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Molecular drivers of wine texture and taste



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1. Abstract

This project focused on the impact of non-volatile compounds on wine texture and taste. The evolution of phenolics and polysaccharides during white wine production was studied. Another primary aim was to identify new texture and taste target compounds in wine. Work on the bitterant tryptophol sulfonate showed that it was more important in white than red wines, with concentrations increasing in response to post-bottling SO_2 additions. A coumaric acid glycoside was initially identified as a potential bitterant, but later shown through sensory analysis not to impart bitterness. A survey of glutamic acid revealed that it was present above detection threshold in most wines, and could impart 'savoury' flavour.

2. Executive summary

This project aimed to create new knowledge on the molecular drivers of taste and texture in wine and how they are affected by various winemaking inputs. Areas explored included compounds responsible for bitterness and savoury character and the impacts of dissolved oxygen management and winemaking steps on phenolics and polysaccharides (and the resultant wine taste and mouthfeel).

Formation of the 'bitter'/'hard' tasting compound tryptophol sulfonate (TSO_3) in wines when using a high-tryptophol-producing yeast was investigated post-bottling in Chardonnay, Riesling and Gewurztraminer wines, and it was found that TSO_3 concentration stabilised 3-6 months post-bottling. The results further confirmed that wine SO_2 concentration during bottle storage was a significant driver of TSO_3 formation. Threshold testing of individuals to TSO_3 revealed a heterogeneous distribution across tasters resulting in only around 1 in 10 tasters being able to taste TSO_3 at the concentrations found in the white wines at 18 months post-bottling. Subsequent formal sensory analysis of the white wines found no significant differences in bitterness between the white wines with different TSO_3 concentrations. Shiraz fruit was fermented using the same high tryptophol-producing yeast and adjusted to two different SO_2 and pH values prior to bottling. Consistent with findings of the white wine study, little TSO_3 was formed during winemaking. However, in contrast to the white wines, TSO_3 in the red wines did not increase post-bottling, regardless of SO_2 concentration or pH. The white wine study implicated SO_2 availability post-bottling as a critical factor in TSO_3 production, which may explain the differences between TSO_3 production in the white and red wine. TSO_3 could not be mitigated through intervention with either protein or polysaccharide agents. The difficulty in removing TSO_3 from wine once formed in bottle suggests that mitigation strategies (particularly the judicious application of SO_2 pre bottling) should be employed to avoid TSO_3 formation.

Phenolic fractions were isolated from a bitter-tasting white wine prepared from hard pressings. A non-targeted metabolomics approach was used to analyse the composition of these fractions and identify compounds that correlated with their perceived bitterness. A glycoside of trans-p-coumaric acid (4-β-d-glucopyranosyl coumarate) was identified as a potential bitterant. The compound was synthesised and purified on a preparative scale with its identity and purity validated by NMR. It was then subjected to formal taste and texture descriptive analysis, presented in model white wine. Contrary to expectations, the sensory assessment indicated that at low concentration the compound suppressed the bitterness of the model wine, which is presumably elicited by ethanol. The hydroxycinnamic acids (of which coumaric acid is a member) are the predominant class of phenolic compounds found in white wine. This result suggests that the presence of glycosylated forms of hydroxycinnamates may positively contribute to the taste of white wine by reducing bitterness.

The term 'savoury' is synonymous with complex, high-quality wines. While the molecular drivers of 'savoury' character in wine are uncertain, the amino acid glutamic acid, succinic acid, and sodium and potassium salts are likely to contribute to savouriness in wine either directly or via their interactions. A survey of the concentrations of these compounds was conducted on 425 Australian

commercial wines. The survey was also extended to include concentrations of sodium chloride in wine, as this salt is known to contribute to mouthfeel characteristics such as viscosity and soapiness. Results indicated that glutamic acid was above sensory detection threshold in most wines, and may impart 'savoury' character. The data generated will be used to design sensory experiments that further explore the interactions between compounds in wine known to produce savouriness, in an effort to gain knowledge that could benefit winemakers seeking to enhance the savoury character in their wines.

In a scoping study to assess possible winemaking influences on glutamic acid and succinic acid concentrations in red wine, the impacts of must sugar concentration, YAN, fermentation temperature, yeast strain and press fraction were determined in two red varieties. Succinic acid concentration was influenced by yeast strain, YAN and fermentation temperature. Glutamic acid production was enhanced with higher fermentation temperatures, and significantly, the glutamic acid concentrations in the pressing wines were three to four times higher than in the free run wines.

In the late 1990s winemakers began using flotation to clarify white juices, as grape solids could be removed more quickly and at a lower cost than cold settling due to its lower energy requirements and continuous nature. Reports on the efficiency of must clarification using flotation are commonplace, but data on the influence of flotation on wine composition and wine sensory properties have not been widely available. A study involving two commercial wineries from the Murray Valley was conducted to compare the composition, taste and mouthfeel properties of Chardonnay and Frontignac wines made from juices clarified using different methods: flotation using nitrogen, cold settling/racking and unclarified. The non-volatile composition including total phenolics, total polysaccharides, phenolic profile and polysaccharide molecular weight profile of the wines produced by flotation were found to be much like those made using cold-settled juices, which was reflected in the wines having similar mouthfeel and taste properties. Both the Chardonnay and Frontignac wines were perceived to be slightly more viscous than the wines made from either the settled or unclarified juice. Perceived viscosity was best correlated with higher pH and higher total phenolic content and specifically with caftaric acid concentration. Surprisingly, higher total polysaccharide concentration was associated with lower perceived viscosity. The cold-settled Frontignac wines were slightly less bitter than the high-solids and low-solids floated wines, but the differences in bitterness could not be attributed to total wine phenolics nor to any of the quantified phenolic compounds including the known bitterants, catechin and epicatechin. This suggested that the compounds responsible for the greater bitterness in the settled wines made from this variety were not captured in this study. Perceived hotness, astringency and acidity were not consistently affected by juice settling methods. Overall, while a few differences in mouthfeel attributes between wines made from floated and cold settled juices were observed, the magnitude of the differences was small. Winemakers can benefit from the known efficiency and cost advantages of switching from cold settling to flotation, while being confident that the sensory and compositional quality of their wines is unlikely to be diminished by changing to the more efficient system of clarification.

The evolution of polysaccharides and total phenolics during the entire process of white winemaking including during short and extended periods of juice settling was determined for the first time in Sauvignon Blanc and Chardonnay. Total phenolics increased in the first 12 hours of settling, suggesting that they were being rapidly extracted from the grape solids. However, at the completion of fermentation and pre-bottling, the total phenolic content of wines was independent of the time taken to settle the juice off solids. The concentration of high molecular weight mannoprotein polysaccharides that assist in protein and cold stabilisation increased significantly following the start of fermentation. The concentration of medium molecular weight arabinogalactan protein polysaccharides (previously shown to contribute positively to white wine texture by reducing alcohol hotness and increasing perceived viscosity) reduced both during settling off grape solids and particularly during fermentation. Longer juice settling time, contrary to expectation, did not have a significant effect on the polysaccharide composition of the settled juices or of the finished wines.

This was reflected in the sensory outcomes where no consistent differences were found between the wines.

Dissolved carbon dioxide (DCO_2) was previously found to be a factor influencing the textural properties of white wines. This work was expanded to assess the influence of DCO_2 on red wine taste and mouthfeel. The impact of DCO_2 concentration on red wine taste and texture in the presence of increased red wine tannin and ethanol was determined in a Shiraz and Cabernet Sauvignon wine. Higher DCO_2 concentration resulted in reduced bitterness and astringency and increased sweetness, while not affecting viscosity, hotness or overall flavour. However, the dominant effect of increased DCO_2 on red wine texture was the addition of a 'spritz' character. A further study investigated the loss of DCO_2 from the act of pouring wine into a glass and leaving it standing. The study found that significant losses in DCO_2 resulted from the act of pouring, and that higher alcohol wines experienced greater DCO_2 losses during standing. Based on the published difference threshold for DCO_2, the loss would have resulted in a perceptible reduction in spritz character, but only after leaving wine in the glass for 15 minutes. This work indicates that winemakers should consider their protocols regarding pouring of samples and the time between pouring and assessment when making decisions at the tasting bench.

Based on the results of the project, winemakers now have a clearer understanding of a range of influences on wine taste and texture that they can incorporate into their practices.

3. Acknowledgements

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External collaborators and contributions

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- Zilzie Wines (Candie Jonsson).

4. Background

The key stakeholders in this project were winemakers and wine consumers, because sensory perception and in-mouth texture define many of the most valuable commercial wines of the world. The molecular drivers responsible for these sensory attributes are however, poorly defined.

It was expected that this project would generate new fundamental knowledge on the molecular drivers of positive taste and texture characteristics, and also of negative attributes such as bitterness, palate hardness, unpleasant acidity, hotness and pungency. Generation of that knowledge would be key to an improved understanding of the impact of various vineyard and winery inputs on wine composition and sensory properties.

This project aimed to identify compounds that may lead to positive and negative taste and texture outcomes, throughout the different stages of wine production, or in response to specific winemaking practices. Such negative characters can occur through different stages of the wine production process, from grapegrowing (temperature and exposure impacts), throughout processing and also post-bottling.

Increasingly the premiumisation of red and white wine is inseparable from the concept of texture as it defines style and typicality (the interaction between terroir and winemaking practice). It has been proposed that in-mouth texture defines the 'typicality' of many of the most valuable commercial wines of the world, for example the creaminess of barrel fermented white Burgundy, the oily texture of Alsatian Pinot Gris made from high solids juices, the oily and drying nature of Viognier made with skin contact from the Northern Rhone, or the rich full-bodied expression of Shiraz produced in the Barossa. It could also be argued that the high value placed on these wines by consumers is the result of a perception of uniqueness of some sensory property, whether it be flavour or texture, associated with a particular region or vineyard site. In terms of taste, many European and new Australian styles of red wines are positively characterised by a savouriness. However, despite knowledge of molecular drivers of savoury/umami flavours in foods, similar compounds have not yet been characterised or their functions defined in wines. Compounds described by mouthfulness, or 'kokumi' have also been characterised in foods but not in wine, although evidence exists that such compounds may be present in wines.

5. Project aims

This project aimed to address the broad aim of creating new knowledge on the molecular drivers of taste and texture and how they are affected by various winemaking inputs.

Deliverables

Year 3 deliverables

- Quantitative data on the dynamics of phenolic and other non-volatile compound evolution and loss during the white and red winemaking process, and how these may be affected or managed/altered by winemaking interventions such as juice preparation, yeast, oak fermentation and maturation, lees stirring, alcohol concentration, additives and fining.
- Knowledge regarding compounds or classes of compounds leading to negative characteristics (such as bitterness, aggressive or unbalanced astringency, palate hardness, unpleasant acidity taste profiles, hotness and pungency).

Year 5 deliverables

- Knowledge regarding compounds or classes of compounds leading to positive taste and textural characteristics (such as texture, creaminess, viscosity, savouriness, mouthfulness, balanced acidity).
- Knowledge of defined risk factors throughout wine production which may contribute to the presence of specific phenolic and other non-volatile compounds in white and red wine associated with negative wine attributes (e.g. possible browning reactions leading to premature ageing of white wines; colour loss in red wines; bitterness; excessive astringency).
- Practical advice and extension materials for winemakers

AOP FY 2017/18:

Deliverables	Activities	Target date
A. Quantitative data on the dynamics of phenolic and other non-volatile compound evolution and loss during the white and red winemaking process, and how these may be affected or managed/altered by	1. Conduct a critical literature review of the molecular drivers of the mouthfeel of white wine and submit manuscript. 2. Draft a publication on a newly identified bitterness compound from white wine. 3. Bottle wines from the vintage 2017 'solids contact settling time' wines, analyse chemistry (polysaccharides, polyphenolics, proteins, volatiles) at appropriate time points, schedule sensory analysis. 4. Design and execute a vintage 2018 winemaking trial to	• December 2017 • December 2017 • June 2018

winemaking interventions such as juice preparation, yeast, oak fermentation and maturation, lees stirring, alcohol concentration, additives and fining (2019).	quantify the dynamics of the non-volatile compounds in red wine previously found to add bitterness and hardness during steps of red wine production.	• June 2018
B. Knowledge regarding compounds or classes of compounds leading to negative characteristics (such as bitterness, aggressive or unbalanced astringency, palate hardness, unpleasant acidity taste profiles, hotness and pungency) (2020).	1. Develop preparative scale methods to isolate and elucidate compounds that contribute to negative characters in white and red wine. 2. Develop methods to assess interactions between sensorially negative compounds of interest with key macromolecules or matrix compounds.	• June 2018 • June 2018

AOP FY 2018/19:

Deliverables	Activities	Target date
A. Quantitative data on the dynamics of phenolic and other non-volatile compound evolution and loss during the white and red winemaking process, and how these may be affected or managed/altered by winemaking interventions such as juice preparation, yeast, oak fermentation and maturation, lees stirring, alcohol concentration, additives and fining	1. Complete analysis of vintage 2018 winemaking trials investigating the evolution of potentially bitter compounds. 2. Analyse collected set of wines for prevalence of sulfonated indole derivatives and other potentially bitter compounds. 3. Design and execute vintage 2019 winemaking trials to assess the factors that contribute to the evolution of potentially bitter, non-volatile compounds in red and white wines. 4. Draft publication(s) on bitter and texture-related compounds, the evolution of sulfonated indole derivatives and potential influencing factors. 5. Complete sensory analysis of vintage 2017 settling/contact time trial and compile results for publication.	June 2019
B. Knowledge regarding compounds or classes of compounds leading to negative characteristics (such as bitterness, aggressive or unbalanced astringency, palate hardness, unpleasant acidity taste profiles, hotness and pungency)	1. Submit publication on the influence of CO_2 on mouthfeel in wine. 2. Optimise preparative scale methods to isolate and elucidate compounds that contribute to negative characters in white and red wine, especially bitter compounds. 3. Assess interactions between sensorially active compounds of interest and wine macromolecules and compare results with sensory analysis. 4. Design and execute an experiment to investigate the influence of yeast-derived or grape-derived compounds on mouthfeel and taste.	June 2019

AOP FY 2019/20:

Deliverables	Activities	Target date
A. Quantitative data on the dynamics of phenolic and other non-volatile compound evolution and loss during the white and red winemaking process, and how these may be affected or managed/altered by winemaking interventions such as juice preparation, yeast, oak fermentation and maturation, lees stirring, alcohol concentration, additives and fining	1. Conduct chemical analyses of white wine samples from vintage 2019 at regular timepoints to measure the evolution of tryptophol sulfonates. 2. Conduct a bench tasting of vintage 2019 wines at least 12 months post-bottling to assess the development of different sensory profiles. 3. Conduct a sensory assessment to compare the bitterness intensities of sulfonated and non-sulfonated indole derivatives or phenolics. 4. Conduct sensory and chemical assessment of white wine made with grape seed powder as a fining agent to assess the impact of the intervention on wine macromolecules, protein stability and wine sensory properties. 5. Draft a manuscript for publication in a peer-reviewed journal.	June 2020
B. Knowledge regarding compounds or classes of compounds leading to negative characteristics (such as bitterness, aggressive or unbalanced astringency, palate hardness, unpleasant acidity taste profiles, hotness and pungency)	1. Complete analysis of trials investigating the impact on macromolecule interactions of wine additives such as polysaccharides on negative wine characters such as bitterness and palate hardness. 2. Compile results for publication.	June 2020
C. Knowledge regarding compounds or classes of compounds leading to positive taste and textural characteristics (such as texture, creaminess, viscosity, savouriness, mouthfulness, balanced acidity) (Year 5 deliverable)	1. Review the literature on potential contributors of positive mouthfeel characteristics, including umami and kokumi. 2. Identify and source wine compounds likely to contribute to positive mouthfeel characteristics. 3. Investigate methods to assess the influence of saliva in modulating positive textures induced by dissolved CO_2 or other significant wine texture components. 4. Design and execute experiments to assess the influence of significant and ubiquitous wine matrix components, such as salt and succinic acid, on positive mouthfeel properties including mouthfulness, umami and kokumi.	June 2020
D. Knowledge of defined risk factors throughout wine production which may contribute to the presence of specific phenolic and other non-volatile compounds in white and red wine associated with negative wine attributes (e.g. possible browning reactions leading to premature ageing of white wines; colour loss in red wines; bitterness; excessive astringency (Year 5 deliverable)	1. Design and execute vintage 2020 winemaking trials to assess the potential risk factors associated with the development of negative wine attributes, such as SO_2 addition and fining, with a focus on bitterness and excessive astringency.	June 2020
E. Practical advice and extension materials for	1. Prepare and present a poster for the 17th AWITC highlighting the factors influencing wine texture and macromolecule	June 2020

winemakers (Year 5 deliverable)	evolution.	

AOP FY 2020/21:

Deliverables	Activities	Target date
A. Knowledge regarding compounds or classes of compounds leading to positive taste and textural characteristics (such as texture, creaminess, viscosity, savouriness, mouthfulness, balanced acidity)	1. Develop a rapid HPLC method with support from Metabolomics Australia to quantify glutamic acid and other amino acids in wine that may contribute to its savoury (umami) character. 2. Conduct a survey of Australian and international wines to determine the concentration range of compounds that either directly contribute to or enhance 'savoury' (umami) character in wine (e.g. glutamic acid, succinic acid, glutathione, salts). 3. Conduct a sensory trial to assess the impact of these compounds and other compounds on 'savoury' character. Analyse and interpret the data arising from the trial. 4. Adopt a model method to assess the influence of saliva and wine compounds in modulating positive wine textures through CO_2 ingress.	June 2021
B. Knowledge of defined risk factors throughout wine production which may contribute to the presence of specific phenolic and other non-volatile compounds in white and red wine associated with negative wine attributes (e.g. possible browning reactions leading to premature ageing of white wines; colour loss in red wines; bitterness; excessive astringency	1. Conduct chemical analyses of vintage 2020 Shiraz wines at regular timepoints to measure the evolution of tryptophol sulfonate. 2. Conduct formal sensory analysis of vintage 2020 wines at least 12 months post-bottling to assess the development of bitterness/hardness in Shiraz wine. 3. Revisit previously identified potentially bitter tasting targets for characterisation, or identify new target compounds for sensory assessment. 4. Compile data on the evolution of tryptophol sulfonate in red and white wines during fermentation and ageing for peer-reviewed publication.	June 2021
C. Practical advice and extension materials for winemakers.	1. Prepare a new roadshow presentation on the role of tryptophol sulfonate in wine bitterness and how they can be avoided. 2. Deliver roadshow presentations on dissolved CO_2 and wine texture, as requested. 3. Publish an industry article on the effect of dissolved carbon dioxide on the sensory properties of table wine.	June 2021

AOP FY 2021/22:

Deliverables	Activities	Target date
A. Knowledge regarding compounds or classes of compounds leading to positive taste and textural characteristics (such as texture, creaminess, viscosity, savouriness, mouth-fullness, balanced acidity).	1. Obtain target compounds (e.g. glutamic acid, peptides containing glutamic acid, succinic acid, and salts) thought responsible for savouriness by preparative extraction from wine, synthesis and/or from external sources, as required. 2. Conduct laboratory-scale winemaking trials to identify winemaking and/or viticultural factors that modulate the formation of compounds that contribute to 'savoury' attributes in wine. 3. Conduct a sensory trial to determine wine compositional factors listed in 1. that contribute directly or cooperatively to wine savouriness. 4. Compile and analyse survey data of Australian and international wines to assess the concentration range for compounds that are expected to either directly contribute to, or cooperatively affect, 'savoury' character. Extend the survey to include concentrations of chloride and its associated cations. 5. Draft a manuscript for peer review on the concentration range of compounds in wine that may elicit, contribute and/or modulate 'savoury' taste and other sensory attributes.	June 2022
B. Knowledge of defined risk factors throughout wine production which may contribute to the presence of specific phenolic and other non-volatile compounds in white and red wine associated with negative wine attributes (e.g. possible browning reactions leading to premature ageing of white wines; colour loss in red wines; bitterness; excessive astringency).	1. Scale up synthesis to obtain 2 g of trans-*p*-coumaric acid glycoside (CG), identified as a potential bitter tasting target compound, for sensory assessment. 2. Conduct benchtop sensory analysis of CG. Subject to the results, adapt the scaled-up synthesis method to include other, more abundant, compounds of the same class. 3. Assess using isothermal calorimetry the nature of interactions between molecules identified as being bitter with polysaccharides previously found to influence wine texture and with fining agents, to assess methods for their removal or taste mitigation.	June 2022
C. Practical advice and extension materials for winemakers.	1. Prepare industry articles summarising key findings of 3.1.3 applicable to winemaking practice. 2. Prepare presentations to be delivered in person or via virtual roadshows or AWRI webinars and extension materials for the 18th AWITC. 3. Contribute articles to AWRI Technical Review, *eNews* and other communication channels, as applicable. 4. Support Projects 2.1.1 (Extension) and 2.1.2 (Communication and awareness-raising activities) through identifying topics and preparing materials for awareness-raising and practice change activities.	June 2022

6. Methods

6.1 A critical literature review of the molecular drivers of the mouthfeel of white wine

A review of 181 articles on the physiological basis of, and the influence on white wine mouthfeel of phenolic compounds, polysaccharides, glycerol, ethanol, organic acids, carbon dioxide and amino acids was conducted and practical implications and future research directions were proposed and discussed.

6.2 Timing of formation and factors affecting tryptophol sulfonate in white and red wines

A white and a red winemaking trial were conducted to assess the effect of SO_2 and acid additions on the formation of tryptophol sulfonate (TSO_3). In the first trial, Chardonnay, Riesling and Gewurztraminer grapes were crushed, the juice was drained off skins and cold settled and adjusted to pH 3.4 by adding tartaric acid, and to a free SO_2 greater than 10 mg/L by adding potassium metabisulfite. The juices were inoculated using a high tryptophol-producing strain of *Saccharomyces cerevisiae* (AWRI 2940) and fermented at 15-16°C. The wines were then divided into 20 L stainless steel kegs and two acid (no addition and + 3 g/L tartaric acid) by two SO_2 (50 and 150 mg/L in the form of potassium metabisulfite) treatments were applied to the divided wines, in triplicate. In the second trial, wines were made from Shiraz grapes sourced from a single vineyard in McLaren Vale, South Australia using two grape maceration methods to produce wines of two phenolic levels. The red musts were fermented using *Saccharomyces cerevisiae* strain AWRI 2940, with other winemaking as described in Bindon et al. (2019). The finished wine was divided into kegs and adjusted by the addition of 0 or 2 g/L of tartaric acid and potassium metabisulfite at 10 or 80 mg/L. The wines were bottled under inert conditions, sealed under screw cap, and stored at 15°C until sensory assessment.

Analysis of TSO_3 was conducted at the completion of fermentation and at 3, 6, 9, 12 and 21 months post-storage by HPLC for the white wines and at the end of fermentation, 3 and 6 months post-storage for the red wines. The HPLC conditions were: Phenomenex Kinetex F5 column (150 x 2.1 mm, 2.6 µm pore size), injection volume 5 µl, column temperature 45°C. Flow rate 0.4 mL/min. Phase A: 0.1% formic acid in water. Phase B: 0.1% formic acid in acetonitrile. Elution gradient: 0%B for 8 min, 5%B at 40 min, 25%B at 49 min, 80%B at 55 min, 80%B at 58 min, dropped to 0%B and held for 15 min. Detection was carried out by FLD with excitation at 280 nm and emission at 350 nm. A TSO_3 standard was synthesised (Arapitsas et al. 2018), and its structure and purity confirmed by HPLC-QTOF-MS/MS.

6.3 Sensory assessment of bitterness of the white wines 21-months post-bottling

Twelve assessors experienced in descriptive analysis of the in-mouth sensory properties of wine were trained by tasting all samples in three one-hour bench tasting sessions whereby bitterness and other in-mouth attributes were selected, the latter to preclude the possibility of sensory dumping effects, which occur when a salient sensory attribute is not included and the tasters' opinion about that attribute is displaced onto another inappropriate attribute (Clark and Lawless 1994). Sensory profiling of each variety was conducted on separate days with all samples being assessed in triplicate. Wine samples (30 mL) were presented to tasters in ISO wine glasses at room temperature in isolated booths and under daylight-type fluorescent lighting. A modified Williams Latin Square random block design determined the presentation order. Attribute intensity was rated using an unstructured 15 cm line scale with anchor points of 'low' and 'high' at 10% and 90%, respectively.

6.4 Taste detection threshold of tryptophol sulfonate

The taste detection threshold of TSO_3 in model wine (pH 3.4, 10% v/v ethanol) was determined according to the ASTM method E 679 using 36 AWRI employees experienced in difference testing. A reference sample of 256 mg/L of TSO_3 was tasted just prior to the formal tasting. Concentrations of 4, 8, 16, 32, 64 and 128 mg/L TSO_3 were presented as triangle tests as 25 mL samples in ISO wine glasses at room temperature and under sodium lighting. Individual best-estimate thresholds were

calculated as the square root of the concentration of the last incorrect identification multiplied by the first concentration of consecutive correct identifications.

6.5 Interactions between tryptophol sulfonate and polysaccharide and fining agents

A trial was conducted to assess whether bentonite (1 g/L) or the proteinaceous fining agent casein (200 mg/L) (Laffort Casei Plus) could remove tryptophol (0.5 g/L) and TSO_3 (5 mg/L) from model wines (12% v/v ethanol, pH 3.0 and 3.4 and at 0 and 100 mg/L SO_2. Isothermal titration calorimetry (ITC) (Microcal Auto ITC 200, General Electric, Northampton, MA, USA) was also used to measure the thermodynamic parameters of interaction – enthalpy (ΔH), entropy (ΔS) and association constant (Ka) by titrating TSO_3 into aqueous solutions of high, medium and low molecular weight polysaccharides extracted from white wine buffered at pH 4.0 using ammonium sulfate.

6.6 A potentially new bitter compound in white wine

A hexose ester of trans-p-coumaric acid (4-β-d-glucopyranosyl coumaric acid) was identified using a metabolomics approach as a potential bitterant in white wine. The compound was synthesised on a preparative scale based on the methodology of Galland et al. (2007), where p-coumaric acid was methylated by dissolving it (10.54 mmol) in MeOH (30 mL) containing 1 mL H_2SO_4, refluxed for 24 h and concentrated under vacuum. The concentrate was diluted with ethyl acetate and washed with 5% aqueous $NaHCO_3$ and water, then dried over anhydrous Na_2SO_4. The resultant methyl p-coumarate was mixed with tetra-O-acetyl-RD-glucopyranosyl bromide (1.5 eq) in anhydrous CH_2Cl_2 (25 mL), methyl cinnamate (5.6 mmol) and tris[2-(2-methoxyethoxy) ethyl] amine (1.5 eq) in 20 mL of 1 M $NaHCO_3$/1 M KCl, (1:1). The mixture was refluxed for 48 h under N_2. After the addition of H_2O (50 mL) and extraction with CH_2Cl_2 (3x20 mL), the combined organic phases were successively washed with 1 M HCl (100 mL) and water (100 mL), dried over Na_2SO_4, concentrated under reduced pressure, and purified by silica chromatography. The resultant compound methyl 4-(2',3',4',6'-tetra-O-acetyl)-β-D-glucopyranosyl coumarate was confirmed by NMR. Deprotection was realised by mixing with 1 M NaOH (8 eq)/H_2O/ MeOH, (1:2:3 v/v/v) (22 mL) and stirring for 8 h at 24°C. The mixture was acidified to pH 1-3 with HCl and concentrated under reduced pressure. A scale-up was performed on 4 g of starting material, and following purification using C18 chromatography, 2.26 g of the hexose ester of the trans-p-coumaric acid (m/z 325.0928) was obtained for sensory analysis, and the structure and purity validated by NMR.

6.7 Sensory assessment of 4-β-d-glucopyranosyl coumaric acid

A tetrad test using 30 experienced tasters who regularly participate in difference testing compared two samples (model wine 8% ethanol, pH 3.4) with the same model wine with 200 mg/L 4-β-d-glucopyranosyl coumaric acid added. Moderate statistical evidence was found (P=0.04) to support a perceptible difference. Therefore, a descriptive analysis was performed to evaluate the effect of the compound at 25, 50, 100 and 200 mg/L on the in-mouth sensory properties of model wine (8% ethanol, pH 3.4). Eleven assessors who responded correctly to a preliminary screening discrimination task were selected to participate. Panel training and formal assessment conditions are described above.

6.8 Survey of tryptophol sulfonate concentration in Australian wines

Bottled commercial Australian wines were sourced from those submitted to AWRI Commercial Services (now Affinity Labs) for routine analysis of SO_2, pH, alcohol and residual sugar. Their grape variety and region of origin were recorded according to information provided on the wine label. A 50 mL aliquot of each wine was sampled and stored at 4°C until analysis, as described above.

6.9 The effect of dissolved carbon dioxide (DCO_2) on the sensory properties of still red wines

The DCO_2 concentrations of a commercially bottled Shiraz and Cabernet Sauvignon wine, sealed under screw cap, were varied by blending nitrogen-sparged and carbonated versions of the same

wine. The wines were then adjusted for ethanol (+1% v/v) and tannin concentration (+200 mg/L oenotannin) (Gawel et al. 2020). The wines were tasted in duplicate by nine trained tasters, using standard descriptive analysis protocols. The tasters quantified the intensity of:

1) the mouthfeel characteristics of spritz, astringency, hotness and viscosity

2) sweetness, acidity and bitterness

3) overall aroma and flavour.

To mimic wine consumption by a consumer, the tasters were presented with 150 mL of wine in 'restaurant'-dimensioned (100 mm height, 80 mm bowl, 60 mm opening) polycarbonate glasses at typical consumption temperatures (23°C). To minimise variations in CO_2 dissipation from the glass after pouring, the wines were tasted within two minutes of pouring. Preliminary work showed that the concentration of DCO_2 in the glass after pouring was 20 to 30% lower than the DCO_2 concentration of the wine when in the bottle (Gawel et al. 2020). For this reason, the DCO_2 concentrations in the glass were measured concurrently with the wines being tasted using an Orbisphere system adapted for in-glass measurement.

6.10 CO_2 ingress through salivary layers

A model mouth was constructed using hydrophilic microscope glass slides and an inner well extending above the glass base to allow the known thickness of salivary layers on the oral mucosa to be applied while also allowing solutions containing dissolved CO_2 to be applied without disturbing the salivary layer. Whole saliva from two subjects (one male aged 43, and one female aged 26) was provided by spitting, and used immediately upon collection. Solutions of water with 0.375, 0.75 and 1.5 g/L of dissolved CO_2 were applied to four thicknesses (0, 0.7, 1.4 and 2.1 mm) of saliva using a randomised design within the two saliva sources. The ingress of CO_2 through the saliva layer of different thicknesses was recorded using a CO_2 optical sensor (Precision Sensing GmbH, Regensburg, Germany).

6.11 The effect of wine composition on CO_2 losses from a wine glass during consumer tasting

A commercially available Chardonnay wine (13.0% v/v ethanol) was used in a study of CO_2 losses from wine glasses. In addition to using the wine in its original state, samples were also adjusted to assess the effect of wine matrix components on DCO_2 loss from the bottle during wine serving and consumption. The treatments chosen were acidity (+2 g/L tartaric acid), ethanol (+2 % v/v) and residual sugar (+5 g/L fructose). Samples of 150 mL of wine at 7°C were poured into four stemless restaurant-style wine glasses (400 mL, 100 mm height, 80 mm bowl, 60 mm opening) and left to stand at room temperature (23°C). A DCO_2 measurement was taken from the first glass immediately after pouring, using a Hach Orbisphere 3658 with a sampling port modified for in-wine-glass measurement (described in Gawel et al. 2020), while the remaining three glasses had 10 mL of wine tipped out to mimic consumption. The DCO_2 in the second glass was measured at 5 minutes, and the remaining two glasses had another 10 mL tipped out. The process was repeated until the DCO_2 concentration of the fourth glass was measured at 15 minutes. For replication, the process was repeated three times for each wine.

6.12 Survey of compositional factors in Australian wines thought to contribute to 'savoury character'

Bottled commercial Australian wines were sourced from those submitted to AWRI Commercial Services (now Affinity Labs) for routine analysis. The grape variety and region of origin of each sample were recorded. Glutamic acid concentration was determined as per Kassara et al. (2019). Succinic acid concentrations were determined using the method of Nissen et al. (1997). Chloride concentrations were quantified as NaCl equivalents using the argenometric titration method (OIV-MA-AS321-02:R2009).

6.13 Winemaking influences on glutamic acid and succinic acid concentration

A 20 kg batch of Shiraz and Pinot Noir grapes was destemmed and partially crushed, with the partially crushed berries separated from the juice by draining. The juice and berries were split into halves by weight, and then combined to produce two 10 kg lots of must with a 1:1 juice to berry ratio. One lot was inoculated with *Saccharomyces cerevisiae* strain AWRI 796 (Redox, Adelaide) and the other with strain PDM (Lallemand, Australia) at 0.24 g/kg of must. The must splitting process of draining, combining skins and juice was then repeated to allow 36 g/L of glucose and fructose (1:1) and 200 mg/L of yeast assimilable nitrogen (YAN) as diammonium phosphate to be applied. Ferments were conducted in triplicate at either 18°C or 28°C in 400 mL containers with the cap being submerged. The ferments were monitored daily using gravimetry and upon reaching stable mass the free-run wine was drained off and pressings obtained by pushing the grape solids against a 1 mm mesh using hand force. The free run and pressings were kept at 28°C for 28 days before being stored at 4°C prior to analysis.

6.14 Impact of different clarification techniques on non-volatile composition and mouthfeel

Chardonnay and Frontignac grapes from the Murray Valley region were processed into juice by different commercial wineries using similar protocols. Pectolytic enzymes were added at crush and the juice drained off skins into a pre-flotation storage tank. Two 20 L juice samples of each treatment were collected for fermentation. These treatments (summarised in Table 1) were:
1) High solids (HS) taken from an upper racking valve of the storage tank to preclude gross solids
2) Low solids by settling (LS-SE) where HS juice was settled at 0°C before being racked off fine solids
3) Low solids by flotation (LS-FL) produced by dosing in-line with bentonite before floating off solids using nitrogen gas at a discharge rate of 30 kL/h.

The juice samples were chilled and adjusted to pH 3.4 by tartaric acid addition and to a free SO_2 of 10-25 mg/L. Juices were inoculated at the same time using *S. cerevisiae* strain EC1118 at 15-16°C. The resultant wines were racked and 60 mg/L of SO_2 was added; and they were then cold stabilised at 0°C using 4 g/L KHT seeding, pad and membrane filtered, adjusted to 35 mg/L free SO_2, and bottled in 375 mL units under screw cap.

Table 1. Summary of winemaking procedures and contact of grape solids with juice and fermenting wine

Treatment	High solids	Low solids-settled	Low solids -floated
Enzyme at crusher	Yes	Yes	Yes
Contact with gross lees	Yes	Yes	Yes
Bentonite addition to juice	No	No	Yes
N_2 addition to juice	No	No	Yes
Contact time with grape solids (from racking off gross lees to yeast inoculation)	3.5 days	3.5 days	<0.1 hrs
Fermentation on high grape solids	Yes	No	No



Figure 1. Grape solids being floated and skimmed off the Frontignac juice used in this study

The solids content of the high solids juices was measured as % wet weight, while the solids content of the floated and settled juices was determined by nephelometry. Total phenolic concentrations were determined using the Folin-Ciocalteu method. A targeted HPLC analysis of phenolic compounds representative of the major phenolic classes found in white wines was conducted by reverse phase C18 HPLC. Total wine polysaccharides were quantified by the phenol-sulfuric method, and the polysaccharide molecular weight distribution was determined by size exclusion chromatography (Gawel et al. 2016).

Nine assessors experienced in descriptive analysis rated the relevant taste and mouthfeel attributes (viscosity, astringency, hotness and bitterness) using an unstructured line scale. A 30 mL aliquot of wine was presented in triplicate in an order and timing determined to account for carry-over effects. The wines were tasted at 22–24°C in isolated booths under daytime lighting.

6.15 Influence of white juice settling time on phenolic and polysaccharide composition and wine mouthfeel

Batches of hand-harvested Sauvignon Blanc and Chardonnay grapes (0.5 t of each variety) were crushed, destemmed and given additions of 50 ppm SO_2 and pectolytic enzyme. The juices were drained, and the skins pressed into 500 L tanks. The tank contents were stirred while being distributed into 9 x 30 L stainless steel fermentation vessels and transferred into a 0°C room for cold settling. Three vessels were randomly selected to settle for 24 h before being racked and inoculated. In another six vessels, the grape solids were remixed with the juice by rolling for 3 min at 24 h, three of which were randomly selected 24 h later for racking and inoculation. The remaining three vessels were remixed in the same way and left to settle before being racked off grape lees at 72 h. Using this protocol, there were three fermentation replicates that had solids contact for 24 h, 36 h and 72 h. Following racking from grape lees, the clarified juices were inoculated with *S. cerevisiae* PDM strain (250 ppm) and fermented at 15°C until the musts reached 3 °Bé and thereafter at 20°C until dryness. The wines were then inoculated with *Oenococcus oeni* strain VP41 (10 ppm) and upon completion of MLF, were protein and cold stabilised using standard commercial practices. The wines were filtered and bottled in 375 mL bottles under screw cap and stored at 15°C. Samples were taken at the start of, and during, settling (t=0, 12 h, 24 h, 48 h and 72 h) as well as after 1, 2 and 4 days post-yeast inoculation, at dryness, post-MLF, and then again after filtration and bottling.

Polysaccharides from the wines were extracted, purified, and their molecular weight distribution obtained using size-exclusion chromatography (Gawel et al. 2016). The concentration and monosaccharide composition was determined (Kassara et al. 2019). Total phenolics were quantified using the Folin-Ciocalteu method.

Differences between wines produced using different juice settling times at 12 months post-bottling were determined using projective mapping (Risvik et al. 1994) with twelve tasters experienced in the method. An additional winemaking replicate from each settling time point was included for sensory replication purposes. Confirmatory tetrad difference tests using 30 tasters and a standard protocol was conducted 18 months post-bottling to determine if an overall difference in taste and texture existed between the wines settled for 24 h and those settled for 72 h.

6.16 Grape seed powder as a potential fining agent

Sauvignon Blanc and Semillon juices were fined for 1 h with grape seed powder (GSP) obtained from Chardonnay grape seeds at 7.5 g/L and 15 g/L. An untreated juice was used as a control. The GSP was removed by cold settling and the juices were fermented in 20 L batches in triplicate by *Saccharomyces cerevisiae* strain EC1118 at 18 ± 2°C until dry. The wines produced from juices untreated with grape seed powder were split into two batches with one batch treated with bentonite to achieve heat stability according to Bentotest®. The wines were filtered (0.45 µm), bottled under screw cap and stored for six weeks at 4 °C before undergoing chemical and sensory analysis. The composition and concentration of haze-forming proteins, protein instability, phenolic and polysaccharide composition, and the appearance, aroma and taste palate profile of the wines were determined (Romanini et al. 2020).

7. Results and discussion

7.1 Year 3 Deliverable. Quantitative data on the dynamics of phenolic and other non-volatile compound evolution and loss during the white and red winemaking process

7.1.1 Literature review

The literature review conducted (Gawel et al. 2018) identified the aspects of white wine composition that influence white wine mouthfeel and elucidated the possible mechanisms that underlie mouthfeel perception in white wine. Alcohol, pH and phenolics were found to influence most aspects of white wine mouthfeel, while polysaccharides were mostly involved in moderating negative mouthfeel attributes. The effect of glycerol on mouthfeel perception of white wine was negligible and cannot be sufficiently modulated by winemaking practices to effect a perceptible change in mouthfeel. The review also pointed to the key role of phenolics in influencing all major aspects of white wine mouthfeel, affecting white wine astringency, viscosity, hotness and bitterness. Areas of future research were identified, the most compelling of which were to develop models to quantify the additivity of bitter tasting compounds found in wine and to better understand the role that saliva plays in wine mouthfeel.

7.1.2 Impact of flotation and solids inclusion on the composition, taste and texture of white wine

7.1.2.1 Impact of flotation and solids inclusion on turbidity

A crucial step in white winemaking is the removal of grape solids from must prior to fermentation, as doing so is thought to enhance varietal expression. Traditionally, solids have been removed by cold settling and racking. More recently, a process called flotation has become a popular alternative to cold settling due to its efficiency (i.e. its high throughput and continuous nature). A study using Chardonnay and Frontignac under commercial conditions was conducted to compare the composition and sensory qualities of wines made from musts clarified using either traditional cold settling or flotation. Clarification by flotation yielded wines of very similar phenolic and polysaccharide composition, and similar textural qualities to wines made from juices clarified using the less efficient method of cold settling. The solids content of the low-solids treatments produced by settling and flotation was similar (Table 2) and the wine compositional parameters were within acceptable ranges (Gawel et al. 2022).

Table 2. Solids content of winemaking treatments (n=2)

	High solids (% wt)	Low solids-settled (NTU)	Low solids-flotation (NTU)
Chardonnay	3.75%	100	97
Frontignac	2.75%	95	90

7.1.2.2 Compositional outcomes of flotation and solids inclusion on wine non-volatiles

The floated Frontignac juice had significantly lower total phenolic concentration than the settled juice (Figure 2A). A similar but non-significant trend for phenolics was observed in the Chardonnay juices. The lower total phenolics in floated juices compared with settled juices was consistent with previous studies (Ferrarini et al. 1995, Sindou et al. 2008) and may be explained by the shorter contact time between grape lees particles and juice experienced during flotation compared with cold settling (Table 2). The total phenolic concentrations of the high-solids juices were not significantly different from the cold-settled juice for both varieties, which was expected as the grape solids were in contact with the juice for the same duration. However, these explanations are speculative as the phenolic content of white grape solids and their phenolic extractability into juice pre-fermentation has yet to be determined. Another possible explanation was that some phenolics may have been removed from the floated juices by the bentonite added to improve the efficacy of flotation. Lastly, it should be noted that the differences in total phenolic concentration between treatments was relatively small compared with the average concentration, suggesting that the majority of total phenolics was extracted into the juices prior to clarification (i.e. during crushing, draining and the settling and removal of coarse solids).

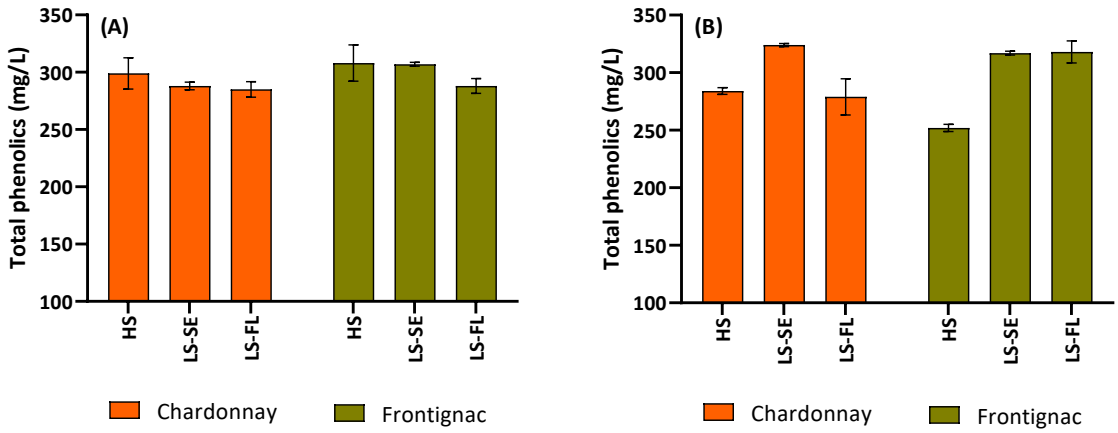


Figure 2. Total phenolics in (A) juice and (B) wine expressed in gallic acid equivalents. HS – high solids, LS-SE – low solids via settling, LS-FL – low solids via flotation. Error bars represent two standard errors.

The effect of juice clarification on total phenolic concentrations in the bottled wines varied between the varieties studied. The total phenolic concentration of the high-solids Frontignac wines was lower than those clarified by settling and flotation (Figure 2B), but the low-solids settled Chardonnay wines were higher in total phenolics than the high-solids and floated wines. Other researchers have also not observed a relationship between juice solids content and total wine phenolic concentration (Singleton et al. 1975). However, here, the significant decrease in total phenolics seen after fermentation on high solids suggests that the solids may have acted as a phenolic 'fining agent', possibly related to non-covalent interactions with polysaccharides, which were also present in higher concentrations in these wines (Figure 2).

Wines made from settled and floated low-solids juices showed similar phenolic profiles, different from those produced with high-solids juices (Figure 3). Specifically, the wines made from low-solids juices had significantly higher concentrations of caftaric acid, and generally lower concentrations of its derivative grape reaction product (GRP) than the high-solids wines. The ratio of caftaric acid to GRP can be influenced by SO_2, which may explain the differences between the two varieties, as the grapes were processed in different wineries with different SO_2 regimes.

The combined concentrations of the monomeric flavan-3-ols, catechin and epicatechin were significantly higher in the settled wines than in the floated and high-solids wines (Figure 3). Ferrarini et al. (1995) also found that cold settling resulted in higher catechin concentrations in white Picpoul wine compared to a flotation treatment. The tyrosol concentrations of the settled and floated wines were similar, but the effect of high solids was variety-dependent (Figure 3). Konitz et al. (2003) reported higher tyrosol concentrations in Riesling wines made from juice floated using air compared with those made from settled juices, a result which may be explained by a healthier yeast fermentation due to must oxygenation.

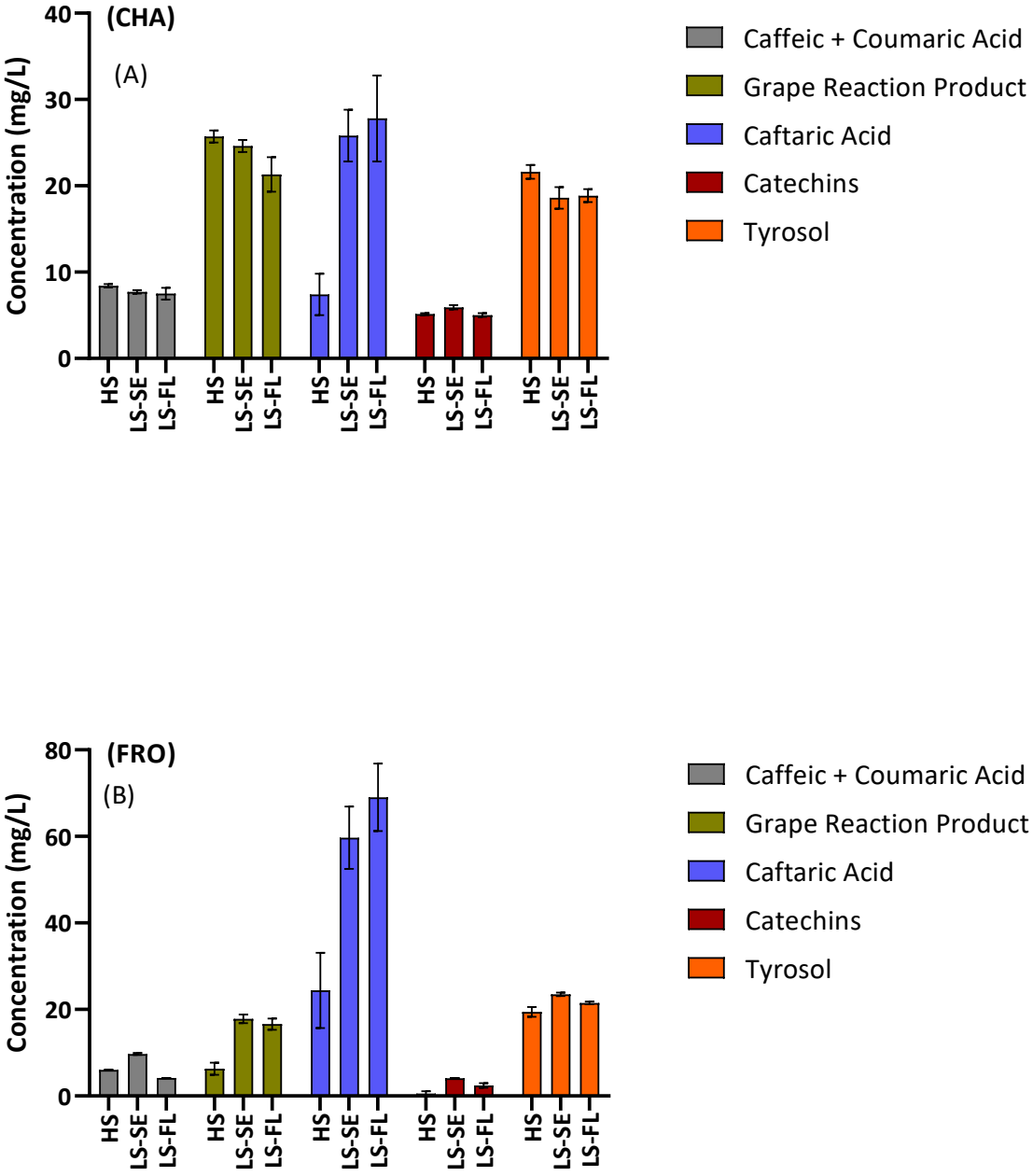


Figure 3. Mean concentration of phenolic compounds in wine A. (CHA) Chardonnay, B. (FRO) Frontignac. Error bars represent two standard errors.

High-solids juices produced wines with a higher total polysaccharide concentration (Figure 4) and a higher proportion of high molecular weight polysaccharides (Figure 5) compared with the wines produced from low solids juice using flotation. Previous work at the AWRI has shown that white wine polysaccharides with molecular weights greater than 93 kDa consist mostly of mannoproteins derived from yeast during fermentation and yeast lees contact. This result was consistent with the knowledge that yeast-assimilable sterols adsorbed on grape solids help improve cell viability and maximum populations of yeast cells, particularly at the end of fermentation, which could promote the production and release of mannoproteins by yeast autolysis.

Wines made from low-solids juices clarified by cold settling and by flotation did not differ in total polysaccharide concentration (Figure 4) or their molecular weight profile (Figure 5). This suggests that the longer clarification time involved in cold settling compared with flotation did not result in significantly greater extraction of polysaccharides from the grape solids prior to fermentation. More information on this study can be found in Gawel et al. (2022).

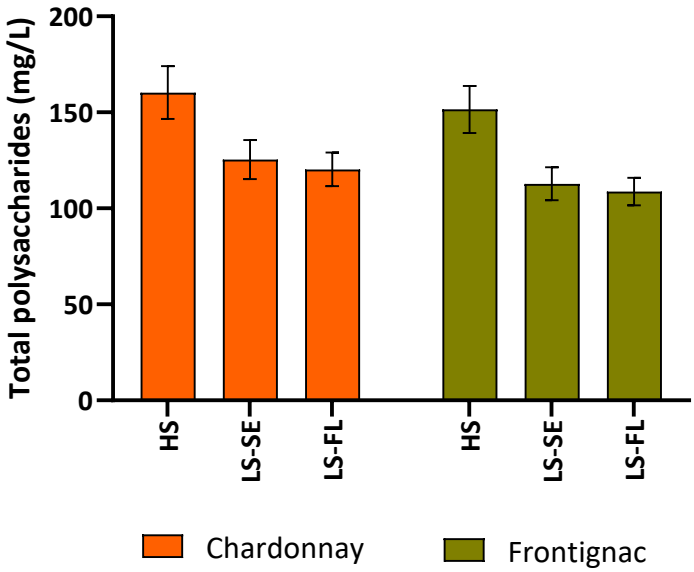


Figure 4. Concentration of total polysaccharides in wine as glucose equivalents. HS – high solids, LS-SE – low solids via settling, LS-FL – low solids via flotation. Error bars represent two standard errors.

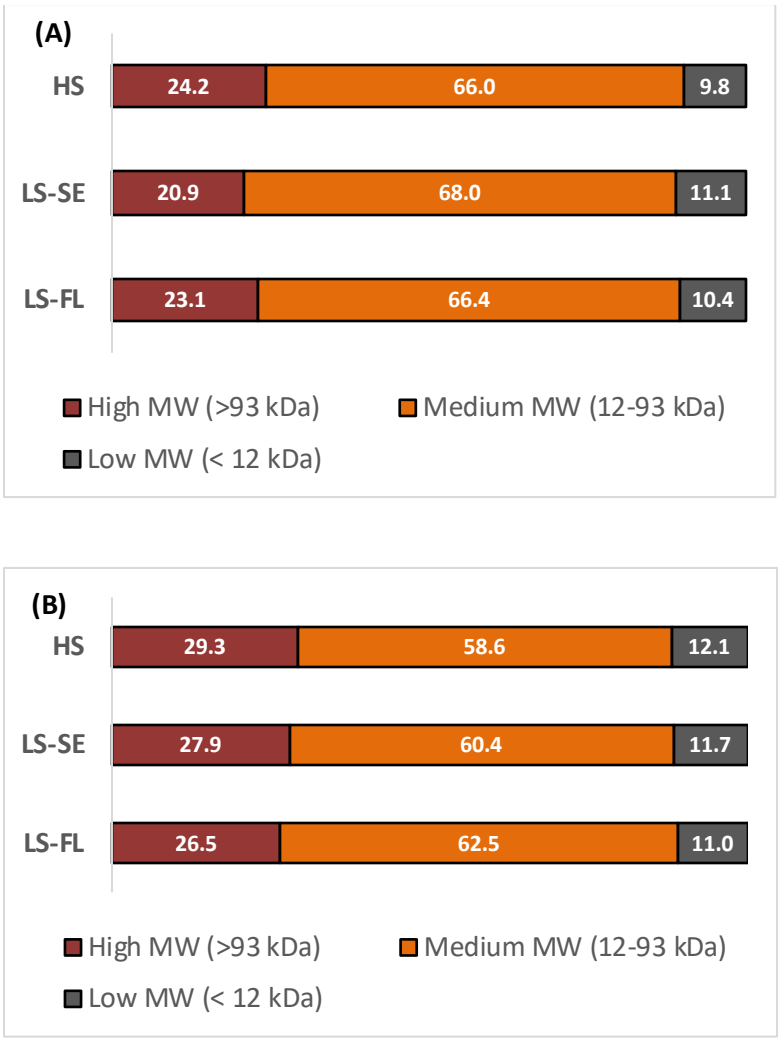


Figure 5. Polysaccharide molecular weight profile (A) Chardonnay, (B) Frontignac. Numbers inside bars represent percentages based on HPLC peak area. HS – high solids, LS-SE – low solids via settling, LS-FL – low solids via flotation

7.1.2.3 Sensory effects of clarification and relationship with composition

There was consistent evidence across both varieties that the wines made from floated juice were slightly more viscous, and in the case of Frontignac, the settled wines were significantly less bitter than the high-solids and floated wines ($P<0.1$). The hotness and astringency of the wines were not affected by the treatments applied.

Perceived viscosity was correlated with total phenolics (Gawel et al. 2013) and specifically with caftaric acid concentration ($P<0.05$) (Gawel et al. 2014) but a stronger positive correlation was observed with wine pH ($P<0.01$) as also reported by others (Runnebaum et. al. 2011). Higher total polysaccharide concentration was associated with a lower perceived viscosity ($P<0.1$) (Figure 6), a result which was contradictory to that of some other studies, where higher concentrations of neutral polysaccharides in white and red wine were found to correlate with increased viscosity (Vidal et al. 2004, Gawel et al. 2016).

The cold-settled Frontignac wines were less bitter than the high-solids and low-solids floated wines ($P=0.05$). The differences in bitterness could not be attributed to total wine phenolics (Figure 2B) nor to any of the quantified phenolic compounds (Figure 3) including catechin and epicatechin, which elicit bitterness, albeit at higher concentrations than found in these wines. This result, together with

the lack of influence of ethanol (which also elicits bitterness) (Figure 6) suggests that the compounds responsible for the greater bitterness in the settled wines were not captured in this study.

	Total Polysaccharides	High MW PS	Med MW PS	Low MW PS	Glycerol	Glucose+Fructose	Ethanol
Viscosity	-0.595	-0.143	0.062	-0.045	0.032	0.159	0.036
Acidity	0.135	-0.102	0.359	0.358	0.061	-0.080	-0.008
Hotness	0.445	0.049	-0.275	0.040	-0.029	-0.102	0.012
Astringency	-0.149	-0.294	-0.062	-0.072	-0.023	-0.011	0.003
Bitterness	0.023	-0.229	0.054	0.061	0.008	0.019	0.009

	Total phenolics	GRP	Caftaric	Catechins	Caffeic+Coumaric	Tyrosol
Viscosity	0.691	0.249	0.574	0.317	-0.094	-0.105
Acidity	0.007	-0.037	-0.161	-0.081	-0.222	-0.192
Hotness	-0.422	-0.150	-0.319	-0.079	0.111	-0.052
Astringency	0.137	0.052	0.079	0.130	-0.183	-0.157
Bitterness	0.063	0.009	-0.026	0.054	-0.242	-0.200

	pH	Total acidity	Tartaric acid	Malic acid	Lactic acid	Succinic acid
Viscosity	0.735	-0.130	-0.252	0.049	-0.118	0.013
Acidity	-0.079	-0.007	0.111	-0.200	-0.227	-0.015
Hotness	-0.371	0.082	0.181	-0.034	0.127	-0.032
Astringency	0.361	-0.124	-0.075	-0.036	-0.015	0.007
Bitterness	0.279	-0.139	0.001	-0.132	0.016	-0.005

Figure 6. Heat map showing the correlation (r) between mouthfeel and taste characters and analytical parameters. Green indicates positive correlation and red negative correlation. Density of colour represents strength of correlation. Correlations outlined with black boxes were statistically significant ($P<0.05$).

To summarise this study, the composition and sensory qualities of Chardonnay and Frontignac wines made from juices clarified using flotation were compared with wines made using the less efficient method of cold settling. The non-volatile composition including total phenolics, total polysaccharides, phenolic profile and polysaccharide molecular weight profile of the wines produced by flotation were similar to those made using cold-settled juices, which was reflected in the wines having similar mouthfeel and taste properties. Both the Chardonnay and Frontignac wines produced by flotation by two different wineries were perceived to be slightly more viscous than the wines made from either the settled or unclarified juice. Perceived viscosity was best correlated with higher pH and higher total phenolic content. More information on this study can be found in Gawel et al. (2022).

7.1.3 Influence of white juice settling time on total phenolic content and polysaccharide composition

The content and structure of polysaccharides are known to affect the efficiency of wine stabilisation processes and their sensory properties. The major white wine polysaccharides vary in molecular weight (MW) and monosaccharide composition and their source during the white winemaking process. They can be classified into three broad categories – mannoproteins (MPs), arabinogalactan proteins (AGPs) and rhamnogalacturonans (RG II). Mannoproteins arise from yeast cell walls either during fermentation or through contact with yeast lees following fermentation (Escot et al. 2001). They are characterised by a high mannose content relative to other sugars (Goncalves et al. 2002) and vary significantly in molecular weight, representing several populations based on their winemaking source (Escot et al. 2001). Arabinogalactan proteins are mostly comprised of arabinose and galactose residues and are localised within the grape intracellular spaces and therefore can be extracted from processing grapes into juice and during the early stages of fermentation (Guadalupe and Ayestaran 2007). Rhamnogalacturonan II is a low MW (<20 kDa) acidic polysaccharide that is released from pectins found in grape cell walls (Pellerin et al. 1996). They are characterised by a high proportion of rhamnose and galacturonic acid and their diagnostic sugar, fucose. During red wine processing, RGs are extracted during fermentation on skins. The source of RGs in white wine where

skin maceration during fermentation does not occur is less clear, but they may be released during the brief period between crushing and draining off skins, or when suspended grape cells are in contact with juice during cold settling. Wine mannoproteins are implicated in the prevention of protein haze (Waters et al. 1994), and, together with RG II, in the inhibition of potassium hydrogen tartrate crystallisation (Gerbaud et al. 1997), therefore potentially allowing for reduced use of bentonite fining and more efficient cold stabilisation practices. The different classes of polysaccharides have also been shown to differentially affect the mouthfeel properties of red and white wines including alcohol induced hotness (Gawel et al. 2014) and perceived viscosity (Vidal et al. 2004). However, all the winemaking studies referred to above involved red winemaking. The evolution of different species of polysaccharides and phenolics during the processing steps used to make white wine has been little understood.

The evolution of polysaccharides and total phenolic content from must to wine was determined for the first time in white wine using Sauvignon Blanc and Chardonnay (Figures 7 – 9). The influence of juice cold settling time (24, 48 and 72 h) on these non-volatile components and effects on wine mouthfeel were also studied. For data interpretation, the contribution of polysaccharide classes was inferred from the concentration of individual monosaccharides characteristic of reported classes of wine polysaccharides and MW ranges (Doco et al. 1999). Mannose was attributed to mannoproteins, arabinose and galactose to AGPs, and the uronic acids and the diagnostic sugar fucose to rhamnogalacturonans (Figure 5). The molecular weight distribution of polysaccharides indicated the presence of three major populations:

1) greater than 94 kDa (high MW)
2) 12-94 kDa (medium MW)
3) 5-20 kDa (small MW).

The high MW class was attributed to high MW MPs; the medium MW class to AGPs (because previous studies suggest the MW of AGPs in wine range from 48 to 262 kDa (Vidal et al. 2003)); and low MW polysaccharides were considered indicative of rhamnogalacturonan type II dimers (RG-II) (Pellerin et al. 1996), AGPs and low MW MPs (Ayestarán et al. 2004) (Figure 8).

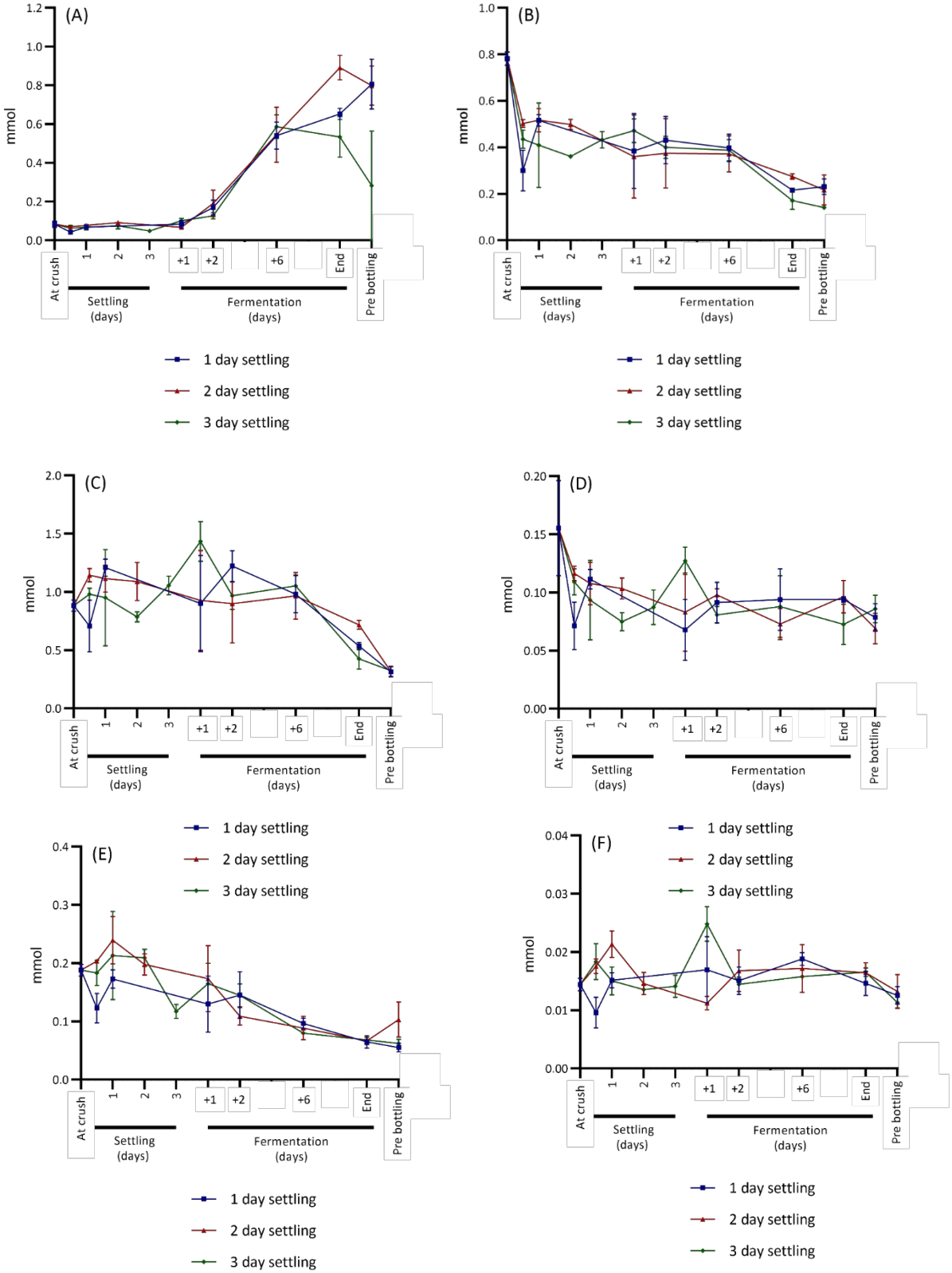


Figure 7. Monosaccharide composition of polysaccharides extracted at different stages of settling, fermentation and prior to bottling of a Sauvignon Blanc juice and wine. (A) mannose, (B) arabinose, (C) galactose, (D) glucuronic acid, (E) galacturonic acid, (F) fucose. (A) is indicative of mannoproteins; (B) and (C) are indicative of arabinogalactan proteins (AGPs); (D) and (E) are uronic acids; and (F) is the diagnostic sugar of rhamnogalacturonic acids.

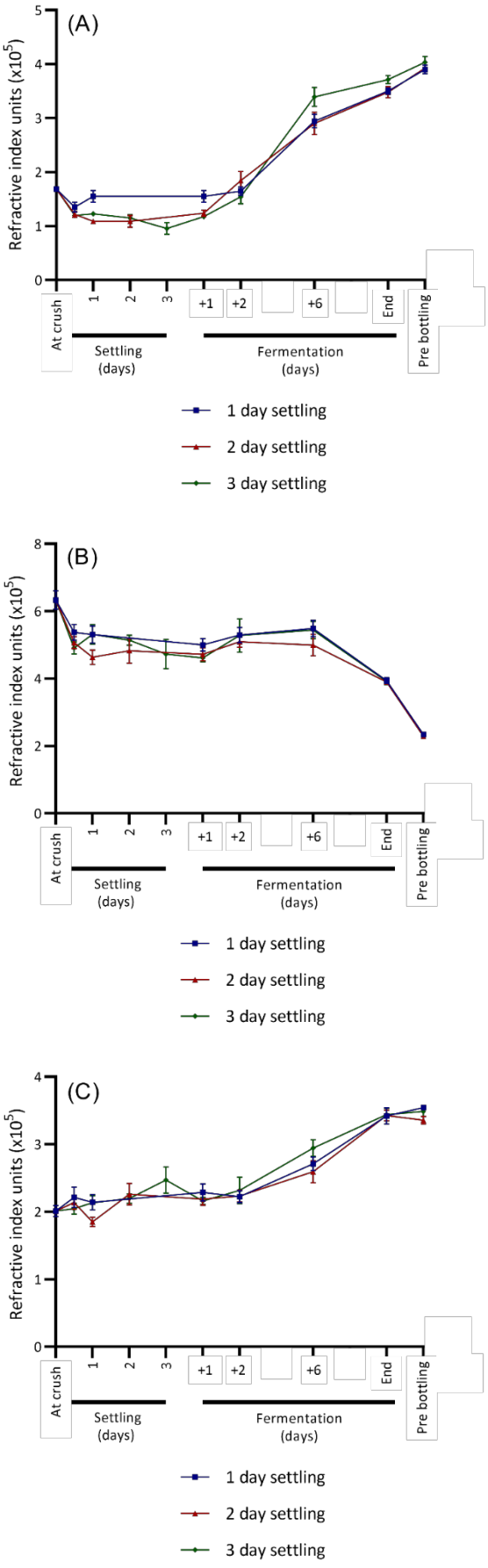


Figure 8. Molecular weight distribution of polysaccharides of a Sauvignon Blanc juice and wine at different stages of settling, during fermentation and prior to bottling (A) High MW (>94 kDa), (B) Medium MW (12-94 kDa), (C) Low MW (<12 kDa)

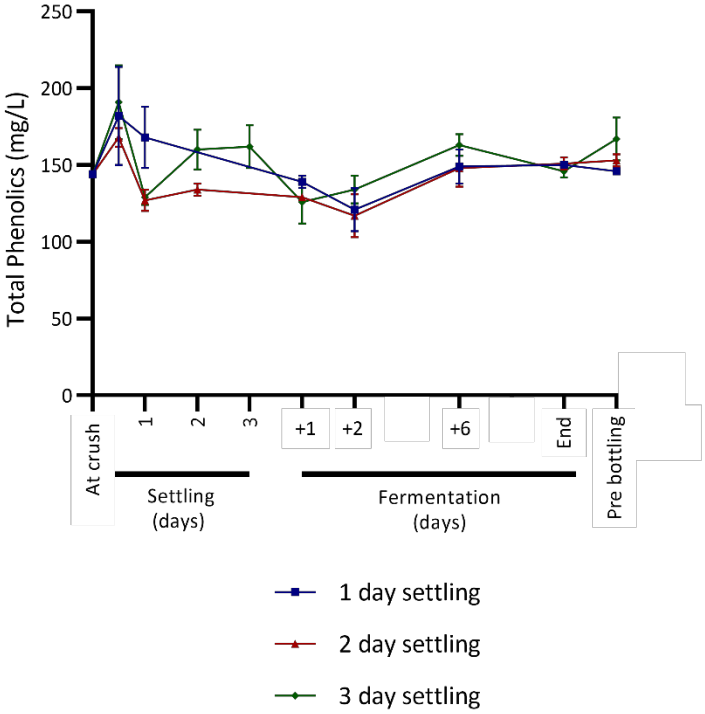


Figure 9. Total phenolics of a Sauvignon Blanc juice and wine at different stages of settling, fermentation and prior to bottling

Mannose residues and high MW polysaccharides increased significantly during fermentation, which suggested that the high MW mannoproteins which assist in protein and cold stabilisation were extracted from yeast cell walls during fermentation. Arabinose and galactose were the most abundant residues at all the white winemaking stages, which was consistent with the findings of Vidal et al (2000). The combined concentrations of the residues indicative of the medium MW polysaccharide AGP were greatest in the juice at crushing, and declined significantly during the early stages of juice settling and then again after the early stages of fermentation. The mechanism driving the loss of AGPs is unknown but the results suggest that during fermentation the precipitation of AGPs exceeded their solubilisation. Nevertheless, AGPs have been shown to contribute positively to white wine texture by reducing alcohol-induced hotness and by increasing perceived viscosity (Gawel et al. 2014) so further investigations into the mechanism behind the loss of AGPs are warranted. The concentration of low MW polysaccharides thought to be extracted from skin fragments increased steadily throughout the winemaking process. However the concentrations of uronic acids decreased, and fucose concentrations remained steady suggesting that low MW mannoproteins were likely present in the low MW polysaccharide fraction.

Total phenolics increased in the Sauvignon Blanc juice after 12 hours of settling, suggesting that phenolics were rapidly being extracted from the suspended grape solids. After one day of fermentation the total phenolic content of the settling treatments stabilised and then increased slightly throughout fermentation. However, at the completion of fermentation the total phenolic content of wines produced using the three settling times did not differ (Figure 9).

Earlier work at the AWRI (Gawel et al. 2015) suggested that the amount of time taken to settle grape solids could affect the polysaccharide content of white wine. The work presented here suggests that this is not the case. Differences in settling time from 24 to 72 h did not affect the polysaccharide composition or phenolic content of the finished wines (Figures 7 - 9) which was also reflected in the results of the sensory projective mapping (Supplementary Figure 19), and the lack of evidence of any overall taste and mouthfeel differences between wines made from juices that had been settled for 24 hours and 72 hours (Supplementary Table 6).

7.1.4 The effect of dissolved CO_2 on wine texture and taste

Red wines that contain insufficient dissolved carbon dioxide (DCO_2) taste 'flat', but excess DCO_2 can elicit a 'spritzy' textural component that is not consistent with consumer expectations of dry red wines. The impact of DCO_2 on red wine taste and texture in the presence of increased red wine tannin and ethanol were determined.

7.1.4.1 The effect of dissolved CO_2 on the taste and mouthfeel of red wines

Higher DCO_2 concentration resulted in reduced bitterness and increased sweetness (Figure 10). The increase in perceived sweetness is consistent with the reduction in bitterness as the two tastes mutually suppress each other, but the cause of the effect of DCO_2 requires further investigation. Higher DCO_2 concentrations in red wines also reduced astringency, while not affecting viscosity, hotness or overall flavour. The finding that increasing DCO_2 resulted in decreased astringency perception was at odds with the views of a significant majority of winemakers attending the workshop on 'Dissolved gas management' at the 17th Australian Wine Industry Technical Conference who tasted two red wines adjusted to different DCO_2 levels using membrane transfer. The reasons for this were not clear, but may stem from the different approaches used to modify DCO_2, which may have resulted in different wine pH and therefore astringency perception. Dissolved CO_2 did not consistently interact with the hotness arising from ethanol or astringency from tannin, implying that the intensity of the spritz sensation elicited by DCO_2 had a compelling effect on the taste and texture of red wine. More information on this study can be found in Gawel et al. (2020).

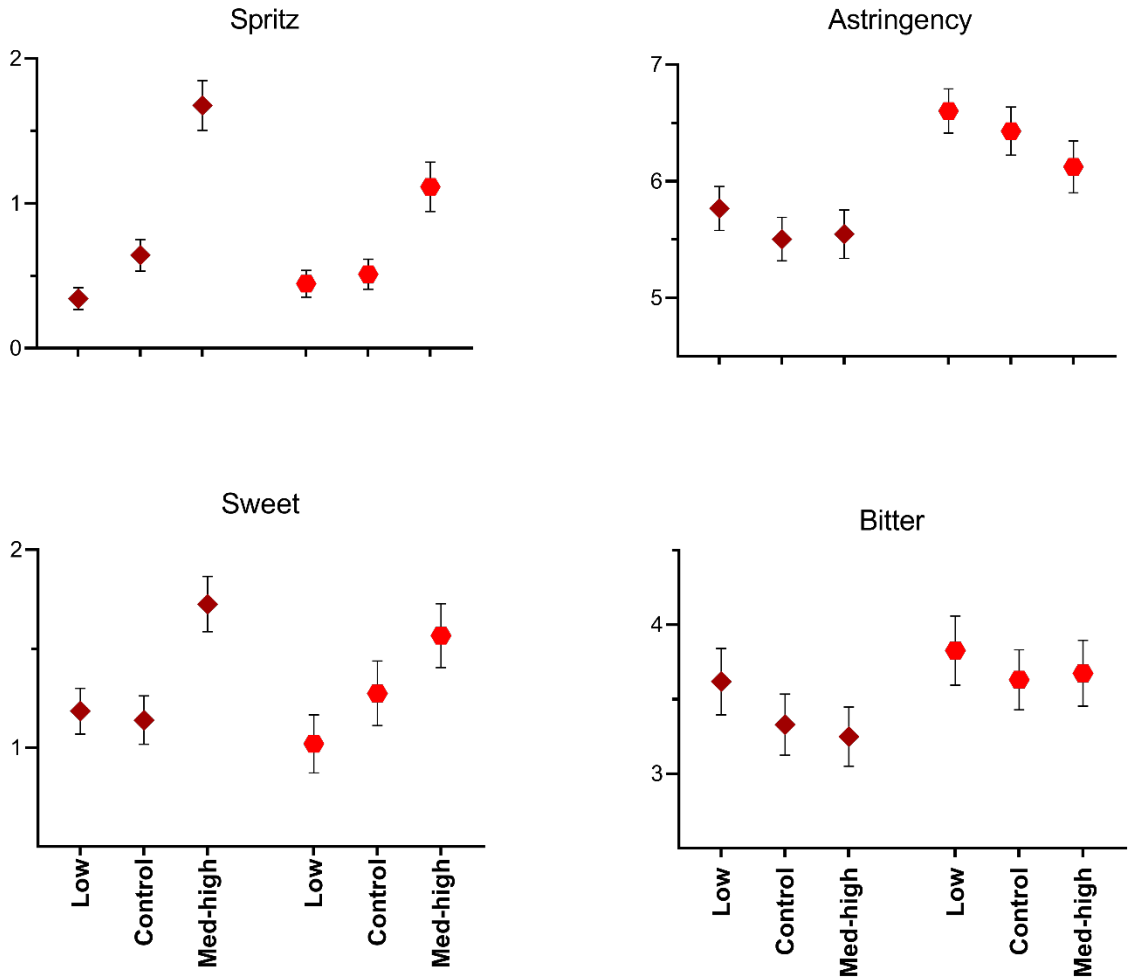


Figure 10. Effect of dissolved CO_2 on perception of tastes and textures in Shiraz (dark red diamonds) and Cabernet Sauvignon (light red hexagons) wines. Low=0.1 g/L, Control=0.5 g/L, Med-high=0.8 g/L (Shiraz) and 0.9 g/L (Cabernet Sauvignon).

7.1.4.2 The effect of wine composition on CO_2 losses from a wine glass during consumer tasting

When wine is consumed, the amount of DCO_2 in the glass is lower than that originating in the bottle, due to the losses that occur due to agitation during pouring, repeated tasting and when wine is standing in the glass before consumption. To investigate whether DCO_2 loss was likely to influence the taste and texture of the wine, a system was developed to directly measure DCO_2 in a wine glass (described in Gawel et al. 2020), allowing the determination of the DCO_2 concentration likely to be experienced by wine consumers. A study quantified the losses of DCO_2 in a white wine during pouring and standing, and determined which compositional factors influenced the rate of loss (Schulkin and Gawel 2021).

Pouring from bottle to the glass reduced bottled DCO_2 concentration by an average of 0.1 g/L (or 10%) (Figure 11). DCO_2 concentration further reduced linearly with losses of between 0.10 and 0.15 g/L over the 15 minutes where the wine was standing in the glass and was being subjected to simulated tasting. Of the compositional factors investigated, only ethanol concentration significantly ($P<0.05$) hastened DCO_2 loss from the glass compared with the control wine (Figure 12).

While sensory work was not conducted on these wines, Le Calve et al. (2010) reported a strong CO_2 related sensory response to changes in DCO_2 concentration within the range of concentrations used in this study, and Gawel et al (2020) found perceptible differences in spritz intensity in white wines in a similar concentration range, suggesting that DCO_2 losses due to pouring and consumption under these conditions are likely to be perceptible. Further work is required to determine the spritz difference threshold of DCO_2 to clarify the sensory impact of the reduction of DCO_2 that occurs during pouring and consumption.

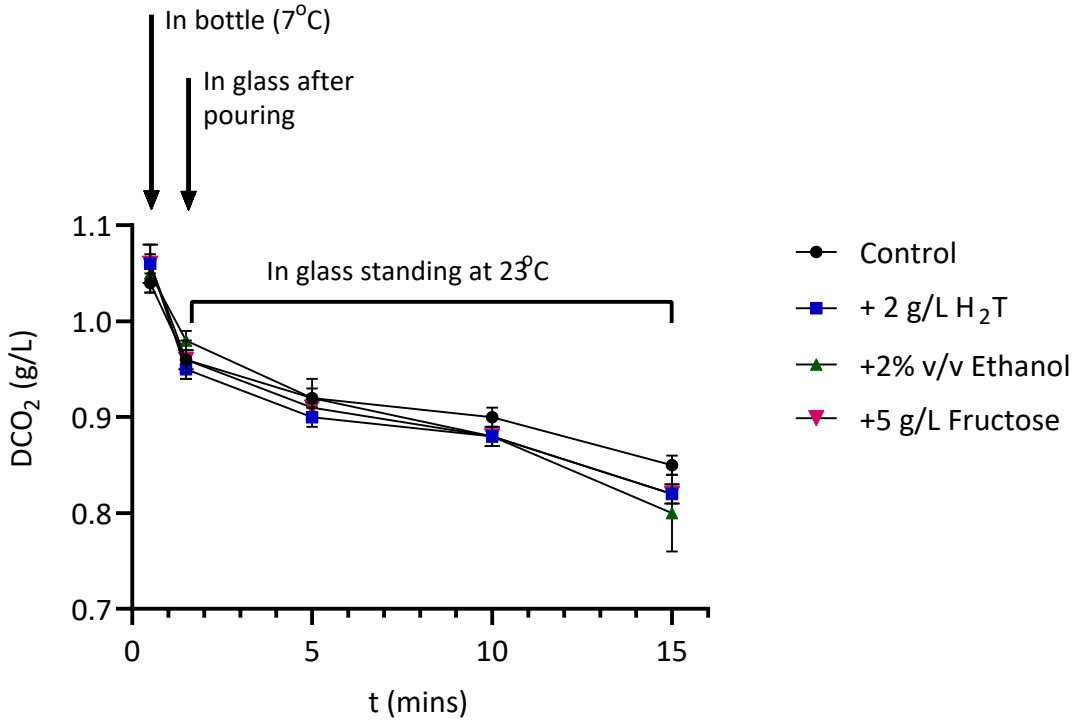


Figure 11. Dissolved CO_2 concentration of white wine in the wine glass during simulated consumption showing the effect of the wine matrix (acidity, ethanol and residual sugar). Error bars are 2 standard errors.

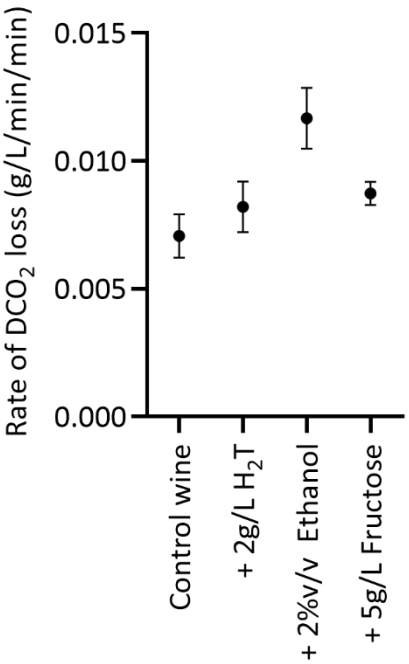


Figure 12. Average rate of DCO_2 loss from a white wine in a wine glass following pouring and during simulated consumption showing the effect of the wine matrix (acidity, ethanol and residual sugar). Error bars are 2 standard errors.

7.1.4.3 Carbon dioxide ingress through the salivary layer using a model mouth system

This basic research explored the effect of salivary layer thickness on DCO_2 perception as it relates to different wine styles. A salivary thickness of 2.1 mm was used as a resting mouth based on the mouth surface area and volume of expectorated saliva (Collins and Dawes 1987). The level of DCO_2 was chosen to represent different wine types with 0.375 g/L to 0.75 g/L DCO_2 representative of red wine and 1.5 g/L DCO_2 representative of still white wine (Gawel et al. 2020). While to our knowledge, the effect of repeat tasting of wine on salivary thickness has not been quantified, it was reasonable to expect that salivary layer thickness would decrease upon repeat tasting. The results of this investigation indicated that saliva might limit CO_2 transport to the mouth surface, probably due to its bicarbonate-buffering capacity. The data suggested that wines with high DCO_2 concentrations should become more 'spritzy' and their taste and textures affected more upon each repeat tasting of the wine, compared to wines with very low concentrations of DCO_2 where spritz, tastes and textures would be little affected (Figure 13). However, this hypothesis is tentative and requires sensory testing.

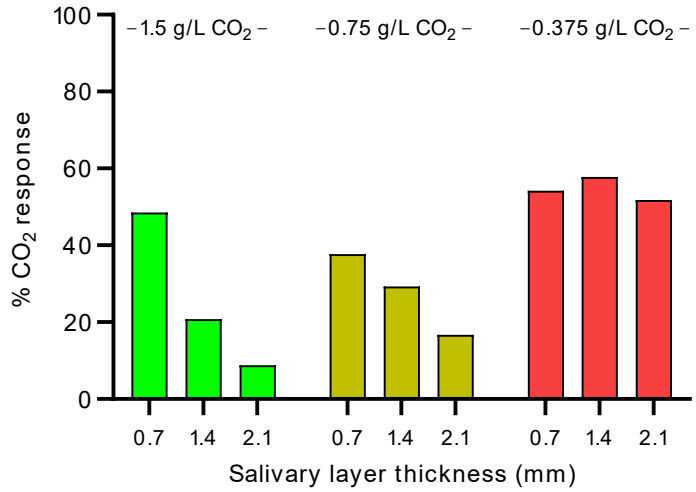


Figure 13. Percentage of CO_2 potentially reaching the mouth surface as a function of salivary layer thickness and dissolved CO_2 concentration. (mean of saliva donated by two individuals).

7.2 Year 3 Deliverable. Knowledge regarding compounds or classes of compounds leading to negative characteristics.

7.2.1 Tryptophol sulfonate evolution during storage

A previous AWRI study established that tryptophol sulfonate (TSO_3) is a component of bitter Australian white wines. A method was developed to synthesise TSO_3 using food grade reagents on a scale and purity necessary for use in both sensory evaluation and as an analytical standard. Sufficient TSO_3 was obtained to establish its textural properties and sensory threshold in model white wine. A fast, straightforward HPLC method was also developed to quantify TSO_3 and its precursor tryptophol, which was applied to establish the typical concentrations ranges of these compounds in Australian wines.

Tryptophol sulfonate and tryptophol were tracked in three white varieties from must, through ferment, and during post-bottling maturation. Very little TSO_3 was formed during fermentation, but

its concentration increased considerably following SO_2 addition and bottling. Winemakers are able to control final wine pH and SO_2 prior to bottling, so an experiment was designed to investigate the influence of these parameters on the evolution of TSO_3 during bottle maturation. A higher SO_2 concentration at bottling resulted in increased TSO_3, but the kinetics of its evolution were unaffected by wine pH.

The concentrations of tryptophol at the completion of fermentation using the high tryptophol-producing *S. cerevisiae* strain AWRI 2940 ranged from 21 to 87 mg/L. The formation of TSO_3 in these wines adjusted with potassium metabisulfite and/or tartaric acid following fermentation when stored and bottled under anaerobic conditions is shown in Figure 14. Consistent with the findings of others (Alvarez-Fernandez et al. 2020, Cordente et al. 2021) the wines at the completion of alcoholic fermentation contained small amounts of TSO_3 (<3 mg/L) relative to concentrations post-storage.

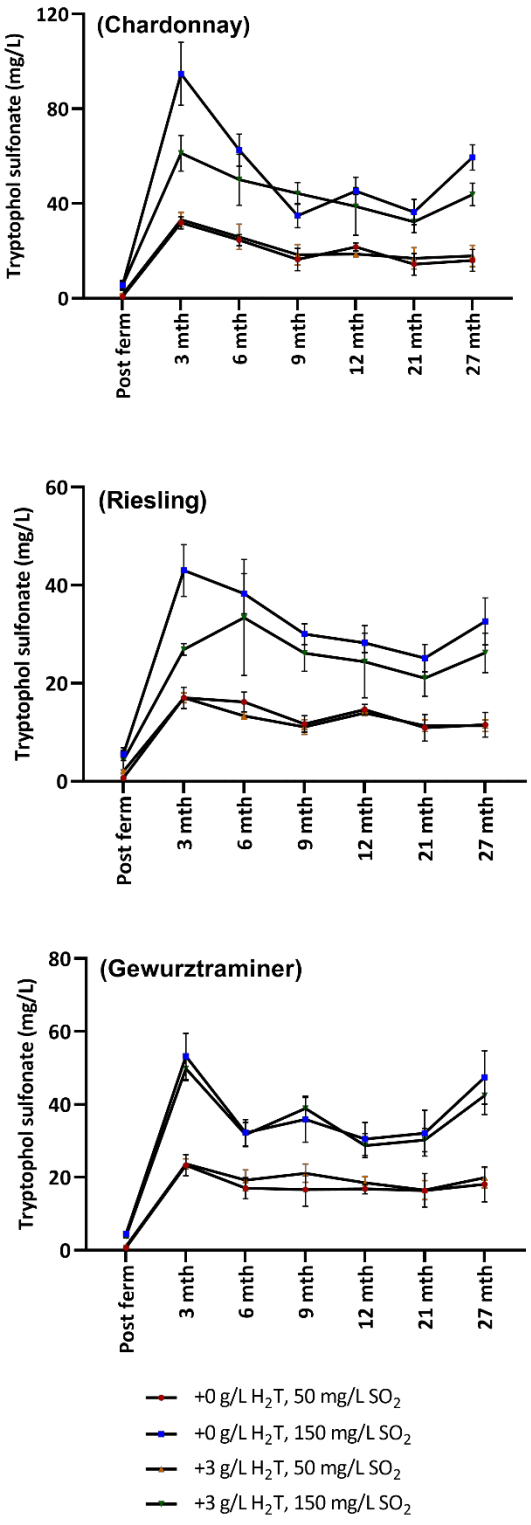


Figure 14. Concentration of tryptophol sulfonate in (A) Chardonnay, (B) Riesling and (C) Gewurztraminer wines from post-fermentation to 24 months storage, with potassium metabisulfite (50 mg/L and 150 mg/L) and tartaric acid (0 g/L and 3 g/L) added prior to storage.

The concentration of TSO_3 generally reached a maximum after three to six months of storage post-bottling for all varieties and decreased thereafter, possibly due to acid hydrolysis. These observations were consistent with a survey conducted by Arapitsas et al. (2018), which showed that young wines had significantly higher concentrations of $TS0_3$ than older wines, and also Cordente et al. (2021) who observed a maximum concentration of TSO_3 in a Chardonnay wine at three months post-bottling.

Wines treated with 150 mg/L potassium metabisulfite prior to bottling and storage had significantly higher TSO_3 concentrations than those receiving 50 mg/L of potassium metabisulfite, for all three varieties ($p<0.001$) (Figure 14). The molar concentration of added HSO_3^- was greater than that of the tryptophol concentrations at the post-fermentation stage for all three wines, and for both rates of bisulfite addition, suggesting that tryptophol was the limiting factor in TSO_3 production. A possible explanation could be that when less HSO_3^- was available, it was more efficiently used to form adducts with acetaldehyde and caftaric acid (Hayasaka et al. 2017). The lower acid wines were higher in TSO_3 at three months post-storage, which can be explained by increased availability of bisulfite, as this form of SO_2 is favoured over molecular SO_2 at higher pH.

The red wines contained very low TSO_3 concentrations at the end of fermentation, but in contrast to the white wines, TSO_3 concentrations did not increase above the levels observed post-fermentation, as the storage period progressed. This may have been the result of a lower availability of bisulfite for TSO_3 production, as bisulfite has a propensity to bind with acetaldehyde, anthocyanins, flavanols and other indolic compounds (Arapitsas et al. 2018).

7.2.2 Bitter intensity of white wines produced under different storage conditions

The individual taste thresholds of TSO_3 in a white model wine were 22 mg/L (n=3), 45 mg/L (n=1), 91 mg/L (n=12) and 181 mg/L and greater (n=20) suggesting a wide distribution of individual taste thresholds, albeit above the maximum concentrations found in the survey of Australian wines (2.6 mg/L red wines (n=66), 8.1 mg/L white wines (n=75)) (Figure 15).

There were no significant differences in the bitterness intensity of the Riesling and Chardonnay wines at 21 months despite significant differences in TSO_3 concentration (Figure 14). The lack of a perceived difference in bitterness intensity was consistent with the detection threshold results, as the greatest difference in TSO_3 concentration between these wines (22 mg/L) was well below the detection threshold of the most sensitive tasters (32 mg/L). The bitterness intensity of the Gewurztraminer wines at 21 months post-bottling was affected by acid and bisulfite addition, even though, like the other white varieties, the greatest difference in TSO_3 concentration was also less than the detection threshold of the more sensitive tasters. This may relate to this pink-skinned variety typically having higher concentrations of flavonol glycosides and hydroxycinnamic acids than white varieties (Samoticha et al. 2018, Liang et al. 2012) the most abundant of which is caftaric acid, which forms bisulfite adducts (Hayasaka et al. 2017). Overall, TSO_3 concentration was negatively correlated with bitterness, and positively correlated with tryptophol concentration ($p<0.05$). The reason for this was unclear, as the detection threshold of TOL is between 10 and 414 mg/L (Van Gemert 2011) with the lowest estimate (10 mg/L) being greater than the difference in TOL concentration in the experimental wines. Further studies to determine the likely additive effects (Keast et al. 2003) of sub-threshold concentrations of phenolic compounds, sulfonated indoles and their glycosides on wine bitterness are required.

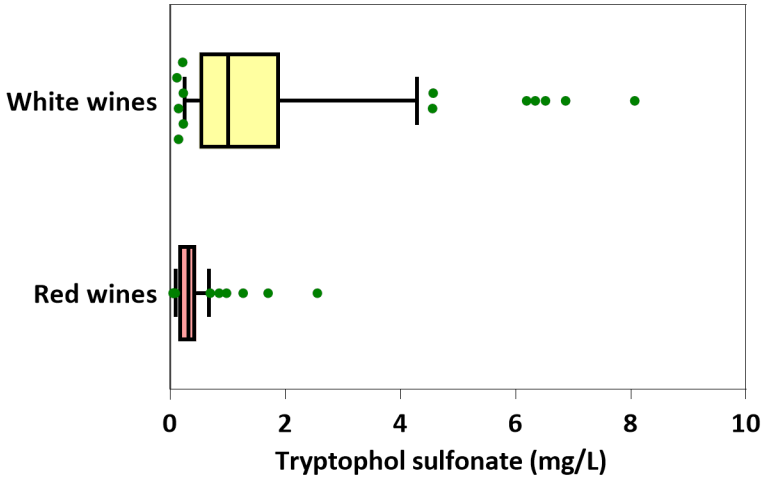


Figure 15. Distribution of TSO_3 concentrations in Australian white (n=75) and red (n=67) commercial wines. Boxes represent the 25th to the 75th quartiles and the whiskers 10% and 90% deciles.

Neither the proteinaceous fining agent casein (used to remove bitter-tasting phenolics) nor bentonite (used to protein stabilise white wine) were effective in removing TSO_3 or tryptophol from model wines (data not shown). Therefore, other possible mitigating influences were considered. The binding isotherms of isothermal calorimetry titrations of TSO_3 into a solution of whole polysaccharides extracted from white wine at pH 4.0 indicated that only a weak binding between them occurred (data not shown), suggesting that polysaccharides in wine cannot be used to mitigate the potential bitterness arising from TSO_3.

7.2.3 Grape seed powder as a potential fining agent

Powdered grape seeds were trialled as a sustainable, grape-derived alternative to bentonite in producing protein-stable white wines. Grape seed powder (GSP) added to Sauvignon Blanc and Semillon juices during fermentation improved the heat stability of the wines, even though high levels of residual protein remained, suggesting that GSP imparts a protective factor. Wines fined using GSP displayed some undesirable sensory traits compared with bentonite-fined wines, being more yellow in appearance and more bitter and astringent, all of which could be attributed to the higher phenolics imparted by the powdered grape seeds. This sensory outcome together with the high concentrations of grape seed powder required to achieve stability (15x more than a typical bentonite addition) suggested that while potentially effective, grape seed powder is not a commercially viable alternative to bentonite. More information on this study can be found in Romanini et al. (2021).

7.3 Year 5 Deliverable. Knowledge regarding compounds or classes of compounds leading to positive taste and textural characteristics

7.3.1 Concentrations of glutamic acid, succinic acid, sodium and potassium cations in Australian wine and their potential to contribute to savoury character in wine.

A review of the literature of compounds that are known to elicit savoury taste in foods and those that are found in wine indicated that glutamic acid is a possible driver of 'savoury' character in wine. The literature also suggested that monovalent cations and the fermentation-derived succinic acid act as savoury taste enhancers. Knowing this, a preliminary tasting by winemakers of mixtures of glutamic acid, succinic acid and sodium chloride in model wine suggested that glutamic acid was needed to elicit a savoury taste, but mixtures containing succinic acid and sodium chloride were

considered more savoury than glutamic acid alone.

More recently, a defined role for glutamic acid in red wine taste was described within the AWRI's flavour project, whereby glutamic acid added at wine-like concentrations (18 to 100 mg/L) to a Shiraz wine containing a baseline concentration of 17 mg/L strongly imparted savoury (umami) taste. Interestingly, it was found that a small augmentation effect in umami taste was imparted by the amino acid proline at moderate concentrations. Although the effect of L-glutamic acid in wine was deemed to be moderate within the concentration range used, it was nonetheless a significant effect. This indicated that a greater understanding of the range of L-glutamic acid in finished red and white wine was required, in addition to other potentially umami (or savoury) wine components.

Subsequently, a large-scale survey of Australian wines was conducted to determine the concentration distribution and range of glutamic and succinic acid, cations and sodium chloride in Australian wine (Table 3, Figure 16, Figure 17).

Table 3. Concentrations (mg/L) of compounds and cations in Australian wines reported to contribute to umami/savoury character

	n	Red wine (mg/L)	n	White wine (mg/L)	P
Glutamic acid	248	31.7 [a]	178	41.2 [b]	<0.0001
Succinic acid	248	603 [b]	154	381 [a]	<0.0001
Na+	187	50.2 [b]	147	33.7 [a]	<0.0001
K+	187	902 [b]	147	440 [a]	<0.0001
NaCl	130	274 [b]	70	151 [a]	<0.0001

Consistent with an earlier survey of succinic acid concentrations in Australian wines (Wilkes 2016), the succinic acid concentrations in the red wines surveyed were significantly higher than those in the white wines (Table 3), but within each wine type there were no significant varietal effects (Figure 14B). The lack of difference among varieties was expected, as succinic acid concentration depends on both must composition and fermentation conditions, including pH (Shimazu and Watanabe 1981), amino acid and vitamins (Castellari et al. 1995, Aragon et al. 1998), yeast strain and fermentation temperature.

Sodium and potassium ion concentrations also showed considerable variation (Figure 17). The variability in wine sodium concentration results from uptake by the vine from the soil, which depends on soil conditions, irrigation practices and rootstock selection (Walker et al. 2004), the use of processing aids such as sodium bentonite and the sodium salt of carboxymethyl cellulose, and from adding skin pressings, which contain higher concentrations of sodium ions (Walker et al. 2010). Potassium in must is affected by viticultural practices that influence vine canopy shading, and by the addition of potassium metabisulfite to achieve wine oxidative and microbiological stability. Potassium concentration in wine is also significantly influenced by practices used to limit the formation and post-bottling precipitation of potassium bitartrate. The removal of potassium bitartrate is pursued more aggressively in white winemaking, which may explain the lower concentrations of potassium in white wines compared with the red wines (Figures 16 and 17).

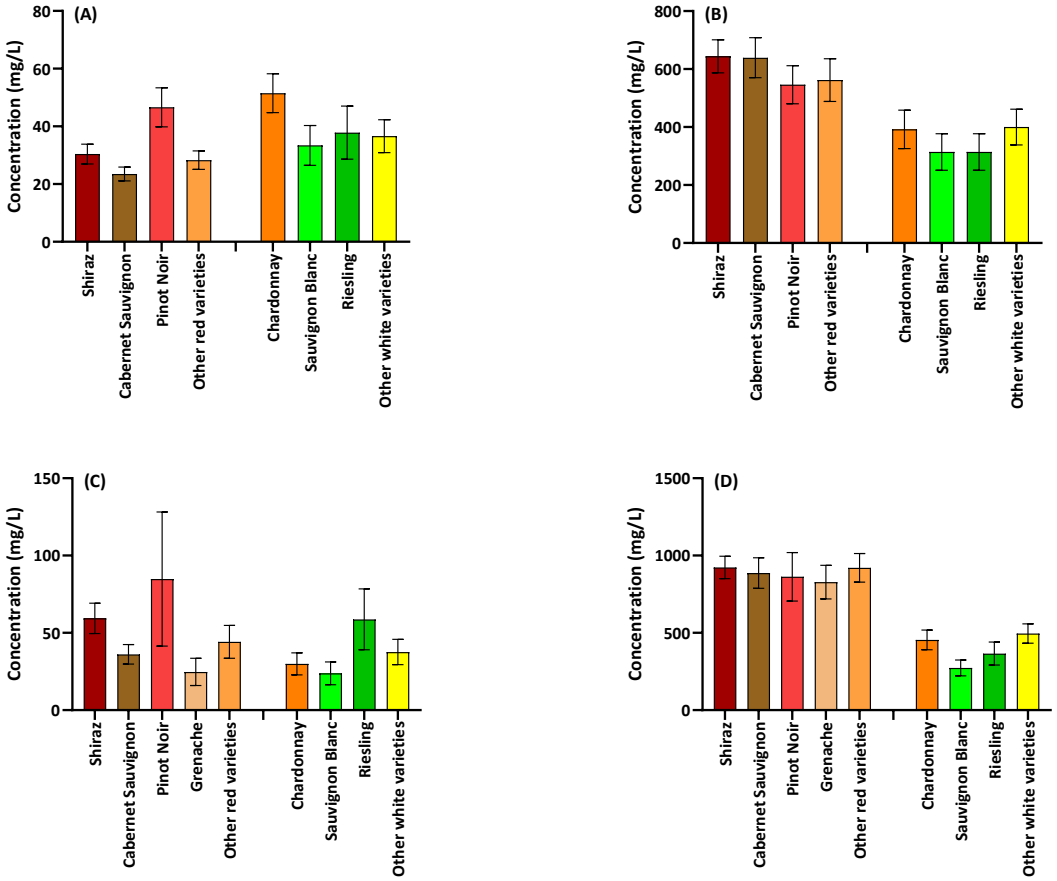
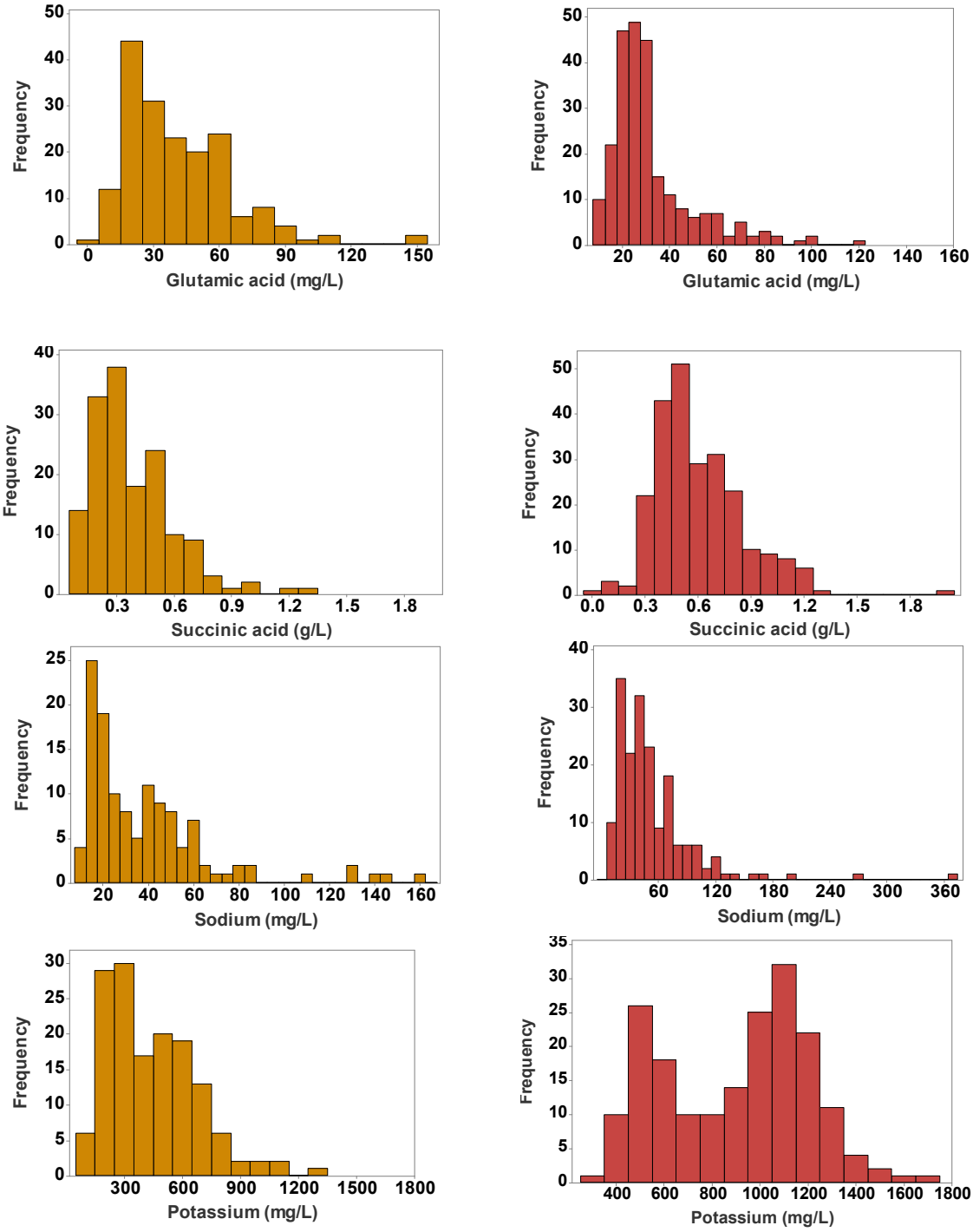


Figure 16. Mean concentrations of (A) glutamic acid, (B) succinic acid, (C) Na^+ and (D) K^+ in surveyed wines. Varieties given when n>15. Error bars represent 2 standard errors.

The white wines contained a significantly higher mean concentration of glutamic acid than the red wines. (Table 3, Figure 16). Others have reported an average glutamic acid concentration in Chardonnay wines of 58 mg/L, which is consistent with the findings reported here (Figure 16A). Here, the Chardonnay wines had significantly higher glutamic acid concentrations than Sauvignon Blanc and Riesling, and other white varieties (Figure 16A). This is possibly due to the greater use of the post-fermentation practices of lees contact and the greater use of malolactic fermentation in Chardonnay wines compared to the other varieties (practices that have been shown to increase glutamic acid concentration (Alcaide-Hidalgo et al. 2008, Prokes et al. 2022)). The Pinot Noir wines had significantly higher mean glutamic acid concentrations than the other red varieties (Figure 16A). This may be the result of the common practice in Pinot Noir winemaking of fermenting on stems, as glutamic acid is the predominant amino acid found in the stem rachis (Gourieroux et al. 2016), or because the surveyed Pinot Noir wines were all grown in cool climate regions (data not shown), a factor known to promote glutamic acid production in grapes (Petropoulos et al 2018).

Sodium chloride has detection thresholds in red and white wine of 0.30 and 0.31 g/L, respectively and recognition thresholds of 2.05 and 1.77 g/L in red and white wine, respectively (de Loryn et al. 2014). This suggests that while none of the surveyed wines would specifically be recognised as being salty, in 35.8% of the red wines and 51.9% of the white wines, sodium chloride could have been detected. The distribution of individuals' detection thresholds for glutamic acid (as a sodium salt) in water has a bimodal distribution with modes at 10.3 and 47 mg/L (Lugaz et al. 2002). Applying these reported glutamic acid thresholds to the distribution of glutamic acid concentrations shown in Figure 17 needs to be done with caution, as glutamic acid thresholds in wine will be higher than in water since sugars, acids and bitter compounds suppress perception of glutamic acid (Kemp and

Beauchamp 1994, Miranda et al. 2021). However, given that 38% of the red wines and 58% of the white wines surveyed contained greater than three times the lower modal threshold of glutamic acid, it is probable that savouriness would be perceived. The taste threshold of succinic acid in water adjusted to a red wine-like pH was found to be 0.11 g/L (Hufnagel and Hoffman 2008), and the umami taste threshold of succinic acid in fermented kimchi was recently reported as 0.083 g/L (Christa et al. 2022). Both threshold values are at the lower range of the succinic acid concentrations in the surveyed wines, suggesting that this contributor to savoury character could possibly be perceived in all but 10 of the 396 surveyed wines. Further work is required to determine the threshold of glutamic acid in wine and quantify the interactions between glutamic acid, succinic acid and cations in determining their potential to elicit savoury character in wine.



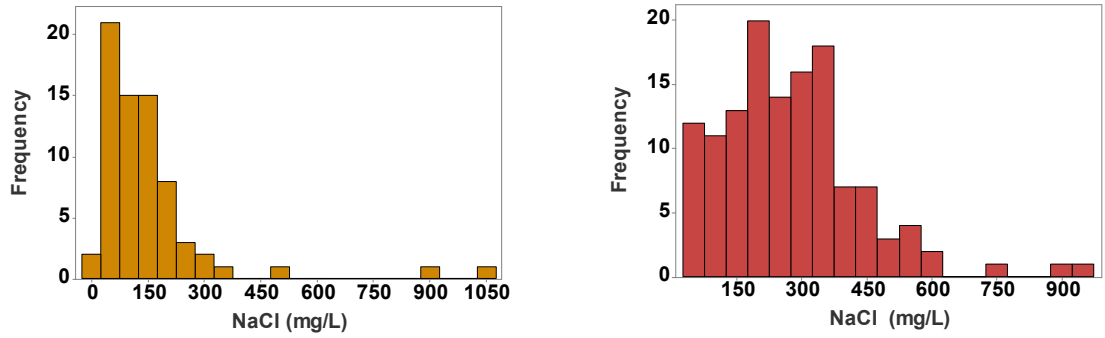


Figure 17. Distribution of glutamic acid, succinic acid, Na^+, K^+ and NaCl concentrations in a survey of red and white wines. Sample sizes are given in Table 3.

7.3.2 Impact of primary fermentation factors on glutamic acid and succinic acid in wine

A red winemaking study was conducted to scope out the possible influences of primary fermentation factors on glutamic and succinic acid concentrations. Increased assimilable nitrogen, a higher fermentation temperature and using the strain AWRI 796 for fermentation resulted in higher succinic acid concentrations in both wines, while in contrast to Shimazu and Watanabe (1981), adding glucose/fructose to the must had no effect on succinic acid concentration. The pressing wines and free run wines had similar concentrations of succinic acid (Figure 18).

Higher must assimilable nitrogen and fermentation temperature resulted in significantly ($p<0.05$) higher glutamic acid concentrations in the free run Shiraz wine. Across both varieties, higher must sugar concentration resulted in higher wine glutamic acid concentration, which is consistent with other published findings that glutamic acid accumulates in the berry during ripening (Arias-Pérez et al. 2022) and that higher must sugar content results in higher glutamic acid concentrations in wine (Prokes et al. 2022). Glutamic acid is a favoured nitrogen source of yeast for fermentation (Bell and Henschke 2005). In this experiment, the amount of supplemented assimilable nitrogen exceeded that required for efficient fermentation (Cejudo-Bastante et al. 2010), resulting in the possible greater conservation of must glutamic acid in the wine. The pressings wines contained significantly more glutamic acid than the free run wines (Figure 18) across all experimental variables, suggesting that glutamic acid is extracted from the grape skins and/or seeds at pressing. Yokotsuka and Fukui (2002) reported that grape skins and (particularly) seeds contain more glutamic acid than the juice. Given that the wines had completed fermentation prior to pressing, this made any further contribution from the fermentation process unlikely. It is therefore probable that the additional glutamic acid in the pressing wines was that which was less available as a yeast nitrogen source during fermentation, potentially being sequestered in grape solids.

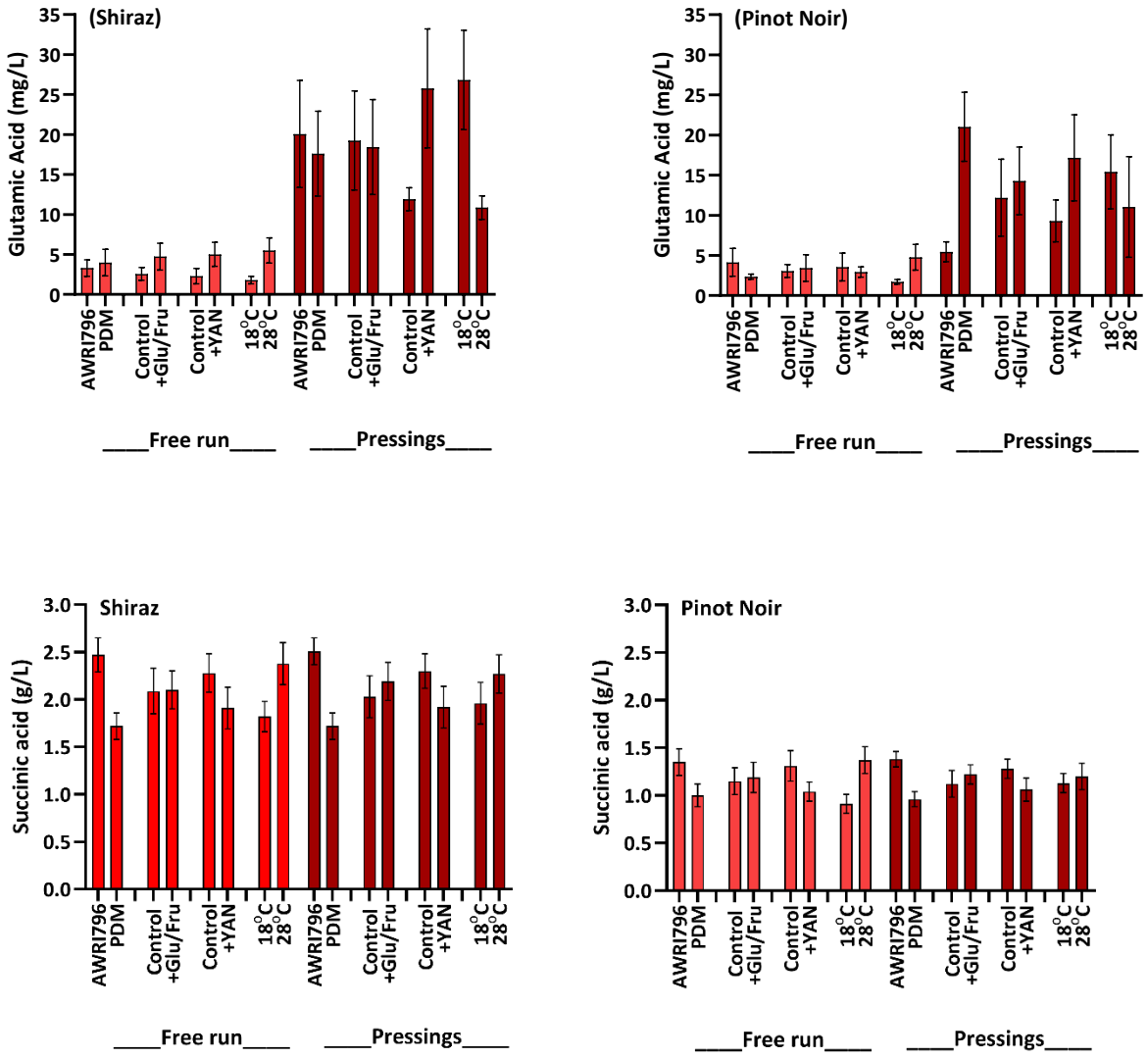


Figure 18. Mean concentrations of glutamic acid and succinic acid in Shiraz and Pinot Noir free run and pressing wines produced using different *S. cerevisiae* strains (AWRI 796 and PDM), fermentation temperatures, must sugar and YAN concentrations

7.4 Year 5 Deliverable. Knowledge of defined risk factors throughout wine production which may contribute to the presence of specific phenolic and other non-volatile compounds in white and red wine associated with negative wine attributes

7.4.1 Identification and sensory assessment of a potential bitter tasting compound 4-β-d-glucopyranosyl coumaric acid

Through a metabolomics LC-MS approach on discrete wine fractions ranked highly for bitterness, 4-β-d-glucopyranosyl coumaric acid was identified as a possible bitterant in white wine. The compound was synthesised on a preparative scale as described in Section 6.5. The effect of added 4-β-d-glucopyranosylcoumaric acid was evaluated at four concentrations (25, 50, 100 and 200 mg/L) to determine its impact on the in-mouth sensory properties of a model wine. It was hypothesised that adding coumaric acid glycoside would influence wine sensory properties, possibly contributing

bitterness, astringency, or 'hardness'. The five samples (one control and four addition samples) were rated for their intensity of taste and mouthfeel attributes by a pre-screened panel.

Adding 25 to 200 mg/L of 4-β-d-glucopyranosylcoumaric acid to model white wine did not significantly affect its basic tastes, astringency, perceived viscosity or hotness (Table 4). From the ANOVA, weak statistical evidence ($P = 0.087$) supported that one attribute, bitterness, was rated by the panel differently among the samples (Table 4). Sample mean values from all the assessors' scores are presented in Table 5. At low to moderate added concentrations the intensity of bitterness decreased, while the control wine and the highest added concentration (200 mg/L) were both rated similarly high in bitterness, possibly indicating a non-linear response. In conclusion, contrary to the hypothesis that the compound was bitter, there was some evidence that it decreased the bitter taste of the model wine when presented at the lower concentrations (25 and 50 mg/L).

Table 4. F-ratios, probability values[†], degrees of freedom (df) and mean square error (MSE) from the of the analysis of variance

Attribute	**Assessor**	**Sample**	**Rep**	**S*A**	**Rep*A**	**S*Rep**	**MSE**
Sourness T	17.02***	0.59	3.17 ǂ	1.00	1.60ǂ	2.30*	1.111
Sweetness T	11.20***	0.69	0.60	1.06	1.81*	0.92	0.901
Bitterness T	22.52***	2.20ǂ	2.35	1.41 ǂ	1.96*	0.81	0.945
Umami T	3.20*	0.36	10.16***	1.11	2.48***	0.74	1.313
Salty T	5.03***	0.61	4.81*	1.64*	2.18**	1.61	0.916
Astringent MF	42.75***	1.10	0.55	0.82	1.00	0.76	0.848
Viscosity MF	17.20***	1.09	2.08	1.75*	1.70*	0.73	0.529
Hotness MF	39.35***	0.55	0.43	0.82	2.11**	0.91	0.557
df	10	4	2	40	20	8	80

T: Taste, MF: Mouthfeel. [†]Significance levels are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; ǂ $P < 0.10$. df = degrees of freedom. S = Sample, Rep = Presentation Replicate, A = Assessor.

Table 5. Mean attribute scores for the control model wine and four samples containing the phenolic glycoside at different rates of addition. (Note: T = taste, MF = mouthfeel)

Attribute	Control	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Sourness T	3.99	4.11	3.89	3.89	4.21
Sweetness T	2.23	2.53	2.35	2.59	2.41
Bitterness T	3.55	2.91	2.90	3.11	3.44
Umami T	2.15	2.07	1.91	2.17	2.24
Salty T	1.62	1.75	1.77	1.63	2.03
Astringent MF	3.16	2.89	3.31	3.11	3.18
Viscosity MF	2.38	2.08	2.04	2.29	2.42
Hotness MF	2.96	2.74	2.92	2.89	2.94

7.5 Year 5 Deliverable. Practical advice and extension materials for winemakers

Further details on extension and communication outputs from this project are shown in Section 10 (Appendix 1 'Communication'). During the course of the project, four roadshow presentations and one webinar were presented on the outcomes of the dissolved CO_2 and bitterness work. Following the webinar on the dissolved CO_2 work, 75% of post-webinar survey respondents indicated that based on the information presented they would reassess their current practices, seek extra information or discuss with peers. A high number of questions were asked during the webinar itself,

suggesting strong industry interest in this work. A workshop presentation on dissolved gas management was delivered at the 17th AWITC, as well as an ASVO seminar on white wine mouthfeel.

From the results of the project, five technical publications and three peer-reviewed journal articles were published. Two further manuscripts have been prepared for peer-review from the work on TSO_3 and the survey of potentially savoury components in red and white wines, respectively. Furthermore, four conference/ASVO seminar/AWITC presentations, an AWRI webinar and four posters were presented on the results generated from studies within this project.

Further details on extension and communication outputs from this project are detailed in Section 10 (Appendix 1 'Communication').

8. Outcome and conclusion

An important element of wine quality from the viewpoint of consumers is the general perception of how a wine feels in the mouth when it is consumed. As such, wine texture is a major product differentiator for wine. Understanding and improving wine texture in a way that is relevant to a desired style of wine depends on knowing the compounds that influence wine texture and understanding their winemaking origins. The knowledge gained during this project has made progress towards enabling winemakers to optimise certain positive textural attributes while minimising negative ones.

The bitterness of a white wine produced by blending other bitter-tasting wines was associated with the concentration of a sulfonated tryptophol. Sensory analysis of this compound, and another analogue, at concentrations higher than are found in wine showed that they are perceptually like the known bitterants, quinine sulfate and (-)-epicatechin. Tryptophol sulfonate (TSO_3) in white wine accumulated after fermentation and pre-storage additions of SO_2 as bisulfite and reached a maximum at three months post-storage/bottling, while in red wine TSO_3 concentrations remained unchanged when stored under the same conditions. In this study, a high tryptophol-producing yeast was used to conduct primary fermentation and the wines were stored under higher bisulfite concentrations than are typically used in commercial winemaking. This resulted in wines at the upper limit of TSO_3 concentration, and in the case of white wine, within the bitterness taste detection range of a small cohort of tasters. This suggests that TSO_3 by itself would not strongly affect the bitterness of white wine for most wine consumers. However, the recently discovered diversity of bitter tasting wine metabolites (Arapitsas et al. 2016), including amino acids and phenolic compounds that can form sulfonates following the typical winemaking practice of bisulfite addition, suggests that tryptophol sulfonates may contribute to overall white wine bitterness in some wine styles through additive sensory effects (Keast et al. 2003).

Winemakers know there is a 'sweet spot' of dissolved carbon dioxide (DCO_2) in red wine, where if DCO_2 is low the wine can appear flabby and lack freshness, but the same wine can be perceived to be 'spritzy' if the DCO_2 is excessive. In a sensory study where the 'in glass' DCO_2 concentrations in two commercial red wines with varying DCO_2, tannin and ethanol concentrations were tightly controlled at the moment of tasting, it was found that increased DCO_2 resulted in increased perceived sweetness and reduced bitterness and astringency, but the perceptions of viscosity, hotness and overall flavour were unaffected. The study resulted in a method to quantify DCO_2 in wine from a wine glass *in situ,* which allowed for a follow-up consumer-focused study to be conducted, investigating the losses of DCO_2 in wines that varied in the key compositional factors of residual sugar, ethanol and acidity. This study was conducted under conditions designed to mimic typical wine consumption (i.e. pouring wine from the bottle, letting it stand in a wine glass and the agitation that happens during repeated tasting). Pouring significantly reduced the 'in bottle' DCO_2 concentration, and following 15 mins of simulated tasting, DCO_2 dropped to a level where the taste and mouthfeel could potentially be affected. Furthermore, of the compositional factors investigated,

only higher ethanol concentration significantly increased the rate of loss of DCO_2 from the glass during the simulated tasting.

The 2019 AWRI Vineyard and Winery Practices Survey revealed that switching from traditional cold settling to floating solids off white juice was the practice nominated as having the second highest positive impact in the previous five years. This project confirmed that the phenolic and polysaccharide composition and the resultant taste and mouthfeel properties of white wines made from juices clarified using flotation and those made using cold settling were similar. Winemakers can therefore have greater confidence in benefitting from the known efficiency and cost advantages of switching from cold settling to flotation, while being sure that the sensory and compositional quality of their wines are unlikely to be diminished by changing to the system of clarification.

Possible drivers of desirable 'savoury' characters in wine (i.e. glutamic acid, succinic acid and sodium and potassium cations) were benchmarked following an extensive survey of Australian red and white wines. Some possible winemaking impacts on the key compounds thought to be responsible for 'savoury' character in red wine were explored in a red winemaking scoping study using two varieties. The study investigated whether *Saccharomyces cerevisiae* strain choice, must sugar concentration, must nitrogen status or fermentation temperature could affect the glutamic acid and succinic acid concentrations in the free run and pressings wines. Succinic acid concentration was positively influenced by lower must YAN, higher fermentation temperature and also by yeast strain. The prototypical driver of savouriness, glutamic acid, was enhanced when the ferment was conducted at a higher temperature and particularly in the pressing wines, which contained three to four times the concentration of glutamic acid than the free run wines, results which provide insights into how winemakers could incorporate greater savouriness in their wines.

9. Recommendations

A recent AWRI study indicated that the prototypical savoury compound, glutamic acid, elicits an umami taste at wine-related concentrations, a result consistent with the outcome of the Australian wine survey and published threshold data presented in this report. However, while savoury character is commonly reported by winemakers in tastings, the concept of savouriness in the context of wine is ill-defined. Therefore, widespread consultation with winemakers is required to firmly establish the scope of what constitutes savouriness in wine. A tighter definition of savouriness is necessary to investigate how glutamic acid interacts with other compounds in wine that are likely contributors to savouriness, including peptides, organic acids, salts and possibly volatiles. A firm definition of savouriness is also necessary to achieve taste-directed discovery of new savoury compounds in wine. Future work should involve discovery of peptides and other compounds that drive savoury character and obtain actionable information on how they interact to impart savoury character. This work should also assess the contribution of fermentation-derived acids to savoury character against their potential to introduce unbalanced acidity or aggressive astringency.

Bitter tasting wines mostly contain bitter-tasting compounds at sub-threshold concentrations, which strongly suggests that bitter taste additivity is occurring, that other bitter compounds are present, or both. Future work should focus on investigating the strength of the additive effects of bitter-tasting compounds found in wine, including TSO_3, to provide mitigation strategies through winemaking practices or other interventions. In commercial wineries, bitterness in red wines at the completion of fermentation is typically removed by proteinaceous fining following tasting by winemakers, despite incomplete knowledge of the dynamics of bitterant formation. A better understanding of the evolution of bitter compounds during winemaking is needed to create new practical approaches to mitigate bitterness, reducing the reliance on the costly practice of using fining agents as a remediation strategy.

New knowledge was generated through this project, showing that the concentrations of arabinogalactan protein (AGP) class of wine polysaccharides was highest post-crushing, but progressively declined through juice settling and then after the early stages of fermentation. These results are important, as the AGP polysaccharides contribute positively to white wine mouthfeel by reducing alcohol-induced hotness, and by increasing perceived viscosity. The mechanism(s) underlying the loss of AGPs are unknown and should be further investigated to create actionable winemaking options that maintain AGP concentrations, thereby facilitating a greater positive impact on white wine mouthfeel.

The new understanding gained about the effects of DCO_2 on the taste and textural properties of red wine will provide guidance to winemakers as to how to appropriately manage DCO_2 in-bottle using gas exchange processes to achieve optimum sensory outcomes for red wine. Dissolved CO_2 was found to play a significant role in the taste and mouthfeel perception of red wine, and significant losses of DCO_2 were found to occur following pouring and during the tasting process. These findings suggest that accurate decision making at the winemaker's tasting bench could be assisted by either normalising or taking into consideration the DCO_2 concentrations of unfinished wines, by applying standardised protocols for pouring and the time between pouring and assessment. The finding that DCO_2 is better retained in wine with lower alcohol may also affect decisions regarding DCO_2 specifications at bottling, in particular for lower alcohol wine products.

10. Appendix 1: Communication

Publications

Gawel, R., Schulkin, A., Smith, P.A., Espinase, D., McRae, J.M. 2020. Effect of dissolved carbon dioxide on the sensory properties of still white and red wines. *Aust. J. Grape Wine Res.* 26: 172-179.

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Schulkin, A., Smith, P.A., Espinase Nandorfy, D., Gawel, R. 2021. A little dissolved CO_2 goes a long way in a wine glass. *Wine Vitic. J.* 36(1): 39-41.

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Gawel, R. Schulkin, A., Espinase Nandorfy, D., Milton, P., Bindon, K., Smith, P.A. 2020. 'White wine texture' presented at the Australian Society of Viticulture and Oenology Managing Phenolics and Texture Seminar, October 2020.

Romanini, E., McRae, J.M., Colangelo, D., Lambria, M. 2019. Grape seed powder as a novel and sustainable bentonite alternative. 17th Australian Wine Industry Technical Conference, Adelaide, July 2019.

Gawel, R., Schulkin, A., Smith, P.A., Espinase, D., and McRae, J.M. 2018. The effect of dissolved CO_2 on the taste and texture of white and red wine. Crush Symposium, Adelaide, August 2018.

Gawel, R., Schulkin, A., Smith, P.A., Espinase, D., and McRae, J.M. 2018. The effect of dissolved CO_2 on the taste and texture of wine. Workshop presentation: Dissolved gas management. 17th Australian Wine Industry Technical Conference, Adelaide, July 2019.

The effect of dissolved CO_2 on the taste and texture of white and red wine. AWRI webinar 9/7/2020. https://www.youtube.com/watch?v=Wxq5HD1L_r0

AWRI roadshow presentations. White wine texture: the interactive effects of phenolics, polysaccharides, acidity and alcohol, Coonawarra, SA, July 2017, Mornington, VIC, June 2022, Clare Valley, SA, September 2022.

AWRI roadshow presentation. The effect of dissolved CO_2 on the taste and texture of white and red wine, Geelong, VIC, June 2022.

Posters

Schulkin, A. and Gawel, R. CO_2 evolution in the wine glass: Implications for winemakers and consumers. 18th Australian Wine Industry Technical Conference, 2022.

Gawel R., Schulkin, A., Espinase Nandorfy, D., Milton, P., Bindon, K., Smith, P.A. 2022. Float or Sink? The effect of flotation and cold settling on wine non-volatiles, taste and mouthfeel. 18th Australian Wine Industry Technical Conference, Adelaide, July 2022.

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Wine Australia Newsletter: Dissolved CO_2 beliefs debunked by new research. 10th November 2018.

CO_2 – an important component of still wines, not just sparklings. AWRI *eNews*: May 2018.

11. Appendix 2: Intellectual property

Program IP has been communicated through presentations and publications and resides in the public domain. Copyright in any reports, posters or presentations is held by the AWRI. Copyright in publications remains with the publisher. No inventions or patentable discoveries have been identified from the work. No Program IP has been commercialised or identified for commercialisation at the time of writing this report.

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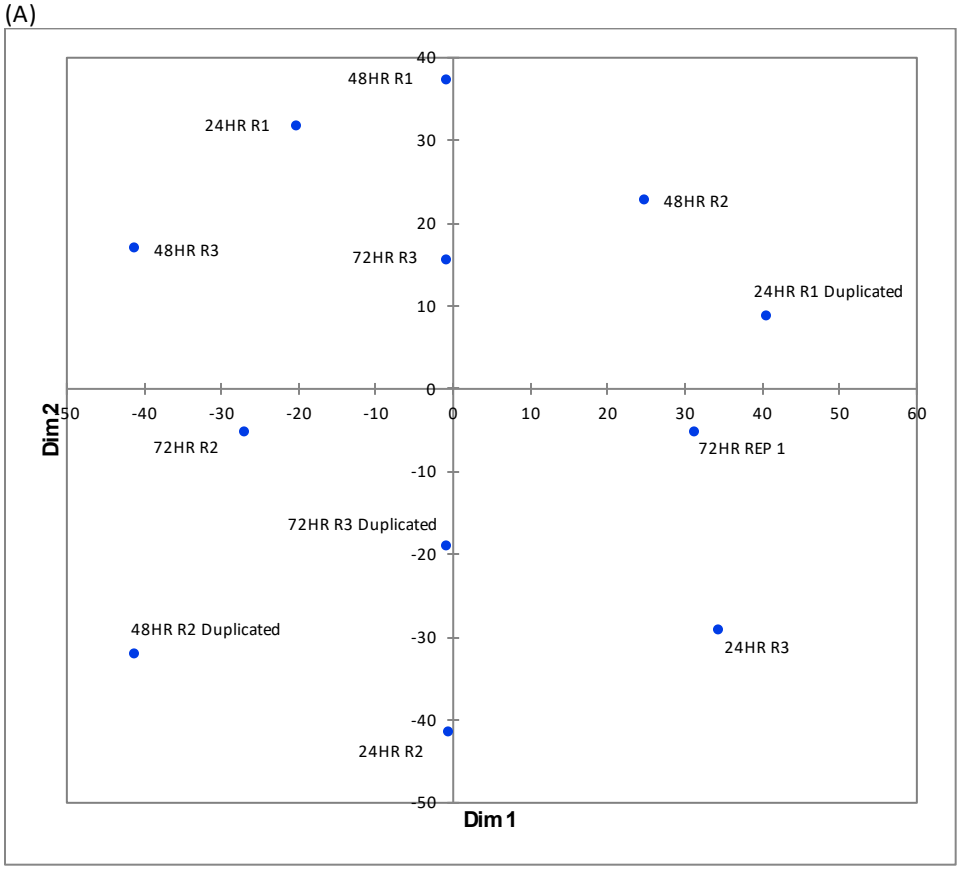
13. Appendix 4: Staff

- Dr Richard Gawel
- Dr Paul Smith (until September 2017)
- Dr Jacqui McRae (until July 2019).
- Dr Keren Bindon (since July 2019)
- Dr Alicia Jouin (from March 2020 until January 2022)
- Mr Alex Schulkin
- Ms Melissa Aitchison (from January 2019 until August 2020)
- Ms Renata Kucera (from November 2020 until May 2022)
- Ms Eleanor Bilogrevic
- Mr Damian Espinase Nandorfy
- Mr John Gledhill

14. Appendix 5: Other supporting material

Figure 19. Perceptual map of palate attributes of wines produced by juices settled for 24, 48 and 72 hours. Sauvignon Blanc (A), Chardonnay (B).

(A)



(B)

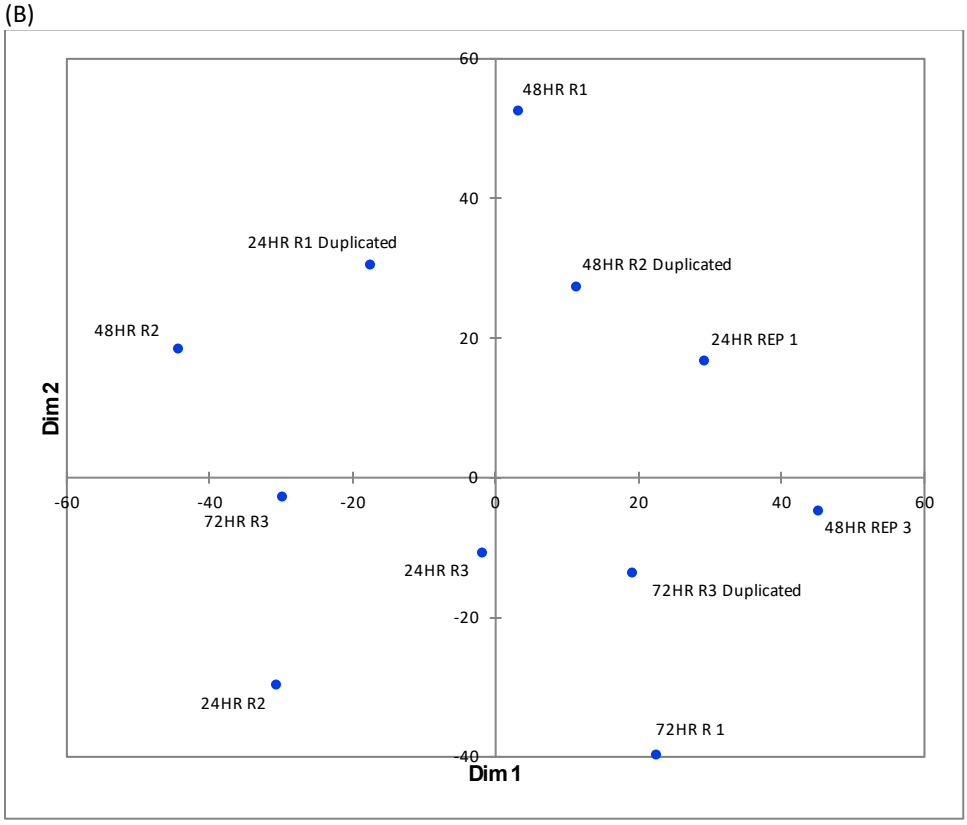


Table 6. Difference tests results. Taste comparisons of wines made from juices settled for 24 hours compared to those settled for 72 hours.

Chardonnay	**P**
72 hr rep 1 vs. 24 hr rep 1	0.79
72 hr rep 2 vs. 24 hr rep 2	0.95
72 hr rep 3 vs. 24 hr rep 3	0.52
Sauvignon Blanc	
72 hr rep 1 vs. 24 hr rep 1	0.24
72 hr rep 2 vs. 24 hr rep 2	0.37
72 hr rep 3 vs. 24 hr rep 3	0.04