrations of epicatechin, gallocatechin, epigallocatechin and syringic acid, the lowest catechin, ferulic, hydrobenzoic and caftaric acids, the second lowest epicatechin gallate, vanilic and caffeic acids. Denavolo was one of three wines that contained quercetin and presented the highest concentrations. Pheasant's Tears contained the highest concentrations of gallic acid, protocatechuic acid, vanilic acid, p-coumaric acid and was one of only three wines that contained quercetin. Higher proportions of gallic acid were observed in commercial wines than research wines and as such, it can be assumed that the different concentrations in commercial wines correspond to different aging periods in oak, along with differences in oak composition *[21]*.

Table 4-8: Analysis of compounds in Fraction 1 of commercial wines by HPLC

Compound	*Muddy Water*	*Valli*	*Vandal*	*Denavolo*	*Pheasant's Tears*
	(mg/L)	**(mg/L)**	**(mg/L)**	**(mg/L)**	**(mg/L)**
Gallic acid	5.04	19.36	5.38	19.91	38.84
Protocatechuic acid	0.36	4.25	2.73	1.52	6.77
Gallocatechin	16.03	18.88	7.19	58.58	42.40
Hydrobenzoic acid	14.52	30.75	11.08	0.72	1.61
Caftaric acid	24.29	58.04	54.72	12.95	51.44
Epigallocatechin	0.02	2.58	2.30	59.19	3.21
Catechin	10.68	14.79	5.39	1.53	3.21
Vanilic acid	0.70	7.18	8.60	2.61	8.92
Caffeic acid	10.01	4.22	3.88	2.21	0.37
Syringic acid	6.52	9.40	7.57	33.08	13.92
Epicatechin	6.47	2.33	2.94	16.82	8.95
Malvidin-3-O-glucoside	ND	1.18	ND	ND	ND
p-coumaric acid	0.47	0.46	1.40	4.70	5.53
Ferulic acid	3.58	0.64	1.61	0.24	0.49
Rutin	1.74	2.74	6.74	1.96	0.74
Epicatechin gallate	15.92	20.34	7.90	2.91	1.44
Quercetin	ND	ND	1.14	3.48	1.04

ND = not detected.

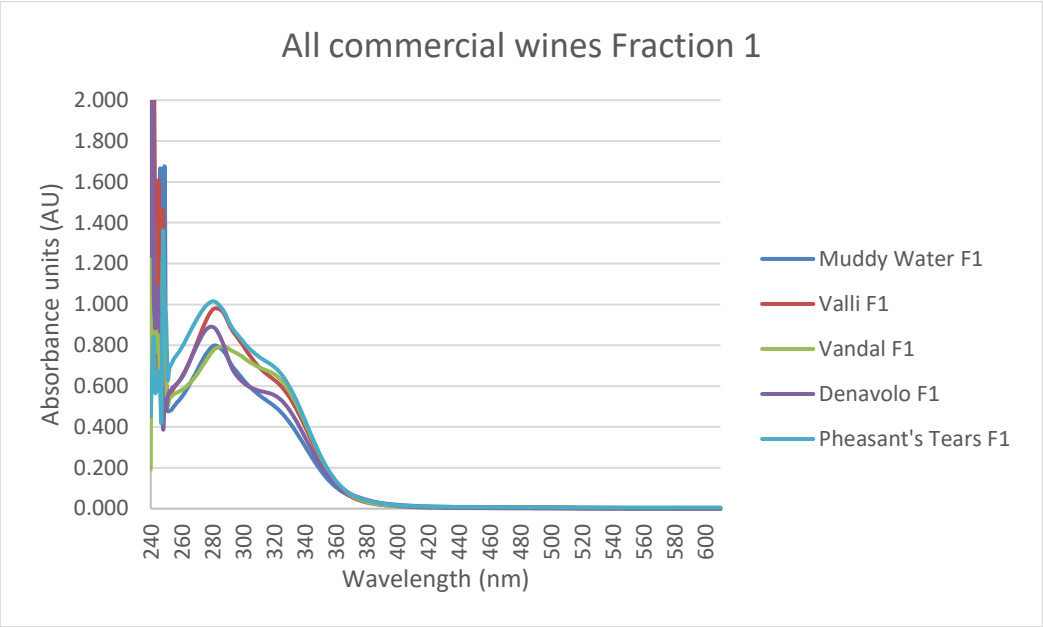


Figure 4-18: Commercial wines Fraction 1 spectral scans

Fractions 2 and 3 of all commercial wines present higher results in fraction 3 of all wines (see Table 4-9). This differs from the data presented on the research wines, in that fraction 2 presents the highest concentrations from all treatments (Table 4-5). Denavolo contains the highest concentrations in both fraction 2 and 3, followed by Pheasant's Tears and Muddy Water. Vandal contained the lowest concentrations in both fractions. Pheasant's tears fraction 3 concentration was approximately 4 times higher than its concentration in fraction 2, the largest increase out of all commercial wines.

Table 4-9: Fractions 2 and 3 tannin measurements of commercial wines

Wine	*Fraction 2 (mg/L)*	*Fraction 3 (mg/L)*
Muddy Water	202	538
Valli	197	272
Vandal	72	103
Denavolo	430	1325
Pheasant's Tears	221	866
P-value	0.000*	0.000*

Tannin is expressed in mg/L epicatechin equivalents.

Figure 4-19: Muddy Water spectral scans of fractions 2 & 3

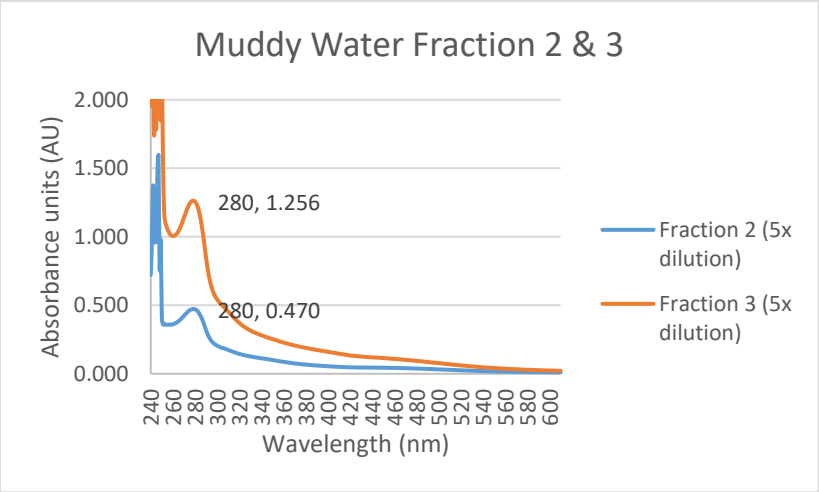


Figure 4-20: Valli spectral scans of fractions 2 & 3

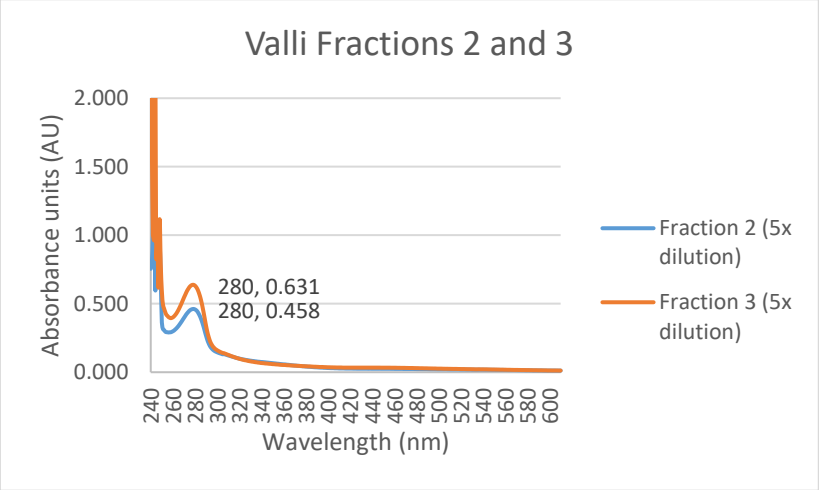


Figure 4-21: Vandal spectral scans of fractions 2 & 3

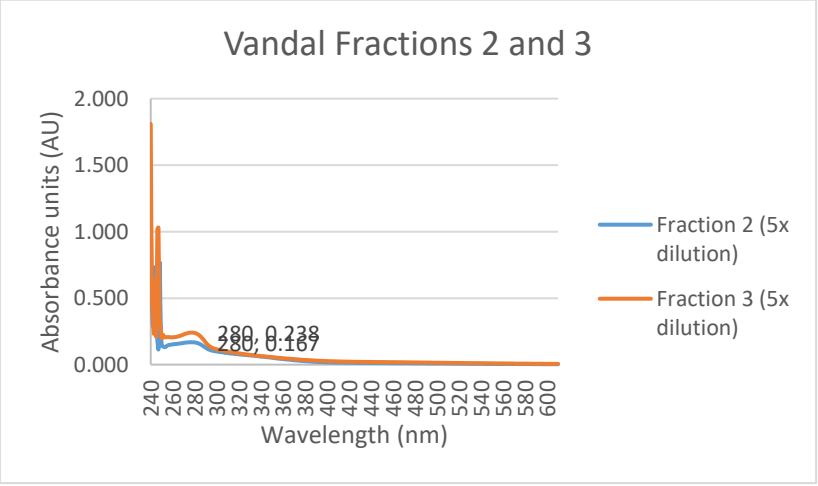


Figure 4-22: Denavolo spectral scans of fractions 2 & 3

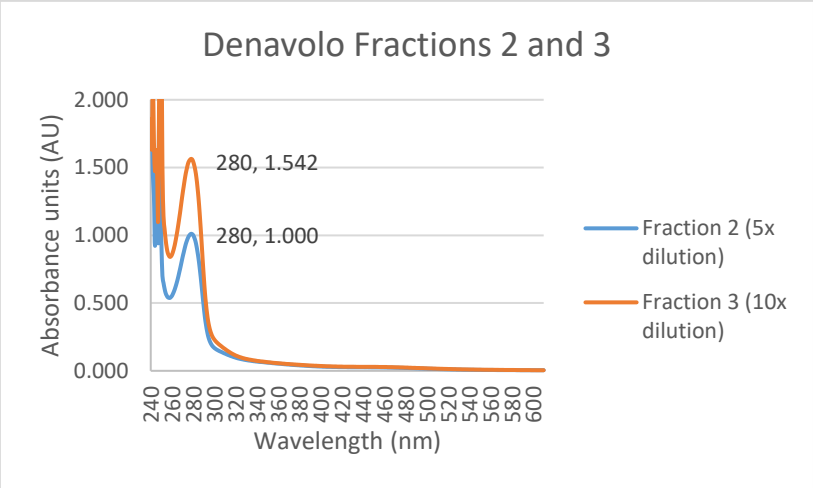
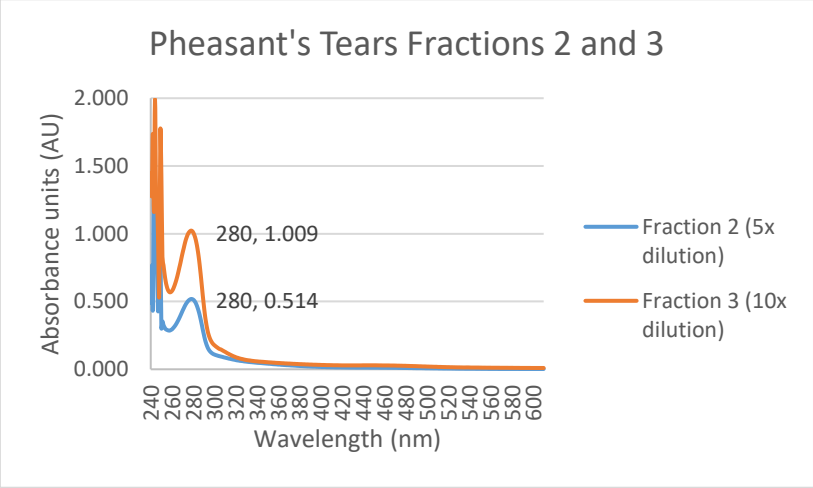


Figure 4-23: Pheasant's Tears spectral scans of fractions 2 & 3



4.2.5 Mean degree of polymerisation (mDP)

Commercial wines presented considerable differences between their mDP (see Table 4-9). Denavolo had the highest mDP, followed by Pheasant's Tears. Vandal and Valli were in the middle, with the lowest mDP exhibited by Muddy Water. Statistical analysis was unable to be performed, as such the standard deviation is noted. The mDP results of the commercially produced wines help to validate the method of analysis and technique used for the research wines through consistent and expected results.

Table 4-10: Commercial wines mDP

	Muddy Water	*Valli*	*Vandal*	*Denavolo*	*Pheasant's Tears*
mDP	8.8	9.5	11.2	17.7	14.3

Standard deviation = 3.66.

Chapter 5

Discussion

5.1 Fermentation kinetics and basic chemical composition of research wines at juice stage, following fermentation (T1) and aging (T2)

The initial analysis of juice data presented significant differences in terms of the pH and SO_2 data. Maceration in wine production has been found to result in a reduction in TA and increase in pH due to the extraction of potassium from skins *[21]*. This is initially observed in the current experiment in the pH of the juice and follows a similar pattern at T1 and T2.These results are consistent with other literature involving white wine skin maceration *[12, 21]*. Other observations are consistent with the literature also, in that lower alcohol percentages are found in white wines produced using skin contact *[12]*. The presence of FSO_2 was observed both in the juice without skins and in the treatments with skins added, increasing with the addition of skins. The SO_2 presence in juice is likely due to SO_2 additions made at the vineyard or weighbridge at the time of sampling, prior to receival by Lincoln University. Increases in SO_2 consistent with increases in skin addition can be attributed to the presence of a preservative sheet containing SO_2, found in the box of table grapes after purchase. This was unfortunately unavoidable, given the parameters of the experiment. However, SO_2 did not persist in the treatments without SO_2 addition post-fermentation.

Skin maceration during the production of white wines have been shown to improve fermentation kinetics through the availability of extra nutrients from skins and suspended solids *[21]*. This helps to explain the earlier start to fermentation observed in all treatments with skins, especially in treatments which had double the amount of skins added (C & E). Fermentation began abruptly in these treatments; 3 days following inoculation with yeast, compared with 3-4 days in treatments B and D and 5 days in treatment A. This may indicate a lack of nutrients in the juice, supplemented by the addition of skins. Yeast assimilable nitrogen was not measured in the juice prior to fermentation.

Results at T1 show the addition of skins has continued to have a significant effect on the pH and also a significant effect on the TA of the wines which was not evident at the juice stage. The process of fermentation may have extracted further potassium from the grape skins which was not evident at the time of sampling. Tannin extraction was shown to be hindered by the use of SO_2, with treatments that had SO_2 additions following similar patterns to those without but at lower concentrations. This is inconsistent with other studies *[14, 21, 36]*, in which the use of SO_2 has been demonstrated to increase phenolic extraction. SO_2 is known to significantly affect yeast metabolism *[21]*, however these differences are more likely due to human error during the MCP analysis.

5.2 Effects of skin contact treatments and oxidation on phenolic composition

Differing levels of skin contact resulted in increasing concentrations of tannin as the skin weight increased. This was found to be limited by the use of SO_2, which is inconsistent with the literature as SO_2 has been found to facilitate further extraction of phenolics *[14, 21, 36]*. However, these studies usually use much higher concentrations of SO_2, as high as 80 – 150 mg/L, and therefore the low dose of SO_2 may not have been enough to produce a similar effect. These studies were also concerned with red wine production, not orange wine, so differences in effects such as these could be expected. Alcohol percentage is not the likely factor for a decreased concentration of tannin, as wines contained the same alcohol % as their skin addition counterparts with no added SO_2. This may indicate that SO_2 has an impact on yeast metabolism and ability to extract tannin from grape material.

Where colour was concerned, treatments without SO_2 showed the strongest indications of browning in colour at T1. This could be attributed to either the enzymatic activity of polyphenol oxidase in juice prior to fermentation of oxidation of phenolic compounds during aging. The oxidation of phenolic compounds such as catechin, epicatechin and hydroxycinnamic acids has been found to result in yellow and/or brown coloured compounds as a result of the polymerisation of *ortho*-quinones *[16]*. The use of SO_2 has been demonstrated to reduce *ortho*-quinones in wine and therefore subsequent browning *[39]*. This was evident at T2 in the wines aged under accelerated conditions also, in which treatments without SO_2 addition showed a substantial increase in brown hue, while treatments with SO_2 additions had a comparatively minimal increase in browning.

During fractionation, it was found that all research wines displayed higher concentrations of phenolics in F2 than in F3. Jeffery, et al. [*22]* found that younger red wines displayed greater concentrations of phenolics in the hydrophilic first polymeric fraction (F2), whereas older red wines displayed greater concentrations of phenolics in the hydrophobic second polymeric fraction (F3). F3 was also found to be the only fraction containing epicatechin gallate, typically found in grape seeds. This helps to explain the differences found between research and commercial wines during fractionation, as research wines did not contain any added grape seed material and therefore could be expected to have a lower concentration in F3. The youngest commercial wines were also at least 2 years old, therefore all commercial wines could be expected to have higher concentrations of phenolics in F3.

Results for research wines mDP were variable and statistical analysis was unable to be performed. This may be due to the very small concentrations of tannin observed which could have proved challenging for accurate analysis. However, it was found that treatments with SO_2 additions also had lower mDP results. This is consistent with other studies, in which the addition of SO_2 has been found to result in a slight decrease in polymerisation in model red wine solutions *[37]*.

In comparison with research wines, the mDP of commercial wines were distinctly different but no clear patterns could be observed. The highest mDP were observed in Denavolo and Pheasant's Tears which also possessed the highest concentrations of tannin, however the lowest was observed in Muddy Water, which had the third highest tannin concentration. This indicates that mDP is not related to overall tannin concentration in wine. Tannins are known to continue to polymerise during wine aging *[8]*. This has been thought to result in a gradual precipitation out of wine when the chains become too large and as such, older wines have been demonstrated to have a lower mDP *[9]*. However, other studies have shown that due to electrophilic aromatic substitution (EAS), in which an electrophile reacts at an unsubstituted position on the benzene ring which results in displacement of a proton, *[51]*, proanthocyanidin chains may be cleaved and reformed under acidic wine conditions which can result in a lower mDP *[49]*. This may help to explain the higher mDP observed in the research wines, as these wines are comparatively young. For the other commercial wines which were all of the same age (2016 vintage), a higher mDP may indicate greater extraction of skin tannin than seed tannin, as seed tannins have been found to have fewer sub units compared with skin tannins, which have been found to have greater amounts of sub units *[10, 26]*.

The presence of anthocyanins in red grapes is known to exhibit a significant effect on tannin extraction and retention during wine production and aging, through the formation of anthocyanin-tannin complexes *[42]*. This may explain the low concentrations of tannin observed in the wines produced in this experiment, however, commercial wines provided for side-by-side comparison displayed much greater concentrations of tannin and significant differences in phenolic composition. Potential explanations for these inconsistencies are addressed below.

5.3 Addressing significant differences in phenolic composition between research wines and commercial wines

As mentioned in the methodology, wine grapes were not able to be used due to the timing of this experiment (September 2017 - March 2018). This resulted in the acquisition of frozen wine grape juice samples from previous vintages and table grape skins being substituted to provide the source of phenolic material. An assumption was made that table grape skins would have a similar phenolic

composition to wine grape skins, (Glen Creasy, pers. comm.). As this experiment did not involve a sensory evaluation, the absence of varietal compounds present in wine grape skins was not considered to be significant. As the table grapes were seedless, seeds were not present in the fermentation or compensated for. This lack of seed phenolic material, along with the comparatively short length of extended maceration, may be a likely explanation for the resulting low phenolic content of the research wines produced, as grape seeds are recognised as a significant source of tannin in wines produced with pomace *[8, 10, 23, 40]*. This was evident in fraction 1 of the commercial wines, in which substantially higher concentrations of epicatechin gallate were observed, as well as a much higher concentration in fraction 3 than in fraction 2. As fraction 2 has been found to be more hydrophilic and fraction 3 to be more hydrophobic *[22]*, it's possible that fraction 3 may decrease over time and fraction 2 may increase as chemical reactions increase the solubility of phenolics through complexing with other compounds. However, these wines would need to have been analysed relatively soon after production to confirm this. As discussed in Chapter 5, 5.2, epicatechin gallate is typically found in grape seeds, while only fraction 3 contains epicatechin gallate *[22]*. This is a consideration that could be compensated for in future by repeating the experiment with fresh wine grapes. A 5 day extended maceration period was allowed for, following the end of fermentation. Time constraints meant longer maceration times could not be considered. Information provided on the commercial wines analysed shows that many orange wines go through an extended maceration for at least a period of multiple weeks, if not months and in extreme cases, years. As all commercial wines present significantly higher tannin concentrations, it could be expected that the short duration of extended maceration in this experiment may have then heavily impacted tannin extraction into the final wines.

Yeast selection may have also played a part in tannin concentrations. Maurivin PDM (*Saccharomyces cerevisiae var. bayanus*), was selected as the yeast for this experiment in order to ensure a reliable and consistent fermentation. However, studies have shown that yeast strain selection can have a significant impact on the tannin concentration in the resulting wines *[11]*. It could therefore be suggested that this experiment be repeated with Maurivin PDM as a control and a selection of different commercially available yeast strains, including non-saccharomyces wine yeasts and wild fermentations to see how tannin concentration is affected in orange wines.

As orange wine is undergoing a re-emergence and is a style that currently has no regulatory framework around production, it is often a source of experimentation for modern winemakers. As such, all the commercial wines provided for side-by-side comparison are inevitably produced from a wide range of different *Vitis vinifera* varietals, some single varietal, some blends (see Chapter 3, 3.8). They have also been produced by a variety of different methods, such as the duration of skin contact on the wine, the type of aging vessel (oak, amphorae, botti), the addition of sulfites (or lack thereof),

the use of fining and/or filtration and bottle aging before release. The different varietals used, along with wide range of different winemaking techniques lends itself to a vast number of variables which can influence the composition of the final wines.

5.4 Implications and recommendations

This research allows a better understanding of the phenolic composition of orange wines, along with whether similar techniques used in red wine production to influence the phenolic composition can be used to gain similar effects. The limitations of this study and recommendations for future work are discussed below.

One of the main limitations of this study was the lack of fresh wine grape material, indicating that results gained during this experiment may have been impeded by the use of table grape skins. This is potentially unable to reflect the actual composition of wine grapes and lacks the standard phenolic material provided by wine grape seeds. Repetition of this study with fresh Pinot Gris fruit from a current vintage would help to support results from this study, or to give a more accurate picture of the phenolic composition of orange wines from a particular variety. YAN could be also be measured at the juice stage, after the additions of skins and SO_2 and prior to fermentation, and adjusted to make all treatments the same in an attempt to have fermentation commence evenly among treatments. The ability to carry out a longer extended maceration to allow further extraction of phenolics could also be of some benefit, as orange wines have been demonstrated to have a much longer period of time on skins following fermentation, at least several weeks. The effects of aging in oak, amphorae or qvevri could be explored, as this is a common practise for orange wines and may change the way phenolic composition develops over time. This experiment could also be carried out with different varietals to compare compositional differences. Blends could also be produced to see how different varieties affect the final wine composition, as orange wines are produced from several *Vitis vinifera* varieties. The influence of yeast selection could also be explored. This experiment could be repeated with the yeast originally selected for this study (Maurivin PDM) as a control, compared with a selection of different commercially available yeast strains, including non-saccharomyces wine yeasts and wild fermentations to see how this affects tannin concentration in orange wines.

Sensory analysis could also be an added consideration, in order to assess the effect of different rates of skin additions and oxidation on the sensory perception of not only phenolics, but also colour, aroma and flavour of the resulting wines.

Chapter 6

Conclusion

From the results presented, it is evident that the use of skin contact and additions of SO_2 have significant effects on basic wine chemical composition, tannin concentrations, fraction proportions and the mean degree of polymerisation. Several conclusions can be drawn:

1. Increasing rates of skin addition result in a higher tannin composition in wine, along with an increase in pH and a decrease in TA and alcohol percentage.

2. The use of SO_2 during the production of orange wines limits tannin development, mean degree of polymerisation and proportions observed in fractions, but protects total phenolics from oxidation over time, especially those present in Fraction 1.

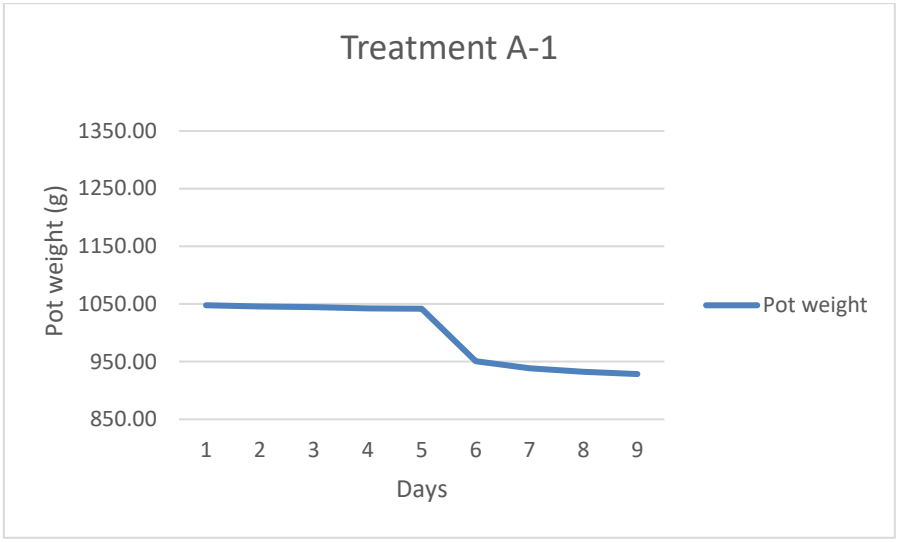
3. The use of SO_2 results in orange wines with a lesser degree of browning due to the reduction of *ortho*-quinones. The presence of SO_2 has been found to limit polymerisation of these compounds and the subsequent production of brown or yellow colours in wine. This trend is therefore observed in orange wines also.

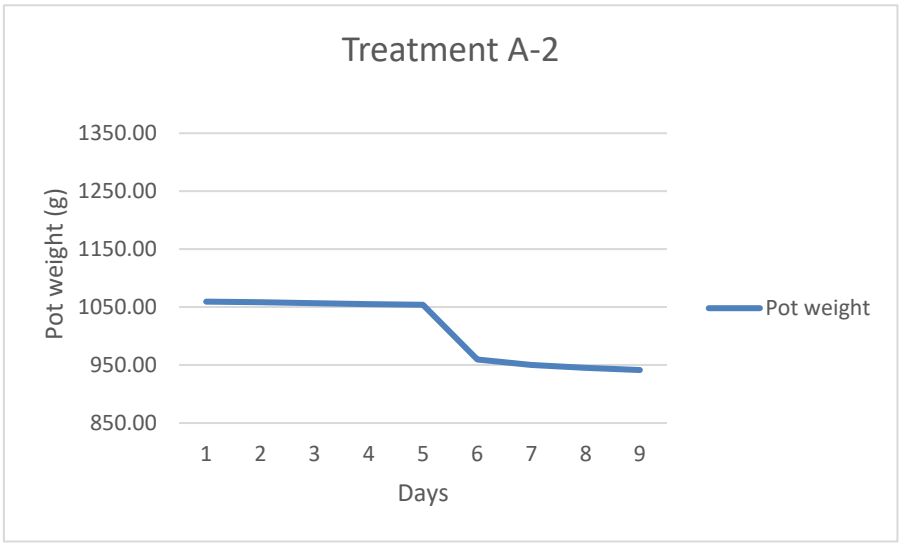
4. Orange wine phenolics develop in a similar way to red wine phenolics and are affected by similar winemaking conditions.

Appendix A

Ferment graphs of all treatments and replicates

Figure A-1: Treatment A pot weights of all replicates during fermentation





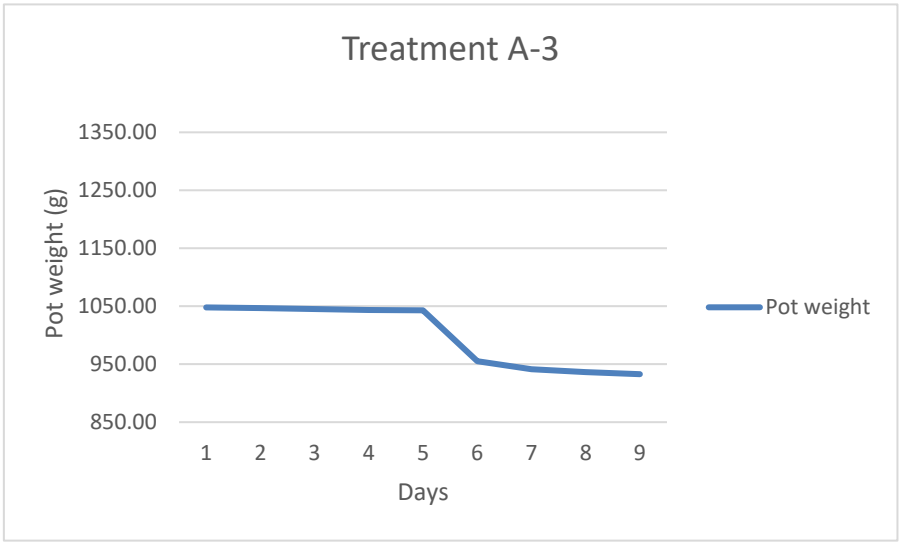
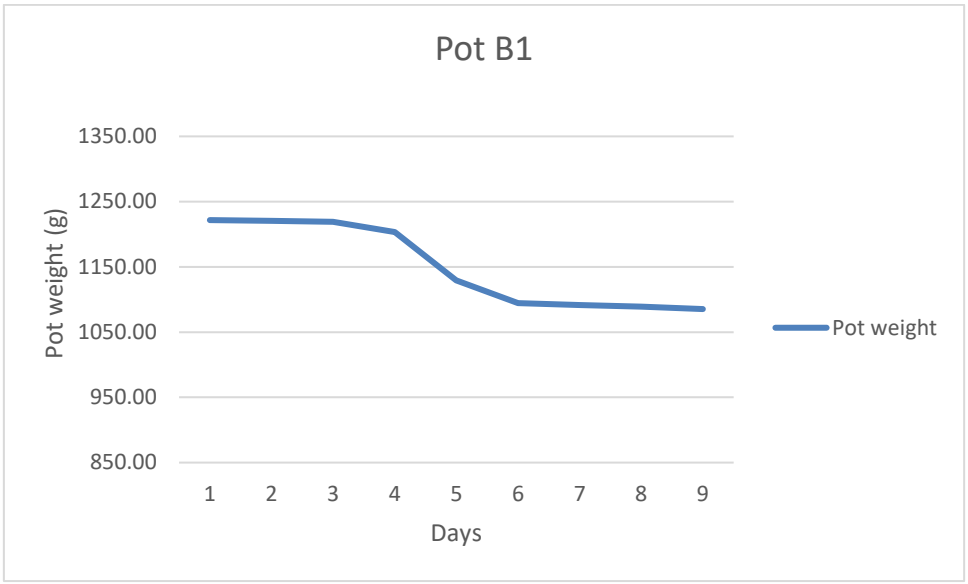
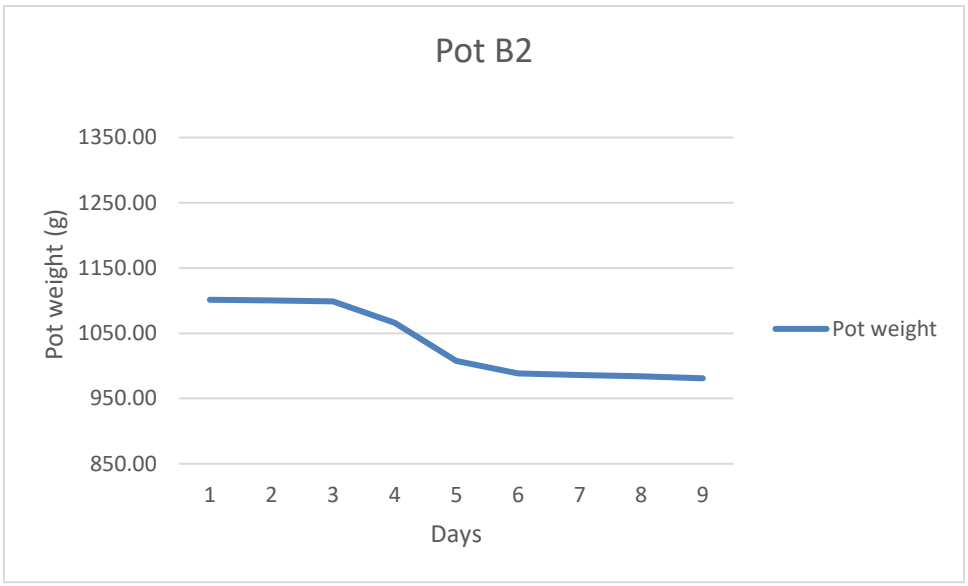


Figure A-2: Treatment B pot weights of all replicates during fermentation





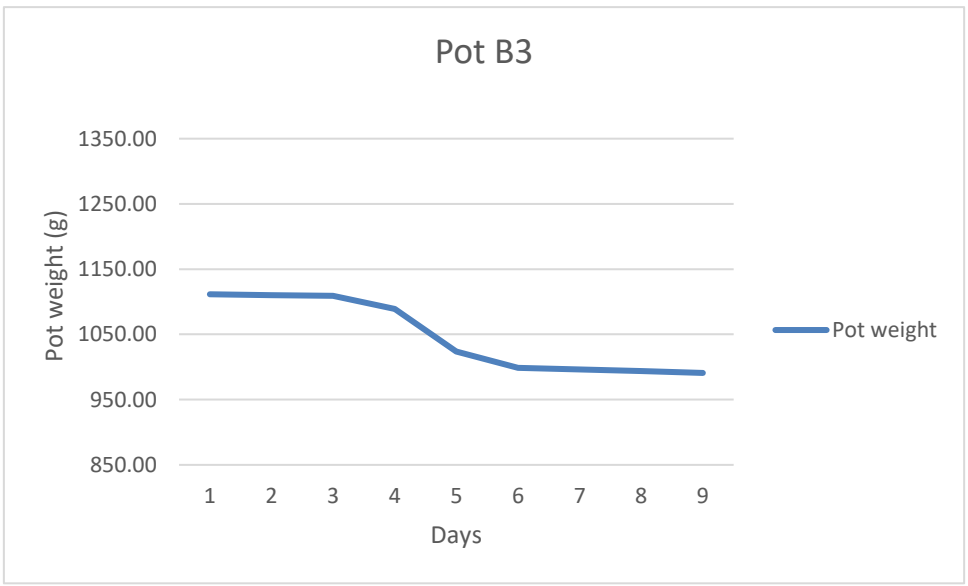
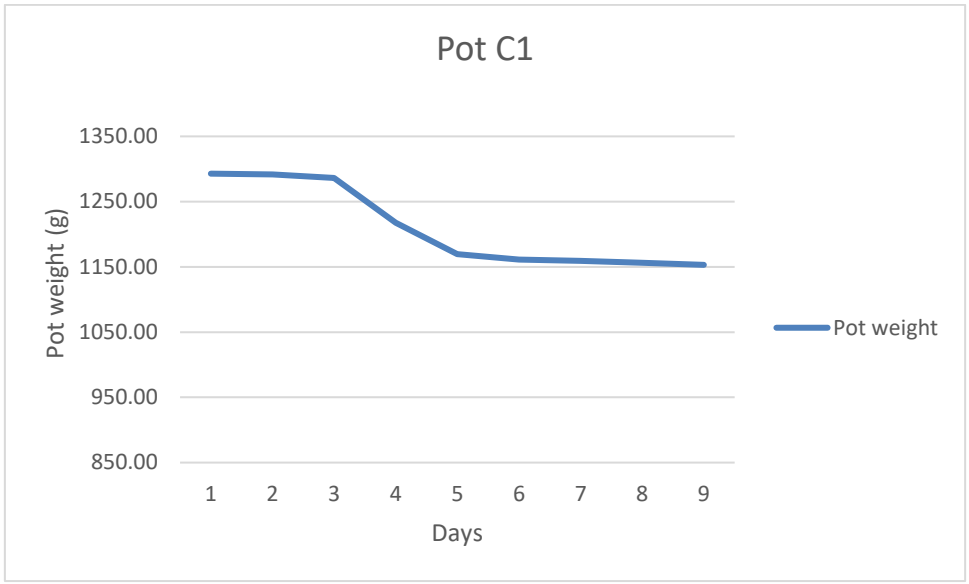
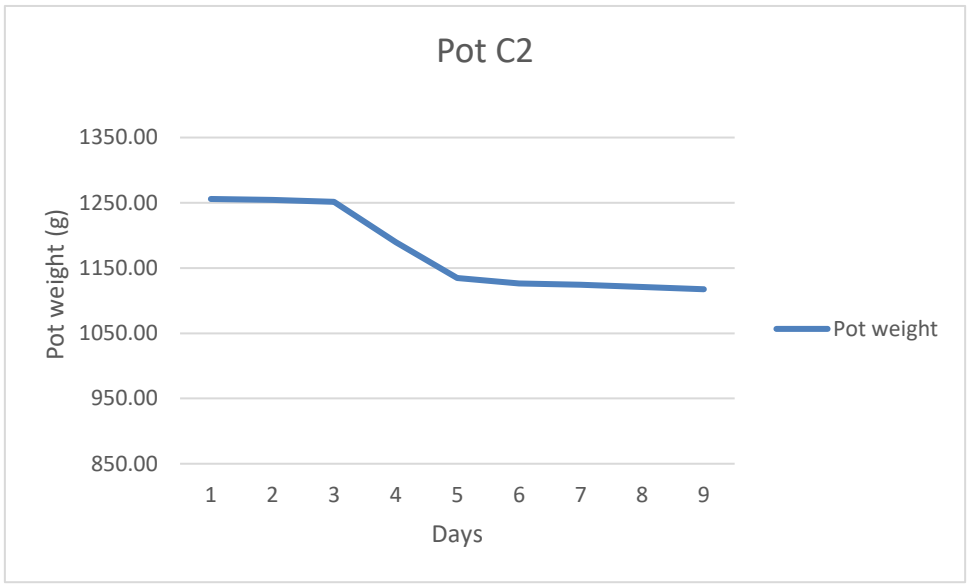


Figure A-3: Treatment C pot weights of all replicates during fermentation





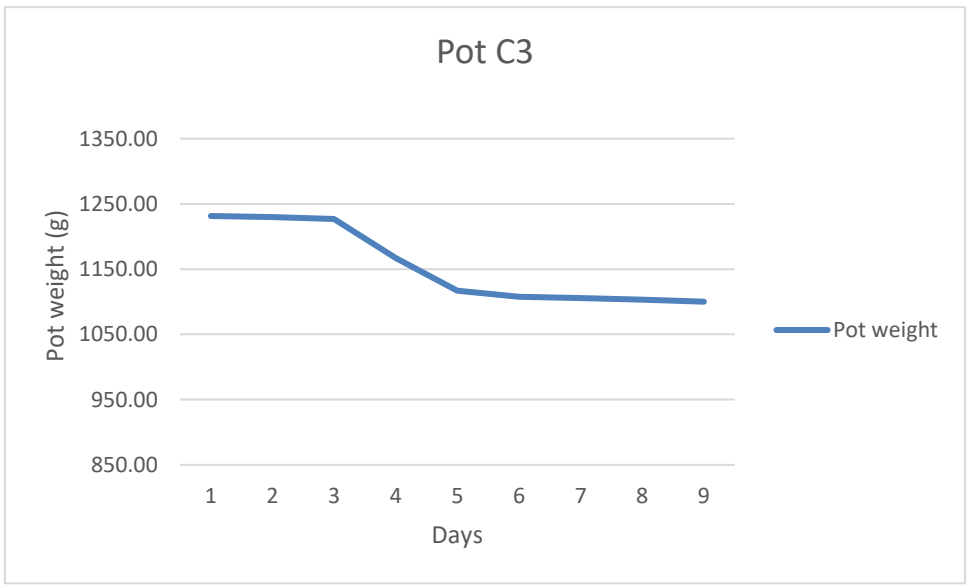


Figure A-4: Treatment D pot weights of all replicates during fermentation

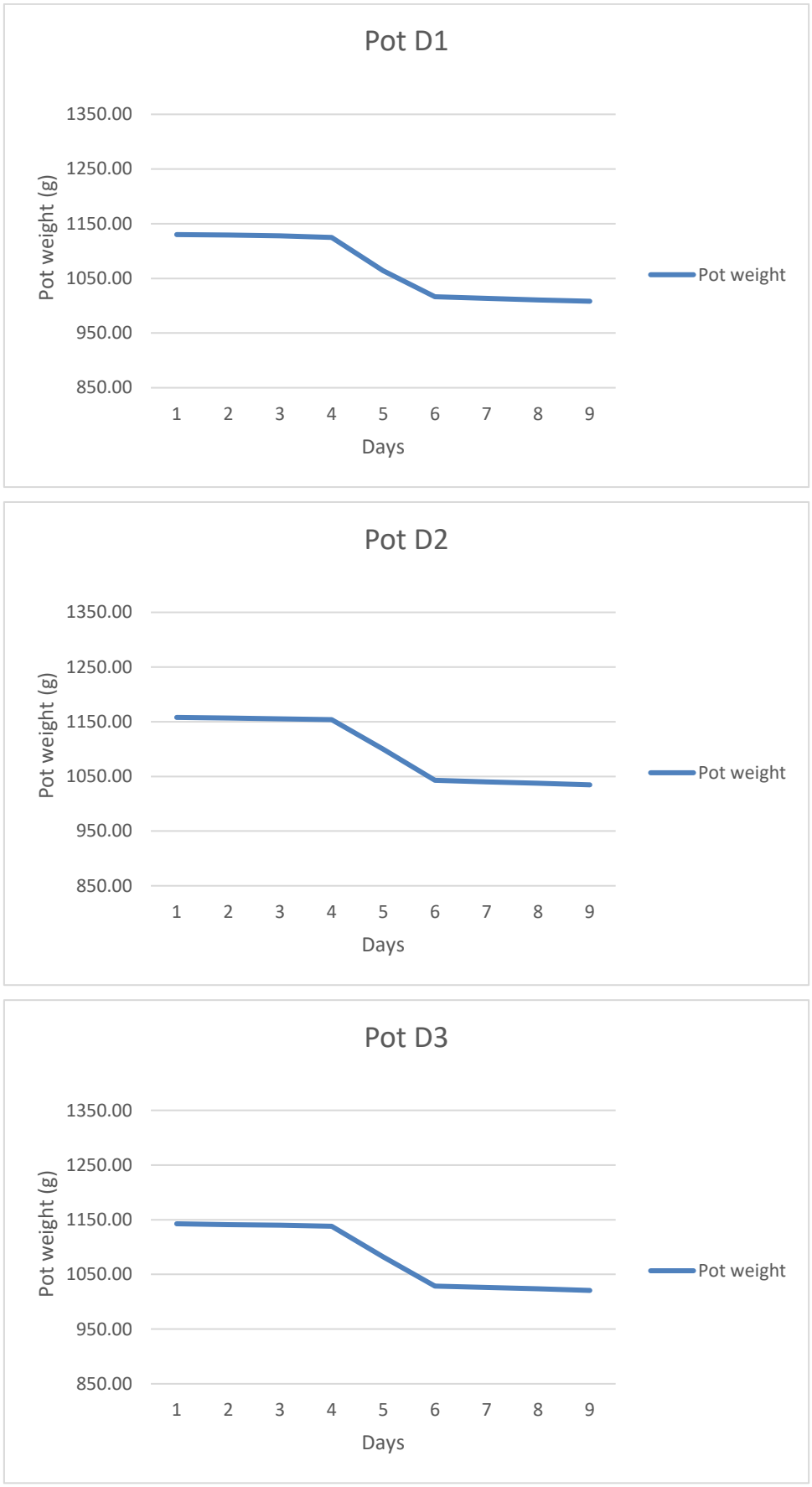
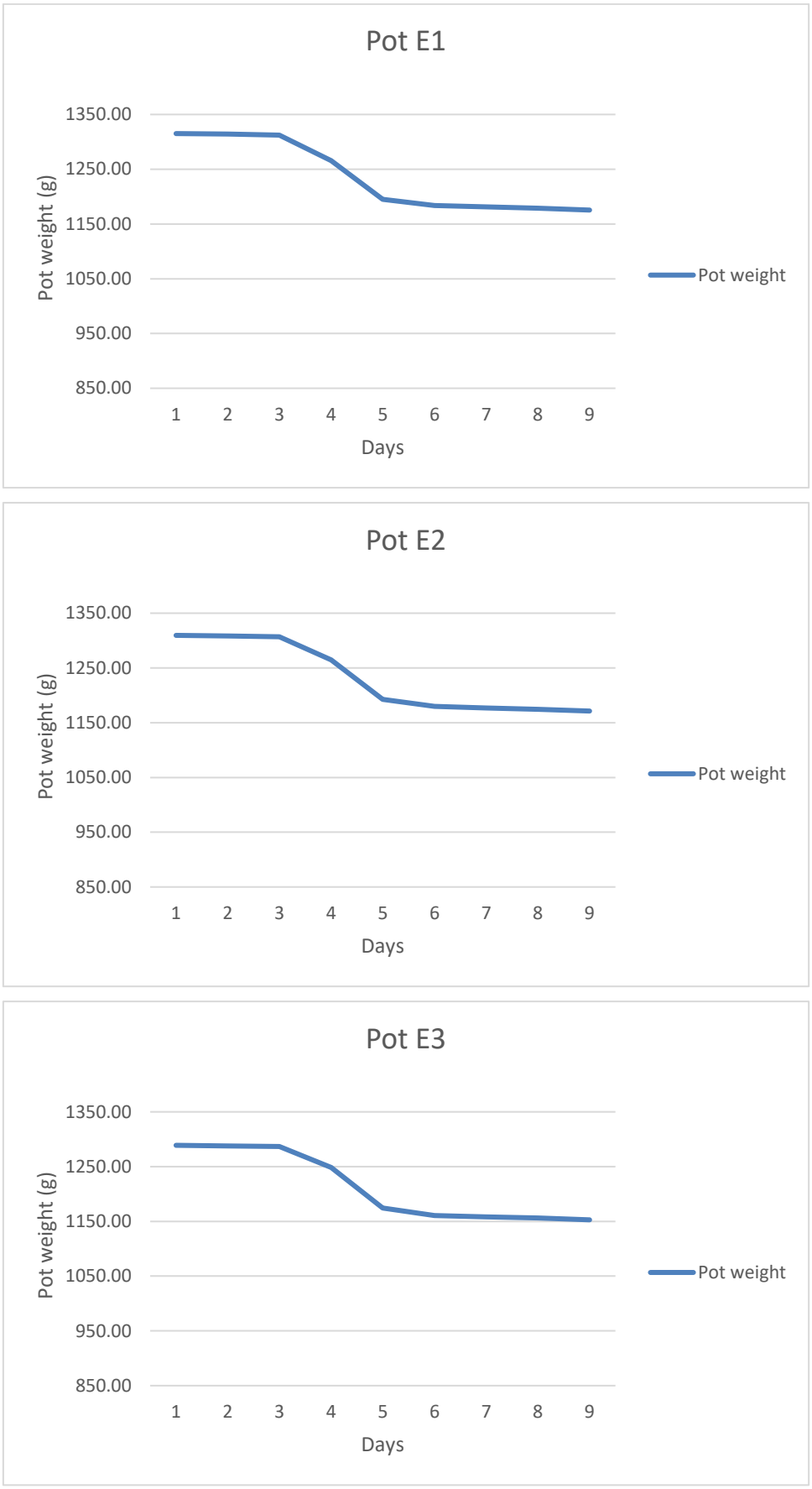


Figure A-5: Treatment E pot weights of all replicates during fermentation



Appendix B

Temperature graphs for ambient cellar aging

Graphs are presented in these appendices to demonstrate how temperature was monitored during ambient cellar aging. For each time period, there are two sets of graphs. One for the temperature monitor placed next to the wines during aging, one for the temperature monitor placed on the other side of the Lincoln University winery, near the main window but not in direct sunlight. The aim was to measure differences in temperature which may occur within the same space and to measure the diurnal shift between day-time and night-time temperatures.

Figure B-1: Minimum and maximum daily temperatures from 20/12/17 to 09/01/18 recorded next to bottles

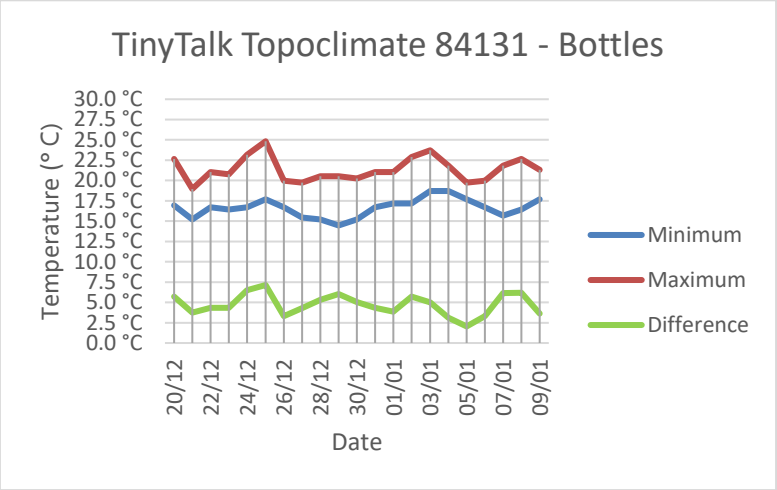


Figure B-2: Minimum and maximum daily temperatures from 20/12/17 to 09/01/18 recorded next to window

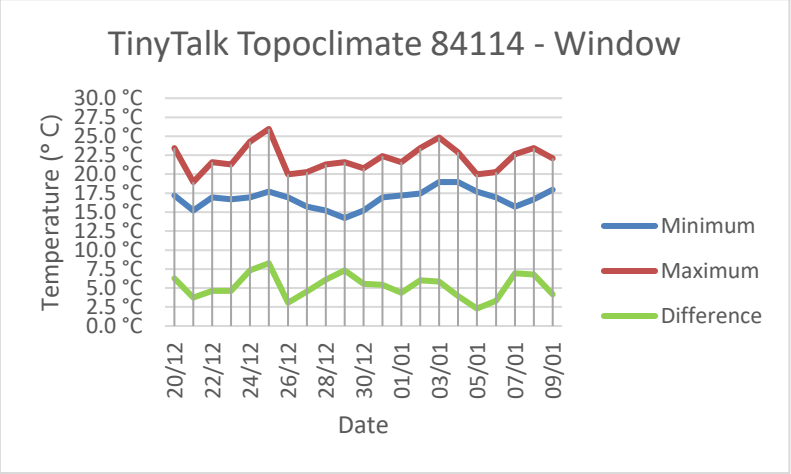


Figure B-3: Minimum and maximum daily temperatures from 10/01/18 to 24/01/18 recorded next to bottles

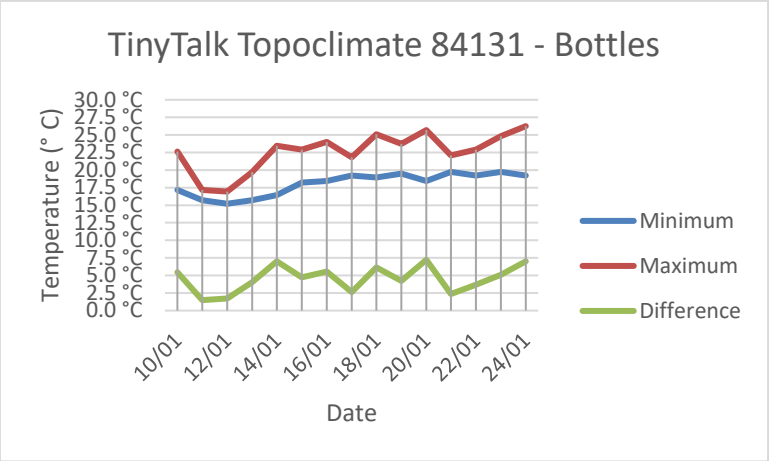


Figure B-4: Minimum and maximum daily temperatures from 10/01/18 to 24/01/18 recorded next to window

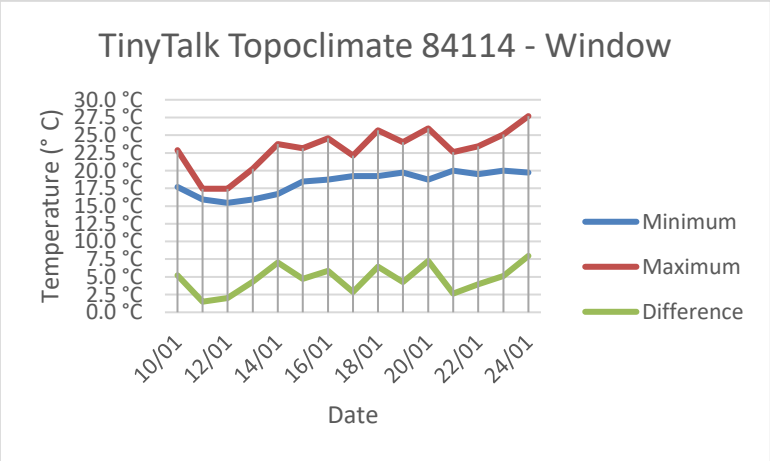


Figure B-5: Minimum and maximum daily temperatures from 25/01/18 to 07/02/18 recorded next to bottles

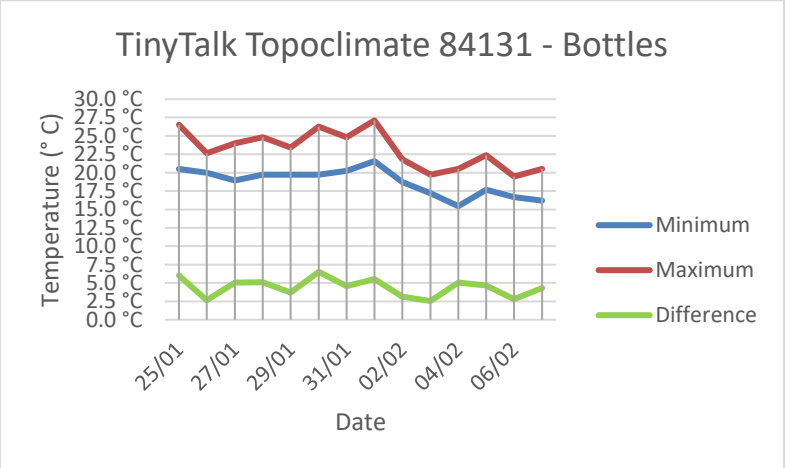


Figure B-6: Minimum and maximum daily temperatures from 25/01/18 to 07/02/18 recorded next to window

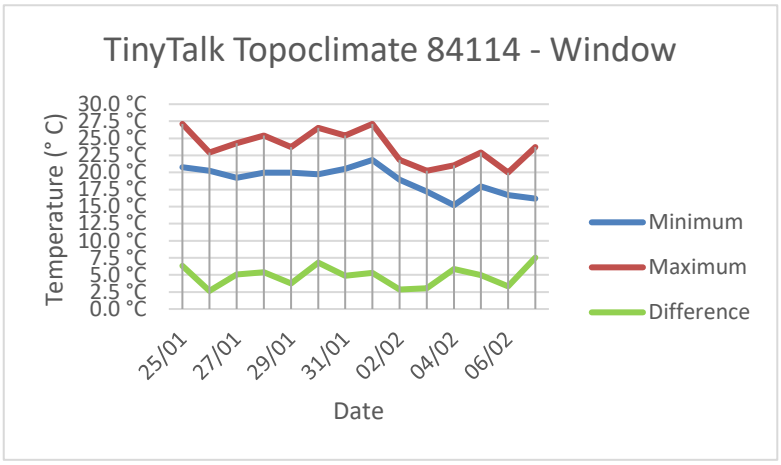


Figure B-7: Minimum and maximum daily temperatures from 08/02/18 to 23/02/18 recorded next to bottles

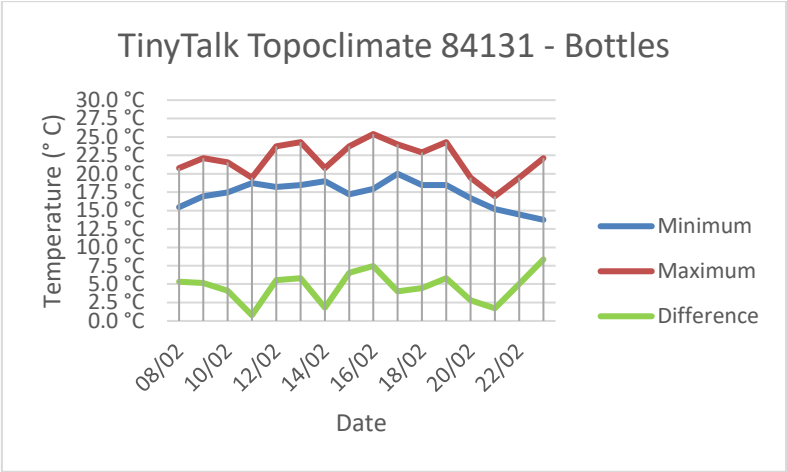


Figure B-8: Minimum and maximum daily temperatures from 08/02/18 to 23/02/18 recorded next to window

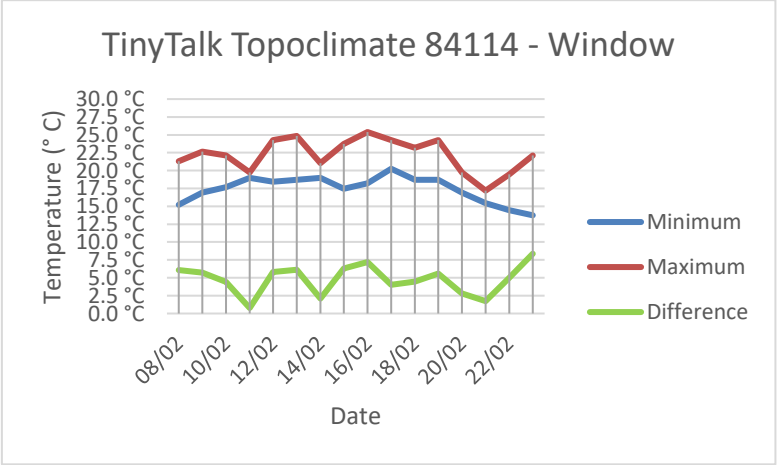


Figure B-9: Minimum and maximum daily temperatures from 24/01/18 to 07/03/18 recorded next to bottles

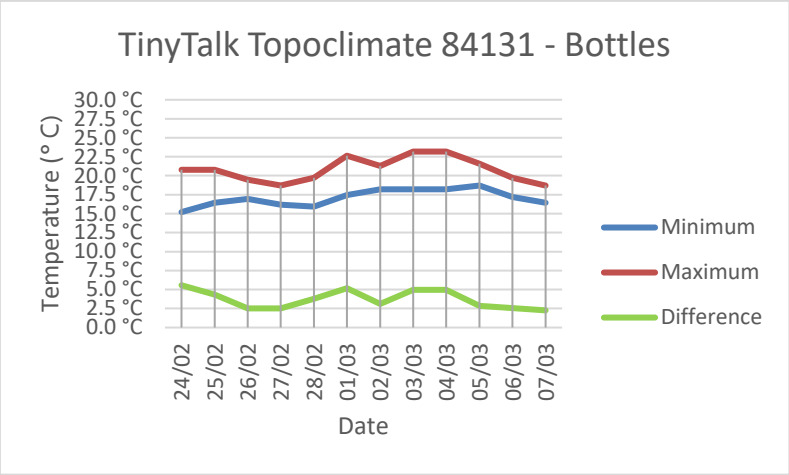
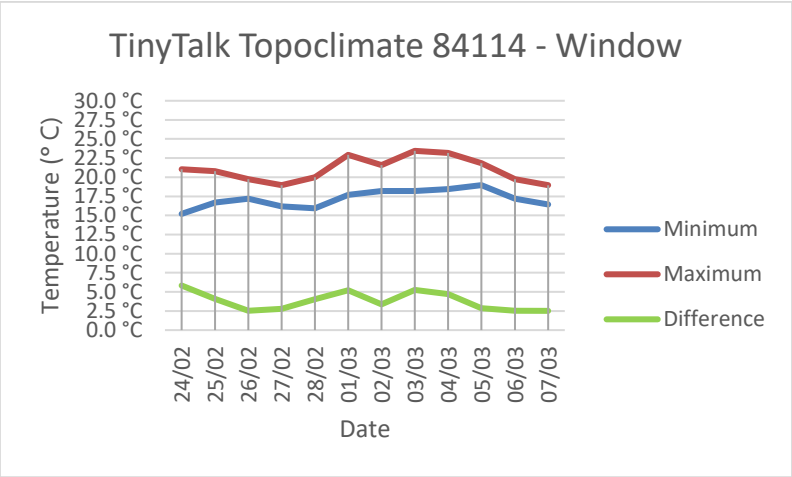


Figure B-10: Minimum and maximum daily temperatures from 24/01/18 to 07/03/18 recorded next to window



Appendix C

Sulfur dioxide additions and analyses

Table C-1: Free and bound sulfur dioxide measurements after fermentation (T1) and aging (T2)

Treatment	*FSO_2 at T1 (mg/L)*	*BSO_2 at T1 (mg/L)*	*FSO_2 at T2 (mg/L)*	*BSO_2 at T2 (mg/L)*
D-1	0.0	34.9	8.2	65.3
D-2	0.0	36.5	11.4	63.7
D-3	0.0	36.5	11.4	68.5
DA-1	-	-	0.0	55.5
DA-2	-	-	4.9	57.1
DA-3	-	-	4.1	49.0
E1	1.7	33.2	7.3	62.0
E2	0.0	28.2	11.4	71.8
E3	0.0	29.9	6.5	68.5
EA-1	-	-	3.3	50.6
EA-2	-	-	3.3	58.8
EA-3	-	-	4.9	58.8

Single letter denotes aging at ambient cellar temperature. “A” following treatment letter indicates aging under accelerated conditions.

Table C-2: Free sulfur dioxide analyses and additions

Treatments	*After juice addition (ppm)*	*After ferment (ppm)*	*Addition (g)*	*1st recheck (ppm)*	*Addition (g)*	*2nd recheck (ppm)*	*Addition (g)*	*At bottling (ppm)*	*Addition (g)*
D1	18.0	0	0.04	13.3	0.02	-	-	3.3	0.03
D2	15.0	0	0.04	11.6	0.02	-	-	13.3	0.03
D3	22.0	0	0.04	13.3	0.02	-	-	11.6	0.02
E1	25.0	1.7	0.04	5.0	0.03	11.6	0.02	5.0	0.03
E2	10.0	0	0.04	5.0	0.03	13.3	0.02	5.0	0.02
E3	20.0	0	0.04	1.7	0.04	15.0	0.02	11.6	0.02

Additions prior to fermentation were made in the form of a 5% SO_2 solution. Additions after fermentation were made in the form of potassium metabisulfite powder dissolved into wine. Treatment D did not receive a second re-check as the first recheck was deigned to be close enough to the target ppm and volumes of treatments were limited.

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