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Building and measuring the quality of fine Australian sparkling wines, through identification of the impact compounds responsible for 'autolytic character' in sparkling wine, and novel winemaking techniques to hasten autolysis



FINAL REPORT TO WINE AUSTRALIA

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Principal Investigator: **Dr Fiona Kerslake**

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1 Abstract

This study investigated innovative practices across the entire production process for growing and making sparkling wine.

Viticulture - we demonstrated impacts on yield, and chemical and sensory properties in aged sparkling wine, associated with pruning (spur, cane), crop load (10 nodes, 60 nodes) and leaf removal treatments.

Pressing - a proof-of-concept prototype was developed to 'read' juice phenolic fingerprint in real-time at the press, and automate press fraction cut off (tank switching), based on UV-Vis reflectance at 240 nm and 290 nm.

Tirage – yeast disruption techniques (microwave, ultrasound, enzyme) were applied to yeast for tirage, and these achieved 'aged' aroma in wines sooner than control yeast treatment.

Cellaring – some of these wines were aged on lees, off lees and on lees at high temperature (25°C). This demonstrated that 'autolytic' character in wines could be achieved through chemical aging, in the absence of yeast. High temperature aging, however, was associated with poor foam stability.

The wide scope of this study identified several avenues for further research, particularly related to impact compounds for sparkling winemaking that might underpin objective measurement of sparkling wine quality.

2 Executive Summary

This project - *Building and measuring the quality of fine Australian sparkling wines, through identification of the impact compounds responsible for 'autolytic character' in sparkling wine, and novel winemaking techniques to hasten autolysis* - was conducted in four parts. The first utilised research wines *sur lie* from a prior project to explore the impact of viticultural practices on aged sparkling wines. The second part examined mechanisms related to sparkling wine quality including 'autolytic character' and associated mouthfeel, texture, and aromas. The third part sought key discriminant factors of phenolics in grape juice at the pressing stage, and developed a proof-of-concept prototype to measure phenolics in-line and in real-time. The fourth part tested if spectral techniques could be used for regional discrimination of juices destined for sparkling wine production.

The Tasmanian Institute of Agriculture at the University of Tasmania led this project, and drew on expertise from the Australian Wine Research Institute, the University of Melbourne and Wine TQ. Several private wine companies supported the research, with major contributions by Hill-Smith Family Vineyard and Josef Chromy Wines. Hill-Smith Family Vineyard generously donated base wines and the time of their team for the autolysis component of the project. Josef Chromy wines generously provided facilities for the larger disgorging at 12 months in the autolysis component, and allowed the research team to install in-line sensors in their press to prototype real-time measurement of juice phenolics. There were numerous other supporting wine companies, predominantly through winemakers' time for sensory appraisal of wines via expert tasting panels.

Viticulture

This study examined the impact of several commonly used viticultural practices on sparkling wine quality over an extended aging period. Practices examined were yield restriction, cane versus spur pruning, and leaf removal at various stages. The yield restriction research revealed that 'high' and 'low' crop load treatments had some impact on wine composition but minimal sensory effect. Hence yield restriction did not produce a discernible impact on sparkling wine quality. Average annual net return per hectare from the 'low' crop load treatment was $1,845, compared with $9,518 for the 'high' crop load treatment. This indicated it would be prudent for vineyard owners and managers to consider managing vines for 'high' crop loads to deliver increased return on investment (ROI.

Yield restriction

…did not produce discernible impact on sensory quality

…higher crop load could deliver greater ROI

Pruning method

…cane pruning associated with compounds that can improve foaming

…spur pruning with compounds that can improve mouthfeel and influence wine aroma

Pruning method impacted several important chemical parameters with relevance for sparkling winemakers. Aged sparkling wines from cane pruned Chardonnay from 2010 and 2011 showed consistently high concentration of medium molecular weight polysaccharides, which play a role in foam stability. Aged wines from 2010 and 2011 spur pruned Chardonnay were consistently higher in hydroxycinnamates, which have mouthfeel effects in sparkling wine. Phenylacetaldehyde, a compound associated with 'honey' and 'floral' aromas, was consistently lower in all spur pruned wines. Phenylacetaldehyde is one of the Strecker aldehydes, which can be markers of white wine oxidation. Oxidation is mediated by the activity of free radical

scavengers, like hydroxycinnamates, which may have played a role in this aroma compound outcome. In the warm, dry vintage 2010, wines from the cane pruned treatment had higher average concentration of Thaumatin-like protein, which can be important for foaming but has also been associated with haze formation. Based on these chemistry findings, vineyard managers might favour cane pruning for blocks where foaming quality of wine has previously proven deficient. Spur pruning may be appropriate for blocks that have previously delivered wine with good foaming quality, but have scope for development of mouthfeel. While pruning method was demonstrated to significantly impact wine chemistry, particularly in the warm dry 2010 vintage, the chemical impacts observed were not sensorily discernible to an expert panel, based on blind tasting and rating of wines via a 20-point wine show rating system. These experts make their living making, tasting, blending, assessing and marketing sparkling wine, so the lack of distinctive sensory outcomes suggests further research would be needed to optimise the pruning effects observed, to justify industry uptake.

…vineyard managers might favour cane pruning for better foaming, and spur pruning for better mouthfeel…

A high proportion of wines from the leaf removal trial were unstable after 5.5 years aging, and hence resulting data was not suitable for rigorous statistical analysis. A Principal Component Analysis of spectral data from the aged wines that were sound did, however, suggest there may have been hydroxycinnamate effects. Analyses of base wines from those trials showed leaf removal wines differentiated from control wines on wavelengths that had been associated with two key hydroxycinnamates: caffeic and ferulic acid. The commonly used spectral measure of 280nm for 'total phenolics' was not found useful for evaluating and differentiating the SPF of juices and base wines from this leaf removal trial. Hydroxycinnamic acids in white wine have been positively associated with viscosity, and some sparkling winemakers actively manage expression of hydroxycinnamates in juice and wine through oxidative handling. Our findings of a possible association between leaf removal and hydroxycinnamates may offer another potential avenue for industry to manage expression in juice and wine of this important class of compounds.

Leaf removal

Leaf removal impacted the concentration of compounds that may improve mouthfeel

Aged Character

The development of 'aged character' in sparkling wines was investigated. Expert sensory appraisal of experimental sparkling wines was undertaken where wines had been aged for 24 months: with and without yeast lees, and at two different cellaring temperatures ('hot' 25°C and 'control' 15°C). While a complex picture emerged, the expert panel observed 'autolytic' character in wines that had been aged off lees. This was a character that could not have arisen from yeast autolysis, but was likely from chemical ageing. Wines aged at 25°C showed higher intensity of several positive aroma characteristics the panel associated with 'aged' sparkling wine (including 'autolytic' character). These two findings together, indicated that chemical kinetics played an important role in development of sparkling wine aged character, in the absence of yeast lysis. While the aroma profile of 25°C wines was judged positive by the sensory

Age or autolysis?

Chemical aging was associated with 'autolytic' character, in the absence of yeast lees

Hot cellaring?

Wines cellared at 25°C showed poor foaming performance

panel at 24 months bottle age, separate experiments showed poor foaming performance by the 25°C wines, possibly associated with hastened biochemical evolution of polysaccharides and proteins. Better understanding of base wine protein status, and the chemical and biochemical pathways affecting protein and polysaccharides might support commercial uptake of 'hot' cellaring.

Disrupted yeast

Addition of disrupted yeast hastened development of 'aged character'

Various techniques were investigated to hasten yeast lysis, and test their use for driving 'autolytic' character development. Visualisation of yeast cells by PhD candidate, Ms Gail Gnoinski, allowed quantification of cell wall integrity changes during time on lees, compared with enzyme, microwave and ultrasound treatment. The treatments were all associated with faster structural breakdown than observed from normal yeast aging on lees. Impacts of disrupted yeast in Chardonnay trial wines were not particularly striking, but Pinot noir wines provided proof-of-concept for yeast disruption as a technique to potentially manage sparkling wine aroma development. According to the sensory panel, the treatments had positively influenced the development of particular aged characters in Pinot noir wines at 24 months. For example, the enzyme treated yeast was associated with wines having more spicy, nutty aroma. Microwave yeast wines were judged more autolytic, nutty, honey and toasty. Findings from this one set of experiments are not widely generalisable, however, they suggest the development of aged character in sparkling wine might be more precisely managed via addition of disrupted yeast at tirage. This approach warrants further research.

Bevscan

BevScan was used on the wines from the viticulture and 'autolytic' character experiments, and the spectral data generated successfully discriminated between wines based on the various treatments applied. This suggests, with further investigation, that BevScan might have a role in an objective, non-destructive measure of sparkling wine quality. This could be a reliable and economical way to monitor the ageing process for sparkling wines in commercial settings.

In-line Phenolics

Press automation

A portable, modular prototype was developed that switched tanks at a pre-determined phenolic threshold

Component parts were identified and trialled for a proof-of-concept prototype to measure juice phenolics in real-time at the press. Practices for deciding juice cut off for premium sparkling wine production are currently labour intensive and imprecise. In the first year, a quartz window and a sample port were installed in a press manifold for juice sampling during pressing, and a portable spectrometer allowed the SPF method to be applied in real-time in the winery. The best prediction method for total phenolics concentrations in juice was identified (240 and 290 nm in reflectance mode). With additional funding from a Science and Innovation Award for Young People in Agriculture, Fisheries and Forestry, Dr Rocco Longo, designed and tested a second prototype including in-line UV-Vis sensing, an actuator to switch feed between tanks, and a digital screen to enter threshold points at which to switch feed. The second prototype was not ready in time for 2018 vintage, but was demonstrated successful using frozen juice. Pathways to developing this technology for industry are being actively explored.

Juice Provenance

Spectral provenance

UV-Vis and NIR scanning combined were not sufficiently powerful or nuanced to distinguish juice origin

The concept of identifying juice origin based on phenolic profiles proved more challenging than first anticipated. Over two vintages, juices were collected and scanned in UV-Vis and NIR regions (200-600nm and 800-2500nm). Findings from the second vintage, where sampling protocol had been refined, showed some regional distinction. The much appreciated additional resourcing of this research, by way of Plumpton College Master student Mr Hugh McCullough, did not clearly identify spectral means of juice provenance, but method refinement recommendations are provided that will potentially guide future work, and may lead to improved classification.

3 Background

3.1 Viticulture

Sparkling wines are intrinsically complex, with specific fruit composition and flavour profile requirements. Recognition of the growth potential for this category, along with the importance of Pinot noir production in cool climates, saw Tasmania as an appropriate site for research investment. This resulted in Wine Tasmania, a consortium of Tasmanian wine producers, UTAS and other contributing companies being awarded $1.8M in 2008 through an AusIndustry Industry Co-operative Innovation Program (ICIP) grant. Of the five components of this program, two were dedicated to sparkling wine viticulture and vinification. This Wine Australia funded project - *Building and measuring the quality of fine Australian sparkling wines, through identification of the impact compounds responsible for 'autolytic character' in sparkling wine, and novel winemaking techniques to hasten autolysis* - is a continuation of that early, and in the vinification space, pioneering ICIP project work.

The viticulture component of this current study is the evaluation of wines produced during the ICIP project. Conversations with, and a survey of, Tasmanian sparkling wine producers in 2009 (Kerslake, Jones and Dambergs, unpublished data) revealed a range of practices which were used in sparkling wine producing vineyards, with three being identified as of particular importance: crop load, pruning method and leaf removal. On this basis, trials were established in 2009 for the 2010, 2011 and 2012 vintages. Within the constraints of the ICIP project, the 2010 and 2011 wines were tiraged. This fortunately delivered wines from a warm and dry (2010), and a cool and wet (2011) season.

3.1.1 Crop load

Crop load is a common parameter used to manipulate fruit quality and may be particularly important in a cool climate where the ability to ripen fruit can be compromised. At the time of trial establishment, there was some thought that fruit destined for sparkling production may be able to carry a higher crop load as lower total soluble solids (TSS) and higher acidity fruit is generally used. The original study aimed to examine fruit and base wine composition when crop load was manipulated by varying node number at winter pruning; winter is the most cost-effective time in the season for crop load adjustment. The current study aimed to evaluate the composition of these wines after 5.5 years *en tirage* and provide assessment by a panel of experienced winemakers.

3.1.2 Pruning method

Anecdotal evidence in the Tasmanian industry suggested that an issue of basal bud infertility limited the success of vines that had been spur pruned. Spur pruning is significantly cheaper to conduct than the widely practiced system of cane pruning. This was despite some well documented advantages of spur pruning, including more uniform shoot growth and higher capacity for the storage of reserves (Bernizzoni et al., 2009). Cane pruning remained the preferred option due to the most fruitful buds being retained, and less dense canopies being considered a lower disease risk, however such a preference was still based on anecdotal or observational evidence (Jones et al., 2018). This project aimed to quantify the differences in yield, juice and base wine quality from Pinot Noir and Chardonnay vines which had either been cane pruned or spur pruned. The current study evaluated the composition of these wines after 5.5 years *en tirage* and provided assessment by a panel of experienced winemakers.

3.1.3 Leaf removal

Leaf removal is a common cool climate practice when fruit is destined for table wines. It is used as a means of influencing the phenolic profile, through exposure to UV light. It has the added benefit of allowing better air circulation and access for spraying for disease control. A meta-analysis of 59 papers that examined pre-bloom leaf removal demonstrated impacts on yield (lower with pre-bloom leaf

removal) and fruit sugar concentration (higher with pre-bloom leaf removal) (Vanderwiede et al 2021). Those authors suggested that leaf removal, applied prior to flowering generated carbohydrate limitation which reduced fruit set (crop load management). As described above, reducing crop load has been viewed as a way to manage, and potentially improve, fruit quality. For sparkling wines, where phenolic quality is important but may be represented by less well understood profiles, little is known about the impact of leaf removal practices. The current study evaluated the composition of these wines after 5.5 years *en tirage* and provided for assessment by a panel of experienced winemakers.

3.2 Autolysis and autolytic character

The most well-regarded sparkling wines are most frequently produced by the traditional method, or *Méthode Champenoise*, requiring two fermentations with the second in-bottle (see Figure 3-1). After secondary fermentation, wines are aged on lees for at least nine months before disgorging and packaging. Aging on lees is intended to allow for breakdown of yeast cells following fermentation, and release of compounds for development of texture, fine bubbles and many organoleptic qualities (Culbert et al., 2017; Alexandre and Guilloux-Benatier, 2006; Kemp et al., 2015). The compounds which impact on quality in white and sparkling wines are poorly defined, if at all, however premium sparkling wines rely on the development of so-called 'autolytic character'. This character is thought to result from a lengthy period of aging on lees, however the question has been raised by industry if this is entirely attributable to yeast lysis or if some of this character is simply aged wine character, given that expert sparkling winemakers were unable to distinguish between, or indeed agree upon, the two in a tasting in 2009 (Donachie et al., 2010). If autolysis is not the mechanism for this character, then alternative processes may be available to streamline winery efficiency. Methods that have been developed and applied by TIA in red wine maceration include ultrasound, microwave (Carew et al., 2014) and endogenous enzymes. In this study, these methods were proposed to drive 'autolytic character' as they had potential to disrupt yeast cell wall integrity. Cell wall disruption might enable earlier release of cell wall components thought to contribute to 'autolytic character' in high quality sparkling wines.

Hastening the development of 'autolytic character' in sparkling wine would offer some potential for wines to be cellared for shorter periods, thereby freeing winery cellar space and providing faster financial return to wineries. The assessment of a sparkling wine's progress towards 'autolytic character' sufficient to be released for sale, requires wines to be opened and tasted by the winemaker. Better understanding of the chemical compounds or array of compounds (fingerprint) associated with 'autolytic character' would allow for potentially more objective and standardised assessment of wines for release to market. An alternative to identifying individual chemical compounds associated with 'autolytic character' would be to develop 'signature fingerprints' to inform in-bottle scanning processes. In-bottle scanning would allow non-destructive or automated evaluation of wine in-bottle.

Better understanding of the chemical compounds, chemical arrays or fingerprints associated with 'autolytic character' in sparkling wines would assist in identification of objective measures of quality, and potentially the 'impact compounds' driving wine quality. As with the identification of rotundone as a key compound in Shiraz wines, identifying impact compounds in sparkling wines would support development of grape growing and winemaking processes that managed the expression of those compounds, for optimal wine quality outcomes.

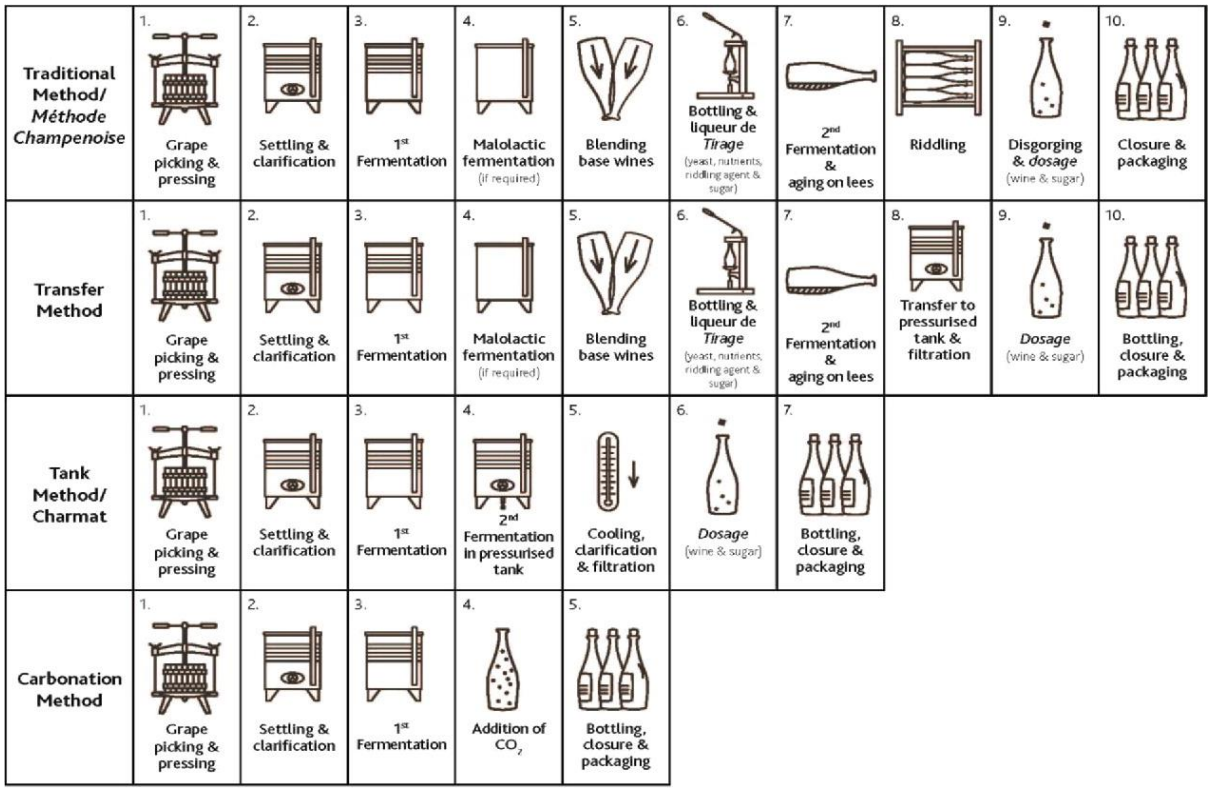


Figure 3-1 Summary of the sparkling wine production methods (Kemp et al., 2018)

3.3 Objectively measuring juice phenolics in-line

(some parts of this section are abridged from McCulloch, 2019)

The process of pressing fruit for sparkling wine production has a substantial impact on the sensory qualities of finished wine. During a pneumatic press cycle, the composition of grape juice changes over the duration of the cycle, as pressure is increased. For sparkling wine, juice is generally fractioned into three classes during the pressing cycle: free-run (as fruit is loaded to the press and prior to pressure being applied), *cuvée*, and *taille*. Decisions about how to run the press and fraction juices vary by winery and winemaker. Because grape berry components (e.g. pulp, skins, seeds) contain a range of organic acids, polyphenols, sugars, polysaccharides and other compounds, press cycle decisions impact on what is extracted into which fraction (Gawel et al., 2014; Marchal et al., 2012; Dowling et al., 2015). For example, a long, gentle press run (many hours) with minimal crumbling of grape solids may deliver different juice outcomes compared with a 90 minute, rough and extractive cycle. During vintage, fast pressing may be necessary due to winery capacity constraints.

Over the duration of a press cycle, specific compounds are extracted. Some are extracted early and others later, as pressure increases, and more grape components lose their physical structure (i.e. get crushed). Over a press cycle, polysaccharide and oligosaccharide content in grape juice decreases (Jégou et al., 2017). These compounds aid in tartrate and protein stability in wines, impact on perception of astringency and may improve sparkling wine mouthfeel and foaming properties (Jégou et al., 2017; Gawel et al., 2016; López-Barajes et al., 2001; Dufrechou et al., 2015). As the press cycle continues, juice pH increases, titratable acidity decreases and more colour is extracted (Gawel et al., 2014; Marchal et al., 2012; Dowling et al., 2015).

Phenolic content in juices increases during the press cycle, with maceration of skins and seeds under higher pressure releasing hydroxycinnamates, flavanols and their derivatives (Gawel et al., 2014; dos Santos Lima et al., 2015; Kerslake, Longo and Dambergs, 2018). Polyphenols are compounds with aromatic rings that contain one or more hydroxyl groups and they can contribute to both astringency and bitterness, texture and mouthfeel, as well as the colour and volatile aroma content of the juice and resulting wine (Patel et al., 2010; Gawel et al., 2014; Alexeindre-Tudo et al., 2017; Cozzolino, 2015a). Some components extracted from the fruit during pressing are caftaric and caffeic acids, both hydroxycinnamate derivatives, which although shown to have some positive mouthfeel attributes in certain concentrations, can also be rapidly oxidised by the enzyme polyphenoloxidase in both juice and wine, causing oxidative browning (Kerslake, Longo and Dambergs, 2018; Ferreira-Lima et al., 2016). This is often undesirable; some producers will saturate juice with oxygen in order to oxidise and then remove larger phenolic compounds by precipitation and racking to improve the quality and mouthfeel of the resulting wine (Gawel et al., 2014).

The complex array of compounds that emerge from grape solids into juice during pressing mean that it is important to understand and manage pressing and press fractioning. It would be impractical in a winery setting, to assess the concentration of each of the juice components discussed above. However, there is clearly value in better understanding extraction, and perhaps identifying indicator compounds, concentrations or juice profiles to support better pressing decisions.

Previous research undertaken at the Tasmanian Institute of Agriculture, with Dr Bob Dambergs, under the auspices of an AusIndustry Industry Co-operative Innovation Program (ICIP) project demonstrated the potential of spectral fingerprinting as an analytical technique that could support sparkling wine production. During the ICIP project, an objective UV-Vis spectral phenolic fingerprint (SPF) measure was shown to discriminate between grape juices and base wines based on viticultural practices (Jones et al., 2018, Kerslake et al., 2013). The research team continued this laboratory based method to validate UV-Vis SPF as an objective measure for discrimination between sparkling wines. The method was also trialled as an objective measure of juice phenolics at the press for the primary purpose of identifying press fractions based on phenolic concentration measurements (Kerslake et al., 2018). Having proven the concept of UV-Vis SPF for in-line, real time juice evaluation at the press, TIA researchers identified currently existing processes and technologies for in-line juice sampling and evaluation. For example (Carew 2016 page 3):

> ‘A custom-designed by-pass on the weigh bridge at Schloss Affaltrach Winery allows real-time assessment of TSS…This by-pass set up and housing may suit assessment of phenolic pick-up for Australian white and sparkling wine making, given appropriate instrumentation (eg. reflectance NIR) and algorithms to interpret data. This may compliment the recently completed work by AWRI on fermentation sensing for sugar levels (AWR 1401).’

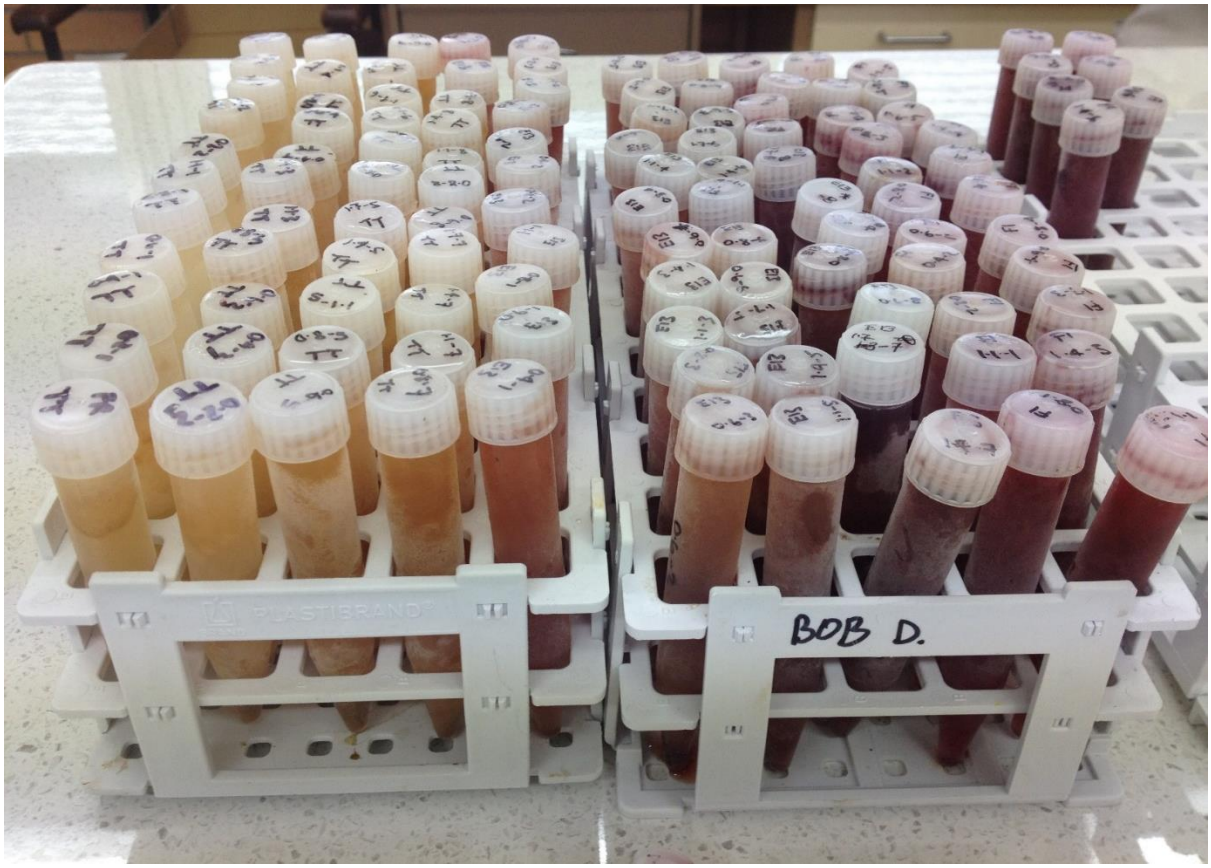


Figure 3-2. samples from Karina Dambergs, Clover Hill winemaker.

3.4 Juice provenance

(some parts of this section are abridged from McCulloch, 2019)

Wine consumers have an interest in the authenticity of the wine they purchase. Regional labelling, the reputation of a winery and information about *terroir* can be important selling points for winemakers (Pomarici, 2016; Picard, Moreira and Loloum, 2018). Studies show that if industry is well placed to highlight these attributes then consumers are likely to purchase more and purchase at higher price points (Danner et al., 2017). At the time of the funding application for this research, provenance had been identified as a key priority area for the Australian wine industry. Much of the drive for this research at the time, was focussed on brand protection, but there was also interest in industry to understand the composition of grapes and juice from high performing sparkling wine regions, and consequently to understand what it was that discriminated these regions from one another. The intention was to open the door for regional classification of juices for authentication of origin, and also for this to be useful for sales and marketing purposes. Most importantly, regional separation could assist winemakers in tailoring harvesting and pressing techniques that best preserve, and highlight, the provenance of their grapes.

Methods to analyse grape juice for production decisions need to be robust, simple and relatively cheap, if they are to be adopted in industry settings. This may be particularly the case for Australian premium sparkling wine production, as there are a number of important smaller producers. Spectroscopy has emerged as an analytical tool for wineries, particularly as the technology has become relatively cheap and is now portable.

With regards to finished wine, studies show regional discrimination of commercially available products by UV-Vis spectroscopy in Australian Shiraz, Argentinian Sauvignon blanc and Spain's north-western Rías Baíxas region (Riovanto et al., 2011; Azcarate et al., 2013; Martelo-Vidal, Domínguez-Agis and Vázquez, 2013). However, there is not yet work in the literature on UV-Vis allowing regional discrimination of grape juices.

NIR spectroscopy is currently used to monitor sugar and alcohol content during wine fermentation. This is one of the most prominent everyday uses for NIR spectroscopy (Cozzolino, Gishen and Cynkar, 2006; Dambergs, Gishen and Cozzolino, 2015). Fermentation has also been successfully monitored via assessment of changing phenolic content (Gishen, Dambergs and Cozzolino, 2005). While measurement through NIR of specific quantities of certain phenolic compounds and other wine parameters has been variable in its effectiveness (as outlined by Dambergs, Gishen and Cozzolino, 2015), there has been some success. Urbano-Cuadrado et al. (2004) noted accurate measures of pH, glycerol, colour, titratable acidity and total phenolics. Other studies have been less successful, suggesting more work is needed before highly accurate techniques can be applied to industry situations (Cozzolino et al., 2011b). With regards to broader assessment of a wine's spectral fingerprint, studies show that NIR can be used for authentication of wine origin. In conjunction with UV scanning, Martelo-Vidal, Domínguez-Agis and Vázquez (2013) successfully discriminated the sub-regions of wines from Spain's Rías Baíxas region. Liu et al. (2007) were not as successful but were able to classify regional distinction between Riesling wines originating in different countries in a high proportion of cases. Regional grouping by spectral fingerprint has also been successful in Cabernet sauvignon and Merlot wines of the Balearic Islands of Spain (González-Cateno et al., 2013). There has not been any work in the literature on regional distinction of grape juices, prior to fermentation.

4 Project Aims and Performance targets

The objectives of this project were spread across four main project parts, with two guiding principles:

1. To deliver practical information for grapegrowers and winemakers to produce the highest quality grapes, and to build, maintain and protect the fine Australian sparkling wine category.
2. To actively engage industry partners in the research, to ensure 'ground-truthing', industry awareness and establish a strong base for uptake, should treatments prove effective.

4.1 Viticulture

To deliver mature sparkling wine composition and sensory outcomes from viticultural trials; including the impact of crop load, pruning method and leaf removal.

4.2 Autolysis and autolytic character

1. To identify compounds related to sparkling wine quality including 'autolytic character' and associated mouthfeel, texture, and aromas.
2. To pursue an objective measure, or spectral phenolic fingerprint (SPF), for sparkling wine.
3. To determine if 'autolytic character' in sparkling wine is due to the assumed mechanism of autolysis or is an artefact of wine aging.
4. To hasten yeast autolysis through application of novel techniques, and differentiate and visualize cell-level impact associated with lees treatment by novel methods (ultrasound, microwave, enzyme).
5. To evaluate and compare the quality of sparkling wines aged on novel treated lees throughout an industry-relevant bottle aging period, and to compare this with standard tirage and base wine aging characteristics.

4.3 Objectively measuring juice phenolics in-line

1. To develop SPF into a real-time assessment method of juice quality during pressing, for increased winery efficiency through automated switching of tank feed.

4.4 Juice provenance

1. To assess the use of UV-Vis and NIR SPF to discriminate the provenance of premium quality juice for sparkling wines.

5 Method

5.1 Viticulture

As part of the previously described ICIP project, trials were carried out in the 2010, 2011 and 2012 vintages to evaluate the effect of three separate viticultural practices on juice and base wines. The three practices were identified from a survey of the Tasmanian sparkling wine industry in 2009 (Kerslake, Jones and Dambergs, unpublished data).

1. **Crop load**
2. **Pruning method**
3. **Leaf removal**

Experiments were conducted at commercial vineyards in northern and southern Tasmania (Figure 5-1).

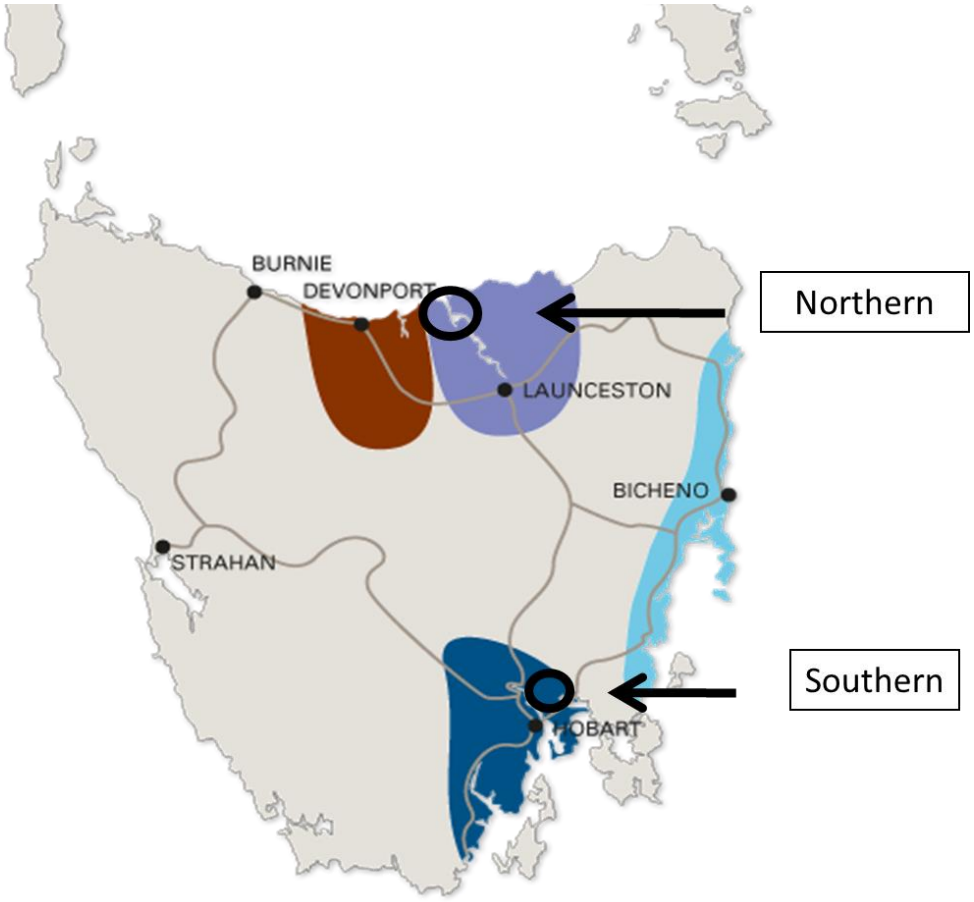


Figure 5-1 Locations of the viticultural experiments in northern and southern Tasmania.

5.1.1 Weather

Weather data was collected on-site for the northern trial site, and from the Australian Bureau of Meteorology weather station approximately 5 km away for the southern site.

5.1.2 Crop load

The crop load trial was conducted on Pinot Noir vines winter pruned to two different node numbers at a Tamar Valley vineyard in the north of the state. 'Low' and 'high' crop loads were achieved by pruning to 10 and 60 nodes per vine, respectively (Figure 5-2). This pruning trial had been running since vintage 2006, giving the vines time to adjust to the pruning level.



Figure 5-2 Example of the pruning in the crop load experiment. Pruned to; 10 nodes per vine for the 'low' treatment (A) and 60 nodes per vine for the 'high' treatment (B).

5.1.3 Pruning method

The pruning method trial was conducted in the Coal River Valley in southern Tasmania on 20-year-old Pinot Noir (D5V12) and Chardonnay (I10V1) vines. Vines were either cane or spur pruned to 20 buds per vine.



Figure 5-3 Example of the pruning in the cane versus spur experiment. (A) Cane pruned vine and (B) Spur pruned vine

5.1.4 Leaf removal

The leaf removal vineyard trial was repeated in the Tamar Valley (north) and the Coal River Valley (south) in Tasmania, on both Pinot noir and Chardonnay. Mature and lateral leaves up to and including the 4th node were removed at three different times in the 2010 and 2011 vintages (pre-flowering, pea sized berries and 50 % veraison, examples in Figure 5-4), excepting the northern site which did not have a pre-flowering leaf removal treatment for 2010.

For each experiment yield, yield composition, juice composition and base wine composition were assessed as part of the original project. A selection of the base wines was tiraged and assessed as

mature sparkling wines after 5.5 years *en tirage*. Wines to be tiraged were selected based on treatments within each trial which showed clear differences in base wine composition, and did not display any influence of small scale winemaking practices. Consequently, this resulted in the determination that it was only worthwhile tiraging the Chardonnay wines, given the minimal influence of leaf removal treatments on the Pinot noir base wines.

During subsequent wine storage and transport for disgorging and analysis, some bottles of wine became unusable, which in turn resulted in a varying number of replicates for each treatment as described in Table 5-1.

Table 5-1 Leaf removal trial aged sparkling wines, number of replicates and treatments

2010 Northern CHA	2010 Southern CHA	2011 Northern CHA	2011 Southern CHA
- 3 replicates of control - 1 replicate of pea-sized - 2 replicates of veraison	- 3 replicates of control - 3 replicates of pre-flowering - 3 replicates of veraison	- 2 replicates of control - 3 replicates of pre-flowering - 4 replicates of pea sized - 4 replicates of veraison	- 4 replicates of control - 4 replicates of pre-flowering - 4 replicates of pea sized - 3 replicates of veraison

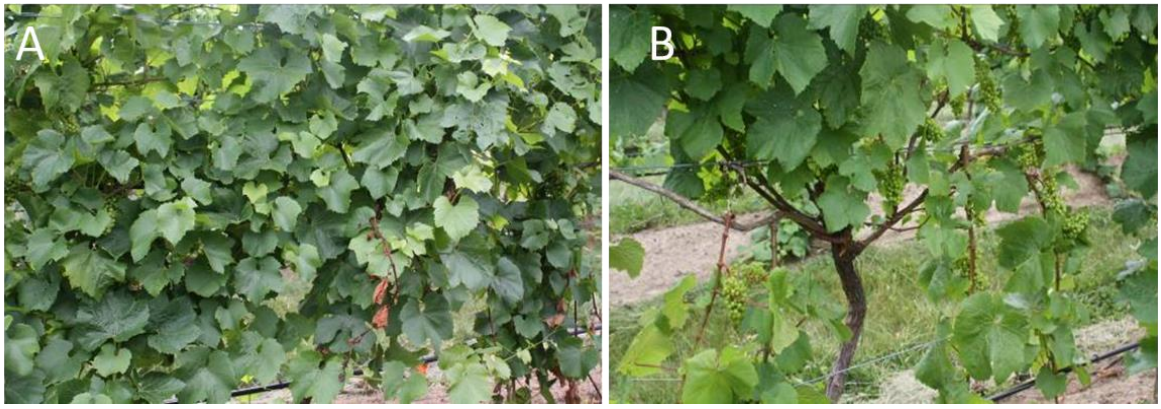


Figure 5-4 Example of a control vine (A), where no leaves were removed and leaf removal treatment of mature and lateral leaves removed up to and including the 4th node (B).

5.1.5 Winemaking

Base wines were made using standard small scale (12 kg) ferments as per the method described in Kerslake et al. (2013), utilising small-scale winemaking equipment such as the custom made flat-bed press (Figure 5-5).



Figure 5-5 Custom made, small-scale, flat-bed press for processing sparkling wine grapes

Base wines were kept separate and hand tiraged 9-11 months later by the research team (Figure 5-6), in conjunction with the Tamar Ridge winemaking team (Figure 5-6), and left *en tirage* for 5.5 years at 15 °C.



Figure 5-6 Hand bottling tirage ferments (Dr Bob Dambergs, 2011)

Wines were hand riddled for 8 weeks at 15 °C, then inverted and stored at 4 °C overnight prior to disgorging (Figure 5-7). Bottle necks were frozen in a salt-based slurry in an upright freezer, then caps removed. After ejection of the yeast plug, the bottle was up righted and minimal additions added to the bottle before resealing with a fresh crown seal. Additions for the 2010 wines were 30 ppm SO_2 and 5 g/L sugar, however after reviewing this with winemakers at the tasting panel, no sugar was added to the 2011 wines as it was deemed unnecessary for industry assessment of the viticultural treatments. The wines were never intended to be finished wines, rather research wines, and were assessed as such.



Figure 5-7 Capping tiraged ferments (Hanna Westmore and Gail Gnoinski, 2016)

5.1.6 Analysis

Wines were analysed in-bottle by post-dispersive diode array visible-near infrared (450-1050 nm) spectrophotometer (BevScan) as described by Dambergs et al. (2015), then tasted by an industry panel of experts at the Effervescence Festival Technical Days. Wines made in 2010 were tasted in November 2016, and wines made in 2011 were tasted in November 2017. Data collected from the tasters included a 20-point wine score (appearance, nose and palate; 3, 7 and 10 points respectively). Other attributes that had been identified by Dr Andrew Pirie and used previously with the Tasmanian wine industry, were rated on a scale of 1-10:

- Autolytic character
- Sweetness
- Acidity
- Bitterness
- Astringency
- Sweetness : astringency balance
- Body
- Flavour intensity/persistence

Three wines were repeated across two flights to 'test the tasters' so as to develop an 'expert panel' of ten tasters out of the full industry panel who participated. Identification of tasters onto the expert panel was based on identifying the ten tasters who delivered the lowest standard deviation of wine show scores for the three repeated wines.



Figure 5-8 Non-destructive, in-bottle sparkling wine analysis with BevScan

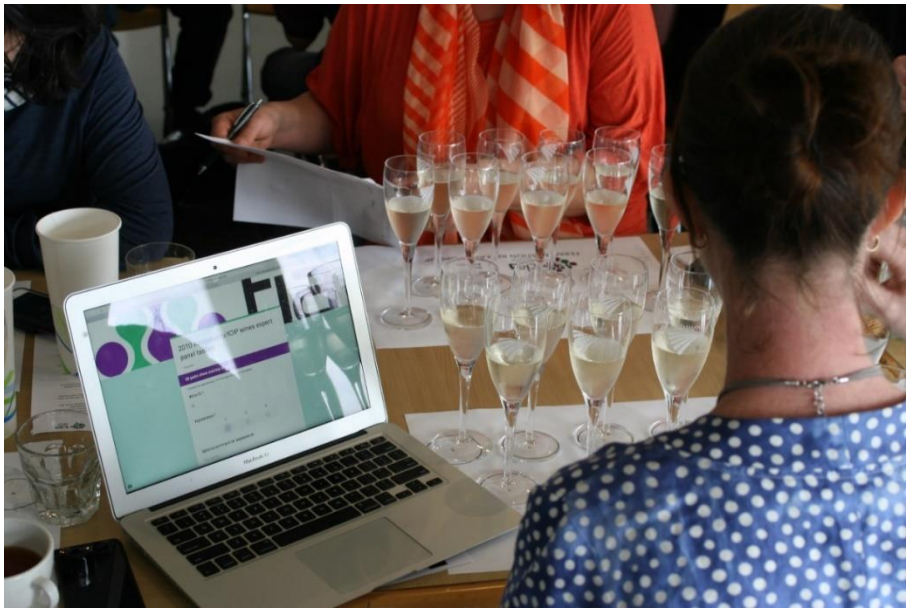


Figure 5-9 Industry expert tasting panel member at the 2016 Effervescence Technical Day

Basic wine matrices were analysed by OenoFoss® at Josef Chromy Wines. Wines were also analysed by UV-Vis SPF as per the method outlined in Kerslake et al. (2018) for validation in degassed sparkling wines. A separate, unopened bottle was sent to the AWRI in Adelaide for analysis of odour compounds outlined in

Table 5-2 and summarised as:

- Aged or oxidised compounds (Mayr et al., 2015), with the inclusion of 2-methylbutanal
- Varietal thiols (Capone et al., 2015)
- Polysaccharides (McRae et al., 2017)
- Proteins (Culbert et al., 2017)
- Norisoprenoids (adapted from Kwasniewski et al. (2010), see 12.1 for further detail)
- Fermentation products (i.e. esters, carboxylic acids and higher alcohols) was performed according to Siebert et al. (2005) with some modifications as outlined in (Sawyer et al., 2019).
- Free aroma (adapted to wine from grapes as performed in Perestrelo et al. (2011), see 12.1 for further detail)

BevScan analytical data was analysed with Unscrambler X, version 10.4.1 (Camo, Norway). All other data was analysed with R 3.3.1 (R Core Team 2016) using ANOVA. When significant, multiple comparisons between treatment means were made on Tukeys HSD using 'agricolae' package (de Mendiburu, 2019).

Table 5-2 Odour descriptors, odour detection thresholds and codes for compounds analysed throughout project.

Code	Compound	Odour descriptor	Odour threshold (µg/L)
		Fermentation products	
Esters			
EE1	Ethyl acetate	Ethereal, fruity	7.5 (mg/L)[†]
EE2	Ethyl propanoate	Ethereal, fruity, rum	5 (mg/L)[‡]
EE3	Ethyl butanoate	Fruity (apple)	20[†]
EE4	Ethyl hexanoate	Fruity (apple peel)	14[§]
EE5	Ethyl octanoate	Fruity, fat	5[§]
EE6	Ethyl decanoate	Fruity (grape)	200[§]
EE7	Ethyl 2-methylpropanoate	Sweet, rubber	15[§]
EE8	Ethyl 2-methylbutanoate		
EE9	Ethyl 3-methylbutanoate	Fruity	3[§]
EA1	Hexyl acetate	Fruity (apple)	480[‡]
EA2	2-Phenylethyl acetate		
EA3	2-Methylpropyl acetate		
EA4	2-Methylbutyl acetate		
EA5	3-Methylbutyl acetate	Fruity (banana)	30[†]
Carboxylic acids			
CA1	Acetic acid	Sour (pungent, vinegar)	200 (mg/L)[†]
CA2	Propanoic acid		
CA3	Butanoic acid	Rancid, cheese, sweaty	173[§]
CA4	Hexanoic acid	Sweaty	420[§]
CA5	Octanoic acid	Sweaty, cheese	500[§]
CA6	Decanoic acid	Rancid, fat	1000[§]
CA7	2-Methylpropanoic acid		
CA8	(R/S)-2-Methylbutanoic acid		
CA9	3-Methylbutanoic acid		
Higher alcohols			
OH1	Butanol	Alcoholic	590[¶]
OH2	Hexanol	Resin, flower, green (cut grass)	8 (mg/L)[†]
OH3	2-Phenylethanol	Honey, spice, rose, lilac	14 (mg/L)[§]
OH4	2-Methylpropanol	Vinous, solvent, bitter	40 (mg/L)[†]
OH5	2-Methylbutanol	Malty, solvent-like	1.2 (mg/L)[¶]
OH6	3-Methylbutanol	Whiskey, malt, burnt	30 (mg/L)[†]
		Compounds associated with ageing and oxidative flavours	
Higher alcohols			
OH7	Methionol	Cooked potato	1000[§]
OH8	Eugenol (phenolic)	Clove	6[§]
Furanones			
FN1	Homofuraneol	Caramel	10[††]
FN2	Maltol		
FN3	Sotolon	Curry, seasoning	15[‡‡]
Aldehydes			
AH1	Hexanal	Grassy, green	2.4[¶]
AH2	Benzaldehyde	Bitter almond	5 (mg/L)[‡]
AH3	Furfural	Sweet bread	14 (mg/L)[§]
AH4	5-Methylfurfural	Sweet, bitter almond	16 (mg/L)[‡]

Code	Compound	Odour descriptor	Odour threshold (µg/L)
AS1	2-Methylpropanal	Malty	6[§§]
AS2	2-Methylbutanal	Malty	16[§§]
AS3	Methional	Cooked potato-like	0.5[¶¶]
AS4	2-Phenylacetaldehyde	Honey, floral	1[§§]
€-2-Alkenals			
[€]L2	(*E*)-2-Nonenal	Fatty, nutty	0.6[§§]
NL4	(*E*)-2-Heptena€		
NL3	(*E*)-2-Octenal		
	Norisoprenoids		
Norisoprenoids			
NP1	B-Damascenone	Fruity	
NP2	α-Ionone		
NP3	β-Ionone		

[†] Determined in 10% w/w aqueous ethanol (Guth 1997); [‡] determined in 14% ethanolic solution (Moreno et al. 2005); [§] determined in 11% v/v aqueous ethanol, 7 g/L glycerol and 5 g/L tartaric acid at pH 3.4 (Ferreira et al. 2000); [¶] determined in water (Czerny et al. 2008); [††] determined in 11% v/v aqueous ethanol and 4 g/L tartaric acid at pH 3.5 (Kotseridis, et al. 2000); [‡‡] Determined in white wine (Martin et al. 1992); [§§] determined in 10% v/v aqueous ethanol and 5 g/L tartaric acid at pH 3.2 (Culleré et al. 2007); and [¶¶] determined in 11% v/v aqueous ethanol, 7 g/L glycerine and 5 g/L tartartic acid at pH 3.4 (Escudero et al. 2000).

5.2 Autolysis and autolytic character

The second component of this project was co-designed with leading sparkling winemakers who wished to better understand the development of the hallmark characteristics of premium sparkling wines. The term 'autolytic character' has frequently been used as a catch-all phrase, however the winemakers had observed there was more complexity to the development of this character than the sole contribution of autolysis by-products. The investigation of the ageing of base wines on and off their lees, in comparison to the sparkling wine was designed to pull apart where 'aged character' and 'autolytic character' came from; from the wine ageing or autolysis, or a combination of these? There was also a desire to understand if these characters could be influenced by winemaking practices, potentially providing greater control over the expression of these characteristics and the amount of time in bottle to achieve the desired level of these characteristics. Methods to hasten development of aged and autolytic character could reduce costs for sparkling winemaking, including; capital required for storage, energy use for temperature controlled time *en tirage* and other overheads associated with holding stock for prolonged periods.



Figure 5-10. The project was co-designed and co-developed between industry and the research team (including Dr Andrew Pirie, Apogee Tasmania – left, Dr Fiona Kerslake, TIA – middle, Natalie Fryar, formerly Hill-Smith Family Vineyards and currently Bellebonne – right)

5.2.1 Winemaking

Two commercial mono-cultivar base wines (*Vitis vinifera* L. cv. Chardonnay and Pinot Noir) were made by Hill-Smith Family Vineyards winery at Angaston, South Australia (Figure 5-11) and lees from the primary ferments retained as outlined in (Sawyer et al., 2021). Eight wines were made from each varietal base wine, in triplicate, comprising of the following treatments:

1. Base wine – no lees, stored at 15 °C
2. Base wine – primary lees added back, stored at 15 °C
3. Control – standard *liqueur de tirage*, stored at 15 °C
4. High storage temp – standard *liqueur de tirage*, stored at 25 °C

5. Ultrasound treated yeast addition – standard *liqueur de tirage* + ultrasound treated yeast, stored at 15 °C
6. Microwave treated yeast addition – standard *liqueur de tirage* + microwave treated yeast, stored at 15 °C
7. Enzyme treated yeast addition – standard *liqueur de tirage* + β-glucanase treated yeast, stored at 15 °C
8. CHA Spike – standard *liqueur de tirage* + highly 'autolytic' Chardonnay (high lees volume, in barrel battonaged wine) addition at disgorging, stored at 15 °C

Wines were tiraged by the Hill-Smith Family Vineyards team in Angaston (Figure 5-11), with treatments applied at this point (



Figure 5-12). Wines were freighted to the TIA laboratories in Launceston, Tasmania for ageing and analysis.



Figure 5-11. Hill-Smith Family Vineyards winery team assisting with small-scale tirage.



Figure 5-12. PhD candidate Gail Gnoinski, adding yeast treatments with Dr Alanna Seabrook at Hill-Smith Family Vineyards, Angaston, South Australia.

5.2.2 Analysis

Wines were analysed at 6-, 12-, 18-, and 24-months post-tirage. This deviated from the original project plan due to an analytical error at the 18-months post-tirage timepoint resulting in an incomplete dataset at this time, which was excluded from data analysis when necessary. In consultation with collaborating winemakers, the proposed 36-month time point was reduced to 24 months, which remained in line with industry timeframes for ageing of sparkling wine. The eighth treatment (autolytic CHA spike described in 5.2.1) did not appear to be distinct from the control based on the analyses conducted, it was excluded from all analyses except from the unsupervised analysis in 6.2.3 (Objective measure of sparkling wine).

Wines were analysed in-bottle by BevScan as described by Dambergs et al. (2015), then tasted by an industry panel of experts for a comparative sensory analysis using a tasting rubric designed in conjunction with Dr Wes Pearson (AWRI), winemakers and the research team (Figure 5-14).



Figure 5-13 (L-R) Karina Dambergs, Dr Andrew Pirie, Curly Haslam-Coates, Ed Carr and Jeremy Dineen tasting autolysis trial wines at 6 month time point in 2017 at Josef Chromy Wines.

Flight 1	Wine 1	Wine show score (out of 20)					
Flight 1	Wine 2	Wine show score (out of 20)					
		Does this wine appear more or less '**autolytic**' than the control?	-10 | Much less	-5 Less	0 Same as control	+5 More	+10 | Much more
		Does this wine appear more or less '**nutty**' than the control?	-10 | Much less	-5 Less	0 Same as control	+5 More	+10 | Much more
		Does this wine appear more or less '**toasty**' than the control?	-10 | Much less	-5 Less	0 Same as control	+5 More	+10 | Much more
		Does this wine display more or less '**honey**' than the control?	-10 | Much less	-5 Less	0 Same as control	+5 More	+10 | Much more
		Does this wine appear more or less '**spicy**' than the control?	-10 | Much less	-5 Less	0 Same as control	+5 More	+10 | Much more
		Does this wine appear more or less '**earthy**' than the control?	-10 | Much less	-5 Less	0 Same as control	+5 More	+10 | Much more

Figure 5-14 Tasting rubric used for comparative sensory analysis. Wine 1 was always the control (standard *liqueur de tirage*, stored at 15 °C) and the treatment wines were compared to wine 1 for the six attributes listed.

Wines were analysed for pH, titratable acidity (TA) and UV-Vis SPF (Kerslake et al., 2018), and a separate, unopened replicate bottle was sent to the AWRI for further analysis. Fermentation products and aged or oxidative off-flavour compounds, 46 analytes in total, were identified and quantified according to Siebert et al. (2005) and Mayr et al. (2015) with some modifications as described in (Sawyer et al., 2019). For the Siebert et al. (2005) method, the samples were heated to 40°C for 5 mins

with agitation initially and the injector temperature was 260°C. The oven temperature started at 40°C and increased to 60°C at 20 °C/min (held for 14 mins). Following this was a series of temperature ramps. First ramp to 70 °C at 10 °C/min, second ramp to 80 °C at 10 °C/min, third ramp to 160 °C at 20 °C/min, and final ramp to 260 °C at 10 °C/min and held for 2 mins. Total run time was 45.5 mins. 2-Methylbutanal was also identified and quantified along with the other aged or oxidative off-flavour analytes described in Mayr et al. (2015).

Dr Dambergs analysed BevScan data using Unscrambler X, version 10.4.1 (Camo, Norway) for Section 6.2.2 (Manipulating aged character/autolysis).

University of Tasmania PhD candidate, Mr Leo Lebanov, analysed BevScan analytical data using R software using the "*mdatools*" and "*randomForest*" libraries as well as the metaboanalyst.ca online R software package for Section 6.2.3 (Objective measures of sparkling wine). For principal component analysis (PCA), the analysis was performed on raw and pre-processed data (by multiplicative scatter correction (MSC) and signal transformation by Savitzky-Golay 1st derivative (SG-1)). For random forest (RF) and partial least squares-discriminant analysis (PLS-DA), the data was pre-processed using MSC and SG1 and Savi^{tz}ky-Golay 2nd derivative (SG2). Double cross-validation of the RF and PLS-DA models was performed by dividing the sample set randomly by 80% of sample set for creating the model and 20% as a test set for validating the model. For RF, resampling was repeated 50 times to calculate overall error of prediction in creating the model and sensitivity for prediction in the test set. Whereas for PLS-DA, the model was created on 80% of the sample set, with random cross-validation with 10 segments. The process was repeated 50 times to calculate average sensitivity and standard deviation for all 50 models. Overall sensitivity achieved in the test set was used to evaluate performance of the PLS-DA models.

Dr Samantha Sawyer analysed all other data using JMP v14.0 (SAS Institute, Cary, NC, USA) by the methods previously described in Sawyer et al. (2019).

5.2.3 Visualising and manipulating autolysis

With the support of a Wine Australia PhD scholarship, another aspect of investigation was added to the autolysis and autolytic character component of this project. PhD candidate, Ms Gail Gnoinski, used the wines described in 5.2.1 for foaming parameter analysis following the method of Condé et al. (2017) in collaboration with Dr Kate Howell and her team at the University of Melbourne (Gnoinski et al., in prep), and for visualisation of what happens to yeast during autolysis. Use of adjuvant in the commercial *liqueur de tirage* prevented successfully viewing the yeast using scanning electron microscopy, so Ms Gnoinski produced another set of wines with similar treatments and also applied flow cytometry as published in Gnoinski et al. (2021b).

Ms Gnoinski also collaborated with Dr Mitchell Gibbs at the Sydney Institute of Marine Science for lipids analysis of the second set of wines (Gnoinski et al., in prep) and had amino acid composition analysed at Metabolomics Australia, and protein concentration quantified by AWRI Commercial Services, as published in Gnoinski et al. (2021a).

5.3 Objectively measuring juice phenolics in-line

With key collaborator, Josef Chromy Wines (Relbia, Tasmania), a quartz window and a sample port were installed in the manifold of their Bucher XPert 250 pneumatic press (Figure 5-16). This setup allowed a series of juice samples to be taken and be subjected to immediate analysis using a portable Jaz UV-Vis spectrometer (Ocean Optics, Florida, USA, Figure 5-17). The portable spectrometer allowed the SPF method (Kerslake et al., 2018) to be applied in real-time at the press (Figure 5-15). After a successful first season trial on the Josef Chromy Wines' Bucher press, a probe was developed to house

the fibre optic cable for use in the EuroPress, which features a wide juice pan (Scharfenberger, Germany).

Juice samples were also analysed for conductivity (DiST® 6, Hanna Instruments, Rhode Island, USA) before being cooled in an eski until the press cycle finished, then refrigerated for additional analysis the following day (usually 8-10 hours). Juice analysis comprised of total soluble solids (TSS), titratable acidity (TA), pH and SPF. A further sub-sample was frozen for later total phenolics analysis by ultra-performance liquid chromatography (UPLC). In the 2018 vintage, small batch wines were also made (Figure 5-20, Figure 5-21 and Figure 5-22).

An additional season of juice samples were collected when Dr Fiona Kerslake travelled to Ontario, Canada. She was hosted by Dr Belinda Kemp at the Brock University, Cool Climate Oenology and Viticulture Institute (CCOVI) and an additional array of analyses were conducted investigating cation and organic acid composition of those juice samples, as presented at the 17th Australian Wine Industry Technical Conference (Kerslake et al., 2019).

Dr Rocco Longo was successful in gaining a Science and Innovation Award for Young People by the Federal Department of Agriculture and Water Resources for a sub-project within this Wine Australia funded research project. Dr Longo's subproject was titled 'A new tool for in-line and real-time grape juice assessments'. This grant allowed Dr Longo to work with local manufacturing and engineering expertise to develop a second, more portable iteration of the in-line sensor (Figure 5-23), despite the challenges presented by COVID-19 and difficulties sourcing parts. These delays and restrictions meant the second iteration was not ready in time for testing in a commercial winery during vintage. Consequently, Dr Longo utilised frozen juice samples later in the year to test the equipment. This second iteration of the inline sensor trialled additional important functionality of being able to automatically switch tanks. Automated switching would mean real-time sensing results could be used to direct the cut off from *cuvee* (high grade juice) to *taille* (lower grade juice).



Figure 5-15. Full setup required for first iteration of the in-line sensor



Figure 5-16. First iteration of the in-line sensor to develop the proof of concept installed at Josef Chromy Wines.

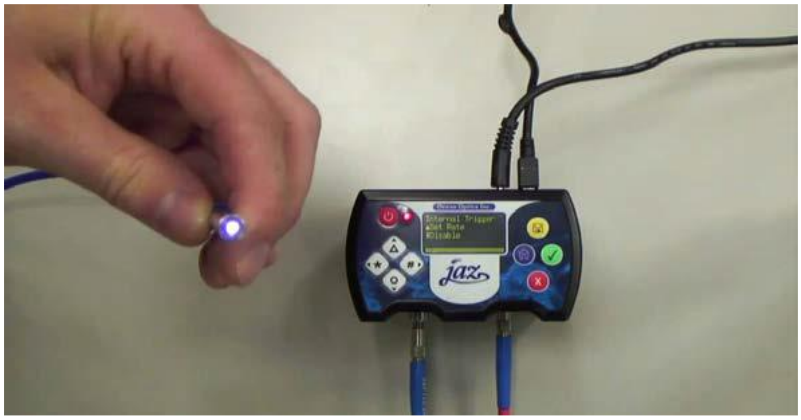


Figure 5-17. UV-VIS-NIR spectrophotometer (Jaz Series, OceanOptics, Florida, USA)



Figure 5-18 Adaptation to the EuroPress open tray with a probe



Figure 5-19 **Juice samples for Ultra Performance Liquid Chromatography (UPLC) analysis**



Figure 5-20 **Pinot noir juice samples for base wines from free run to hard pressing (left to right)**



Figure 5-21 **Small-scale winemaking under temperature-controlled conditions**



Figure 5-22 **Small-scale wines**



Figure 5-23 **Second iteration of portable, in-line sensor which could be fitted to any press**

5.4 Juice provenance

5.4.1 Juice sampling

The objective of this research was to see if there were regionally distinct phenolic profiles discernible in juices used for production of Australian sparkling wine. At the outset of this project, a number of sparkling wine producers were contacted to participate in the collection of juice samples from commercially pressed Chardonnay, Pinot noir and Pinot Meunier fruit. It was requested that 50 mL juice samples be taken before fining and/or additions to the juice, and that the sample be frozen directly. The frozen samples were then to be shipped at the conclusion of vintage. Producers were asked to supply the following information for each juice sample they collected:

- Grape variety
- Region grapes were harvested in
- If grapes were hand or machine harvested
- Baume of grapes
- pH of grapes
- TA of grapes
- Press fraction (*cuvee* or *taille*)
- Date of sample collection

For the 2017 vintage, 148 juice samples were collected from five wineries across Tasmania, Victoria and South Australia with each winery providing juice samples from a range of grape growing regions. Frozen samples were thawed and analysed in the TIA laboratories for pH, total soluble solids (TSS) and spectral phenolic fingerprint (SPF) (Kerslake et al., 2018). A sub-sample was taken from each of the 148 juices, refrozen and later analysed by near-infrared (NIR) spectroscopy at the UTAS Central Science Laboratory. It was determined that samples from 2017 were unable to be analysed statistically in the manner required for this project, as they had thawed during transportation and had not been adequately labelled by the participating wineries. Therefore, the data and findings for 2017 are not reported. That year's juice collection did, however, support a refined juice sampling protocol for 2018 vintage.

For the 2018 vintage, the research team benefitted from the contribution of visiting Plumpton College MSc student, Hugh McCullough, who focussed on the Tasmanian samples for his research project (McCullough, 2019). In that vintage, 175 juice samples were received in good condition, but 70 of those were excluded from analysis as they were either field blend samples, the sample was a *taille* sample (this analysis was only applied to *cuvee*), or fruit had been machine picked. These decisions were based on results from the 2017 analysis that had shown machine harvesting was associated with poor differentiation between *cuvee* and *taille* juice samples, possibly due to variable phenolic pick-up or juice oxidation during handling and transportation of machine harvested fruit from vineyard to pressing facility.

Groups of samples were separated by winery and by press cycle where possible in order to minimise interference from different handling processes (see Table 3.1). Winery 1 processed the majority of samples analysed. It is a large facility (for Tasmania) and processes fruit for a number of clients that originates from all around the state, except from the East Coast. As a result, most displayed results are from Winery 1 and originate in three of the four largest producing regions (i.e. missing the East Coast).

Several samples were removed from certain sets so that picking technique (hand) and press fraction (cuvée) were the same. No statistical analysis was run on taille samples as there were too few to be representative or relevant. Winery 3 provided only 4 samples, all falling into different categories; they

were not used. The trial was originally designed to enable industry participation, with a very applied nature so as to increase engagement with the process and outcomes. This led in turn to some of the inconsistencies described.

For the 2019 vintage, the protocol for this sub-project was amended to eliminate some of the variables observed in 2017 and 2018. In 2019, Dr Rocco Longo sampled 10 kg batches of fruit from picking bins ready for processing at Josef Chromy Wines. This fruit was pressed individually in a small-scale flat-bed press (Figure 5-5) and then the juice sampled. This resulted in 17 Chardonnay and 16 Pinot noir samples, in triplicate, from four regions (Tamar Valley, Pipers Brook, Coal River Valley and North-West Tasmania). This juice was not analysed during the lifetime of this project due to the team running out of time.

The outcomes of 2017 and 2018 juice sampling supported development of the above-described method for collecting samples that might show regional variation. Refinement of the sampling protocol was a useful learning from this project, which could inform future research into juice provenance fingerprinting via spectral methods.

5.4.2 Juice spectrophotometry

(abridged from McCullough, 2019)

Frozen samples of Chardonnay and Pinot noir juice were thawed overnight at 4℃ prior to initial analysis at the Tasmanian Institute of Agriculture's Mount Pleasant Laboratories, Launceston. Samples from the 2018 vintage were analysed in May-June of that year.

Samples were centrifuged using a 5804 Eppendorf (Hamburg, Germany) at 3350 radial centrifugal force (RCF) for 15 minutes for clarification. Measurements were taken for pH and total soluble solids (TSS) by autotitrator and digital refractometer respectively.

A proportion of each sample was returned to storage at -18℃ for Near-Infrared analysis at a later date.

UV- Vis Analysis

Following analysis of pH and TSS, temperature-equilibrated samples were diluted 1:5 with 1M HCl (Merck, Darmstadt, Germany) and dark incubated for one hour at ambient laboratory temperature (22℃). Samples were analysed using a Genysys 10S UV-Vis scanning spectrophotometer (Thermo Scientific, Waltham, MA, USA with an absorbance reading taken every 2 nm from 200 to 600 nm inclusive for the spectral phenolic fingerprint (SPF). Samples were analysed using disposable 10mm quartz cuvettes (Brand-GMHB, Wetheim, Germany), so wavelengths below 250 nm were discounted due to interferences.

NIR Analysis

Frozen samples of Chardonnay and Pinot noir juice were thawed overnight at 4℃ and then equilibrated to laboratory temperature (22℃) for approximately one hour prior to analysis at the University of Tasmania Central Science Laboratory, Hobart. A Bruker MPA instrument (MPAII, Bruker, Karlsruhe, Germany) was used to scan the samples, with no prior centrifugation, which consisted of a fibre-optic probe with a 0.23mm sample measurement gap before a reflective metal tip. The probe houses an interferometer with a gold-plated cube corner mirror and CaF_2 beam splitter. Light is reflected by the samples and generation of an interferogram occurs by a gold-plated sphere and a semiconductor detector over sixty-four scans and a resolution of eight wave numbers. The interferogram is then converted to frequency by Fourier transform and reported as absorbances corresponding to

wavenumbers between 4000 and 12,500 cm^{-1}. OPUS spectroscopy software (OPUS, 2006, Stuttgart, Germany) was used to pre-process the spectra, for conformity testing and for data presentation.

Background environment scans were performed prior to sample analysis and then repeated every hour to ensure consistent operation of the instrument. Full scans were taken and collected by inserting the fibre-optic probe directly into the samples (c.50ml), as the sample is being homogenized by a magnetic stirrer. Between each reading, the probe was cleaned with deionized water, dried and then pre-rinsed with the next sample to be analysed, in order to avoid dilution with either water or juice.

5.4.3 Statistical analysis

(abridged from McCullough, 2019)

Analysis of Variance (ANOVA) testing was used in order to assess statistical differences between regions in total phenolic content (TPC), total hydroxycinnamate content (THC), pH, titratable acidity (TA) and sugar level (°Baumé) (Hair et al., 2014; Dytham, 2003). Residuals plots were consulted to confirm the suitability of the data to the test. TPC and THC were calculated by using the standard absorbance measurements at 280 and 320nm respectively (Mercurio et al., 2007; Somers and Ziemelis, 1985; Aleixandre-Tudo et al., 2017; Kerslake, Longo and Dambergs, 2018; Iland, 2013).

Principal Component Analysis (PCA) was used as a multivariate test to assess the relationships between regions among multiple dependent variables (Hair et al., 2014; Dytham, 2003). Pre-processed spectral data, stored as OPUS files, was imported into The Unscrambler X software (ver. 10.4.1, Camo, Norway, 2016) for PCA analysis. Data was evenly weighted and cross-validated with 20 segments and 2 samples per segment. PCA was performed using the Non-linear Iterative Partial Least Squares algorithm (NIPALS).

6 Results and Discussion

6.1 Viticulture

6.1.1 Weather

The two growing seasons for the viticultural trials could generally be characterised as warm and dry (2010) and cool and wet (2011) as shown in Table 6-1. The growing degree days were very similar between the northern and southern trial sites, and around 200 GDD warmer in 2010 compared to 2011. At the northern trial site, this translated to 1 °C warmer growing season temperature (GST). At both sites, mean January temperature was relatively similar, but in cool climates mean February temperature is generally more relevant as veraison most often occurs during this month and 2011 was markedly cooler than 2010 at both trial sites.

Table 6-1 Seasonal weather conditions during viticulture trials (2010 and 2011 seasons)

Site	Vintage	GDD (Sep-Mar)	GST (Sep-Mar)	Rainfall (Sep-Mar)	MJT (°C)	MFT (°C)
North	2010	1190.0	15.6	414.0	17.8	19.6
	2011	983.6	14.6	676.0	17.6	16.5
South	2010	1150.5	15.2	349.4	17.5	18.0
	2011	987.2	14.5	372.4	17.7	16.5

Figure 6-1 provides a visual representation of the cool finish to the 2011 season at the northern trial site as the downward slope indicates the deviation from the long term (8 year) mean from mid-February onwards. A cool finish to December is also evident, whereas the relatively parallel line during January represents the similarities in mean January temperature (Table 6-1).

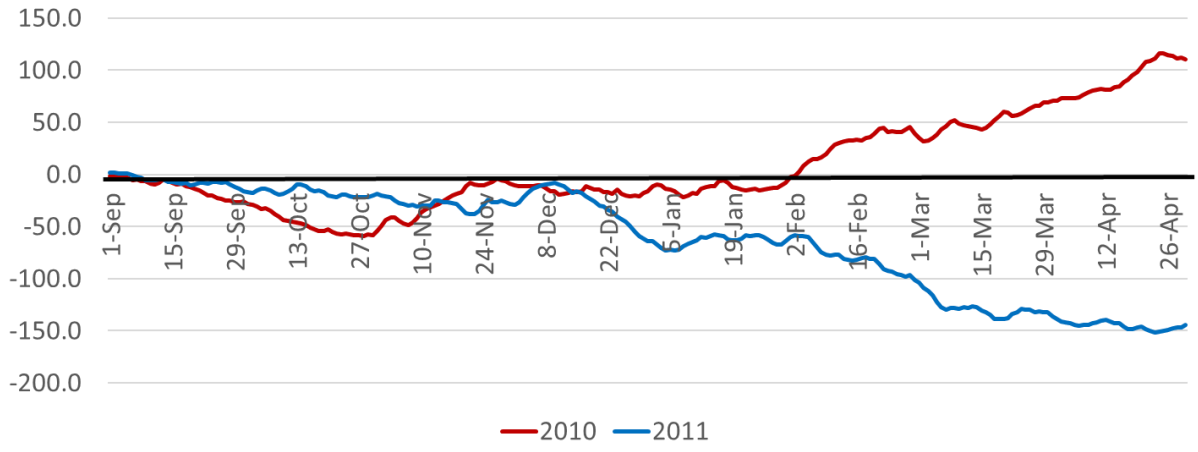


Figure 6-1 Growing degree day accumulation at the northern trial site throughout 2010 and 2011 season in comparison to the eight-year mean

6.1.2 Crop load

6.1.2.1 Fruit

Unsurprisingly, Pinot noir crop load adjusted by winter pruning produced significantly different yields between the two treatments, with yield being more than doubled in both seasons when leaving 60 nodes per vine (high), compared to 10 nodes per vine (low) (Table 6-2). Both treatments were harvested on the same day in each season, with reduced total soluble solids in the high crop load in both years, and lower pH in 2011. The higher yields in 2011 were as a result of the ideal weather conditions at flowering in 2010 (Nov-Dec), with temperatures similar to the long-term average (Figure 6-1).

Table 6-2 Pinot noir crop load trial yield, total soluble solids (TSS), pH and titratable acidity (TA)

	Yield (t/ha)		TSS (°Be)		pH		TA (g/L)	
	2010	**2011**	**2010**	**2011**	**2010**	**2011**	**2010**	**2011**
Low	5.5 a*	8.2 a	10.5 a	10.9 a	2.97	3.27 a	13.1	12.0
High	13.3 b	18.4 b	10.0 b	10.2 b	2.96	3.17 b	12.6	12.9

***** different lowercase letters across rows represent significant difference by post-hoc analysis using Tukey's HSD (*P<0.05*), within each season for each parameter.

6.1.2.2 Base wines spectral phenolic fingerprint (SPF)

Prior to making the trial wines, all Botrytised fruit was intended to be discarded, but it is clear from Figure 6-2 this was not completely successful as demonstrated by the high crop load outliers circled, and also these wines had a distinctly marmalade aroma character. The clear primary separation of the wine data points in Figure 6-2 is the vintage, with the 2010 wines all clearly on the left of the plot and the 2011 wines on the right. These data points were generated from almost 300 wavelength absorbance readings from the UV-Visible spectrophotometer, or the spectral phenolic fingerprint (SPF) (Kerslake et al., 2013, Kerslake et al., 2018). The wavelengths driving this vintage separation are indicative of low molecular weight phenolics, such as hydroxycinnamates, due to loadings around 260 and 330 nm (Kerslake et al., 2013, Kerslake et al., 2018).

When analysed by PCA, the strongest driver of the clustering of wines into groups of similarity was season (Figure 6-2), which reflected the difference in weather during the two seasons (Table 6-1). Within each seasonal cluster of the wines, clear secondary clusters of treatments with vertical separation indicated a consistent treatment effect, albeit it far more pronounced in the warm and dry 2010 season compared to the cool and wet 2011 season.

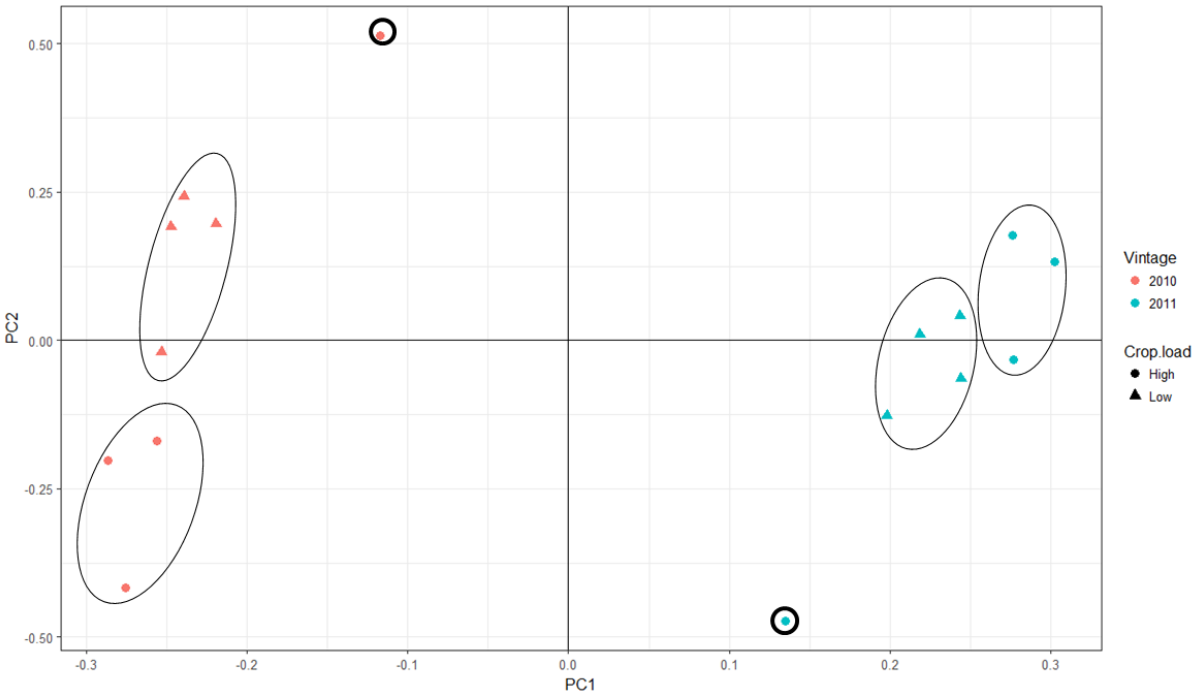


Figure 6-2 SPF of Pinot noir base wines from crop load trial

6.1.2.3 Aged sparkling wine basic composition

Wines were not formally tasted when base wines, rather they were tiraged and then aged on lees for 5.5 years for evaluation as an aged sparkling wine and as can be seen in Figure 6-3, little effect of treatment on sparkling wine composition was observed, rather vintage had more of an effect, similar to the base wines.

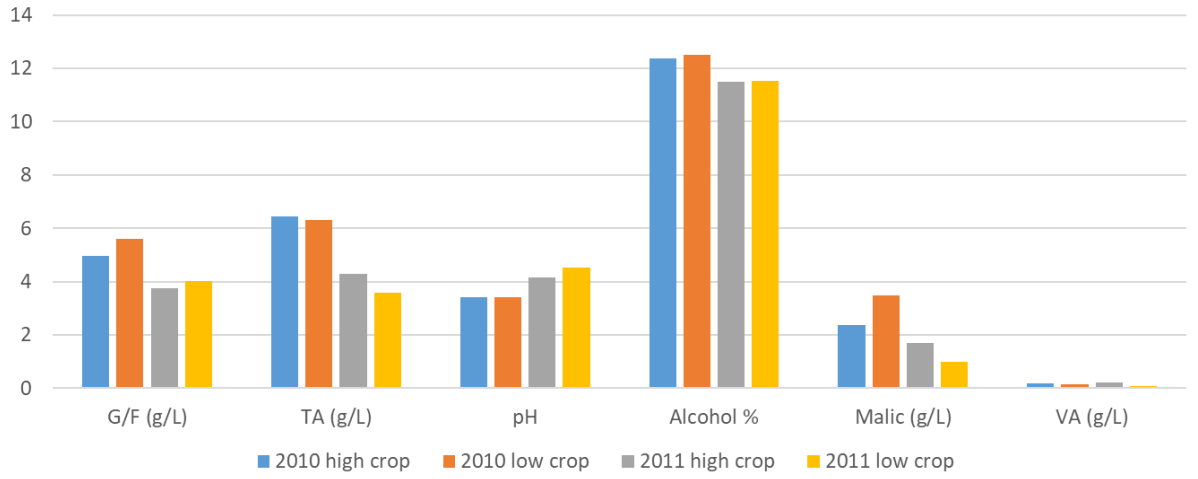


Figure 6-3 2010 and 2011 Pinot noir sparkling wine analyses in 2016 and 2017 respectively, after 5.5 years on lees. Ratio of glucose:fructose (G/F), titratable acidity (TA), pH, alcohol, malic acid and volatile acidity (VA)

6.1.2.4 *Aged sparkling wine spectral phenolic fingerprint (SPF)*

When the SPF's of the aged sparkling wines were analysed, a very similar, distinct separation was observed in the 2010 wines, with much less of a difference in the 2011 wines from that cool and wet season. The research team acknowledge the primary limitation of this work, being the small sample size, but this indicated that the difference between wines from high and low crop loads from a warm and dry were still evident after 5.5 years ageing on lees, however this was not the case for the wines from the 2011 vintage. As for the base wines, it is most likely that hydroxycinnamates were driving the differences between the wines from 2010, given the similarities between the SPF of caffeic and ferulic acids and the 2010 high crop load wines as reported in Kerslake et al. (2018).

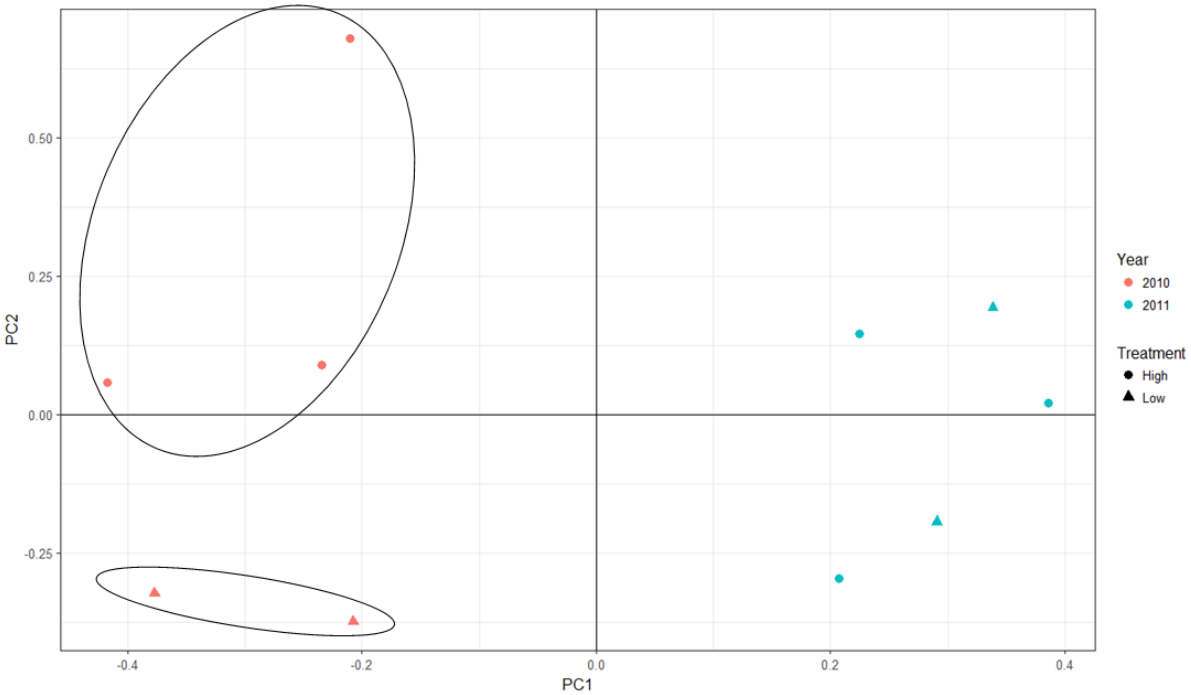


Figure 6-4 Spectral Phenolic Fingerprint (SPF) of Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years

We measured coumaric and caftaric acids in these wines (Table 6-3), from both vintages, and higher levels of coumaric acid were recorded in the 2010 wines as compared to the 2011s, however there was no crop load treatment effect.

Table 6-3 Phenolic acid analysis of the Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years

	2010		2011		Vintage (V)	Treatment (T)	V*T
	Low	High	Low	High	p-value	p-value	p-value
Caftaric acid (mg/L)	29.51	18.10	9.51	13.90	0.077	0.400	0.180
p-Coumaric acid (mg/L)	0.61	0.59	0.25	0.20	0.000	0.373	0.788

6.1.2.5 *BevScan*

BevScan proved to be a useful tool to discriminate treatments by scanning the wines in-bottle without destructively sampling. Pinot noir crop load treatments (high or low crop load) were also discriminated by BevScan, although with less separation between treatments, partially due to the high number of bottles scanned for the high crop load treatment (Figure 6-5). Three principal components were required to drive this clustering, combined this explained 85 % of the variability in the data.

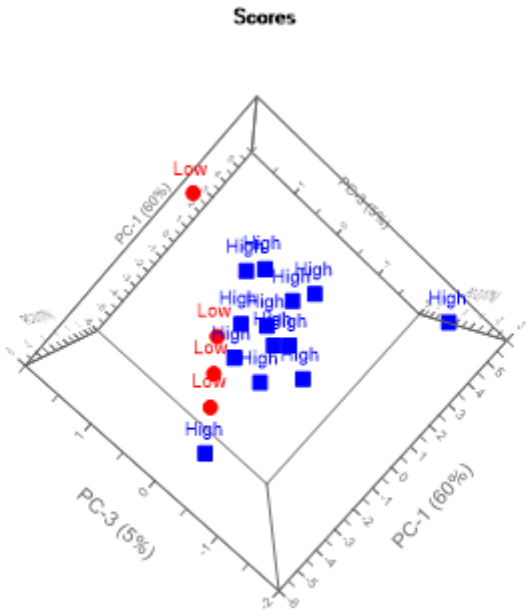


Figure 6-5 Principal component analysis of the BevScan in-bottle scanning data from the Pinot noir crop load trial.

6.1.2.6 *Polysaccharides*

Polysaccharides were analysed in the wines, and greater totals were observed in the low crop load 2010 wines when compared to the high crop load wines from 2010, however in the cool and wet 2011 season the inverse was observed with high crop load wines recording higher total polysaccharide content. Interestingly, the high crop load wine total polysaccharide content was similar between both years.

The high (~185 kDa), medium (~24-48 kDa), and low (~5.8 kDa) molecular weight polysaccharides approximately correspond to yeast mannoproteins, arabinogalactan proteins, and rhamnogalacturonans respectively (McRae et al., 2017, Bindon et al., 2013b). Only the high molecular weight fraction showed a difference related to crop load and mannoproteins have been shown to affect volatility of wine aroma and flavour compounds (Chalier et al., 2007) and accumulation of phenolic acids, such as p-coumaric acid (Sartor et al., 2019). While cropping had an effect, the concentrations of high molecular weight polysaccharides were generally higher in 2010 compared to 2011 and were associated with a similar increase in p-coumaric acid (Table 6-3). The overall

differences in high molecular weight polysaccharides indicate that the changes in juice composition due to the vintage and cropping treatment may have influenced fermentation and yeast autolysis.

Table 6-4 Polysaccharide analysis of the Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years.

	2010		2011		Vintage (V)	Treatment (T)	V*T
	Low	High	Low	High	p-value	p-value	p-value
High molecular weight polysaccharides (mg/L)	44.91 a	40.23 ab	15.37 c	25.56 bc	<0.001	0.042	0.030
Medium molecular weight polysaccharides (mg/L)	44.73	36.36	25.85	43.16	0.184	0.121	0.110
Low molecular weight polysaccharides (mg/L)	17.92	15.92	12.69	23.65	0.997	0.146	0.175
Total poly–accharides - (mg/L)	113.64	98.59	60.00	98.44	0.043	0.096	0.091

6.1.2.7 Proteins

Thaumatin-like proteins and chitinases, arising from grapes, persist following the fermentation process and both can play a major role in haze formation in white wines (Marangon et al., 2014, Marangon et al., 2011). The 'low' crop load wines in each season showed significantly higher concentration of total proteins than 'high' crop load treatment wines, driven by thaumatin-like proteins (Table 6-5Table 6-5). Although proteins are generally found in low quantities in wines, they contribute to the body, sweetness, roundness, and mouthfeel of sparkling wine (Gawel et al., 2014). Aroma compounds are better retained when proteins are at higher levels, and astringency reduced (Alexandre and Guilloux-Benatier, 2006). Importantly for sparkling wines, foam properties are improved in sparkling wines with higher protein content due to surfactant qualities (Gawel et al., 2014, Andres-Lacueva et al., 1997, Kemp et al., 2015)

Table 6-5 Protein analysis of the Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years

	2010		2011		Vintage (V)	Treatment (T)	V*T
	Low	High	Low	High	p-value	p-value	p-value
Total protein (mg/L)	41.32 a	10.72 b	52.33 a	22.17 ab	0.299	0.046	0.870
Thaumatin-like proteins (mg/L)	38.62 a	9.19 b	45.00 a	19.17 ab	0.416	0.045	0.965
Chitinases (mg/L)	2.70	1.53	7.33	3.00	0.036	0.075	0.157

6.1.2.8 Aged wine volatiles analysis

The next stage to understanding the influence of crop load on sparkling wines was to analyse the sparkling wines and have industry taste them. Compounds which were identified as being potential contributors to sparkling wine character included those which are indicators of oxidation, or ageing, norisoprenoids and products of fermentation such as higher alcohols, acids and esters. Figure 6-6 is the output of principal component analysis (PCA) of these compounds which were detected in the wines, reflecting again the more distinct separation of the high and low crop load sparkling wines from the warm and dry 2010 vintage (pink), as opposed to the cooler and wetter 2011 (blue). Not all compounds measured are favourable contributors to wine character, however as a starting point for research of this kind, the research team chose to work with known compounds.

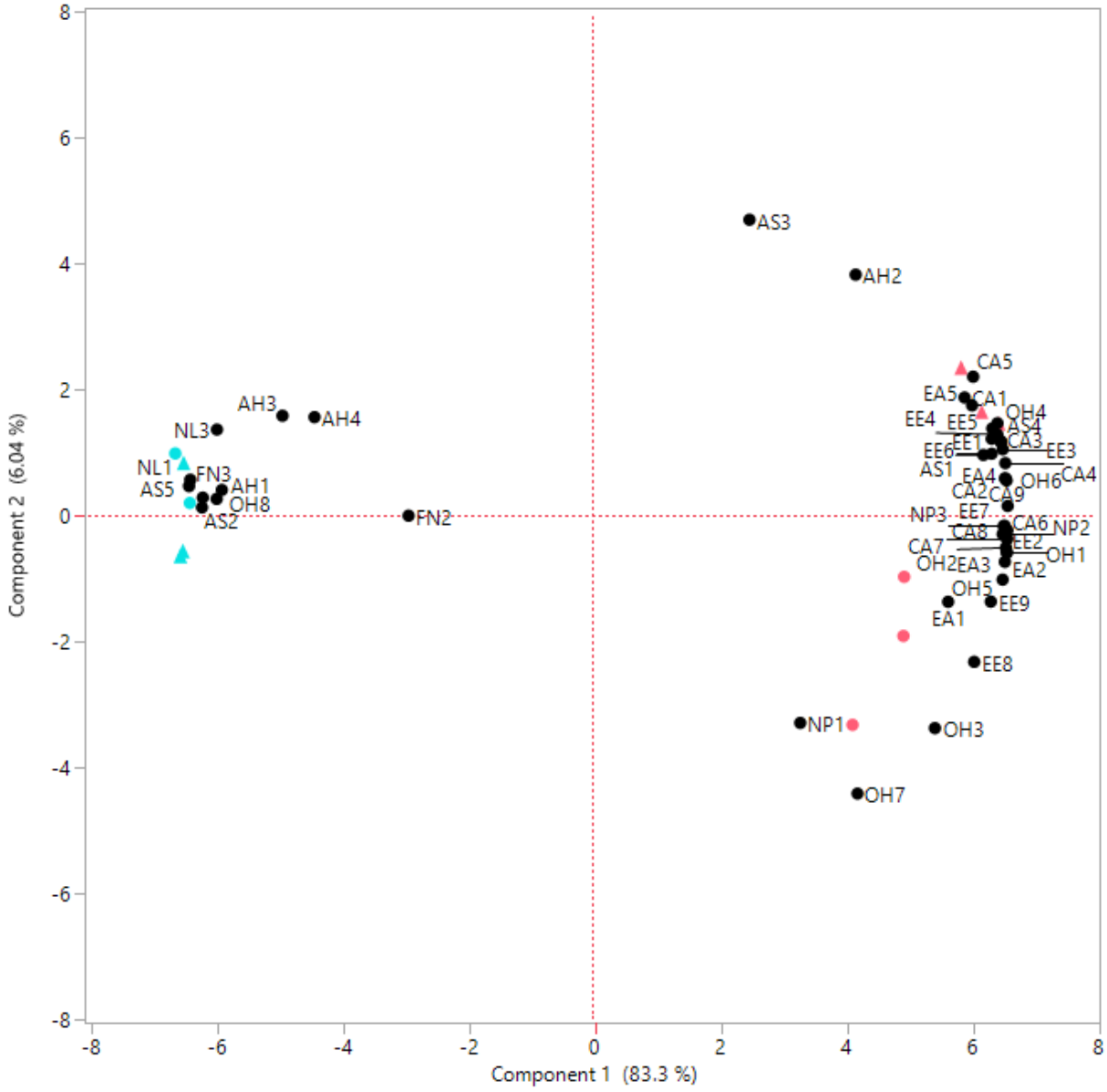


Figure 6-6 Volatile compound analysis PCA biplot with scores and loadings for Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years. Vintage 2010 is in pink, 2011 is in blue. High crop load is circles, low crop load is triangles. The key to the loadings is in Table 6-6.

During aging, ester concentrations vary according to the nature, branching and length of the side chains. Generally, ethyl esters of branched chain acids increase; acetate esters decrease; and ethyl esters of straight chain acids respond in variable ways (Pozo-Bayón et al., 2009, Ruiz-Moreno et al., 2017, Welke et al., 2014, Torrens et al., 2010, Makhotkina et al., 2012, Martínez-García et al., 2017). The pattern of esters suggests 2010 wines (warm and dry season) were more developed than the 2011 wines (cool and wet season) over the same aging period.

Compounds associated with oxidative flavours separated according to vintage as well. The Strecker aldehydes, 2-methypropanal and phenylacetaldehyde were present in higher concentrations in the 2010 wines. These impart malty, honey and floral aromas respectively (Mayr et al., 2015). In contrast, the (E)-2-alkenals and hexanal were present in higher concentrations in the 2011 wine though the (E)-

2-hexenal and hexanal concentrations were also crop load-dependent. (E)-2-Hexenal, (E)-2-octenal, and hexanal impart green apple, fatty and nutty, and grassy aromas respectively (Mayr et al., 2015), but were present at concentrations below their respective odour thresholds in the 2010 wines (Table 6-6). In the 2011 wines, the (E)-2-alkenals were both present above their respective odour thresholds. Both the Strecker aldehydes and (E)-2-alkenals often appear in long-aged wines, although wines exposed to oxygen generally have more (E)-2-alkenals (Culleré et al., 2007).

Interestingly, there were several compounds for which crop load effects were also vintage-dependent. For example, ethyl acetate, propanoic acid, and 2-methylpropanol were highest in the low crop load wines from the warm year (2010). These compounds impart ethereal, pungent and rancid, and solvent-like aromas, respectively (Guth, 1997, Culleré et al., 2007, Ferreira et al., 2000) but only ethyl acetate was present at concentrations above its odour threshold. The results imply that the profiles of the high crop load wines from the warm year and the wines from the wet year (2011), regardless of cropping, were more similar with respect to these compounds than the low crop load wines from the warm year. Climate change projections indicate the sector faces a higher frequency of warm vintages (Remenyi et al., 2019), so yield restriction practices that result in significantly higher ethyl acetate, propanoic acid, and 2-methylpropanol concentrations in wines from warm years, may be detrimental to quality.

The distinct differences in the aroma composition of the two wines based on vintage effects illustrates the significant influence of the climatic variation between the two years. The juice composition varied significantly to affect the fermentation conditions (primary and secondary) and aging of the wines, creating two distinct profiles. While vintage had a greater effect on the overall aroma and flavour profile, there were distinct effects from cropping, which are often dependent on the climatic conditions. Interestingly, these compounds were lower molecular weight products of fermentation and are generally more volatile. The fact that the distinct compounds are from fermentation rather than compounds associated with oxidative flavours suggests crop load is affecting the fermentations (primary and/or secondary) more than the aging of the wines.

Table 6-6 Compositional analysis of variance of Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years.

			2010		2011		Vintage (V)	Treatment (T)	V*T
Compounds	**Code**	**Odour threshold (µg/L)**	**Low**	**High**	**Low**	**High**	**p-value**	**p-value**	**p-value**
Esters									
Ethyl acetate	EE1	12 (mg/L)	48.7 a	31.6 b	23.7 c	26.3 bc	0.000	0.014	0.002
Ethyl propanoate	EE2	45 (mg/L)	264.0	251.7	38.6	42.1	0.000	0.892	0.311
Ethyl butanoate	EE3	20	299.3	218.9	218.8	185.0	0.001	0.010	0.158
Ethyl hexanoate	EE4	14	746.8	533.3	322.0	290.5	0.000	0.076	0.118
Ethyl octanoate	EE5	5	493.0	351.0	462.2	377.6	0.513	0.238	0.946
Ethyl decanoate	EE6	200	58.26	43.85	58.09	41.41	0.598	0.176	0.540
Ethyl 2-methylpropanoate	EE7	15	125.1	115.2	51.9	62.8	0.002	0.536	0.411
Ethyl 2-methylbutanoate	EE8	18	12.0	16.5	10.7	16.3	0.800	0.040	0.486
Ethyl 3-methylbutanoate	EE9	3	19.9	22.7	14.8	20.9	0.138	0.065	0.413
Hexyl acetate	EA1	480	1.8	2.4	5.8	5.2	0.097	0.787	0.644
2-Methylbutyl acetate	EA4	1083	9.6	8.2	20.4	19.9	0.000	0.343	0.668
3-Methylbutyl acetate	EA5	30	155.7	108.2	251.2	182.5	0.068	0.543	0.502
Carboxylic acids									
Acetic acid	CA1	200 (mg/L)	174.2	117.6	433.0	394.6	0.003	0.271	0.881
Propanoic acid	CA2	8.1 (mg/L)	1.5 a	1.2 b	0.9 b	1.0 b	0.002	0.065	0.030
Butanoic acid	CA3	173	1.5	1.1	1.9	1.6	0.011	0.044	0.783
Hexanoic acid	CA4	420	6.6	5.1	7.7	6.9	0.033	0.123	0.681
Octanoic acid	CA5	500	2.6	1.9	11.4	10.1	0.000	0.406	0.543
Decanoic acid	CA6	1000	456.6 c	432.1 c	897.1 a	667.1 b	0.000	0.019	0.029
2-Methylpropanoic acid	CA7	2.3 (mg/L)	1.1	1.0	1.3	1.2	0.200	0.407	0.996
2-Methylbutanoic acid	CA8	2.2 (mg/L)	604.1 a	553.0 ab	487.0 c	514.8 bc	0.005	0.327	0.027
3-Methylbutanoic acid	CA9	33.4	585.4 a	498.4 ab	460.5 b	526.1 ab	0.053	0.610	0.027

Table 6-6 (cont.) **Compositional analysis of variance of Pinot noir crop load trial sparkling wines having been aged on lees for 5.5 years.**

			2010		2011		Vintage (V)	Treatment (T)	V*T
Compounds	**Code**	**Odour threshold (µg/L)**	**Low**	**High**	**Low**	**High**	**p-value**	**p-value**	**p-value**
Higher alcohols									
Hexanol	OH2	8 (mg/L)	1.0	1.0	1.0	1.0	0.564	0.367	0.881
2-Phenylethanol	OH3	14 (mg/L)	15.2	26.0	22.4	28.7	0.014	0.059	0.934
2-Methylpropanol	OH4	40 (mg/L)	26.9 a	18.7 b	17.9 b	16.6 b	0.000	0.002	0.005
2-Methylbutanol	OH5	1.2 (mg/L)	28.1	30.2	25.0	28.0	0.079	0.228	0.434
3-Methylbutanol	OH6	30 (mg/L)	124.4	102.1	143.3	139.9	0.014	0.224	0.327
Methionol	OH7	1000	320.68	437.77	258.14	206.22	0.006	0.412	0.059
Eugenol (phenolic)	OH8	6	2.7	2.6	12.7	11.4	0.009	0.522	0.798
Pyranone									
Maltol	FN2	5000	20.00	20.00	21.07	25.16	0.299	0.522	0.427
Aldehydes									
Hexanal	AH1	20	3.3 c	2.5 c	14.0 a	8.0 b	0.000	0.011	0.025
Benzaldehyde	AH2	2000	119.1	51.4	23.1	47.0	0.056	0.470	0.185
2-Methylpropanal	AS1	6	11.9	9.69	5.17	5.00	0.001	0.543	0.227
Methional	AS3	0.5	2.9 a	1.8 b	1.9 b	2.2 ab	0.105	0.100	0.015
Phenylacetaldehyde	AS4	1	7.5	5.3	1.0	1.0	0.000	0.054	0.101
(E)-2€kenals									
(E)-2-Hexenal	NL1	4	0.6 c	0.2 c	14.9 €10.5 b	0.000	0.018	0.013	
(E)-2-Octenal	NL3	3	0.2	0.1	4.1	6.5	0.002	0.111	0.168
Norisoprenoid									
β-Damascenone	NP1		1.6	2.7	1.1	1.3	0.224	0.584	0.478

6.1.2.9 Industry tasting panel

There was no significant difference discerned between high and low crop load treatments nor vintage based on overall preference rating using a 20-point wine show scoring system. Again, there was no significant difference observed between the treatments, nor the vintage, when the 26-member expert sensory panel rated each of the wines on eight sensory attributes: autolytic character, sweetness, bitterness, astringency, body, flavour intensity and persistence, acidity, and the balance between sweetness and astringency. The sensory panel was comprised of wine industry experts who are involved in making decisions about the quality bracket, wine maturity and retail price point for Tasmanian sparkling wine.

Interestingly both high and low crop load wines were perceived as sensorially similar, despite some compositional effects, which refutes the idea that yield restriction via crop load management has a positive and discernible impact on sparkling wine attributes and quality. Similar findings reported for table Pinot Noir wines concluded higher yields could be achieved and sustained in premium wine regions, such as Oregon USA (Uzes and Skinkis, 2016). Despite the compositional differences seen in the volatile compounds and polysaccharides, sensorially, the high crop load wines were equivalent quality to low crop load wines indicating yield restriction did not deliver a discernible quality improvement in the low crop load treatment, just an overall lower tonnage of harvested grapes.

6.1.2.10 Financial analysis

Based on crop load data from this study, the gross return per hectare under four scenarios was calculated: (1) 'low crop load in a warm season', (2) 'high crop load in a warm season', (3) 'low crop load in a wet season', and (4) 'high crop load in a wet season'. Gross margin (return on investment each season) and average return were also calculated across the two seasons. The average return simulates the financial impact for a vineyard of adopting a longer-term strategy of pruning for 'low' compared to 'high' yield. This scenario assumed there was no vintage-to-vintage decline in productivity associated with long-term 'high' cropping (Coppolani, 1994), given trial vines had been pruned to these node numbers since 2005. The financial analysis was based on industry information on the following costs during the 2010 and 2011 Tasmanian vintages:

- Fixed costs for cultivation ($/ha) – these were assumed the same for both 'high' ($3,000) and 'low' ($3,000) crop load treatments.
- Crop load-dependent costs for cultivation ($/ha) – these are assumed higher for 'high' ($700) crop load due to higher labour for shoot positioning, and higher inputs of fertilizer and spray, compared with 'low' ($500) crop load.
- Fixed costs for harvest ($/t) – this is assumed identical between crop load treatments ($400)
- Crop load-dependent costs for harvest ($/t) – the relative cost of harvest was assumed higher for 'low' ($200) crop load due to those hand-harvesting having to cover greater ground to harvest grapes and comparatively higher transport/tractor cost for moving fruit from vineyard to winery, compared with more densely grown fruit in the 'high' ($150) crop load treatment.
- Average price of grapes ($/t) – in the relatively wet season of 2011, grapes were in greater supply and the price per tonne was lower ($1,300) than in 2010 ($1,500). These prices are very conservative and are definitely not indicative of the price of sparkling wine grapes in the year of writing this report (2021), which is indicated to be around three times higher.

Averaging across the two years of the trial to account for vintage-to-vintage variability, the estimated costs were two-fold higher for the 'high' crop load treatment than for the 'low' crop load treatment (Table 6-7). But the 'high' crop load treatment delivered an average four-fold higher net return. Thus, the average gross margin for 'high' cropping was 44 % as opposed to 19 % for 'low' crop load. Between

2012 and 2015, just over 30 % of cool climate growers in Australia were 'profitable' and over 40 % were operating at a loss (Australian Grape and Wine, 2015). The costs and return in Table 6-7 indicate that Pinot Noir crop load management has substantial financial implications for vineyard owners and managers. While there were compositional differences observed, the sensory impact was minimal with the expert industry panel unable to distinguish between the crop load treatments based on overall wine show score nor out of eight sensory attributes. This study shows that the option of targeting 'high' crop load may be a practical approach for vineyard owners and managers to increase return on their investments and achieve greater financial stability in a challenging agricultural business.

Table 6-7 Estimate of different financial returns to grape growers with high and low crop load, in wet and dry seasons.

	2010		**2011**		**Annual average**	
	Low	**High**	**Low**	**High**	**Low**	**High**
Winter pruning (nodes/vine)	10	60	10	60	10	60
Equivalent grape yield (t/ha)	5.5	13.3	8.2	18.4	6.9	15.9
COSTS						
Fixed cultivation costs ($/ha)	$3,000	$3,000	$3,000	$3,000	$3,000	$3,000
Crop load-dependent cultivation costs ($/ha)	$500	$700	$500	$700	$500	$700
Fixed harvest costs ($/t)	$400	$400	$400	$400	$400	$400
Crop load-dependent harvest costs ($/t)	$200	$150	$200	$150	$200	$150
TOTAL COSTS ($/ha)	$6,800	$11,015	$8,420	$13,820	$7,610	$12,418
RETURNS						
Average price for grapes ($/t)	$1,500	$1,500	$1,300	$1,300	$1,400	$1,400
Gross return ($/ha)	$8,250	$19,950	$10,660	$23,920	$9,455	$21,935
NET RETURN ($/ha)	$1,450	$8,935	$2,240	$10,100	$1,845	$9,518
Gross margin	18 %	45 %	21 %	42 %	19 %	44 %

6.1.3 Pruning method

This trial examined the impact of pruning method on Chardonnay and Pinot noir fruit, base wine and aged sparkling wine. The viticultural trial work was conducted in the Coal River Valley during 2010 and 2011 by Dr Joanna Jones.

6.1.3.1 Fruit

The influence of pruning method treatments (cane or spur pruning) at the same node number (20/vine) have been previously reported in (Jones et al., 2018). The findings can be summarised as pruning method had no significant impact on yield, total soluble solids (TSS) or titratable acidity (TA) for Pinot noir or Chardonnay across the three vintages (2010, 2011 and 2012) of the original trial. Bunch number and weight was influenced by pruning method with spur pruning producing more, but smaller, bunches for both varieties.

6.1.3.2 Base wines spectral phenolic fingerprint (SPF)

The influence of pruning method treatments (cane or spur pruning) at the same node number (20/vine) have been previously reported in (Jones et al., 2018). More, smaller bunches as a result of spur pruning influenced the total phenolics of the base wine in the first vintage (2010), with no impact in subsequent vintages, nor in Chardonnay in any season. The more nuanced spectral phenolic fingerprint (SPF) of the base wines indicated that spur pruning resulted in higher quantities of hydroxycinnamates developed in base wines, rather than the more bitter flavonoids, as indicated by spectral phenolic fingerprints, when compared to cane pruning (Jones et al., 2018).

6.1.3.3 Aged sparkling wine basic composition

Comparing cane and spur pruned vines, there was no interactive effect of vintage and treatment, nor a main effect of treatment for each of the six parameters measured (Table 6-8 and Table 6-9) for either Chardonnay or Pinot Noir. However, five of the six parameters (excepting volatile acidity), were significantly different between vintages.

Table 6-8 Chardonnay wine matrix analysis for cane and spur pruned wines after 5.5 years en tirage.

	2010		2011		p values		
	Cane	Spur	Cane	Spur	Vintage	Treatment	V*T
Glucose + fructose (g/L)	5.3	5.1	1.5	1.8	<0.001	0.626	0.786
Titratable acidity (g/L)	6.1	6.7	4.1	3.9	<0.001	0.426	0.153
pH	3.6	3.5	4.4	4.6	<0.001	0.961	0.204
Alcohol %	11.6	11.4	10.9	10.7	<0.001	0.275	0.454
Malic acid (g/L)	4.7	4.4	1.2	1.2	<0.001	0.116	0.165
Volatile acidity (g/L)	0.08	0.17	0.09	0.09	0.190	0.208	0.255

Table 6-9 Pinot noir wine matrix analysis for cane and spur pruned wines after 5.5 years en tirage.

	2010		2011		p values		
	Cane	Spur	Cane	Spur	Vintage	Treatment	V*T
Glucose + fructose (g/L)	4.9	6	2.3	2.3	<0.001	0.576	0.381
Titratable acidity (g/L)	5.3	6.1	3.7	4.1	<0.001	0.085	0.466
pH	3.8	3.6	4.3	4.3	<0.001	0.650	0.202
Alcohol %	11.5	12.1	11.1	10.8	0.030	0.993	0.213
Malic acid (g/L)	4.3	4.1	0.75	0.87	<0.001	0.803	0.257
Volatile acidity (g/L)	0.15	0.13	0.09	0.08	0.241	0.745	0.811

6.1.3.4 Aged sparkling wine hydroxycinnamates

Caftaric acid and other hydroxycinnamates, such as coumaric acid, are the major phenols other than flavonoids in grapes and wines (Nagel et al., 1979). Caftaric and coumaric acids were analysed as an indicator of the influence of pruning method on hydroxycinnamates, in addition to the SPF analysis, as SPF of base wines had already indicated separation of treatments according to wavelengths in the 300-330 nm region, similar to caffeic acid, another hydroxycinnamate (Jones et al., 2018).

An interaction between vintage and treatment was observed in the aged Chardonnay sparkling wines from the pruning method trial, whereby similar low levels of caftaric acid (4.1 mg/L) were recorded for cane pruning in the warm and dry season of 2010, and spur pruning in the cool and wet 2011 season (Table 6-10). Similarities between canopy parameters reported in Jones et al. (2018) do not assist in explaining this interaction, as it could have been possible that the lower hydroxycinnamates could have been the result of a denser canopy, as hydroxycinnamates have been reported to be produced in response to UV-B exposure of fruit (Kolb et al., 2003). A similar response was not observed for the Pinot noir wines (Table 6-11), where the only significant effect was that of vintage on coumaric acid, which would likely be positively received by winemakers due to the increased likelihood of *Brettanomyces* spoilage with higher coumaric acid levels (Chatonnet et al., 1992).

Table 6-10 Aged Chardonnay sparkling wine pruning method trial hydroxycinnamate analysis

	2010		2011		p values		
	Cane	**Spur**	**Cane**	**Spur**	**Vintage**	**Treatment**	**V*T**
Caftaric acid (mg/L)	4.1 a	9.5 b	8.3 b	4.1 a	0.588	0.726	0.012
Coumaric acid (mg/L)	0.54	0.53	0.65	0.71	0.151	0.749	0.775

Table 6-11 Aged Pinot noir sparkling wine pruning method trial hydroxycinnamate analysis

	2010		2011		p values		
	Cane	**Spur**	**Cane**	**Spur**	**Vintage**	**Treatment**	**V*T**
Caftaric acid (mg/L)	14.6	12.3	18	33.8	0.078	0.168	0.285
Coumaric acid (mg/L)	0.49	0.51	0.26	0.31	0.003	0.477	0.667

6.1.3.5 Polysaccharides

For polysaccharides in aged Chardonnay and Pinot noir sparkling wines, by molecular weight class or as a total, no interaction between vintage and treatment was observed (Table 6-12 and Table 6-13). Cane pruned Chardonnay vines resulted in aged sparkling wines with higher medium molecular weight polysaccharides, however this was not observed in the Pinot noir wines of the same age. Total polysaccharides have been shown previously to affect foam stability time but not the other foamability parameters (Martínez-Lapuente et al., 2015). The medium molecular weight polysaccharides are consistent with arabinoglucan-proteins from grapes (McRae et al., 2017, Bindon et al., 2013a) suggesting the cane-pruning treatment may have improved the foam stability time.

Table 6-12 Chardonnay polysaccharides analysis – aged wine from cane and spur pruned vines from two vintages (2010, 2011)

	2010		2011		p values		
	Cane	Spur	Cane	Spur	Vintage	Treatment	V*T
High molecular weight polysaccharides (mg/L)	30.5	29.2	24.3	19.5	0.004	0.069	0.460
Medium molecular weight polysaccharides (mg/L)	38	32.8	36.1	26.2	0.132	0.005	0.365
Low molecular weight polysaccharides (mg/L)	16.9	13.8	19.7	13.9	0.091	<0.001	0.284
Total polysaccharides - (mg/L)	91.5	81.9	86.2	65.7	0.080	0.007	0.355

Table 6-13 Pinot noir polysaccharides analysis – aged wine from cane and spur pruned vines from two vintages (2010, 2011)

	2010		2011		p values		
	Cane	Spur	Cane	Spur	Vintage	Treatment	V*T
High molecular weight polysaccharides (mg/L)	39.9	36.3	18.1	19.5	<0.001	0.739	0.131
Medium molecular weight polysaccharides (mg/L)	40.5	36.7	27.2	25.7	<0.001	0.253	0.541
Low molecular weight polysaccharides (mg/L)	12.1	13.6	12.4	10.6	0.080	0.411	0.106
Total polysaccharides - (mg/L)	98.6	92.7	63.7	61.9	<0.001	0.359	0.498

6.1.3.6 Proteins

While proteins can improve foaming properties, they also play a major role in protein haze formation. In the warm and dry 2010, cane-pruned Chardonnay vines had higher thaumatin-like proteins than spur-pruned vines (Table 6-14). But there were no statistically significant treatment differences in the cool and wet 2011 season. The cane-pruned Pinot noir vines produced wines with higher concentrations of chitinases in 2010 (Table 6-15). The 8 mg/L reported in these wines is around the threshold reported in the literature for haze formation, particularly in association with addition of sodium sulphate during winemaking (Marangon et al., 2011).

Table 6-14 Chardonnay proteins analysis – aged wine from cane and spur pruned vines from two vintages (2010, 2011)

	2010		2011		Vintage (V)	Treatment (T)	V*T
	Cane	Spur	Cane	Spur	p-value	p-value	p-value
Total proteins (mg/L)	33 a	23.3 ab	22.2 b	24.4 ab	0.066	0.392	0.044
Thaumatin-like proteins (mg/L)	33 a	23.3 b	21 b	23 b	0.021	0.336	0.038
Chitinases (mg/L)	0	0	1.2	1.4	<0.001	0.321	0.782

Table 6-15 Pinot noir proteins analysis – aged wine from cane and spur pruned vines from two vintages (2010, 2011)

	2010		2011		Vintage (V)	Treatment (T)	V*T
	Cane	Spur	Cane	Spur	p-value	p-value	p-value
Total proteins (mg/L)	43.2	35.9	12.2	11.6	<0.001	0.066	0.056
Thaumatin-like proteins (mg/L)	35.2	32.4	9.7	8.6	<0.001	0.446	0.995
Chitinases (mg/L)	8 a	3.5 b	2.6 b	2.9 b	0.007	0.072	0.007

6.1.3.7 Aged wine volatiles analysis

Again, vintage had the greatest impact on the volatile composition of the wines for Chardonnay and Pinot noir (Figure 6-7 and Figure 6-8, respectively). As was seen in the crop load study, the wines from 2010 showed more treatment-based separation based on PCA, whereas there was more variability overall and overlap in 2011. The emerging picture may be that vines were more stressed under 2010 conditions and showed greater response to viticultural treatments under stress conditions.

The wines showed vintage-associated variation in volatiles after 5.5 years in bottle. In the Chardonnay wines, the acetate esters, hexyl acetate, 2-methylbutyl acetate and 3-methylbutyl acetate, were all present at higher concentrations in the 2011 Chardonnay wines than the 2010 wines (Table 6-16). Considering these compounds are often markers of young sparkling wines (Francioli et al., 2003, Riu-Aumatell et al., 2006) because they decrease in concentration over the length of the ageing period (Riu-Aumatell et al., 2006, Torrens et al., 2010, Ruiz-Moreno et al., 2017, Ubeda et al., 2019), it is interesting these acetate esters were present in 2011 at concentrations more than double those of 2010. The 3-methylbutyl acetate was also present at concentrations above the odour threshold both years. This could mean the acetate ester concentrations were particularly high in the wines from 2011 to start with or that the ageing process was different and thus the acetate esters did not decrease in concentration at the same rate as in the 2010 wines. This could be due to different climatic concentrations in both years resulting in different fermentation starting materials.

For Pinot noir wines (Table 6-17), where concentration was significantly different between vintages, the esters were mostly lower in 2011 than 2010 wines. Whereas, the carboxylic acids and higher alcohols tended to be higher in 2011 wines than 2010. This is a similar pattern to the Chardonnay wines, and might suggest strong vintage (climatic) impacts on initial concentration of acetate esters.

Where pruning treatment impacts were identified by ANOVA across the two-vintage dataset, the 2010 cane pruned Chardonnay wines showed significantly higher concentration of 2- methylpropanol (wine, solvent, bitter), 3-methybutanol (whisky, malt, burnt), and phenylacetaldehyde (honey, floral) than spur pruned treatment wines. The concentration of 3-methylbutanol was over the published odour detection threshold, however the odour threshold was exceeded in wines from both pruning treatments so pruning treatment could not be proposed as a means to moderate or manage expression of this aroma compound in finished wines. The compound phenylacetaldehyde is a cinnamic acid derivative (Ilc et al., 2016). Phenylacetaldehyde concentrations are generally higher in grapes compared with wines and tend to be reduced by yeast to 2-phenylethanol. The Chardonnay wines in this trial demonstrated an interaction between vintage and pruning treatment for hydroxycinnamates (see Table 6-10 above) and the significant effect observed here for phenylacetaldehyde concentration signals the need for closer examination of pruning impacts and flow-on effects, on the expression of hydroxycinnamates and derived compounds in the aged Chardonnay wine matrix.

Chardonnay wines from the warm and dry 2010, showed higher concentration of β-damascenone (floral, fruit) in wine from spur pruned vines, as compared to cane pruned vines. The concentration in the wines did not exceed the odour threshold, but some studies suggest β -damascenone has a potential 'aroma enhancing' role in wine (Tomasino and Bolman, 2021) and its concentration has been linked to canopy management (Ristic et al. 2010). Damascenone is thought to be converted from the plant carotenoid into an aroma precursor via degradation and enzymatic conversion (Robinson et al., 2014). Mature grapes exposed to sunlight showed lower concentration of carotenoids, compared to grapes that were subjected to shade conditions in latter stages of maturation (Mendes-Pinto, 2009). The evolution of damascenone to the aroma active compound, occurs via an acid-catalysed reaction (Robinson et al., 2014). It can also be hydrolysed during wine ageing, which decreases the concentration of damascenone in aged wine. As there was little effect in the cool and wet 2011, our findings here add to this understanding of damascenone by suggesting this important aroma compound might be managed through Chardonnay pruning decisions, but the effect is likely to be mitigated by vintage-to-vintage climatic conditions.

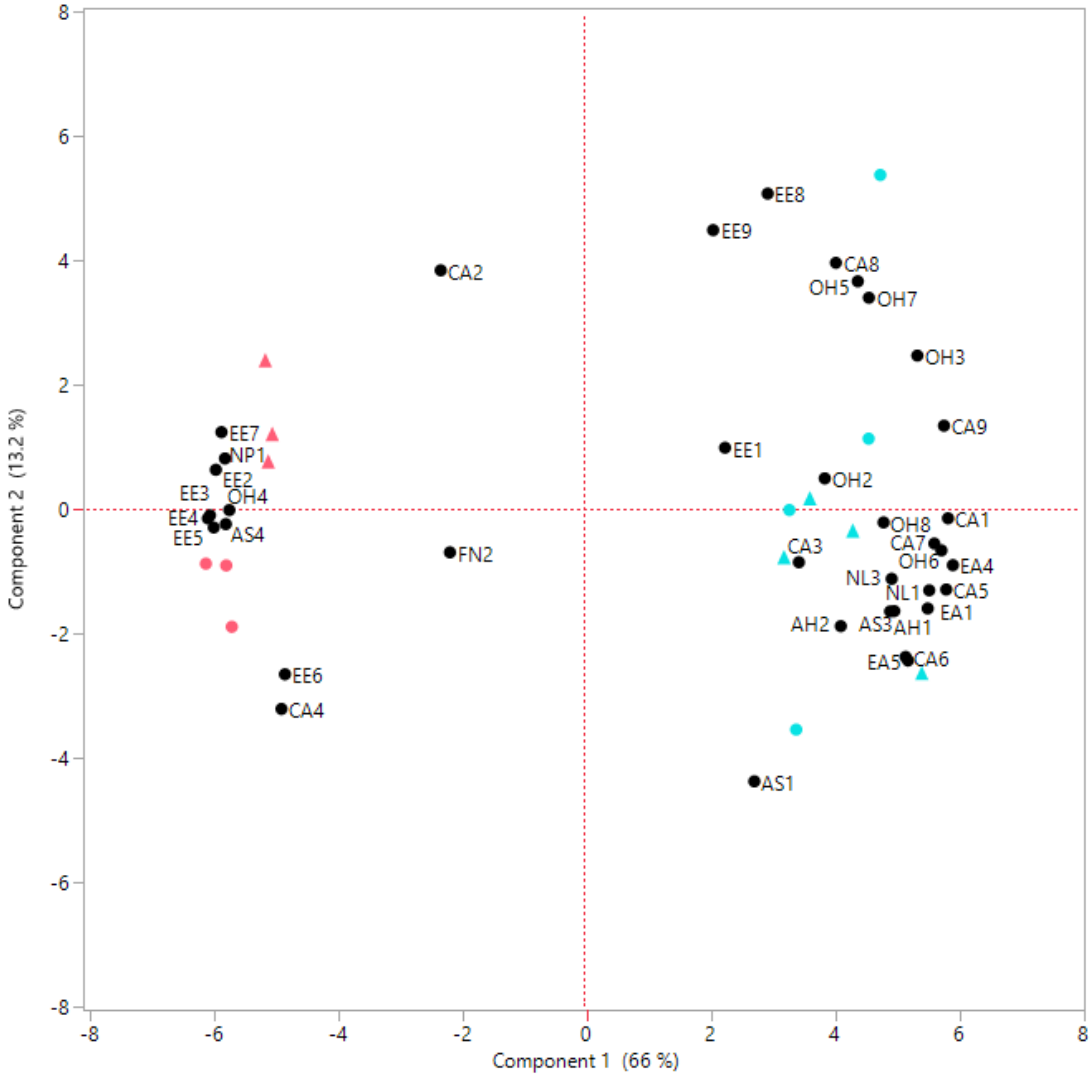


Figure 6-7: CHA PCA biplot with scores and loadings of volatiles. Cane pruned indicated with circle, spur pruned with triangle. 2010 indicated with pink, 2011 indicated with blue. The key to the loadings is in Table 6-6.

Where pruning treatment impacts were identified by ANOVA across the two-vintage dataset, the 2010 wines from the dry warm vintage showed volatile aroma compound differences associated with pruning treatment. The spur pruned Pinot noir wines showed significantly higher concentration of ethyl propanoate, but this was below the odour detection threshold. Hexanol and phenylacetaldehyde concentration was significantly higher in aged wines from the cane pruned, compared with the spur pruned vines. The latter compound (phenylacetaldehyde) has been associated honey and floral aromas in wine, and this result may be important as the concentration of phenylacetaldehyde in the cane pruned 2010 Pinot noir wines was two-fold higher than in wines from spur pruned treatment, and well above the odour detection threshold. This finding is also of interest because the cane pruned Chardonnay from 2010 was also significantly higher in this compound. As discussed above, pruning may be interacting with hydroxycinnamate concentration to deliver this outcome. Another aspect of the development of phenylacetaldehyde is wine oxidation (Ugliano et al, 2018), which is a complex process, in part mediated by the presence of phenolics in the wine matrix.

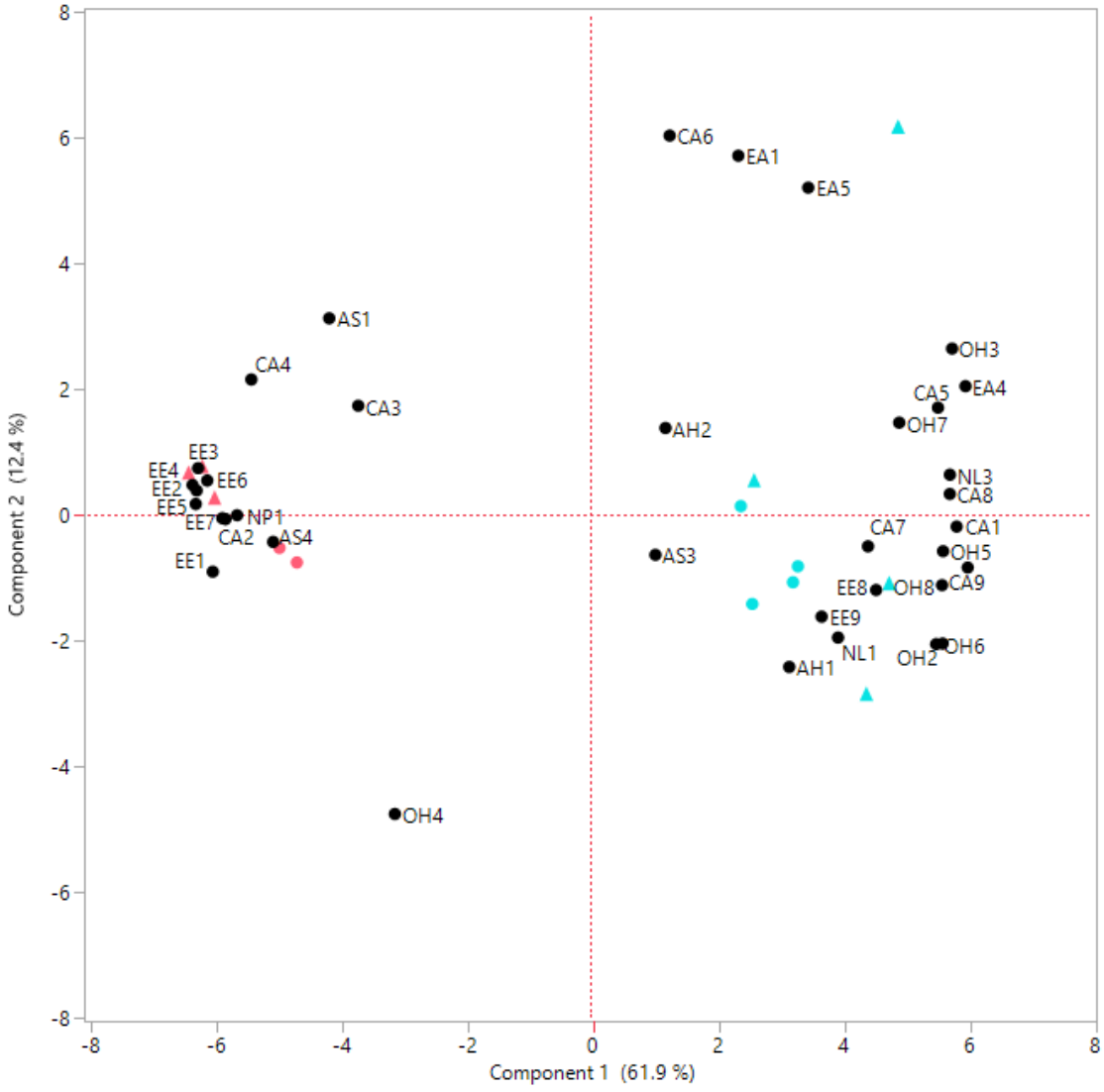


Figure 6-8: PIN PCA biplot with scores and loadings of volatiles. Cane pruned indicated with circle, spur pruned with triangle. 2010 indicated with pink, 2011 indicated with blue. The key to the loadings is in Table 6-6.

Table 6-16 Compositional analysis of variance of Chardonnay pruning method trial sparkling wines having been aged on lees for 5.5 years

			2010		2011		Vintage (V)	Treatment (T)	V*T
Compounds	**Code**	**Odour threshold (µg/L)**	**Cane**	**Spur**	**Cane**	**Spur**	**p-value**	**p-value**	**p-value**
Esters									
Ethyl acetate	EE1	12 (mg/L)	13778	11766	15357	13104	0.243	0.068	0.783
Ethyl propanoate	EE2	45 (mg/L)	225	252	46.3	41.9	<0.001	0.351	0.102
Ethyl butanoate	EE3	20	267	270	152	148	<0.001	0.908	0.232
Ethyl hexanoate	EE4	14	715	660	194	190	<0.001	0.299	0.294
Ethyl octanoate	EE5	5	388	396	168	168	<0.001	0.621	0.541
Ethyl decanoate	EE6	200	40.8	35.1	25.6	24.2	0.006	0.582	0.853
Ethyl 2-methylpropanoate	EE7	15	108 a	119 a	54.5 b	48.1 b	<0.001	0.865	0.037
Ethyl 2-methylbutanoate	EE8	18	9.9	13.1	14.5	13.2	0.144	0.902	0.170
Ethyl 3-methylbutanoate	EE9	3	13.3 b	17.7 a	17.6 a	16.2 ab	0.185	0.339	0.008
Hexyl acetate	EA1	480	4.1	3.3	18.8	21.6	<0.001	0.284	0.554
2-Methylbutyl acetate	EA4	1083	6.5	5	21.9	23.6	<0.001	0.663	0.161
3-Methylbutyl acetate	EA5	30	129	70.4	238	273	<0.001	0.823	0.074
Carboxylic acids									
Acetic acid	CA1	200 (mg/L)	40756	42590	333188	357612	<0.001	0.763	0.736
Propanoic acid	CA2	8.1 (mg/L)	1380	1483	1524	1509	0.008	0.104	0.019
Butanoic acid	CA3	173	7001 b	6384 ab	5500 a	5810 ab	0.002	0.870	0.163
Hexanoic acid	CA4	420	3157	3273	9429	10378	<0.001	0.049	0.511
Octanoic acid	CA5	500	468	479	949	1276	<0.001	0.040	0.170
Decanoic acid	CA6	1000	463	518	595	590	0.061	0.790	0.647
2-Methylpropanoic acid	CA7	2.3 (mg/L)	348	405	545	565	<0.001	0.151	0.442
2-Methylbutanoic acid	CA8	2.2 (mg/L)	1256	1412	1254	977	0.126	0.288	0.281
3-Methylbutanoic acid	CA9	33.4	958	961	1467	1396	<0.001	0.458	0.775

Table 6-16 (cont.) **Compositional analysis of variance of Chardonnay pruning method trial sparkling wines having been aged on lees for 5.5 years**

			2010		2011		Vintage (V)	Treatment (T)	V*T
Compounds	**Code**	**Odour threshold (µg/L)**	**Cane**	**Spur**	**Cane**	**Spur**	**p-value**	**p-value**	**p-value**
Higher alcohols									
Hexanol	OH2	8 (mg/L)	1068 b	1183 ab	1271 a	1197 a	0.011	0.869	0.034
2-Phenylethanol	OH3	14 (mg/L)	14875	21518	39385	38366	0.002	0.671	0.427
2-Methylpropanol	OH4	40 (mg/L)	21548 a	19393 b	15265 c	14977 c	<0.001	0.031	0.043
2-Methylbutanol	OH5	1.2 (mg/L)	22111	24412	29158	28676	0.027	0.914	0.664
3-Methylbutanol	OH6	30 (mg/L)	87823a	74710b	124576c	121239c	<0.001	0.017	0.072
Methionol	OH7	1000	335	376	567	504	0.010	0.618	0.367
Eugenol (phenolic)	OH8	6	4.1	3.2	8.6	6.9	<0.001	0.112	0.282
Pyranone									
Maltol	FN2	5000	24.8	20	20	20	0.334	0.334	0.244
Aldehydes									
Hexanal	AH1	20	4.3	4.1	17.6	25.2	0.006	0.378	0.393
Benzaldehyde	AH2	2000	1.6	1.4	46.7	30.9	0.008	0.368	0.566
2-Methylpropanal	AS1	6	6.4	5.5	6.6	8	0.105	0.642	0.208
Methional	AS3	0.5	2.7	1.4	3.8	4.3	0.003	0.541	0.063
Phenylacetaldehyde	AS4	1	5.1 a	3.6 b	<LOQ c	<LOQ c	<0.001	0.020	0.008
(E)-2-Alkenals									
(E)-2-Hexenal	NL1	4	0.79	0.75	21.7	28.9	<0.001	0.366	0.387
(E)-2-Octenal	NL3	3	0.55	0.57	5.8	4.7	0.002	0.430	0.736
Norisoprenoid									
β-Damascenone	NP1	4	1.4 b	1.8 a	0.11 c	0.11 c	<0.001	0.013	0.004

Table 6-17 Compositional analysis of variance of Pinot noir pruning method trial sparkling wines having been aged on lees for 5.5 years

			2010		2011		Vintage (V)	Treatment (T)	V*T
Compounds	**Code**	**Odour threshold (µg/L)**	**Cane**	**Spur**	**Cane**	**Spur**	**p-value**	**p-value**	**p-value**
Esters									
Ethyl acetate	EE1	12 (mg/L)	28870	28543	16996	15830	<0.001	0.402	0.884
Ethyl propanoate	EE2	45 (mg/L)	212 b	276 a	44.2 c	43.6 c	<0.001	<0.001	<0.001
Ethyl butanoate	EE3	20	267 a	308 a	156 b	132 b	<0.001	0.945	0.022
Ethyl hexanoate	EE4	14	715 b	828 a	207 c	170 c	<0.001	0.482	0.010
Ethyl octanoate	EE5	5	530 a	624 a	276 b	195 b	<0.001	0.487	0.011
Ethyl decanoate	EE6	200	64.1 b	89.3 a	34.4 c	24.7 c	<0.001	0.626	0.012
Ethyl 2-methylpropanoate	EE7	15	88.9 b	126 a	59.6 c	58.5 c	<0.001	0.072	0.013
Ethyl 2-methylbutanoate	EE8	18	8.8	12.3	15.7	16.1	0.012	0.582	0.726
Ethyl 3-methylbutanoate	EE9	3	12	17.3	19.9	20.4	0.037	0.456	0.513
Hexyl acetate	EA1	480	<LOQ	<LOQ	<LOQ	<LOQ			
2-Methylbutyl acetate	EA4	1083	<LOQ	5.3	14.4	16.2	<0.001	0.372	0.688
3-Methylbutyl acetate	EA5	30	26.7	75.4	95.2	142	0.074	0.142	0.728
Carboxylic acids									
Acetic acid	CA1	200 (mg/L)	78858	67668	272636	317669	<0.001	0.257	0.479
Propanoic acid	CA2	8.1 (mg/L)	1230	1428	985	923	<0.001	0.931	0.064
Butanoic acid	CA3	173	1483 b	1774 a	1576 ab	1458 b	0.057	0.588	0.011
Hexanoic acid	CA4	420	6756	6777	5537	5008	0.004	0.543	0.208
Octanoic acid	CA5	500	3705	4469	9005	8307	<0.001	0.919	0.102
Decanoic acid	CA6	1000	511	637	617	703	0.477	0.361	0.638
2-Methylpropanoic acid	CA7	2.3 (mg/L)	1044	1147	1538	1550	0.032	0.712	0.663
2-Methylbutanoic acid	CA8	2.2 (mg/L)	439 b	444 b	531 ab	599 a	<0.001	0.049	0.016
3-Methylbutanoic acid	CA9	33.4	410 b	370 b	528 a	594 a	<0.001	0.291	0.010

Table 6-17 (cont.) **Compositional analysis of variance of Pinot noir pruning method trial sparkling wines having been aged on lees for 5.5 years**

			2010		2011		Vintage (V)	Treatment (T)	V*T
Compounds	**Code**	**Odour threshold (µg/L)**	**Cane**	**Spur**	**Cane**	**Spur**	**p-value**	**p-value**	**p-value**
Higher alcohols									
Hexanol	OH2	8 (mg/L)	1069 b	655 c	1389 a	1319 ab	<0.001	0.008	0.011
2-Phenylethanol	OH3	14 (mg/L)	11854	11450	23243	28546	0.001	0.228	0.344
2-Methylpropanol	OH4	40 (mg/L)	20678	20700	19876	18023	0.031	0.059	0.898
2-Methylbutanol	OH5	1.2 (mg/L)	24472 ab	22353 b	27238 a	28522 a	0.001	0.724	0.033
3-Methylbutanol	OH6	30 (mg/L)	106698	103951	137948	132791	<0.001	0.119	0.802
Methionol	OH7	1000	230	265	396	500	0.022	0.350	0.422
Eugenol (phenolic)	OH8	6	3.6	3	9	9.3	<0.001	0.611	0.256
Pyranone									
Maltol	FN2	5000	20	20	20	20	0.160	0.362	0.205
Aldehydes									
Hexanal	AH1	20	7.4	3	26.4	11.6	0.039	0.058	0.700
Benzaldehyde	AH2	2000	47.4	4.2	27	25.1	0.631	0.177	0.096
2-Methylpropanal	AS1	6	8.8	10.5	6.9	6.5	0.072	0.789	0.455
Methional	AS3	0.5	4	1.7	2.8	2.7	0.756	0.108	0.052
Phenylacetaldehyde	AS4	1	9.8 a	4.4 b	<LOQ c	<LOQ c	<0.001	<0.001	<0.001
(E)-2-Alkenals									
(E)-2-Hexenal	NL1	4	1.4	0.85	29.8	13.6	0.009	0.056	0.441
(E)-2-Octenal	NL3	3	1.2	0.69	5.7	5.7	0.001	0.686	0.636
Norisoprenoid									
β-Damascenone	NP1		2.2	2.8	1.2	0.92	0.002	0.896	0.376

6.1.3.8 Industry tasting panel

Neither pruning treatments, nor vintage, had significant influences on the 20-point wine show score of Chardonnay or Pinot noir aged sparkling wines. When the 26-member expert sensory panel rated each of the wines on eight sensory attributes: autolytic character, sweetness, bitterness, astringency, body, flavour intensity and persistence, acidity, and the balance between sweetness and astringency, there were no conclusive differences identified between the wines. This outcome suggests that while pruning method impacted wine chemistry, particularly in the warm dry 2010 vintage, the chemical impacts observed were not discernible to experts who make their living making, tasting, blending, assessing and marketing sparkling wine.

6.1.4 Leaf removal

Chardonnay vines at two Tasmanian sites were subjected to leaf removal treatments preceding 2010 and 2011 vintages. The two sites were Tamar Valley (northern Tasmania) and Coal River Valley (southern Tasmania). Leaves were removed at pre-flowering, when fruit was pea-sized, and at veraison, and fruit, wine and aged wines from each treatment were compared with control (no leaf removal).

6.1.4.1 Fruit

In 2010, fruit from the trial vines in Northern Tasmania was harvested early due to impending rain. Early harvest was repeated in 2011 for that site, which was also a season of high disease pressure with 250 mm greater rainfall in that year compared with 2010, and 200 growing degrees days difference between the two years. As reported in Kerslake et al. 2012, few treatment effects were observed in the Chardonnay fruit composition (Table 6-18 and 6-19); in 2011, TSS was higher for southern fruit where leaves had been removed at peas-sized and veraison, compared with control fruit. These results are contradictory to the outcomes that might be predicted from early and late leaf removal; a meta-analysis of 59 papers that examined pre-bloom leaf removal demonstrated impacts on yield (lower with pre-bloom leaf removal) and fruit sugar concentration (higher with pre-bloom leaf removal) (Vanderwiede et al. 2021). The lack of significant difference in yield across sites, vintages and treatment, combined with higher TSS observed for the 2011 southern late season leaf removal fruit, was counter to the mechanisms underpinning leaf removal as described by Vanderwiede and others.

> 'Although there is a large variation in floret sensitivity to abscission among grape cultivars,…[pre-bloom leaf removal]… led to a significant reduction in yield per vine… due to the decrease in fruit set that occurs when a large percentage of the carbohydrate source (leaves) is removed from the plant during the period of strong vegetative growth, drastically reducing the carbon portioning to the reproductive organs' (grapes) (Vanderwiede et al. 2021; page 6).

Northern Tasmania

Vintage	GDD (Sep-Mar)	Rainfall (Sep-Mar) (mm)	GST (Sep-Mar) (°C)	MJT (°C)	MFT (°C)
2010	1190.0	414.0	15.6	17.8	19.6
2011	983.6	676.0	14.6	17.6	16.5
2012	1161.1	457.8	15.4	17.7	17.6

Southern Tasmania

Vintage	GDD (Sep-Mar)	Rainfall (Sep-Mar) (mm)	GST (Sep-Mar) (°C)	MJT (°C)	MFT (°C)
2010	1150.5	349.4	15.2	17.5	18.0
2011	987.2	372.4	14.5	17.7	16.5
2012	1120.7	314.4	15.1	18.7	17.9

Table 6-18 **Northern Tasmania leaf removal Chardonnay, fruit analysis**

	Yield (t/ha)		TSS (°Be)		pH		TA (g/L)	
	2010	2011	2010	2011	2010	2011	2010	2011
Control	15.7	10.4	9.2	9.8	2.84	3.01	13.6	14.9
Pre-flowering	-	8.2	-	9.9	-	2.91	-	15.3
Pea-sized berries	14.0	12.4	9.2	9.7	2.84	2.96	13.3	14.8
Veraison	15.1	8.4	9.1	9.7	2.79	2.95	14.2	15.1

Table 6-19 **Southern Tasmania Chardonnay leaf removal**

	Yield (t/ha)		TSS (°Be)		pH		TA (g/L)	
	2010	2011	2010	2011	2010	2011	2010	2011
Control	4.5	7.7	11.0	9.78 a	3.00	3.10	10.71	12.58
Pre-flowering	6.0	8.3	10.9	9.9 ab	3.00	3.04	10.97	14.69
Pea-sized berries	5.5	8.1	10.9	10.1 b	2.97	3.10	10.88	13.45
Veraison	5.2	7.9	10.9	10.0 b	3.03	3.05	10.61	13.36

6.1.4.2 Base wines SPF

When the spectral fingerprints of the base wines from the northern Tasmania Chardonnay leaf removal trial were analysed by PCA, 91 % of the variance in the data was driven by vintage along PC1 (Figure 6-8). Northern Tasmanian base wines from 2010 showed no cluster separation, but the 2011 data revealed reasonably distinct separation on PC2 between base wines from the 'control' versus 'pre-flowering' treatment, and loadings analysis showed this separation was driven by absorbances around 260 and 330 nm. The 'traditional' measure of total phenolics at 280 nm was not a distinctive feature of the loadings plot for northern 2011 base wines. A similar pattern was observed of strong vintage-to-vintage separation between base wines from the southern Tasmanian Chardonnay leaf removal trial (Figure 6-10). From the southern Tasmanian site, base wines showed some cluster separation between 'control' and 'pre-flowering' treatment for the 2011 wines. Loadings analysis

showed no particular role for 280 nm, rather the seasonal variation was driven by the loadings around 260, 310 and 330 along PC1.

As discussed in Kerslake et al. (2012), the spectral fingerprint of the base wines from this leaf removal study indicated peaks at wavelengths other than those for the total phenolic measure which has been associated with 280 nm. This suggests that total phenolics, as measured spectrophotometrically, may not be reliable for assessing impact on phenolics of leaf removal practices under these circumstances. It also potentially indicates need for a wider consideration of phenolic compounds that might influence the finished wine matrix, and hence sensory outcomes.

Overall, the SPF results were not strongly compelling in terms of clustering and differentiation between clusters. It is possible that the limited number of samples in this study led to indistinct outcomes, or that SPF was insufficiently sensitive as a method to capture phenolic differences between sets of base wines. Alternately, leaf removal treatments may not be particularly effective for phenolics moderation – this finding would contradict the industry practice of leaf removal to achieve 'dappled' light to influence grape phenolic development (Kerslake et al. 2012).

Of greater interest are the wavelengths indicated by loadings analysis (Figure 6-9 and Figure 6-11). Absorbance at these wavelengths may be influenced by low molecular weight compounds, such as hydroxycinnamates or coumarates. Unpublished analysis by Kerslake and others demonstrated loadings for two important hydroxycinnamates in white wine: caffeic and ferulic acid showed characteristic troughs and peaks at 260, 310 and 330 nm, when assayed in a wine-like matrix (Figure 6-7). Hydroxycinnamates can effect both mouthfeel and flavour in finished wines. For example in a major study of phenolics in white wine, hydroxycinnamic acids were positively associated by a trained sensory panel with viscosity (Smith and Waters, 2012). Winemakers can adjust expression of hydroxycinnamates (caffeic acid and Grape Reaction Product) in juice and wine through oxidative handling (Smith and Waters, 2012). Our findings here of a possible association between viticultural practices and hydroxycinnamates is important because it offers another potential method for industry to manage expression in juice and wine of this class of compounds.

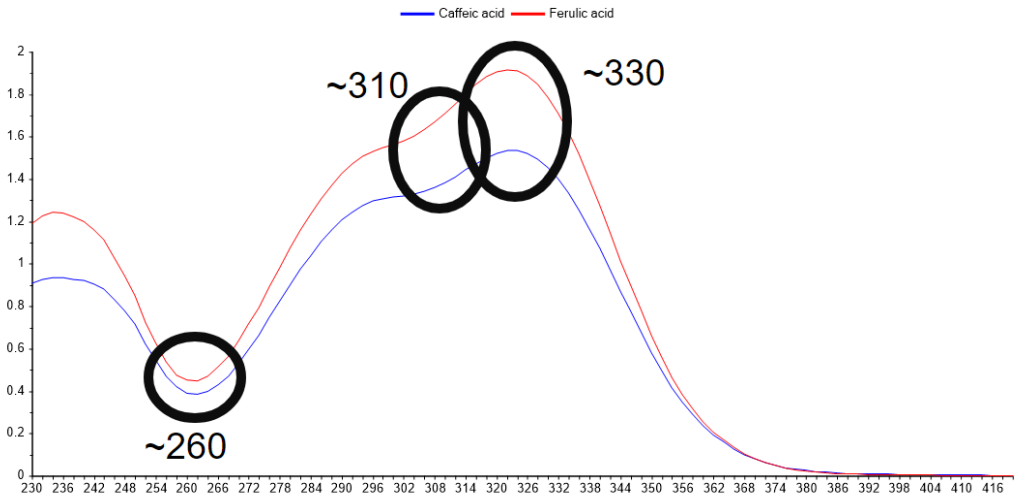


Figure 6-7: UV spectral profiles of ferulic acid (red) and caffeic acid (blue), as ethanolic solutions, dilluted in 1M HCl.

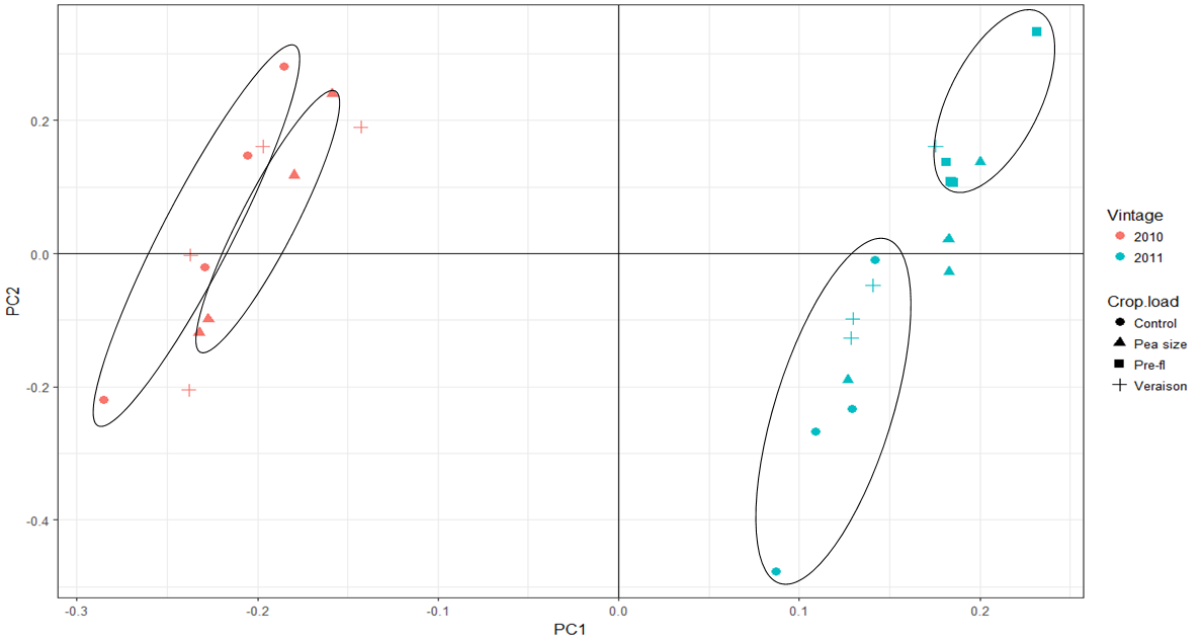


Figure 6-8: Northern Tasmanian leaf removal Chardonnay – SPF of base wine

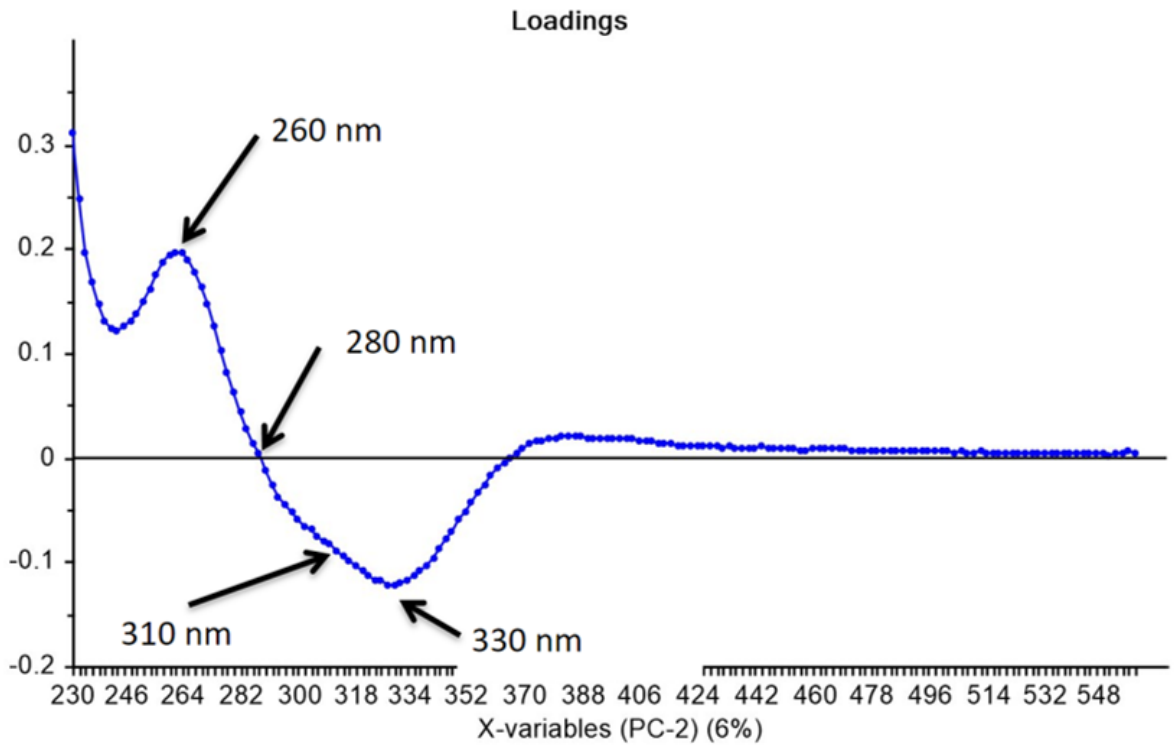


Figure 6-9: Northern Tasmanian leaf removal Chardonnay – base wine spectral loadings PC2

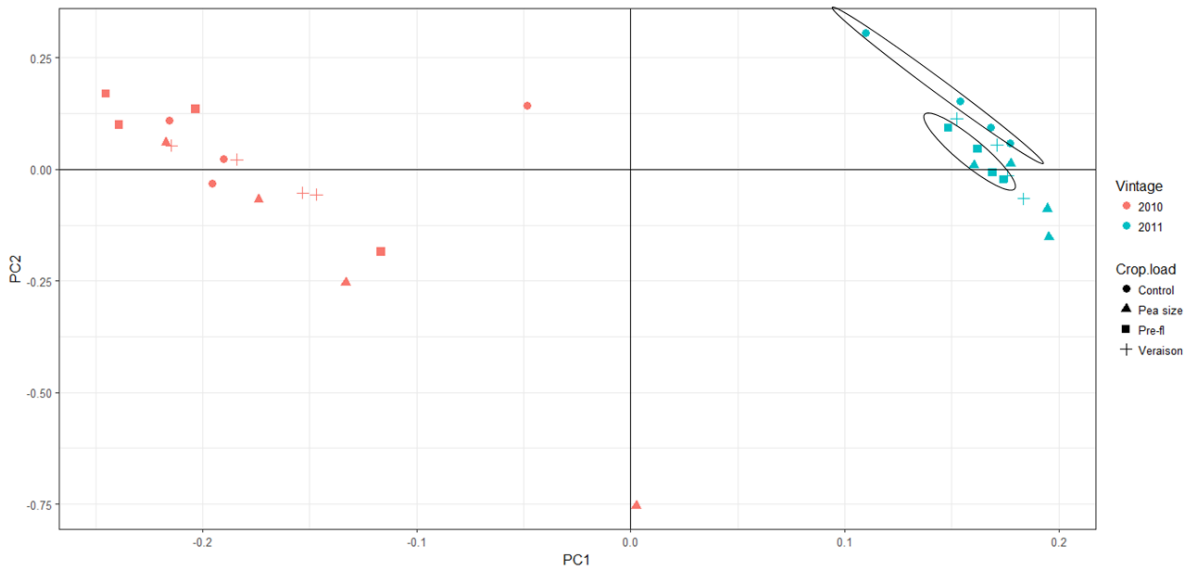


Figure 6-10 Southern Tasmania Chardonnay leaf removal – SPF base wine

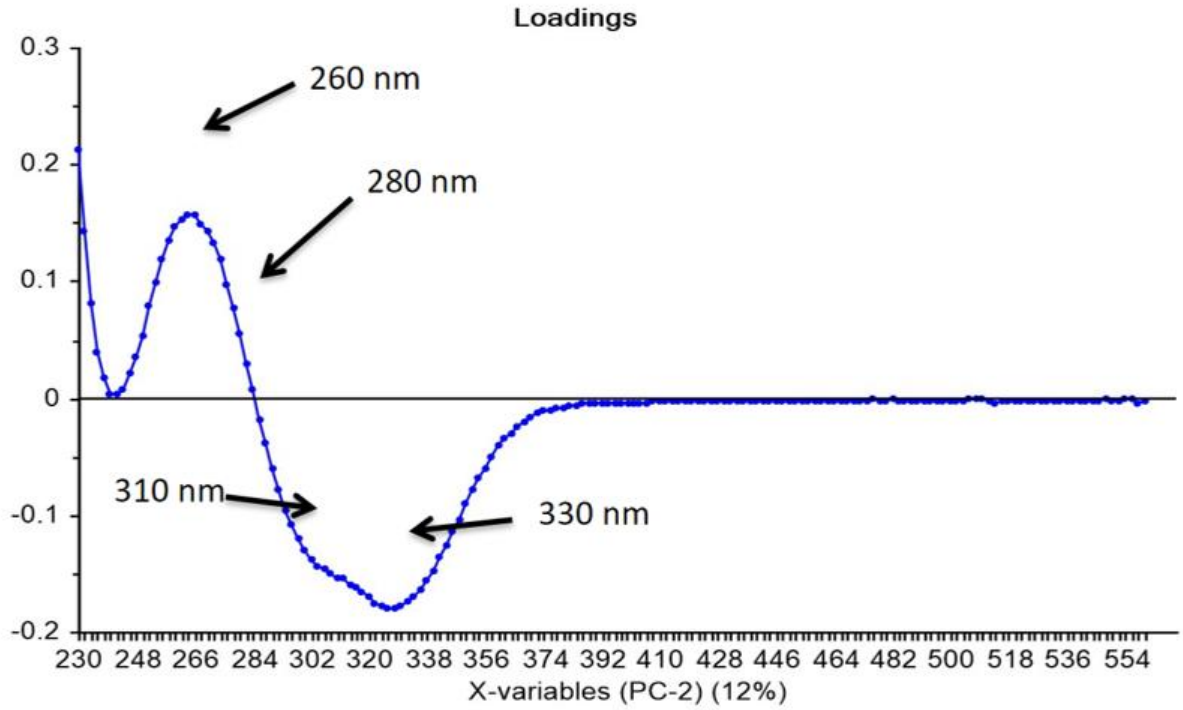


Figure 6-11: Southern Tasmanian leaf removal Chardonnay – base wine spectral loadings PC2

6.1.4.3 Aged sparkling wine SPF

During the 5.5 years that leaf removal treatment wines were aged, approximately half of the wines became unstable and were not suitable for analysis or tasting. This means that not all wines were assessed or analysed. For those bottles that were sound, and hence suitable for analysis, the chemical results and sensory appraisal were not amenable to statistical analysis due to unbalanced design and lack of replication. This means that leaf removal effects in the aged wines are examined using PCA and

tasting data for northern and southern sites was aggregated. The data reported herein is therefore described in terms of 'trends' or 'observations' rather than 'findings'.

Analysis of sparkling wines using SPF after wines had been bottle aged for 5.5 years revealed a similar pattern to the base wines. Seasonal variation was still the strongest factor driving separation of wines from each other (Figure 6-12 and Figure 6-13), and this separation was mostly driven by wavelengths indicating low molecular weight hydroxycinnamates, as opposed to 'total phenolics' (280 nm).

In terms of treatment effects, these were not strong. There may have been some separation of 2010 wines based on treatment with 2010 control wines from the north showing some clustering and separation from veraison treatment, although this is based on a very small number of samples. Similarly, there may have been some clustering and separation for 2010 aged wines from the south, but the effect was not clear or compelling.

Analysis of the aged wines from this trial by SPF did not deliver particularly strong or compelling evidence of impact on wine phenolics associated with the leaf removal treatments applied, but this may have been due to limited sample numbers.

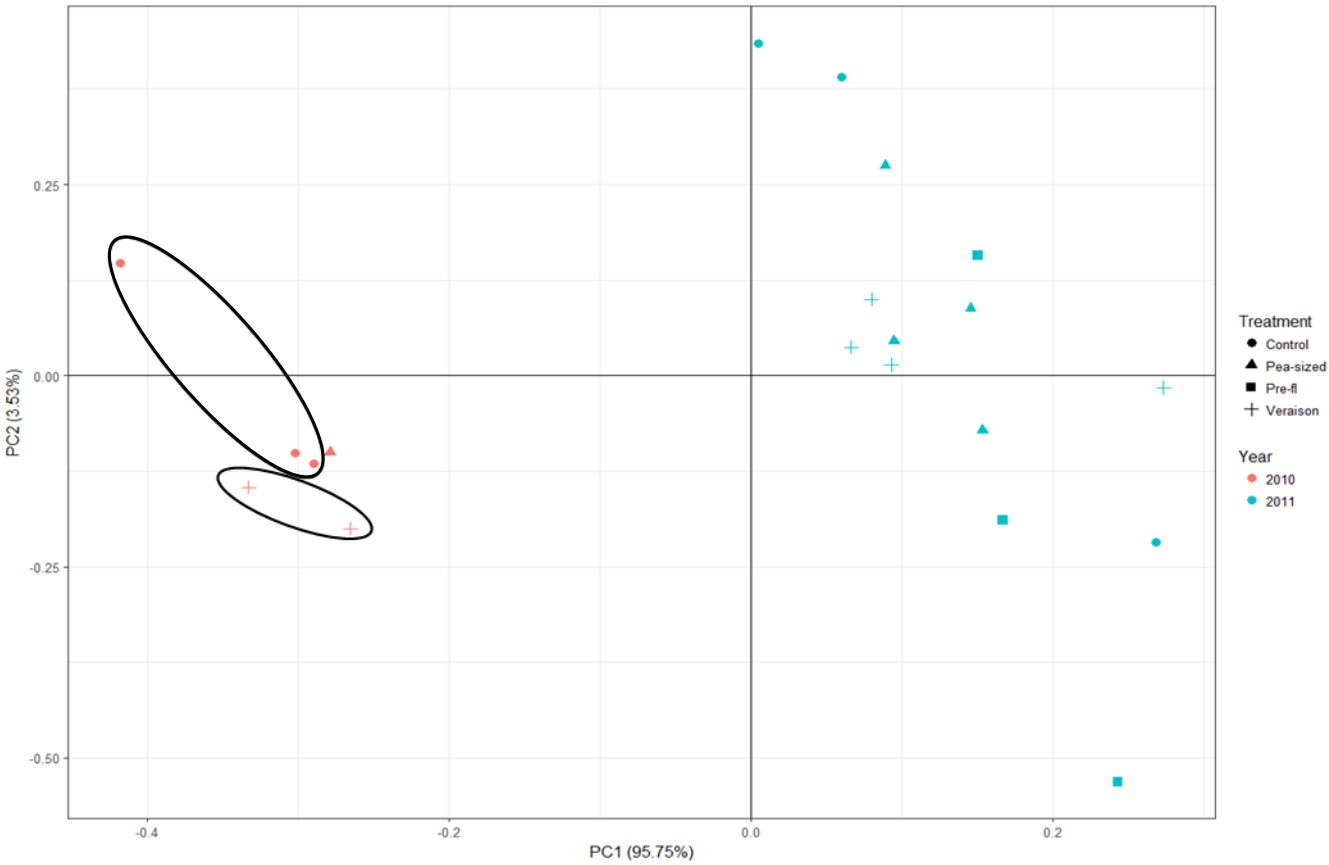


Figure 6-12 **Northern Tasmania Chardonnay leaf removal – SPF aged sparkling wine**

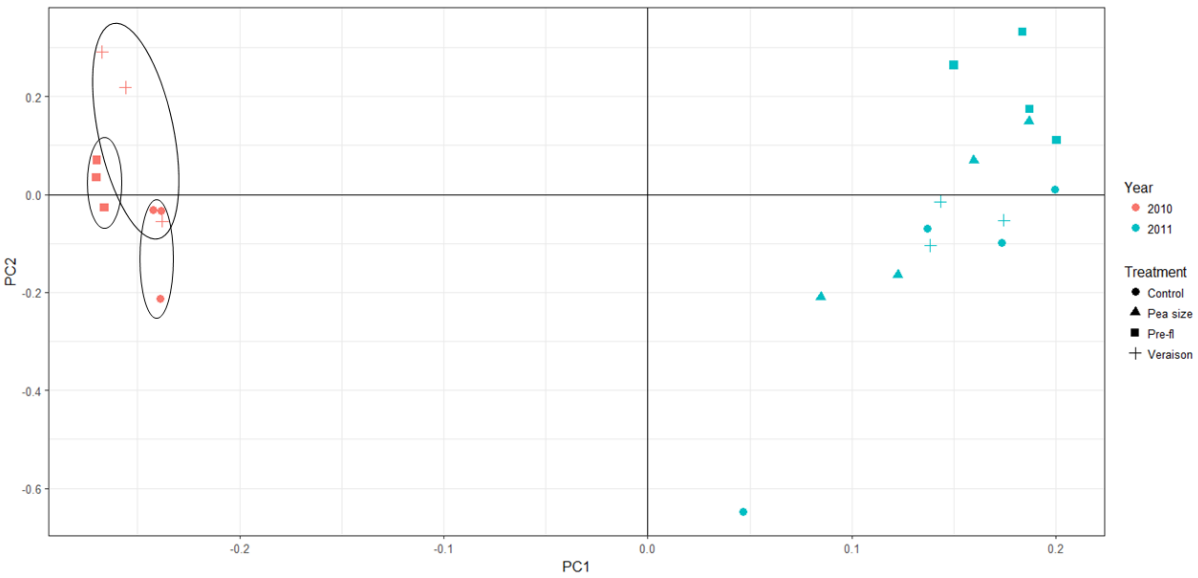


Figure 6-13 **Southern Tasmania Chardonnay leaf removal – SPF aged sparkling wine**

6.1.4.4 Volatiles

Quantification of aroma compounds at 5.5 years bottle age for those wines that were sufficiently sound to analyse showed that vintage was the main driver of difference between the trial wines, for both north and south sites. The leaf removal treatments applied, appeared to have some impact on the aroma profile of the aged wines.

Chardonnay wines from control fruit (no leaf removal) in 2010 from the northern Tasmanian trial site showed separation on PC2 associated with volatile compounds with fruity (hexyl acetate and ethyl butanoate), banana (3-methylbutyl acetate) aroma descriptors. In contrast, aged wines made from leaf removal fruit in that year were associated with aroma compounds that provide floral/rosy, honey and cabbage aromas in wine (2-phenyl ethyl ethanol, phenylacetaldehyde, methionol, respectively).

Wines made from 2011 fruit out of the northern vineyard showed no clustering or separation based on the leaf removal treatment applied; wines from that vintage separated from 2010 wines on PC1 based on compounds associated with green apple, spicy, fishy and dirty aromas (hexanal, eugonol, heptenal, octenal, respectively). Several of these compounds have been associated with ageing and oxidative flavours in white wines, so this may indicate seasonal conditions had predisposed 2011 wines to prematurely achieving 'aged character' (Figure 6-15).

Similar to the northern trial wines, the aged Chardonnay wines from the south showed greatest separation based on vintage (PC1) with 2010 and 2011 wines differentiated from each other. The 2010 wines showed some differentiation associated with leaf removal treatment. The veraison leaf removal wines from that year separated to some extent from control wines on PC2; leaf removal wines were associated with 2-phenyl-ethyl-ethanol (floral/rosy) and acetic acid (sour). Like the northern trial wines, southern 2011 wines were associated with compounds generally associated with aged or oxidised wines (Figure 6-15).

As with the other viticultural treatments examined in this study (eg. crop load, cane or spur pruning), the impact of leaf removal treatment appeared greater under the warm, dry vintage conditions of 2010. This continues the theme that Chardonnay aroma might be managed through leaf removal timing decisions, but the effect is likely tied to vintage-to-vintage climatic conditions.

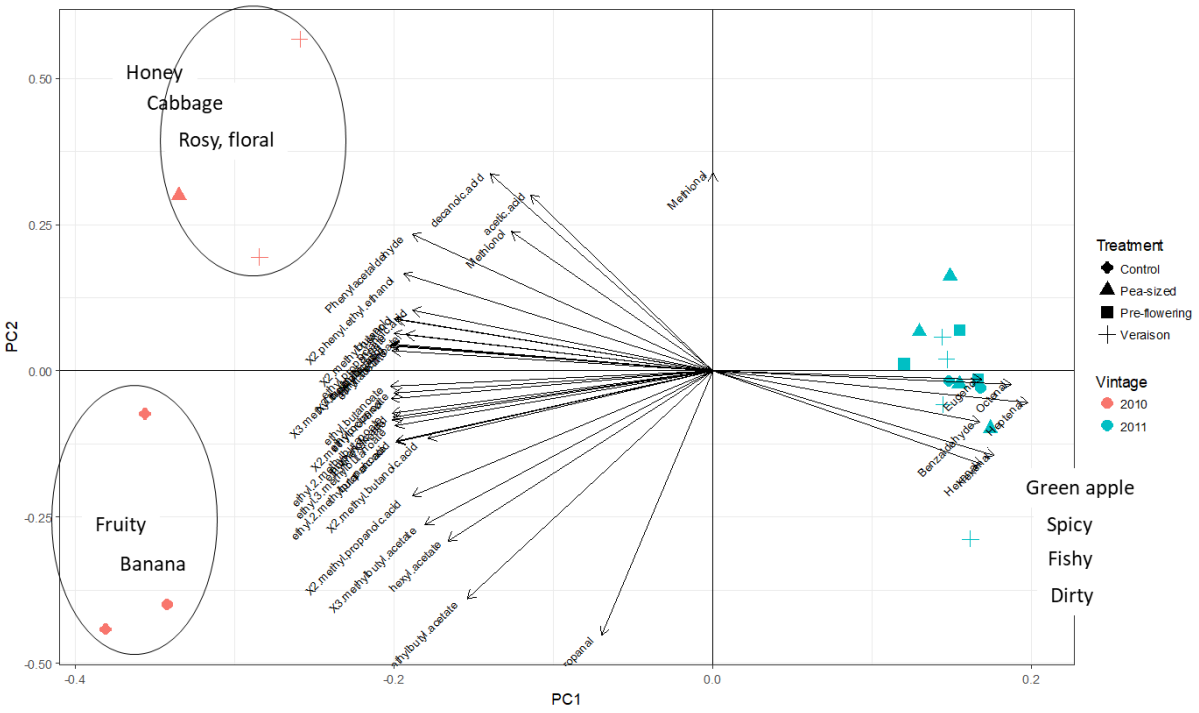


Figure 6-14 Volatile compound – northern Tasmania Chardonnay leaf removal

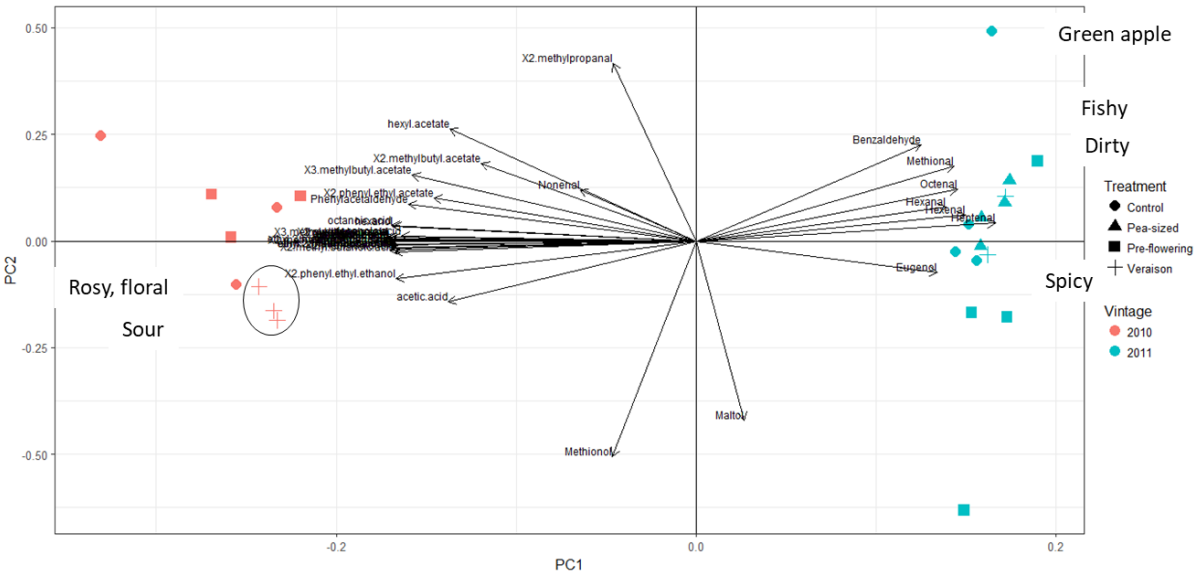


Figure 6-15 Southern Tasmania Chardonnay leaf removal – volatile compounds

6.1.4.5 Industry tasting panel

After 5.5 years aging en tirage, Chardonnay wines from the northern and southern leaf removal treatment trial were blind tasted by an expert industry panel. Due to some wines having become unstable under storage conditions and time constraints at the tasting event (Effervescence Technical Day 2016), only three sets of trial wines could be scheduled for sensory assessment. The 2011 northern wines were tasted, as were southern 2010 and 2011 wines. Judges were asked to indicate preference.

- 2011 Northern wines - pea-sized > veraison > pre-flowering > control
- 2010 Southern wines - veraison > pre-flowering > control
- 2011 Southern wines - veraison > pea-sized berries > control > pre-flowering

Due to the limited numbers of wines that were stable and limited tasting time, show score data for wines from southern and northern trials over both vintages were aggregated into 'leaf removal treatment' categories. Results from the treatment 'pea-sized' were excluded as that treatment was not applied in 2010 southern vintage. Averaged show judging score according to leaf removal treatment is shown below (Figure 6-16) and indicated a preference by the expert panel for wines made when leaves were removed from the basal four nodes at veraison. These wines scored an average show score of 15.8/20, compared with control wines that scored an average of 14.4/20.

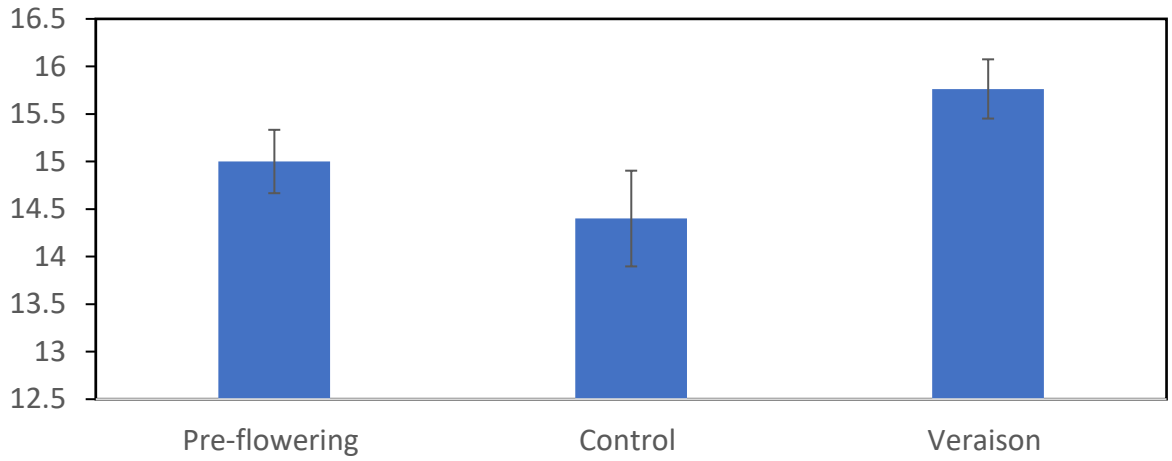


Figure 6-16 Effervescence Technical Day industry expert panel (n=10) - Wine show score out 20. Bars are standard deviation.

Aggregated ratings for each of the eight sensory characteristics are presented below, with trial site and vintage lumped together, and pea-sized removed due to lack of southern 2010 data. This allowed outcomes to be presented as one graph for each sensory characteristic. Panel members rated each wine on eight sensory characteristics: A. sweetness, B. autolytic character, C. bitterness, D. acidity, E. sweetness:astringency balance, F. astringency, G. flavour intensity and persistence, and H. body. The most compelling findings from the sensory evaluation of characteristics were in the contrast between control and veraison leaf removal treatment wines. Compared with control wines, the expert panel judged the veraison leaf removal wines to have (Figure 6-17):

- More autolytic character (b)
- Less bitterness (c)
- Greater flavour intensity and persistence (g)
- More body (h)

In essence, based on the small selection of wines examined in this part of the study, it appeared that leaf removal at veraison was associated with sensorially superior wine outcomes, compared with wines not subjected to leaf removal (control). Further research would be required to elucidate the possible mechanism for this result. The vine, having set fruit based on a reasonable expectation of carbohydrate resources to fully deliver its genetic potential (grapes), may have exhibited a stress

response to the untimely removal of its carbohydrate manufacturing capacity (leaves). From a more prosaic perspective, leaf removal may allow vineyard managers to improve wine sensory quality, with the added advantages of greater spray exposure, and potentially better ventilation to manage disease pressure.

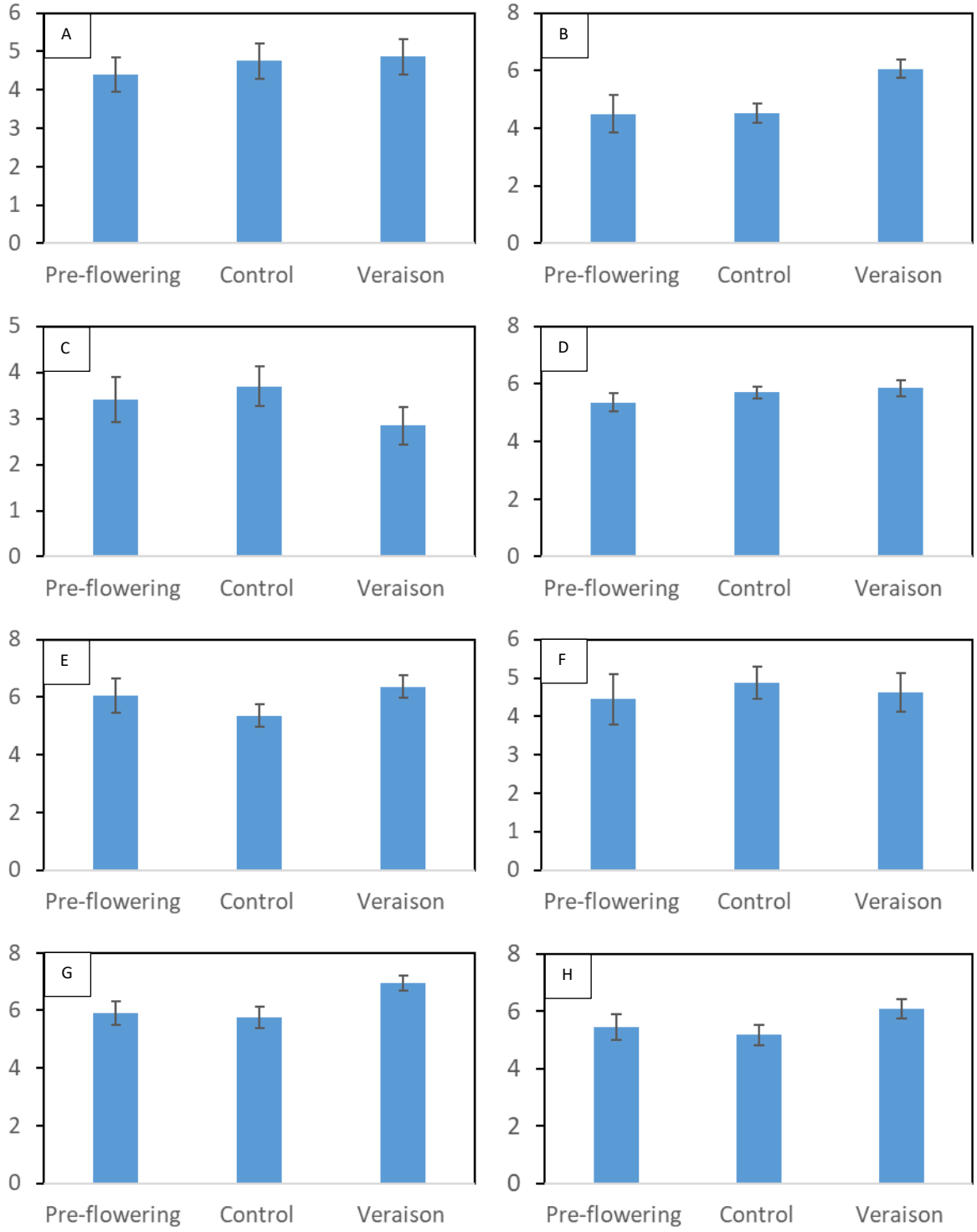


Figure 6-17 Effervescence Technical Day industry expert panel (n=10), A. sweetness, B. autolytic character, C. bitterness, D. acidity, E. sweetness:astringency balance, F. astringency, G. flavour intensity and persistence, H. body. Bars are standard deviation.

6.2 Autolysis and autolytic character

6.2.1 Age or autolysis experiment

Yeast autolysis is considered to be a primary driver for favourable and complex aromatic development in traditional method sparkling wines during wine ageing. To determine the major factors responsible for the development of 'autolytic character', compositional and sensory analyses were conducted. The three treatments were applied to Chardonnay and Pinot noir base wines. The treatments were:

- (1) base wine bottled and aged off lees
- (2) base wines bottled and aged on lees and
- (3) base wines bottled and aged after addition of *liqueur de tirage* (a mix of base wine, sugar and cultured yeast)

After ageing at 15°C for 6, 12, and 24 months, 46 compounds associated with fermentation and the development of oxidative flavours were quantified for each treatment. Comparative sensory analysis was performed at the same ageing intervals, using six aroma descriptors associated with the maturation of sparkling wines: 'autolytic', 'nutty', 'toasty', 'honey', 'spicy', and 'earthy'. Only the 12- and 24-month post-bottling wines were assessed by comparative sensory as yeast autolysis generally takes 6 months to commence, post-tirage (Alexandre, 2019, Kemp et al., 2015).

Age appeared to be the dominant determinant of compositional analyses in both Chardonnay and Pinot noir (Figure 6-18), regardless of treatment, with 6-month, 12-month and 24-month wines forming distinct compositional clusters based on the 46 compounds examined. Treatment-related differences between wines only appeared when each timepoint was considered individually. At 24 months, the Pinot Noir base wine aged off lees and the sparkling wine were more similar than the base wine aged on lees. By contrast, the Chardonnay base wine aged on lees and sparkling wine were more similar than the base wine aged off lees. Generally, there was greater variability between treatments in Chardonnay than Pinot Noir. This was also reflected in the sensory analysis where more treatment-related differences were observed in Chardonnay than Pinot Noir based on the sparkling wine maturation-associated attributes assessed in this study (Figure 6-19).

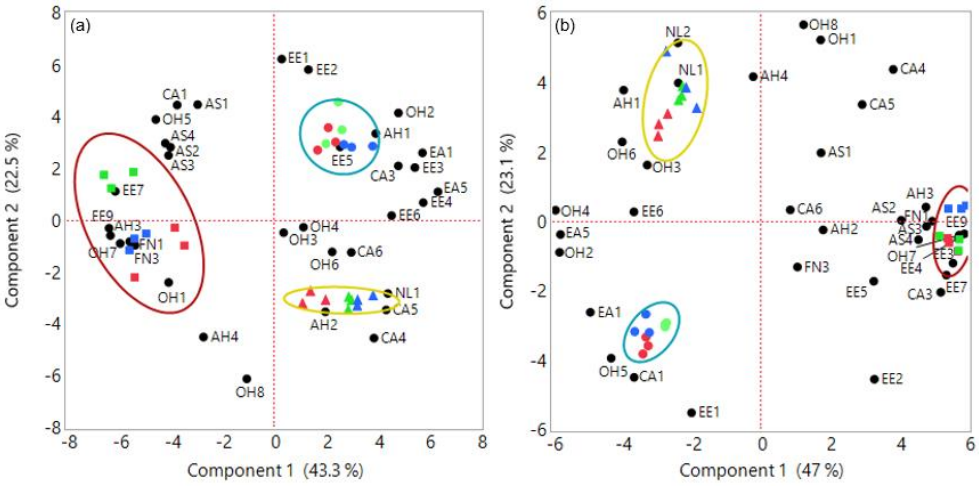


Figure 6-18 PCA biplot of volatile compounds in (a) Chardonnay and (b) Pinot Noir wines after 6 (☐), 12 (▲) and 24 months (■) post-bottling. Base wine aged off lees (☐), base wine aged on lees (☐) and sparkling wine (☐). The 95% confidence ellipses are based on grouping of time points. Chemical codes are as stated in Table 5-2.

Comparative sensory analysis of the Chardonnay wines showed the base wine aged on lees (treatment 2) was perceived as sensorily very similar to the *tirage* wine (3), with no significant differences associated with any of the sensory attributes assessed at 12- or 24-months (Figure 6-19a,b) except for a more intense 'honey' character at 24 months. In contrast, all attributes aside from 'earthy' were perceived as more intense in the base wine aged off lees (1) compared with the *tirage* wine (3) at 12-months age. The difference in perception was less prominent at 24-months with only 'nutty' and 'honey' characters continuing to be perceived as more intense in the 'off lees' treatment wine (1) than *tirage* wine (3). This might suggest that the presence of yeast in Chardonnay wines stopped, limited or curtailed the development of 'nutty' and 'honey' aromas. In comparison, the presence of yeast appeared to delay or slow the development of 'autolytic', 'spicy', 'earthy' and 'toasty' aromas. These results suggest that yeast plays a mediating, limiting or slowing role in the development of aged characters in Chardonnay wine. This raises two questions: Could the yeast additions have 'slowed' the chemical aging process in these wines? Could yeast have been acting as a 'biosorbent', and adsorbing aroma compounds?

Regardless of bottle age, the Pinot noir base wine aged off lees (1) was perceived as very similar to the *tirage* wine (3) based on the sensory attributes examined in this study (Figure 6-19 c,d). The Pinot noir wines bottled on lees (2) showed no significant differences from the on lees (1) and *tirage* wines (3), apart from significantly higher 'honey' aroma at both time points assessed. At 24-months , there was a slightly greater 'autolytic' character in the base wine aged on lees than the base wine aged off lees. These results may suggest the presence or absence of yeast and secondary ferment of the Pinot noir wines had less impact on the development of the aged characters examined in this study, than the use of yeast did in the Chardonnay fermentations. In other words, the impact of yeast was mediated by the composition (or variety) of base wine.

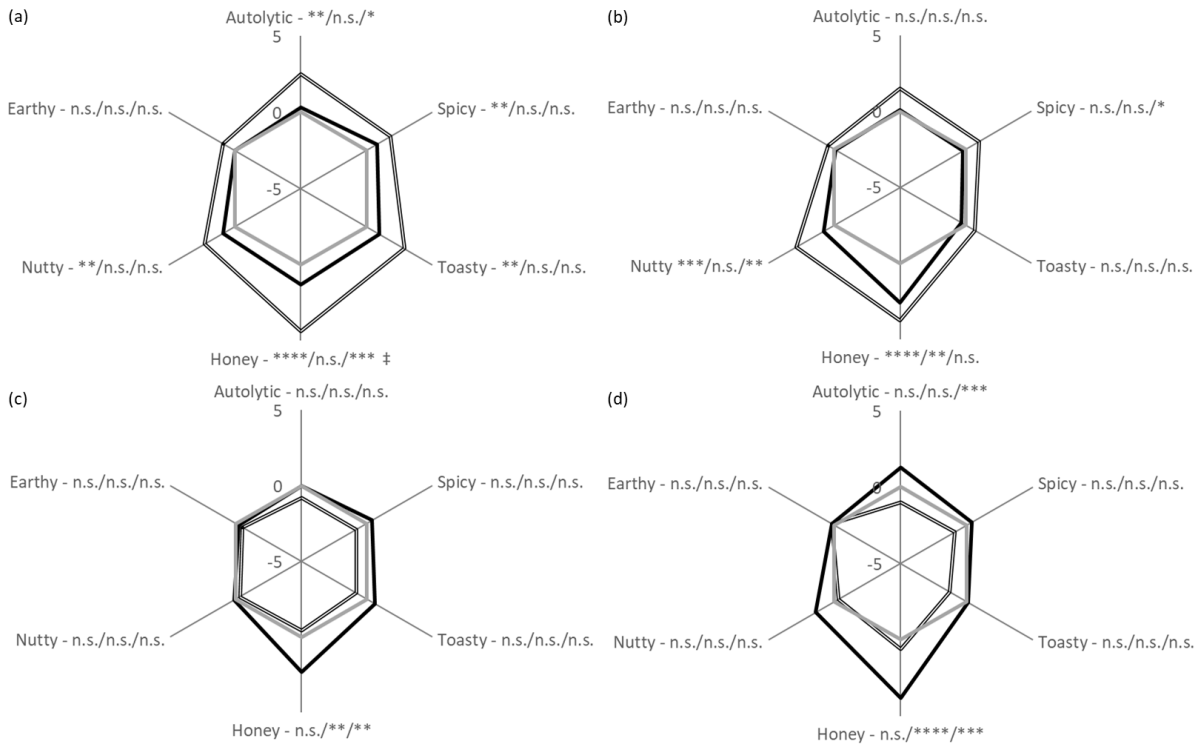


Figure 6-19 Comparative sensory scores of (a,b) Chardonnay and (c,d) Pinot Noir wines at (a,c) 12 months and (b,d) 24 months post-bottling. Tirage wine ( ), base wine aged off lees ( ), and base wine aged on lees ( ). *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; n.s., not significant indicate statistical significance of treatments (base wine aged off lees, base wine aged on lees) compared to the Control (sparkling wine) using Student's t-test and between the two base wine treatments based on three-way ANOVA (considering all two-way interactions), respectively. ‡ Treatment*replicate interaction by three-way ANOVA.

The perception of wines by the sensory panel provided a benchmark against which to consider what chemical cues might be driving or combining to deliver the distinct sensory experiences seen in Figure 6-19. Aldehydes have been shown to contribute to more complex, aged sensory attributes in aged wines and Champagnes such as 'yeasty', 'caramel', 'toasty' and 'malty' characters (Pozo-Bayón et al., 2009, Torrens et al., 2010, Makhotkina and Kilmartin, 2012, Welke et al., 2014, Ruiz-Moreno et al., 2017). The Strecker aldehydes were present at concentrations more than two-fold higher in the base wine aged off lees than the tirage wine, and at concentrations above their respective odour thresholds (Culleré et al., 2007, Escudero et al., 2000). These aldehydes have been associated with 'malty', 'cooked potato-like', 'honey' and 'floral' characters (Riu-Aumatell et al., 2006, Torrens et al., 2010) and may account for the more intense 'honey' characters observed in the wines.

Aside from methional which was not influenced by treatment, the Strecker aldehydes were present at higher concentrations in the Pinot noir base wine aged off lees (113-210%) and base wine aged on lees (23-97%) compared to the tirage treatment wine. While this alone does not account for the different sensory outcomes of the base wine aged on lees compared with tirage wine and base wine aged off lees, the higher concentrations of acetic acid, octanoic and decanoic acids likely contributed to variations in sensory perception and complexity. Strecker aldehyde formation is dependent on the oxidative potential of the wine and the presence of amino acid and higher alcohol precursors (Escudero et al., 2000, Pripis-Nicolau et al., 2000, Silva Ferreira et al., 2002, Bueno et al., 2016). The

prominence of these aldehydes after 24 months suggests the redox potential and/or precursor concentrations varied across treatments and base wine varietal, and that this variation had stronger influence on wine sensory character over time.

Secondary fermentation and subsequent ageing on lees have been associated with the release from yeast cells of potential precursors to aroma compounds (Ruiz-Moreno et al., 2017). However, ageing of the base wine on its lees and tiraging did not result in a significantly different wine from the base wine aged off its lees, based on the compounds analysed and the timeframe of this study. This suggests that many of the precursors and conditions required to induce the volatile changes observed during maturation of sparkling wine may already be present in the base wine and remain even after racking off the primary yeast lees. This implies that so-called 'autolytic character' may not be solely derived from the secondary ferment or subsequent aging on yeast lees.

For the compounds and sensory attributes assessed in the timeframe of this study, this finding may warrant further research into manipulating base wine to alter the composition of sparkling wine.

Based on the limitations of this study around the chemical compounds assessed, and the time wine was aged post-tirage, further studies could also consider grape-derived volatile compounds and their role on final sensory development of sparkling wines, oxidative potential in base wines and its potential impacts, and the effect of manipulating base wines on other organoleptic properties.

Some practical 'take home' messages for winemakers from the findings reported in Section 6.2.1:

- The use of different lees treatments in winemaking had an impact on the development of 'aged character' in sparkling wines;
- Different lees treatments did not have consistent impacts on aroma profile - impacts varied by base wine varietal and the bottle age of wine at sampling;
- 'Aged character' in sparkling wine was not solely a result of yeast autolysis;
- Some wines showed greater 'aged character' having been aged off lees.

In conclusion, this experiment showed that the nature and development over time of 'aged character' in sparkling wine may by impacted by a range of factors including: grape- and primary fermentation-derived aroma precursors, oxidative potential of base wines, base wine varietal, chemical aging of wine in bottle, post-bottling yeast-mediated aroma modification (including yeast metabolic by-products, aroma compound adsorption/ desorption, physical breakdown of yeast cells), and metabolites from malolactic fermentation bacteria.

6.2.2 Manipulating aged character/autolysis

The ability to manipulate the development of aged character in sparkling wines is desirable to control the flavour profiles of premium sparkling wines but also to potentially reduce the time spent ageing on lees. The cost impost of long ageing is in the form of energy, space, and other operational costs. This further increases the time between monetary outlay and return. There is also the risk associated with not being able to control the profile of the final product. Four autolysis-promoting techniques were investigated for their impact on volatile compounds and sensory attributes of sparkling wines made using Chardonnay and Pinot noir base wines. The treatments examined were:

- (1) control wine – standard tirage and ageing on lees at 15°C
- (2) enzyme – standard tirage + enzyme treated yeast and ageing on lees at 15°C
- (3) microwave – standard tirage + microwave treated yeast and ageing on lees at 15°C
- (4) ultrasound – standard tirage + ultrasound treated yeast and ageing on lees at 15°C
- (5) 25°C – standard tirage and ageing on lees at 25°C

It is important to note that treatments (2), (3) and (4) involved the addition of more yeast. The additional dose of yeast in these treatments meant there was a greater amount of yeast material in these wines, compared with treatments (1) and (5).

Wines were assessed by an expert sensory panel for the following aroma characteristics ('aged characters'): autolytic, nutty, honey, spicy, earth, toasty. Wines of both varieties that had been aged on lees at 25°C were perceived to be highest in all six of the aged characters assessed in this study, at both of the time periods examined (Figure 6-20). The 25°C treatments showed particularly high perceived intensity of 'autolytic', 'nutty', and 'toasty' characters, compared with the other aged aroma characters assessed. None of the aroma descriptors was, however, judged to be overly strong or overpowering in relation to other aromas, and the sensory panel judged the 25°C treatments wines to be within acceptable aroma limits (eg. not faulty). This suggests that elevated temperature during ageing on lees could result in earlier development of aroma profiles associated with 'age', without negatively impact overall wine aroma. This finding is in contrast with other literature suggesting elevated temperatures may cause organoleptic defects (Cebollero et al., 2005) including an overly 'toasty' aroma profile.

Comparison between the control wine sensory results, and sensory results from wines with addition of treated yeast (2), (3) and (4) showed more variable effects.

In Chardonnay, the addition of ultrasound treated yeast resulted in increased perceived intensity of 'autolytic', 'toasty', 'honey', and 'earthy' characters at 12 months, and 'nutty', 'honey', and 'spicy' at 24 months, when compared to the control wine. The β-glucanase and microwave treatment wines were perceived as very similar to the control Chardonnay wines, at both 12 and 24 month tastings, with no statistically significant differences in any of the assessed sensory attributes. This result shows that simply 'adding more yeast' did not result in predictable or consistent increase in aroma intensity. The variability observed suggests there were differential effects from the yeast disruption techniques applied. Only the ultrasound treatment resulted in tangible aroma modification of Chardonnay wines at 24 months.

While there were some differences between Pinot noir control and treatment wines at 12 months, by 24 months, wines showed a range of interesting aroma differences according to the sensory panel assessment. While this is only one study and the results would need to be tested and replicated in a range of vintage and winery conditions, if the findings here are valid they suggest that winemakers using a Pinot noir base wine could potentially select and apply the yeast disruption technique associated with the profile they wanted in their finished wines. For example, if a winemaker wanted more spicy and nutty wine, they could add enzyme treated yeast at tirage. For more autolytic, nutty, honey and toasty wine, they might apply microwave treated yeast. For more of everything but earthy aroma (ultrasound), or more of all the aged character aromas examined here, winemakers might cellar their wines as 25°C. These ideas are not conclusive, but the sensory results reported above suggest development of aged character in sparkling wine might be more precisely managed via better understanding of the potential offered by addition of disrupted yeast at tirage, and the use of high temperature cellaring.

It is notable that the sensory results here suggested that the Chardonnay base wine matrix showed less malleability by disrupted yeast treatment than the Pinot noir wine matrix. This contrasts with sensory results from Section 6.2.1 where the presence or absence of yeast lees had less impact on sensory aroma variation for Chardonnay, than for Pinot noir wines. The two sets of results indicate that selection of technique to influence aroma profile or development of 'aged character' might be guided by base wine varietal.

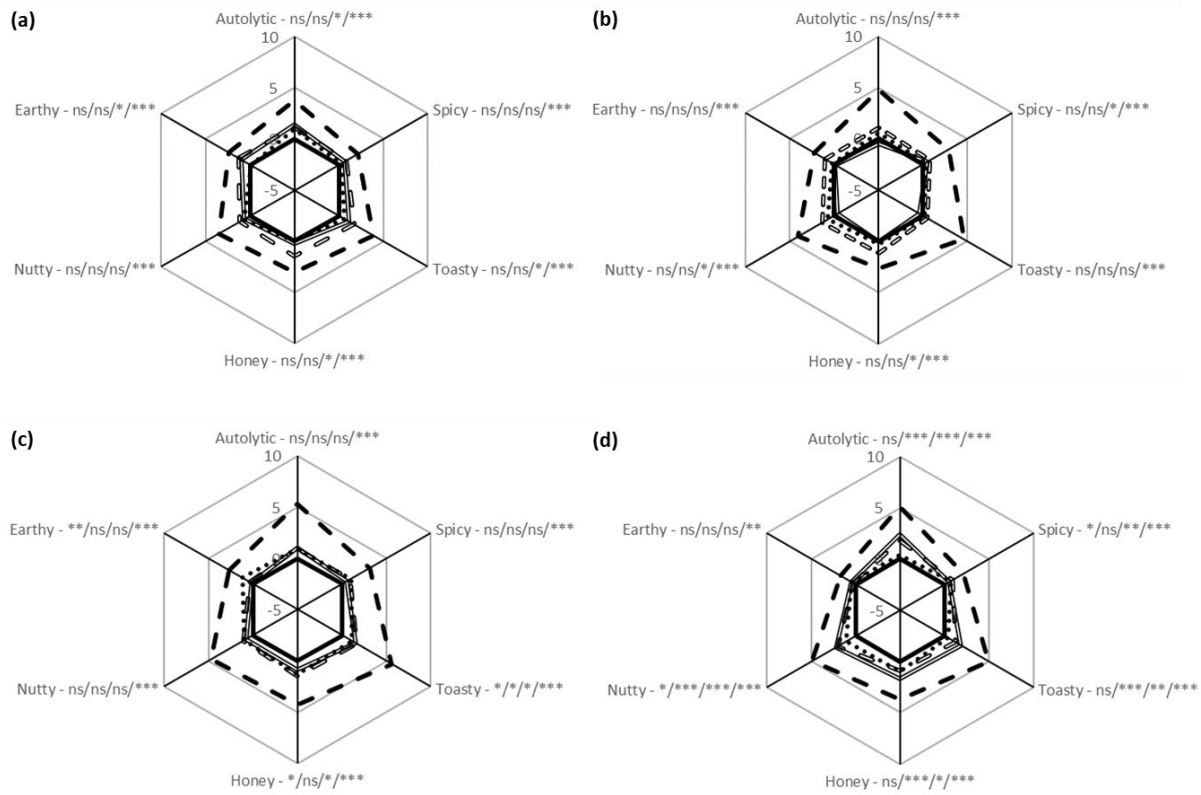


Figure 6-20 Comparative sensory scores of Chardonnay (a,b) and Pinot Noir (c,d) sparkling wines at 12-months (a,c) and 24-months (b,d) post-bottling. Control wine ( ), enzyme-treated ( ), microwave-treated ( ), ultrasound-treated ( ), and 25 °C *sur lie* ( ) wines. *: p<0.05; **: p<0.01; ***: p<0.001. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; n.s., not significant indicate statistical significance of treatments (enzyme, microwave, ultrasound, and 25 °C *sur lie*, respectively) compared to the Control (standard tirage aged at 15 °C) using Student's t-test.

Treatment wines were analysed for volatiles composition. The volatiles composition of the wines was strongly associated with treatment in both Chardonnay and Pinot noir wines based on PCA discriminant analysis (Table 6-20). All the samples that were stored at 25°C were correctly predicted, with confusion only observed between the other treatments and the control (83-92% prediction accuracy) leading to an overall prediction accuracy of 90%. Modelling the variance of the volatile composition profile using two principal components accounted for 83.0-83.6% and 81.8-88.2% of the variability in Chardonnay and Pinot Noir, respectively (Figure 6-21 and Figure 6-22). Only compounds which exhibited statistically significant changes were modelled and sensory attributes were overlaid as supplementary variables. In both varieties, the 25°C ageing treatment separated from the rest of the treatments at 12 months and the separation appears to be driven by the aldehydes, furfural, 5-methylfurfural, 2-methylpropanal, and methional, as well as the two ethyl esters, ethyl 2-, and 3-methylbutanoate. After 24 months of post-tirage, the 25°C ageing treatment separated again from the rest of the treatments in both varieties, but the other treatments also separated from the control with the microwave and ultrasound treatments forming their own cluster. This could account for some of the sensory differences observed. The aldehydes, furfural, 5-methylfurfural, and 2-methylpropanal, were consistently associated with the 25°C ageing treatment in both varieties. These three compounds were positivity correlated with all sensory attributes except 'earthy' in Chardonnay ($0.26 < r < 0.46$, $p<0.01$; $0.16 < r < 0.39$, $p<0.05$; $0.18 < r < 0.54$, $p<0.05$; respectively) and all sensory attributes in Pinot Noir ($0.27 < r < 0.62$, $p<0.0005$; $0.26 < r < 0.61$, $p<0.0007$; $0.27 < r < 0.62$, $p<0.0006$;

respectively). These three aldehydes share a common formation pathway via a Schiff base formed from amino acids and reducing sugars. All three aldehydes are associated with 'yeast-like', 'caramel', 'toasty' and 'malty' flavours (Comuzzo et al., 2006, Torrens et al., 2010, Czerny et al., 2008). Furfural and 5-methylfurfural, in particular, are highly correlated with aroma development in aged cuvée wines (Le Menn et al., 2017) and sparkling wines (Tominaga et al., 2003). Thus, the elevated storage temperature appears to have accelerated wine ageing and the development of aroma.

The β-glucanase treatment had no significant sensory impact compared to the control in Chardonnay and its impact on Pinot Noir was only observed at 24 months. The application of β-glucanase to yeast has been shown to result in an autolysate rich in vitamins, amino acids and macromolecules (Rodriguez-Nogales et al., 2012, Torresi et al., 2014) which should have resulted in increased concentration of precursors to aroma compounds associated with aged characters. The finding of limited sensory difference between control and enzyme treated wines suggests that the enzyme application did not release sufficient amounts of precursor aroma compounds associated with aged character. Alternately, based on the 24 month bottle age PCA results (Figure 6-21 and Figure 6-22) it is possible that enzyme yeast disruption released yeast cellular components but they were not converted to aroma-active forms. Another conjecture would be that the enzyme treatment disrupted production of cellular components associated with secondary fermentation (such as hydrolases), or the enzymes are only active in intact cells (Nunez et al., 2005, Kemp et al., 2015). Setting aside the sensory findings, Figure 6-22 b shows enzyme treated Pinot noir wines at 24 months were more closely associated with 25°C wines in terms of the volatiles composition analysed. This might suggest the enzyme wines were more 'chemically aged' than control, microwave and ultrasound wines. It may also suggest that many of the volatiles assessed were not sensorily significant in these wines.

The evolution of microwave and ultrasound wines between 12 months and 24 months bottle age is shown in the PCA results. In terms of the volatile compounds analysed, all treatment wines were closely associated at 12 months bottle age, and were separated from control wines on PC1, which explained approximately 60% of the separation. By bottle age 24 months, the microwave and ultrasound treatment wines for both Chardonnay and Pinot noir were distinctly separate from the control wines, and the enzyme and 25°C wines, with separation across both PC1 and PC2. Enzyme and 25°C wines appeared to have become more closely associated by 24 months bottle age, in terms of volatile fingerprint. It could be inferred that wine volatile profiles were evolving at different rates during the bottle aging period. One hypothesis is that volatiles composition (as measured in this study) was dependent on yeast degradation, and that the rate of yeast degradation was greatest with high temperature cellaring (25°C), followed by enzyme treatment, whereas microwave and ultrasound treatment only moderately increased the rate of degradation, compared with control.

Two volatiles - 2-Phenylethanol and Ethyl 2-methylproponoate – were associated with ultrasound Chardonnay at 24 months, and with both microwave and ultrasound Pinot noir wines at 24 months bottle age. Honey and nutty characters were judged by the sensory panel to be sensorily significant in ultrasound Chardonnay wines at 24 months, and in both microwave and ultrasound Pinot noir wines at 24 months. Previously, 2-Phenylethanol has been associated with 'honey, spice, rose, lilac' aroma (Rice et al. 2018) and Ethyl 2-methylproponoate has been associated with 'sweet, fruity' aromas in wine (Francis et al. 2005). Further research may be justified into 2-Phenylethanol and Ethyl 2-methylproponoate as potential impact compounds, possibly associated with 'honey' aroma.

Overall, the aroma and flavour maturation of traditional method Chardonnay and Pinot noir sparkling wines was manipulated by elevating storage temperature from 15°C to 25°C. More intense maturation associated characters, 'autolytic', 'spicy', 'honey', 'earthy', 'nutty' and 'toasty' were achieved at a

relatively young bottle age (24 months). The yeast treatments also had a positive impact on perception of maturation associated characters but to a lesser extent. In a separate study of the wines made in this experiment, Gnoinski (2021c) showed that the benefits of 25°C treatment in terms of precocious 'aged character' may be offset by impacts on foaming, compared with control, microwave and enzyme treatment wines:

> '…wines had similar foaming properties, except for the high temperature (25°C) storage treated wines and ultrasound treated wines, in both Chardonnay and Pinot noir. High temperature storage of wine effected the lowest total proteins concentration and an associated decrease in average foam lifetime (foam stability)…'

It is possible that amino acids and reducing sugars in the 25°C treatment wines were Schiff base to form the aldehydes which our analysis of the wines has associated with 'aged character' (furfural, 5-methylfurfural, 2-methylpropanal). Amino acids would hence not be available to support good foaming outcomes for wines aged at 25°C. In contrast, the enzyme and microwave treatment wines in the Gnoinski et al. (2021) study showed promising foaming outcomes.

Considering the impact of elevated storage temperature and alternate yeast treatments on sparkling wine aged character development, further chemical and sensory studies are warranted. Particular focus would be important on optimising the foaming properties of warm cellared wines. Further research could examine impacts on the rate and degree of aged character development from applying a wider range of microwave, enzyme and ultrasound regimes (degree of yeast disruption applied, amount of yeast added at tirage). It would also be useful to understand if greater addition of yeast at tirage in the 25°C wines resulted in 'protective' effects for foaming quality (more amino acid residual) or simply resulted in greater production of aldehydes.

Table 6-20 Confusion matrix (actual class in columns, predicted in rows) of discriminant analysis to predict treatment class using volatile analysis.

	Control	Enzyme	Microwave	Ultrasound	25°C
Control	**11**	**0**	**0**	**0**	**0**
Enzyme	**1**	**11**	**1**	**0**	**0**
Microwave	**0**	**1**	**10**	**2**	**0**
Ultrasound	**0**	**0**	**1**	**10**	**0**
25°C	**0**	**0**	**0**	**0**	**12**
Class % correct	**92**	**92**	**83**	**83**	**100**
Overall % correct	**90**				

Input x-data was normalised and 8 PCA scores (94 % explained variance) were used for discriminant analysis with a quadratic function.

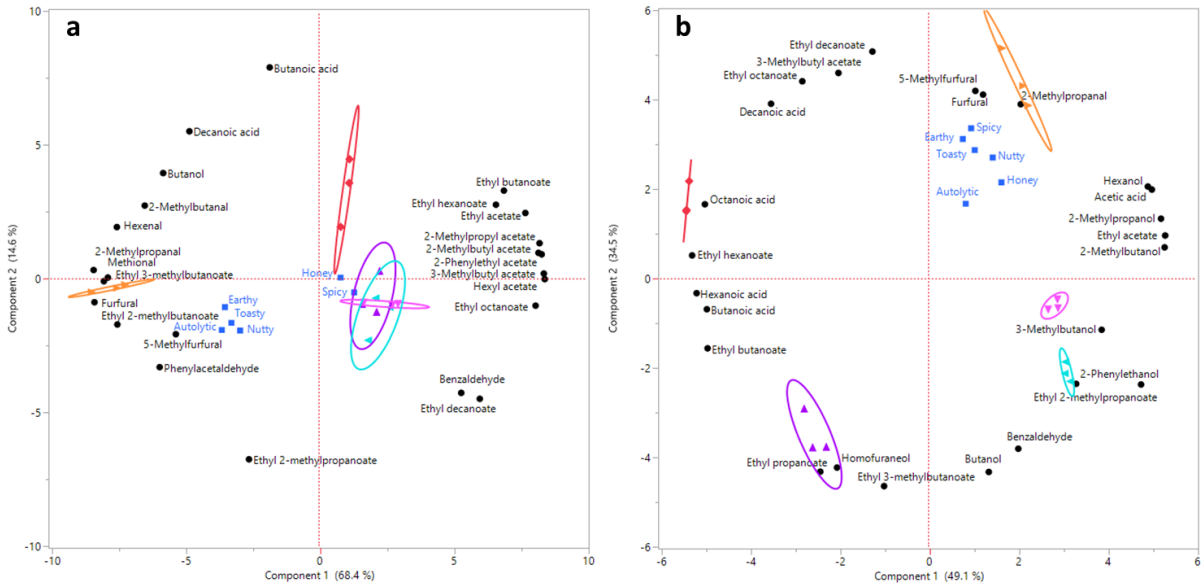


Figure 6-21 PCA biplot of volatile compounds in Chardonnay sparkling wines aged for 12-months (a) and 24-months (b). Sensory attributes (dark blue) have been added as supplementary variables to the biplot. Red symbols are control wines aged at 15 °C, orange symbols are wines aged at 25 °C, and the purple, pink, and light blue symbols are wines with additional enzyme-, microwave-, or ultrasound-treated yeast, respectively. The 95% confidence ellipses are based on grouping of time points.

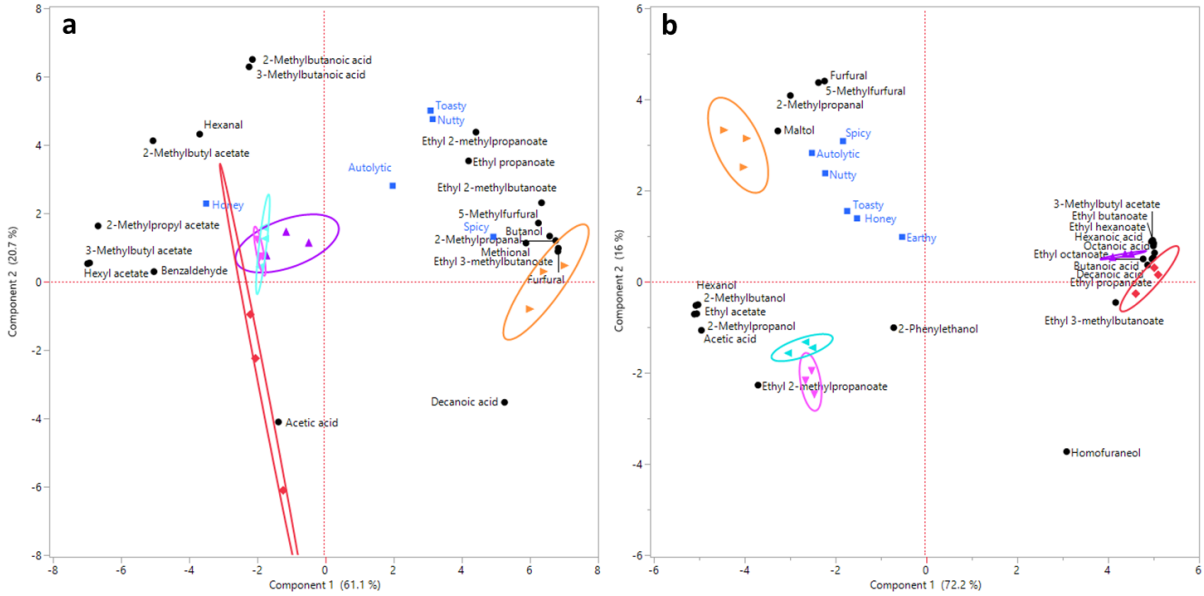


Figure 6-22 PCA biplot of volatile compounds in Pinot Noir sparkling wines aged for 12-months (a) and 24-months (b). Sensory attributes (dark blue) have been added as supplementary variables to the biplot. Red symbols are control wines aged at 15 °C, orange symbols are wines aged at 25 °C, and the purple, pink, and light blue symbols are wines with additional enzyme-, microwave-, or ultrasound -treated yeast, respectively. The 95 % confidence ellipses are based on grouping of time points.

6.2.3 Objective measurement of sparkling wine

In an industry setting, it is expensive and impractical to destructively sample sparkling wines to, for example, assess product quality, authenticate provenance or check on the development of aged character. Earlier work at TIA had demonstrated proof of concept for UV-Vis SPF fingerprinting in discrimination between juices and wines. A non-destructive bottle scanning was undertaken using

BevScan, and UTAS PhD candidate Leo Lebanov assessed the suitability of three different statistical techniques to differentiate wines using their BevScan-derived spectral fingerprints. The statistical techniques assessed were: principal component analysis (PCA), random forest mode (RF) and partial least squares-discriminant analysis (PLS-DA). The 6-, 12-, and 18-months post-tirage Chardonnay and Pinot noir wines from the autolysis and aging experiments were examined. The dataset was initially examined as a whole, with data from all eight treatments used ('Unsupervised Analysis'). Data were then divided into two datasets to reduce complexity of the analysis. The two datasets were called 'age or autolysis' (as discussed in Section 6.2.1 above) and 'manipulating aged character/autolysis' (as discussed in Section 6.2.2 above).

Unsupervised Analysis

Unsupervised pattern recognition analysis (PCA) on the raw data was initially used to assess the whole dataset for Chardonnay (Figure 6-24 a,b) and Pinot Noir (Figure 6-24 a,b). There was some separation based on bottle age (time post-tirage) but no clear discrimination of treatments. Pre-processing of the data sets did not improve discrimination (Figure 6-24 c,d and Figure 6-24 c,d).

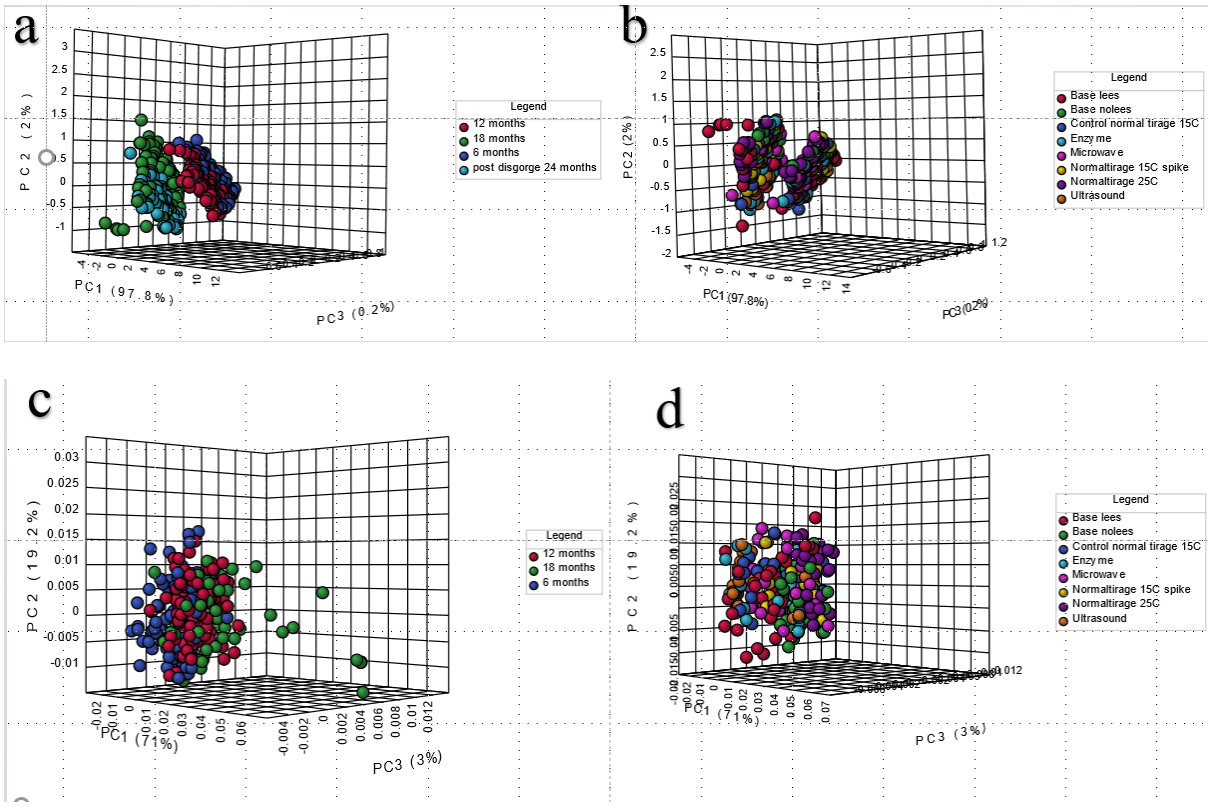


Figure 6-23 PCA on Chardonnay dataset for (a) age, (b) treatments discrimination based on raw data sets, (c) age, (d) treatments discrimination based on pre-processed data (MSC+SG1)

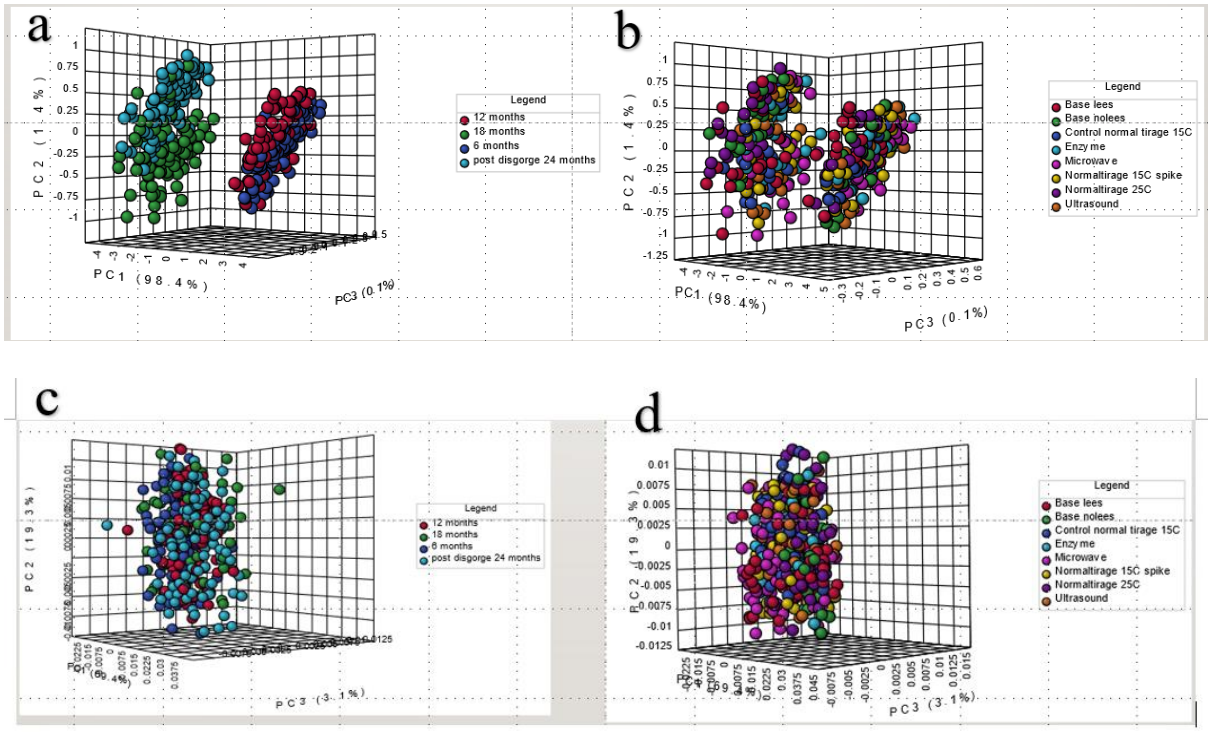


Figure 6-24 PCA on Pinot noir dataset for (a) age, (b) treatments discrimination based on raw data sets, (c) age, (d) treatments discrimination based on MSC+SG1 processed data.

Supervised Analyses

The effectiveness of two supervised methods, RF and PLS-DA, to discriminate wines based on bottle age (time post-tirage) and the eight winemaking treatments was assessed, for Chardonnay and Pinot noir wines. These methods were also applied to sub-datasets where data has been separated into results from the 'age or autolysis' treatments, and 'manipulating aged character/autolysis' treatments. RF was performed on pre-processed data (MSC and SG2) and PLS-DA was performed on the raw dataset.

Both RF (Table 6-21) and PLS-DA (Table 6-22) methods were able to discriminate the wines based on bottle age, even when the whole dataset was included in the model. This included data from all eight treatment wines (including control and tirage wines, base wines with and without lees, additional yeast added to the manipulating aged character/autolysis treatment wines, and high cellaring temperature treatment. These results complement the findings from the age or autolysis experiments which observed the bottle age of the wines was associated with change to the chemical and sensory characteristics of the wines (Gnoinski et al. 2021a).

Sensitivity is the term used to denote 'the number of correct positive predictions divided by the total number of positive predictions'. Overall, the sensitivities of both RF and PLS-DA (Table 6-23) were very high, meaning both techniques were able to use BevScan spectral data to correctly discriminate the wines by bottle age with 99.4 to 100% accuracy.

Table 6-21 RF confusion matrix for wines based on bottle age

	6 months	**12 months**	**18 months**	**Classification error**
Chardonnay				
Whole dataset				
6 months	87	0	0	0
12 months	0	89	0	0
18 months	0	0	89	0
Manipulating aged character/autolysis				
6 months	62	0	0	0
12 months	0	64	0	0
18 months	0	1	61	0.016
Age or autolysis				
6 months	37	0	0	0
12 months	0	37	0	0
18 months	0	0	40	0
Pinot Noir				
Whole dataset				
6 months	88	0	0	0.000
12 months	0	87	0	0.000
18 months	0	0	86	0.000
Manipulating aged character/autolysis				
6 months	63	0	0	0.000
12 months	0	61	0	0.000
18 months	0	1	61	0.020
Age or autolysis				
6 months	38	0	0	0.000
12 months	0	39	0	0.000
18 months	0	1	36	0.030

Table 6-22 Sensitivity for the PLS-DA for age discrimination created on raw dataset

	Sensitivity total	**6 months**	**12 months**	**18 months**	**Sensitivity in test set**
Chardonnay					
Whole dataset (11 LVs)	0.998±0.002	1.0±0.0	0.995±0.007	1.0±0.0	0.999
Manipulating aged character/autolysis (8 LVs)	0.999±0.002	1.0±0.0	0.998±0.007	1.0±0.0	1.000
Age or autolysis (6 LVs)	0.994±0.007	0.983±0.020	1.0±0.0	1.0±0.0	0.999
Pinot Noir					
Whole dataset (12 LVs)	0.999±0.002	0.998±0.005	0.998±0.005	1±0	1.000
Manipulating aged character/autolysis (11 LVs)	0.998±0.003	0.995±0.009	1±0	1±0	1.000
Age or autolysis (5 LVs)	0.998±0.005	0.994±0.014	1±0	1±0	0.999

Table 6-23 Comparison of sensitivities in test set for RF and PLS-DA models

	Sensitivity in test set	
	Random Forest	**PLS-DA**
Chardonnay		
Whole dataset	1.000	0.999
Manipulating aged character/autolysis	0.996	1.000
Age or autolysis	1.000	0.999
Pinot Noir		
Whole dataset	0.994	1.000
Manipulating aged character/autolysis	0.996	1.000
Age or autolysis	0.997	0.999

A reasonably high level of accuracy discriminating control wines from the different treatment wines was observed when the dataset set was split into the two experiments ('age or autolysis', and 'manipulating aged character/autolysis'). The ability of RF (Table 6-24) and PLS-DA (Table 6-25) to discriminate treatment wines from the manipulating aged character/autolysis experiment found accuracy appeared to be somewhat variety dependent. Sensitivity was higher in Pinot Noir for both RF and PLS-DA (Table 6-26). In comparison, both RF (Table 6-27) and PLS-DA (Table 6-28) were able to discriminate the wines from the age or autolysis experiment (Table 6-29). Where treatment wines were confused with each other, it is possible these wines had a similar spectral profile because of the treatments that had been applied. For Chardonnay base wines, confusion observed between enzyme and ultrasound treatments in the later stages of aging (Table 6-24) may indicate those wines were similar to each other from the point of view of their evolving spectral fingerprints. While we cannot rely on BevScan results to explain wine chemistry precisely, the findings suggest the treatments that were applied may have created phenolic matrices in the wines that were not able to be distinguished by BevScan. Similarly in Pinot noir base wines, there was confusion observed at 12 months bottle age between enzyme and ultrasound treatment, and at 18 months between ultrasound and microwave treated wines. Recalling the 'evolution ' of volatile species that was observed for ultrasound and microwave treated Pinot noir (Table 6-25) we could speculate that the BevScan results reinforce the idea of wines evolving in a similar arc; from ultrasound and enzyme wines being 'more phenolically alike' at 12 months, to the microwave and ultrasound wines being 'more phenolically alike' at 18 months.

When data from the 'age or autolysis' experiment was examined independently, the RF model consistently and accurately discriminated between Pinot noir wines 'aged on lees', 'aged off lees' and 'aged after tirage' treatment, for all three of the bottle age periods examined (Table 6-27). In contrast, wines made from a Chardonnay base and aged on lees could not be reliably discriminated from wines aged off lees. The Chardonnay wines that had been tirage treated, however, were reliably discriminated at each bottle age. These findings suggest the varietal wine matrix may 'sponsor' variation in the way yeast and wine phenolics interact as wines bottle age. For example, the wine matrix may impact on yeast metabolic activities that directly or indirectly impact phenolic species and concentration in wines like oxidation, hydrolysis, esterification, polymerization (Echave et al. 2021; Gutiérrez-Escobar et al. 2021). In this series of experiments, the Pinot noir wine base enabled a range of phenolic fingerprints that allowed wines to be distinguished by treatment, whereas the Chardonnay

wine base did not enable wine on lees and wine off lees to develop phenolic profiles that could be reliably distinguished by BevScan.

These results demonstrated that BevScan shows promise for assessment of sparkling wine in-bottle, under some circumstances.

- Coupled with RF and PLS-DA, the **age of wines** could be discriminated with high accuracy. BevScan may be useful for distinguishing wine 'age'.
- The method was potentially reliable for **distinguishing control wines from treatment wines** where the wine matrix or the method of treatment allowed distinctive phenolic profile to arise**.**
- The lack of reliable discrimination between some treatment wines suggests BevScan may not be sufficiently nuanced/precise to always distinguish between wines made from the same base, but using different winemaking methods. This may be exacerbated by wine base (eg. Chardonnay appears more likely to be confused).

These findings make a strong case to continue investigating BevScan as a non-destructive analytical tool for tracking the development of sparkling wine in-bottle. They also demonstrate that base wine matrices have a strong impact on wine evolution post-primary ferment, and that the phenolic fingerprint of sparkling wines can be altered to a significant extent by post-primary fermentation handling.

Table 6-24 Confusion matrix for the RF models for treatment discrimination in 6m, 12m, 18m ageing sub-datasets and for the whole dataset

	Control	**Enzyme**	**Microwave**	**25°C**	**Ultrasound**	**Classification error**
Chardonnay						
6 months						
Control	12	0	0	0	0	0.000
Enzyme	0	12	0	0	0	0.000
Microwave	0	0	6	5	2	0.538
25°C	1	0	2	6	3	0.500
Ultrasound	0	0	1	3	9	0.308
12 months						
Control	11	0	1	0	0	0.083
Enzyme	1	5	0	0	6	0.583
Microwave	0	0	12	3	0	0.200
25°C	1	0	1	11	0	0.154
Ultrasound	0	5	0	0	7	0.417
18 months						
Control	13	0	0	0	0	0.000
Enzyme	0	2	0	0	10	0.833
Microwave	0	0	13	0	0	0.000
25°C	0	0	0	12	0	0.000
Ultrasound	0	6	0	0	6	0.500
All timepoints						
Control	37	0	0	0	0	0.000
Enzyme	0	19	0	0	17	0.472
Microwave	0	0	28	11	2	0.317

25°C	0	0	6	28	3	0.243
Ultrasound	0	14	4	2	17	0.540
Pinot Noir						
6 months						
Control	13	0	0	0	0	0.000
Enzyme	0	12	0	0	0	0.000
Microwave	0	0	10	0	3	0.230
25°C	0	0	0	13	0	00.000
Ultrasound	0	0	4	0	8	0.333
12 months						
Control	13	0	0	0	0	0.000
Enzyme	0	4	0	0	8	0.667
Microwave	0	0	12	0	0	0.000
25°C	0	0	0	12	0	0.000
Ultrasound	0	10	0	0	2	0.833
18 months						
Control	13	0	0	0	0	0.000
Enzyme	0	12	0	0	0	0.000
Microwave	0	0	10	0	2	0.167
25°C	1	0	0	11	0	0.083
Ultrasound	0	0	2	0	11	0.154
All timepoints						
Control	37	0	2	0	0	0.051
Enzyme	0	28	1	0	7	0.222
Microwave	0	0	32	0	5	0.135
25°C	1	0	1	35	0	0.054
Ultrasound	0	7	5	1	24	0.351

Table 6-25 Sensitivity for the PLS-DA for treatment discrimination created on raw dataset

PLS-DA	Overall sensitivity in valid step	Control	Enzyme	Microwave	25°C	Ultrasound	Sensitivity in test set
Chardonnay							
6 months (8 LVs)	0.633±0.046	0.642±0.085	0.816±0.090	0.507±0.136	0.608±0.074	0.607±0.088	0.702
12 months (10 LVs)	0.717±0.043	1±0	0.344±0.152	0.948±0.064	0.898±0.081	0.328±0.130	0.780
18 months (8 LVs)	0.683±0.040	1±0	0.280±0.137	0.882±0.058	1±0	0.683±0.040	0.682
All timepoints (17LVs)	0.670±0.031	0.881±0.033	0.452±0.067	0.776±0.047	0.780±0.055	0.444±0.073	0.672
Pinot Noir							
6 months (13LVs)	0.915±0.025	1±0	0.988±0.032	0.833±0.084	1±0	0.748±0.090	0.938
12 months (10 LVs)	0.724±0.047	0.935±0.066	0.342±0.104	1±0	1±0	0.320±0.141	0.742
18 months (12LVs)	0.812±0.039	0.891±0.077	0.990±0036	0.648±0.078	1±0	0.547±0.139	0.868
All timepoints (17LVs)	0.783±0.20	0.881±0.033	0.765±0.048	0.799±0.044	0.973±0.021	0.489±0.062	0.801

Table 6-26 Comparison of sensitivities in test set for RF and PLS-DA models for treatment discrimination

	Sensitivity in test set	
	Random Forest	**PLS-DA**
Chardonnay		
6 months	0.782	0.702
12 months	0.724	0.780
18 months	0.802	0.682
All timepoints	0.721	0.672
Pinot Noir		
6 months	0.908	0.938
12 months	0.752	0.742
18 months	0.940	0.868
All timepoints	0.830	0.801

Table 6-27 Confusion matrix for the RF models for treatment discrimination in 6m, 12m, 18m ageing sub-datasets and for the whole dataset

	Base wine aged on lees	**Base wine aged off lees**	**Tirage wine**	**Classification error**
Chardonnay				
6 months				
Base wine aged on lees	10	3	0	0.231
Base wine aged off lees	2	10	0	0.167
Tirage wine	0	0	12	0.000
12 months				
Base wine aged on lees	11	2	0	0.154
Base wine aged off lees	3	9	0	0.250
Tirage wine	0	0	12	0.000
18 months				
Base wine aged on lees	14	0	0	0.000
Base wine aged off lees	0	13	0	0.000
Tirage wine	0	0	13	0.000
All timepoints				
Base wine aged on lees	37	3	0	0.075
Base wine aged off lees	4	33	0	0.108
Tirage wine	1	2	34	0.081
Pinot Noir				

6 months				
Base wine aged on lees	12	0	0	0.000
Base wine aged off lees	0	13	0	0.000
Tirage wine	0	0	13	0.000
12 months				
Base wine aged on lees	12	1	0	0.077
Base wine aged off lees	0	13	0	0.000
Tirage wine	0	0	13	0.000
18 months				
Base wine aged on lees	12	0	0	0.000
Base wine aged off lees	0	12	0	0.000
Tirage wine	0	0	13	0.000
All timepoints				
Base wine aged on lees	37	0	0	0.000
Base wine aged off lees	7	31	0	0.184
Tirage wine	0	0	39	0.000

Table 6-28 Sensitivity for the PLS-DA for treatment discrimination created on raw dataset

PLS-DA	**Overall sensitivity in valid step**	**Base wine aged on lees**	**Base wine aged off lees**	**Sparkling wine**	**Sensitivity in test set**
Chardonnay					
6 months (5 LVs)	0.897±0.024	0.847±0.042	0.854±0.064	0.994±0.024	0.917
12 months (6 LVs)	0.952±0.026	0.924±0.033	0.934±0.055	1±0	0.947
18 months (6 LVs)	0.999±0.004	1±0	0.998±0.012	1±0	1.000
All timepoints (9 LVs)	0.938±0.017	0.886±0.037	0.931±0.039	1±0	0.929
Pinot Noir					
6 months (6LVs)	0.994±0.014	0.998±0.013	0.984±0.042	0.998±0.013	1.000
12 months (12 LVs)	0.913±0.040	0.878±0.062	0.863±0.086	0.998±0.013	0.933
18 months (5LVs)	0.987±0.019	1±0	0.998±0.014	0.965±0.048	0.990
All timepoints (9LVs)	0.957±0.009	0.972±0.010	0.896±0.025	1±0	0.956

Table 6-29 Comparison of sensitivities in test set for RF and PLS-DA models for treatment discrimination

	Sensitivity in test set	
	Random Forest	**PLS-DA**
Chardonnay		
6 months	0.873	0.917
12 months	0.913	0.947
18 months	0.983	1.000
All timepoints	0.888	0.929
Pinot Noir		
6 months	0.983	1.000
12 months	0.963	0.933
18 months	0.993	0.990
All timepoints	0.919	0.956

6.2.4 Visualising yeast during autolysis

The term 'autolysis' has been used to describe and explain development of aged character in sparkling wine during bottle aging. Findings reported herein suggest the development of aged character is a more complex process than simply 'the destruction of yeast cells by their own enzymes'. In a project associated with the work described in this report, UTAS PhD candidate Gail Gnoinski investigated the mechanisms of autolysis.

Yeast Morphology Changed with Bottle Age

Yeast cell integrity changes in sparkling wine were assessed over 18 months. Ms Gnoinski developed visualisation and categorisation techniques to document yeast cell changes. Morphological changes were categorised according to the descriptors in Table 6-30. Ms Gnoinski found the control yeast cells were generally smooth and flat at tirage (Figure 6-25). Over time in bottle, progressive degradation of the cell structure was observed, consistent with previous studies (Piton et al., 1988, Tudela et al., 2012). By 6 months bottle age, yeast samples showed almost complete loss of smooth cells, an increase in the proportion of cavitated cells and the appearance of 'doughnut'-shaped cells. Progressively over 12 months' bottle aging, more smooth cells were lost and there was an increase in cells showing cavitated-like features (> 90% of cells) and the first appearance of pitted cells and cell fragments was noted. From 12- to 18-months' bottle age, the range of yeast morphological features did not substantially change, but the impact on cellular structures continued up to 18 months; there was a steady increase in the proportion of cavitated yeast cells and a corresponding decrease in the proportion of cells that appeared flat. While changes to the yeast cell structure began 6 weeks post bottling, yeast cell wall modifications began at 6 months with the removal of the inner cell layer (Piton et al. 1998). These findings were reported in full, in a paper published by Ms Gnoinski and others (2021b).

Table 6-30 Categorisation of *S. cerevisiae* whole cell features (Gnoinski et al., 2021b)

Category	Description
Smooth	Whole-cells with smooth external surfaces, mother-daughter cells, and budding, generally 1 to 5 µm size diameter
Cavitated	Modification of the cell surface to varying degrees creating an uneven cell surface
Flat	Yeast cells positioned flat on the SEM sample holder. Such cells were not classified into the 'smooth' or 'cavitated' or 'doughnut'-shaped categories
Pitted/Porous	Indentations in the cell surface giving an impression of holes in the surface
Doughnut-shaped	Whole cells where the centre appears to have been removed, a cell shaped like a doughnut or bagel
Fragments	Irregular small features thought to result from breakages or remains of cells that are smaller than whole cells, generally less than 1 µm size diameter

Morphological Change in Treated Yeast Cells and Bottle Aging

Ms Gnoinski subjected cultured yeast to disruptive treatment by microwave, ultrasound and β-glucanase, to investigate whether artificially disrupting cell integrity might assist production of precocious 'aged character'. Those treated yeast cultures were used to dose wines (tirage), and then wines were bottle aged alongside control wines.

The microwave, ultrasound and β-glucanase treated yeast cells at tirage generally had features more consistent with the control yeast after it had aged (Figure 6-25). Notably, the proportion of smooth cells in the microwave and ultrasound treatments was significantly lower than in the control (54% and 77% lower, respectively). However, there was no significant difference between the proportion of smooth cells in the β-glucanase treated cells and the control yeast sample, at tirage.

There was a greater incidence of cavitated features in the β-glucanase treatment yeast, compared to the control (68%). This higher incidence of cavitation was similarly observed in the ultrasound (129%) and microwave (91%) treatments.

Yeast cell fragments were observed in all treatments but there was no significant difference between treatments, nor between treatments and the control.

The range and incidence of morphological features observed in the microwave and ultrasound treated yeast cells at tirage, was similar to the range of features observed in control yeast cells after six months of sparkling wine bottle ageing (Figure 6-26), particularly the relative proportion of cells that appeared cavitated or flat. Over time, more yeast cells in the control sample appeared cavitated, and less appeared flat. This might imply a 'progression'; yeast following a degradation pathway from smooth>flat>cavitated. If this progression is valid, the ultrasound and microwave treatments that were applied appeared to hasten the progressive degradation of yeast cells, in a similar way to that observed under bottle aging conditions (but faster). The β-glucanase treated yeast samples showed a similar, but more moderate, pattern of degradation to microwave and ultrasound treated cell samples.

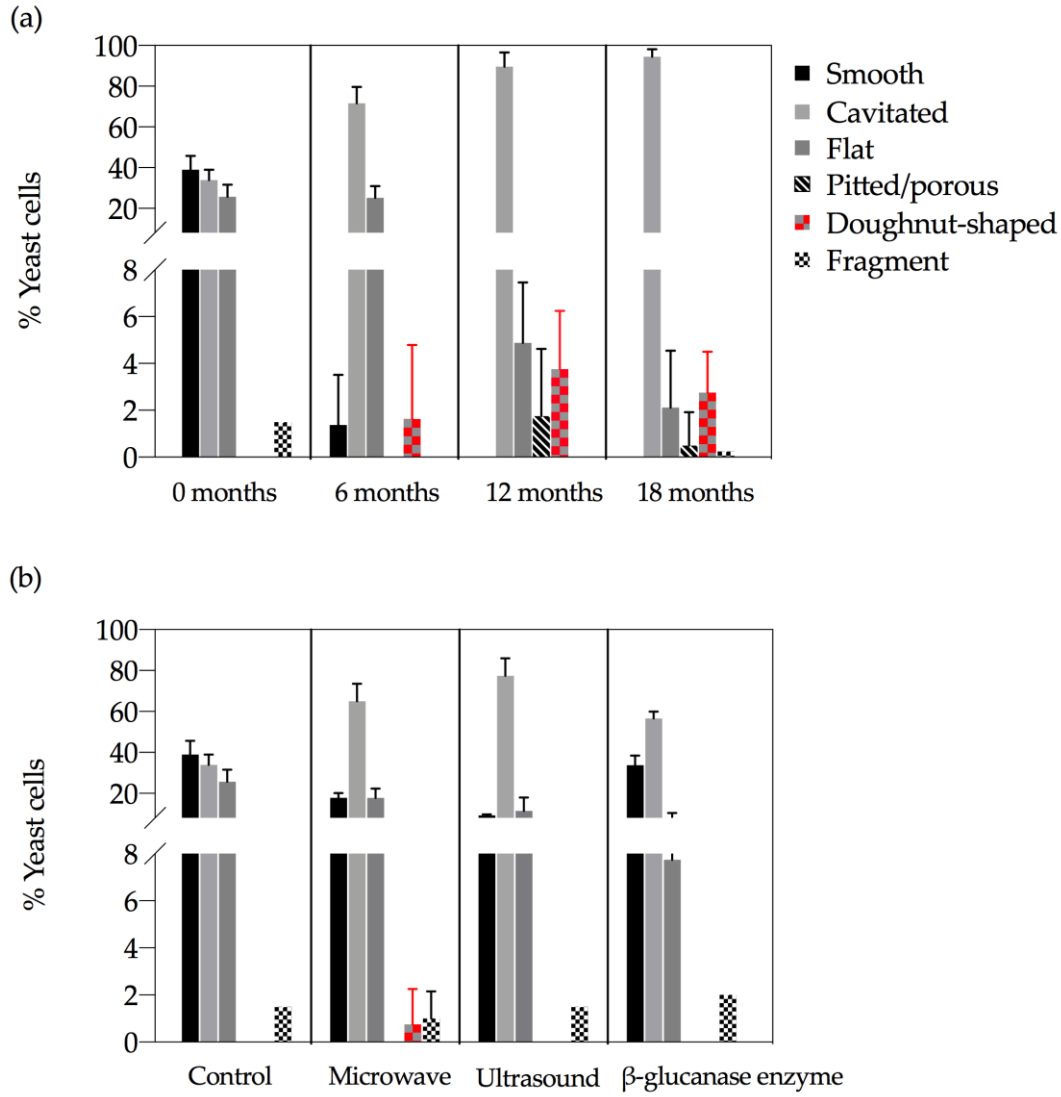


Figure 6-25 "(a) Development of surface features over time on S. cerevisiae whole-cells in the control, and (b) Proportions of surface features observed in the control and the treatments at tirage (time 0)." (Gnoinski et al., 2021b)

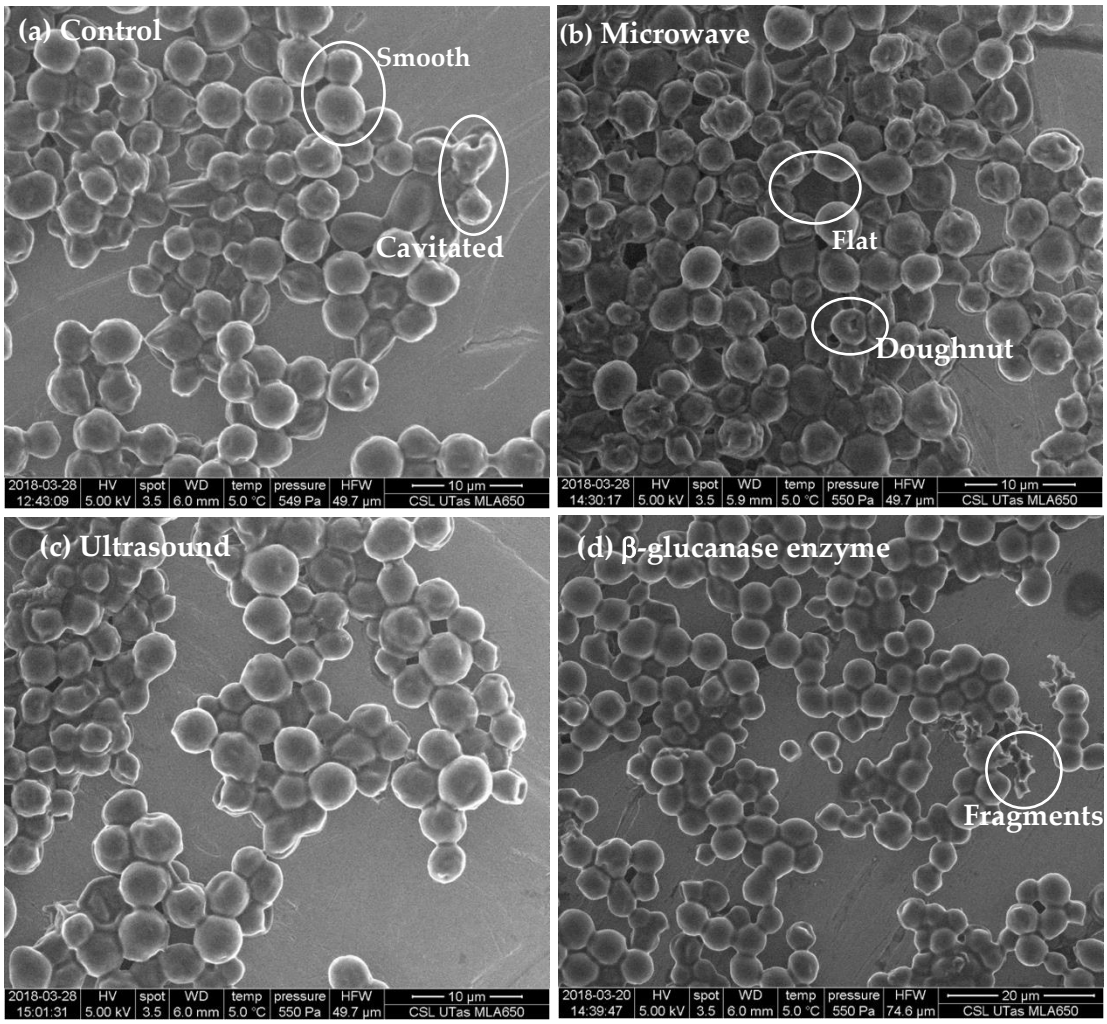


Figure 6-26 "SEM images of S. cerevisiae whole-cells at tirage (time 0) exhibiting surface features of: (a) Control (untreated) cells exhibit 'smooth' external surfaces, budding, mother-daughter cells and 'cavitated' features on some cells; (b) Microwave treated cells displaying 'flat' and 'doughnut'-shaped features; (c) Ultrasound treated cell surfaces displaying 'cavitated' features and (d) β-glucanase enzyme treated cells showing 'cavitated' features and 'fragments'." (Gnoinski et al., 2021b)

Comparison of Cell Wall Impacts of Treatment, and Bottle Aging

It has been suggested that 'autolytic character' in wine results from degradation of the yeast cell wall, induced during sparkling wine aging on lees. To investigate this, changes to cell wall integrity in the control yeast were examined over an 18 month bottle aging period. Flow cytometry analyses monitoring *S. cerevisiae* strains FEM111 and FEM 22 during sparkling wine production reported by Guzzon and Larcher (2015) found an association between decrease in the intensity of propidium iodide fluorescence as wine age, and a reduction in nucleic acid. In our study, there was significant loss of nucleic acid from yeast cells over the 18 months of the study, with much of the propidium iodide interactive material lost within the first 6 months. This loss was possibly due to increased cell permeability, since loss of cytoplasmic contents has been found in other studies to be a common feature of extended maturation and natural autolysis during aging of sparkling wines (Alexandre, 2019, Martinez-Rodriguez et al., 2001). This explanation is supported by cell damage that was observed when interactions of flow cytometry side scatter and forward scatter were considered, particularly at 12 months of bottle age. At this timepoint, SEM indicated there were elevated levels of pitted/porous

features on the control yeast cells, as well as an increase in the proportion of 'doughnut'-shaped cells in that sample.

The microwave-treated yeast had a 4-fold higher propidium iodide fluorescence intensity compared with the control, and elevated intensity compared to the other treatments, suggesting there had been more cell disruption relative to the ultrasound or β-glucanase treatments. Interactions of propidium iodide fluorescence, side scatter and forward scatter parameters indicated there were distinct clusters of cell population for each of the treatments; the microwave treatment population of cells was most distinct from the control and other treatments. Thus, these results show there were distinct treatment effects on yeast morphology. This is consistent with studies investigating impacts of ultrasound (García Martín et al., 2013), heat treatment (Guzzon and Larcher, 2015), and β-glucanase treatments (Feuillat and Charpentier, 1982) on yeast cultures. The changes to cell morphology indicated that microwave, ultrasound or β-glucanase were each effective treatments to initiate faster release of yeast cell compounds into wine.

This study was prompted by the widely-held view that 'autolysis' is important for the development of aged character in sparkling wine. Ms Gnoinski established a 'baseline' understanding of yeast maturation in bottle and found that:

- yeast cells under normal bottle aging conditions were initially smooth, and showed a range of 'degraded' morphologies or species, including: flat, cavitated, donut, fragmented, and pitted/porous;
- the species and incidence of degraded cells changed over time, with a decreasing incidence of smooth and flat cells, and an increasing incidence of cavitated cells over a typical 18-month bottle aging period;
- several findings pointed to loss of cell wall integrity as the main mechanism of yeast cell degradation as wines bottle aged.
- it was inferred that nucleic acids leached from yeast cells (as indicated by loss of propidium iodide interactive material) into the wine.
- the species and incidence of degraded yeast cells in microwave, ultrasound and β-glucanase treatment wines at bottling was similar to the yeast profile observed in control wines at 6 months bottle age.
- yeast that had been microwave treated showed significantly higher propidium iodide fluorescence intensity, than control yeast. Ultrasound and β-glucanase treatment yeast also differentiated from control to a lesser degree.

These findings suggest the application of treatments designed to disrupt yeast cell integrity were effective, with microwaved yeast showing the greatest changes in terms of cell morphology and likely leakage of cellular nucleic acids into wine. Further investigation is warranted into the oenological impacts of these yeast treatments on sparkling wines. If yeast degradation, lysis or leakage are important to the development of aged or 'autolytic' character in sparkling wines, then microwave, ultrasound or β-glucanase treatment may have a role to play. These treatments have potential to more effectively or rapidly achieve aged character for winemakers who want to release their wines sooner.

6.3 Objectively measuring juice phenolics in-line

The concentration of phenolic compounds in press fractions for sparkling wines is a significant focus for winemakers (Chamkha et al., 2003, Culbert et al., 2017). Fining agents are often used to reduce the concentration of phenolics in juice, but at the expense of aroma compounds and aroma precursors (Gambuti et al., 2016). Winemakers often taste juice to evaluate change in the intensity of phenolics during grape pressing. But inter-individual variability and the unreliability of human senses are considerable limitations to achieving optimal juice fraction quality (Hayes and Nolden, 2016). An objective, real-time method to quantify phenolics could improve consistency and provide data-driven decision-making for more precise grape juice press fractioning.

6.3.1 Developing a reflectance model

During 2017 vintage, juice absorbance and reflectance data was collected at Josef Chromy Wines through the first iteration of 'in-line' sampling (see Methods section 5.2). Total phenolic concentration, as determined using UPLC-DAD analysis, for each of the Pinot noir press fractions from the Bucher XPert 250 pneumatic press were used as the reference values for modelling reflectance and absorbance measurements in-line and by UV-Vis spectroscopy in the laboratory, respectively. As expected (Kerslake et al., 2018), the concentration of total phenolics increased significantly from 0.42 ± 0.02 in the free run juices, up to 0.69 ± 0.02 g/L (F=87, p=<0.001)., in the hard pressing juice fractions. Seven press fractions were analysed in both reflectance and transmission mode in the UV-Vis range (Figure 6-27 and Figure 6-28). The reflectance spectra showed higher reflectance peaks in the UV regions between 250 and 260 nm in the late press fractions but higher peaks associated with the early press fractions in the Visible region of 490-540 nm (Figure 6-27). In contrast, the absorbance spectra after clarification showed higher absorbances across the whole wavelength range for the later press fractions (Figure 6-28). The peak at ~280 nm is most likely associated with hydroxybenzoates, stilbenes and flavan-3-ols (Aleixandre-Tudo et al., 2018), while a peak at 330nm may be from hydroxycinnamates (Kerslake et al., 2018, Verette et al., 1988) and the feature at 540nm in the hard pressing is likely due to greater anthocyanin extraction under higher pressure conditions (Versari et al., 2013).

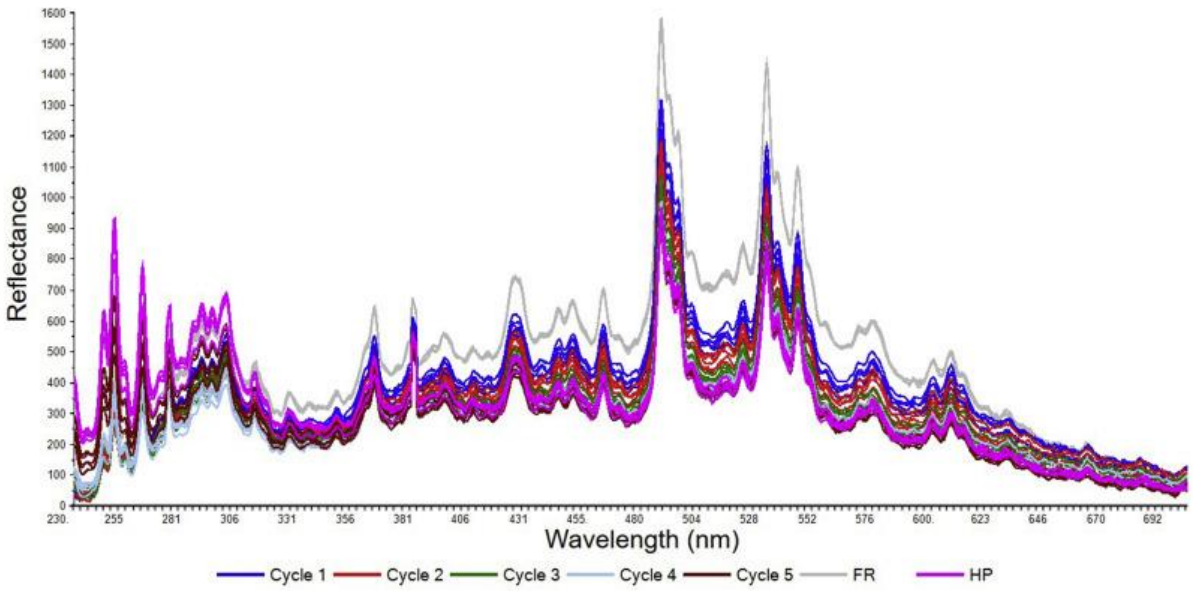


Figure 6-27 Raw UV–Visible reflectance spectra of Pinot Noir press fractions, in-line by the JAZ instrument discriminating free run (FR), Cycles 1–5 and hard pressing (HP).

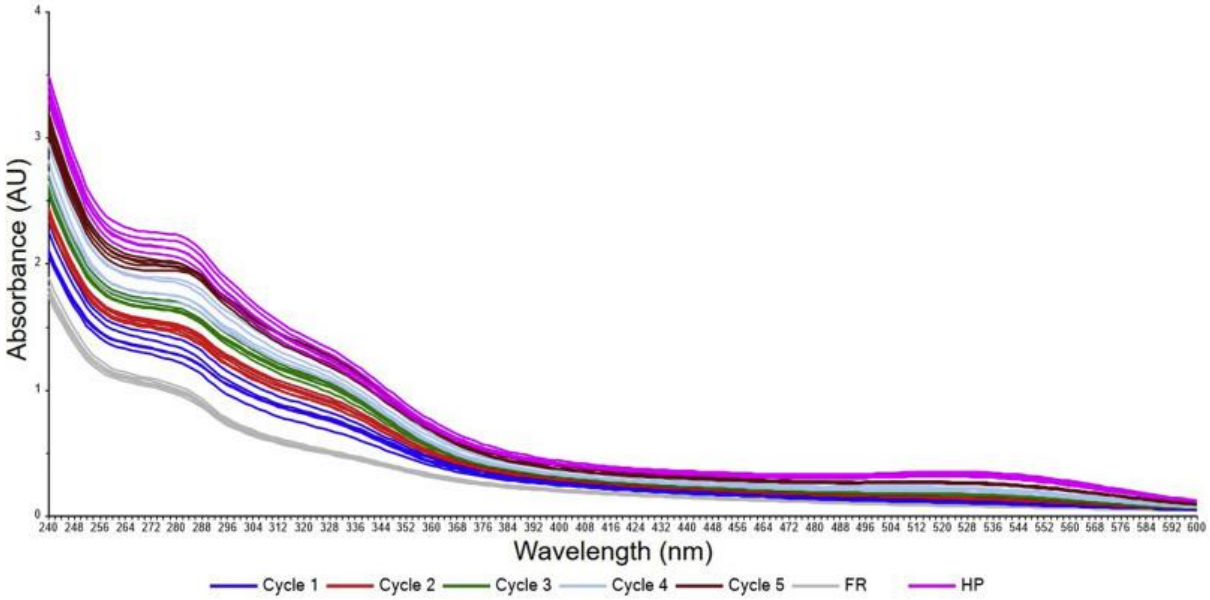
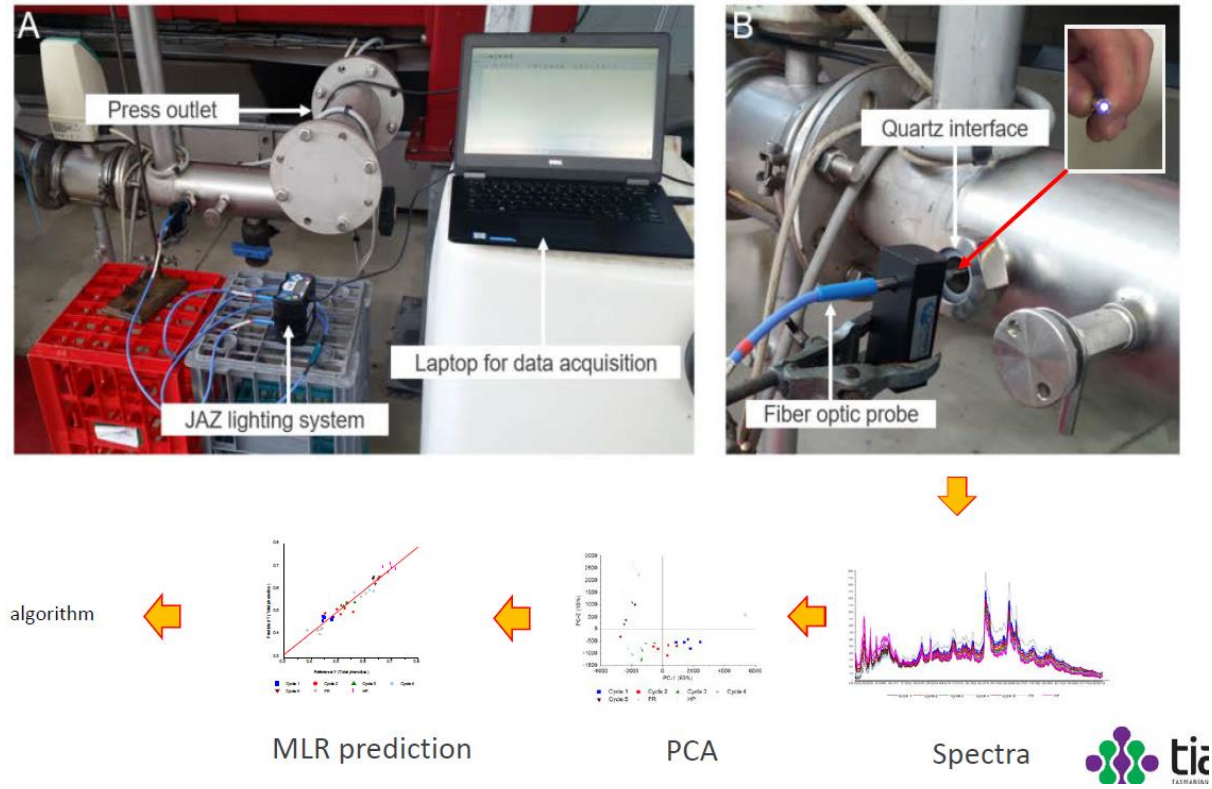


Figure 6-28 Raw UV–Visible absorbance spectra of Pinot Noir press fractions, in-line using a benchtop spectrophotometer, discriminating free run (FR), Cycles 1–5 and hard pressing (HP).



Comparison of three predictive model algorithms, partial least squares (PLS) regression, multiple linear regression (MLR) and linear regression (LR), for modelling in-line and laboratory approaches is summarised in Table 6-31. The best prediction of total phenolics concentrations in press fractions was obtained using 240 and 290 nm in-line in reflectance mode (R^2val = 0.95; SECV = 0.023g/L). The worst prediction was from linear regression using 280 nm collected in transmission mode (Table 6-31). The calibrations were robust and not over-fitted across all models as indicated by the similar R^2 and standard error for calibration and cross-validation values.

Table 6-31 Summary statistics of "in-line" and laboratory UV–Vis calibration and validation models built for phenolic measurements in the different press fractions.

Instrument	Algorithm	Wavelength (nm)	*N*	PLS Factors	R^2cal	R^2val	SEC (g/L)	SECV (g/L)	CV (%)
In-line	PLS	230–700	35	3	0.95	0.92	0.020	0.025	4.7
In-line	MLR	240, 290	35	–	0.94	0.95	0.021	0.023	4.2
Laboratory	PLS	240–600	35	2	0.94	0.93	0.023	0.026	4.7
Laboratory	MLR	280, 520	35	–	0.94	0.93	0.022	0.023	4.4
Laboratory	LR	280	35	–	0.92	0.91	0.026	0.028	5.1

PLS: Partial least square; MLR, multiple linear regression; LR: linear regression; N: number of samples; R^2cal: coefficient of determination in calibration; R^2val: coefficient of determination in validation; SEC: standard error of calibration; SECV: standard error of cross-validation; CV (%): coefficient of variation, calculated as SECV/mean*100.

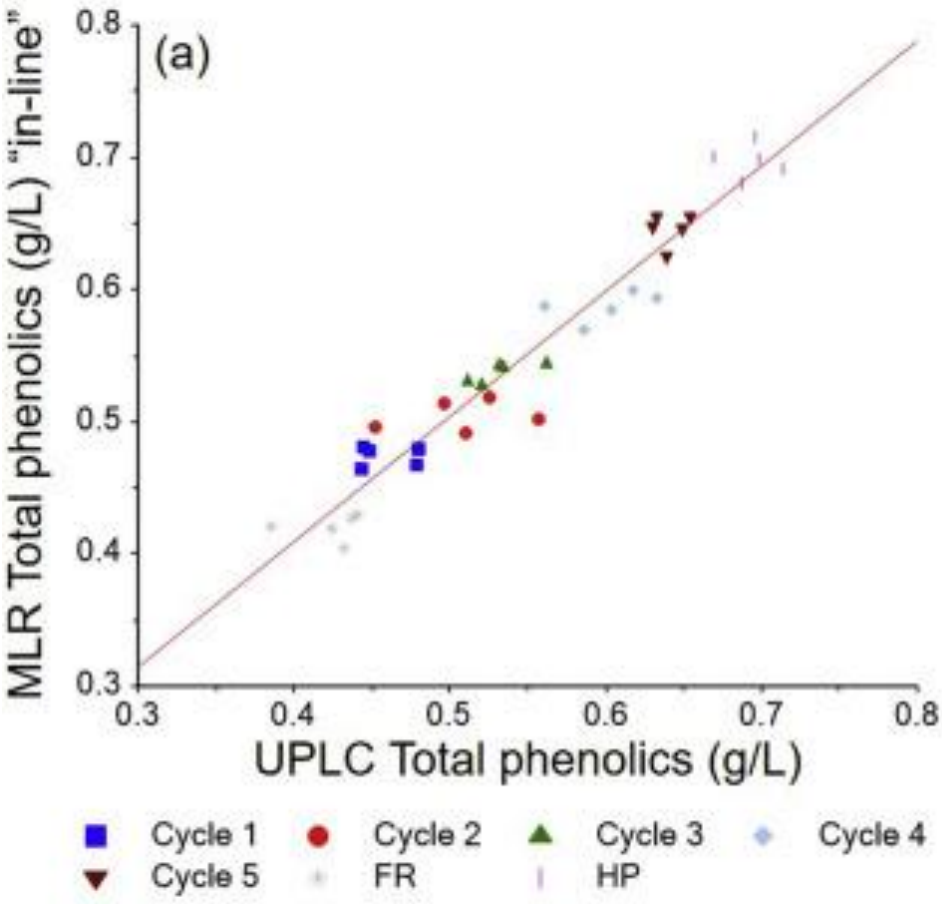


Figure 6-29 Correlation between total phenolics measured by UPLC and total phenolics predicted with MLR based on measurements collected "in-line" at 240 and 290 nm wavelengths. Predicted data was used for cross-validation. R^2val = 0.95; SECV = 0.023 g/L.

The use of the reflectance model for prediction of total phenolics in juice was informally trialled at a Canadian winery through TIA's partnership with the Cool Climate Oenology and Viticulture Institute (CCOVI) at Brock University. The trial was conducted at Tawse Winery (Vineland, Ontario, Canada) on

a EuroPress (Scharfenberger, Germany) which is a grape press with a longer, slower cycle than the presses used to develop the reflectance model. At the Tawse Winery, fruit was packed into the press at higher densities than is customary in Tasmanian winemaking. These two factors may explain why the Tawse first juice press fraction was high in total phenolics, compared with earlier trial data from Tasmania. Data collected was insufficient for statistical analysis, however the Canadian trail demonstrated the reflectance model could be applied in wineries and regions with winemaking equipment and practices different to those used in Tasmania.

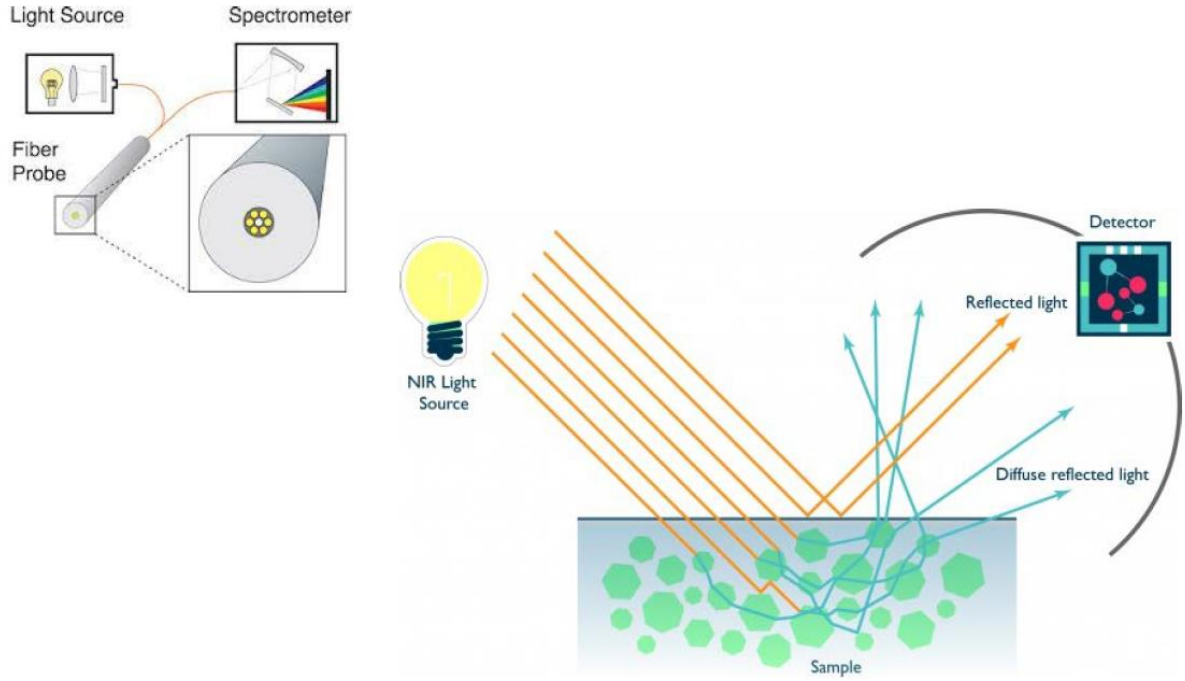


Figure 6-30. Schematic illustrating reflectance-based analyses. NIR light is 'bounced' off the sample, and the profile of wavelengths which are reflected back to the probe provide a spectral fingerprint to feed into the reflectance model.

6.3.2 Second prototype in-line sensor

Dr Rocco Longo was awarded a Science and Innovation Award for Young People in Agriculture, Fisheries and Forestry to build and test a second iteration prototype in-line system to measure phenolics in grape juice during pressing for sparkling wine. The prototype was intended for use in a commercial winery setting, and the work was undertaken within the Wine Australia-funded project but under a sub-project titled 'A new tool for in-line and real-time grape juice assessments'. To improve the functionality of the system, the second iteration was designed with a local manufacturing company to be:

- more portable
- compatible with different types of presses
- able to automatically switch tanks when a predetermined phenolic threshold has been reached.

Photo 1 shows the second iteration in-line sensor and its main components:

- UV-VIS sensor (protected in a plastic box with a peristaltic pump sampling the juice) (Photo 6-2a)

- actuator which switches the valve based on UV-VIS absorbance analysis of grape juice (Photo 6-2b)
- digital screen enclosed in a box and switches with which UV-VIS threshold levels can be set (Photo 6-2c).

The second prototype sensor was tested using Pinot noir and Chardonnay grape juices that had been obtained during vintage, and stored frozen while the prototype was developed. The juices had been pressed at two different pressures resulting in juice samples with 'low' and 'high' phenolic concentration for each grape variety. As there was not sufficient volume of juice to simulate a commercial press run, for this proof-of-concept the sampling inlet and outlet for the sensor was setup to sample the thawed juices from beakers. Bulk juice was simulated using water (in black bins in Photo 6-1).

During testing of the prototype, the sampling inlet was moved from the 'low' phenolic concentration beaker of juice, to the 'high' concentration beaker. This was done to test whether the prototype could detect higher phenolic concentration, and respond by triggering the tank switching mechanism.

We found that when the intake was moved from 'low' to 'high' concentration juice, the actuator was effectively triggered (Photo 6-2b) and automatically switched the valve (obscured by the actuator), thus directing the simulated bulk juice to the relevant tank (represented by the black bins in Photo 6-1).

Prior to testing of the prototype, phenolics concentration in each thawed juice solution was quantified using spectrophotometric analysis at 260-280nm (Figure 6-31). For the prototype test, a slight dilution was applied due to the high amount of phenolics in the juice samples and the relatively 'low' power of the sensor.

At the time of writing this report, 100-1000 times more powerful sensors are under development. Unfortunately, due to COVID-19 restrictions and delays, testing this process during a commercial press run at a winery was not possible. The prototype trial reported here, however, demonstrated proof of concept for tank switching on the basis of real-time inline phenolic sensing. This is an important breakthrough with potential to substantially alter the quality, accuracy and cost-effectiveness of juice pressing for both white and sparkling wine production in Australia, and internationally.



Photo 6-1 Testing the instrument with the Cold Harbour technician under 'typical' grape-pressing conditions using defrosted grape juice, water, and a commercial pump.

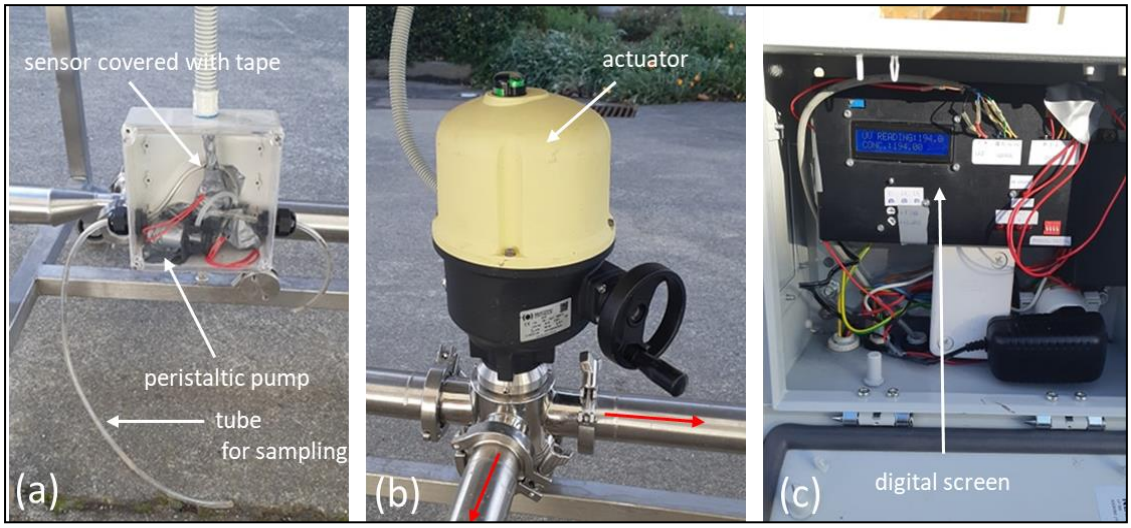


Photo 6-2 (a) Plastic box containing the UV-VIS sensor to measure at 280 nm (i.e. wavelength where phenolics absorb the light) and a peristaltic pump to sample the juice while flowing in the stainless-steel tube; (b) actuator which can direct the flow of the juice in two directions (red arrows) when triggered by the sensor; (c) box for controlling the instrument and set up the UV-VIS levels. The box has a lid with a window which allow the electronic parts to protected by any liquid.

A qualified electrical service certified the electrical work complies with AS/NZ 3000 regulations

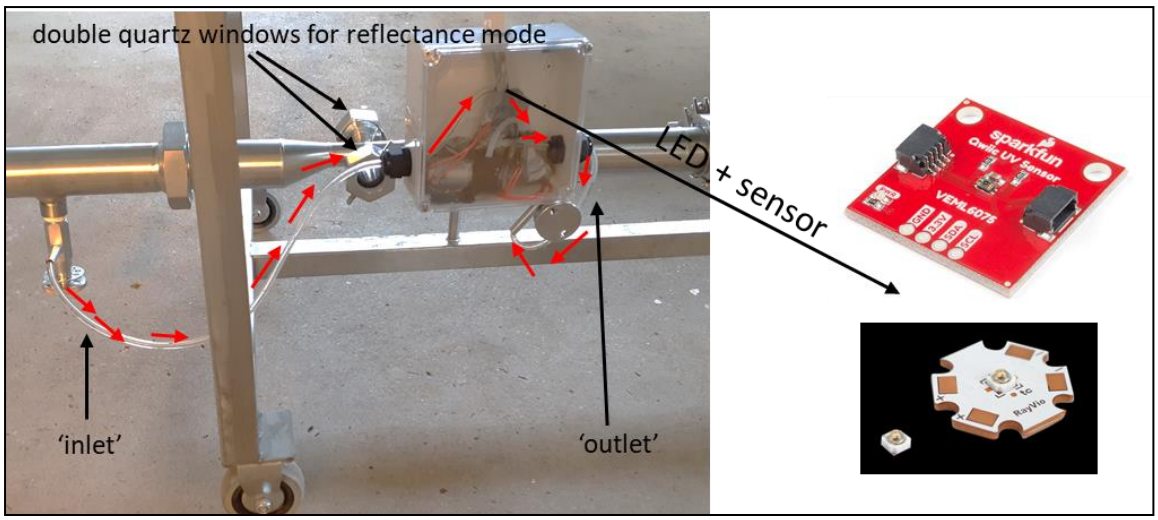


Photo 6-3 This photo shows how the tubes for sampling ('inlet' and 'outlet') should be positioned at when the instrument is used at commercial scale. The red arrows indicate the flow of the juice. The LED and the sensor are protected by a grey tape inside the plastic box (a photo of those parts is provided on the right of the Photo 3).

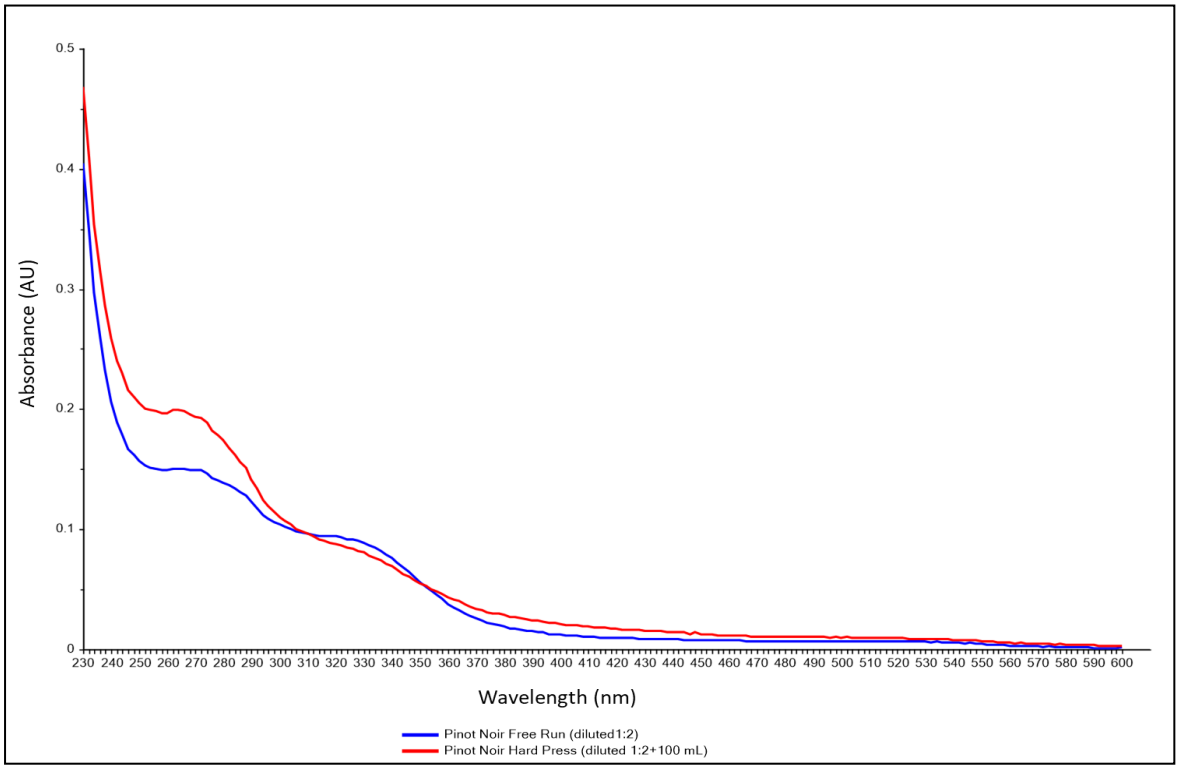


Figure 6-31 Raw UV-VIS (230-600 nm) spectra of the Pinot noir free run and hard press juices used in the experiment.

As illustrated by Figure 6-32 to Figure 6-35 Progression of organic acid extraction from Chardonnay grapes during press cycle from Longo et al. (2019)., traditionally used juice composition measures were examined during the development of real-time juice evaluation processes. These measures included TSS, pH, TA, organic acids (malic, tartaric and citric) and cations (sodium,

magnesium, calcium and potassium). With the possible exception of potassium, none of the juice composition measures changed in concentration in a predictable trajectory over the life of the pressing cycle. Potassium concentration did increase over the life of the cycle, but its accumulation was demonstrated to be highly variable and hence unsuited as a guide to press fraction cut-off. This supports the use of spectral fingerprinting to underpin automation of pressing.

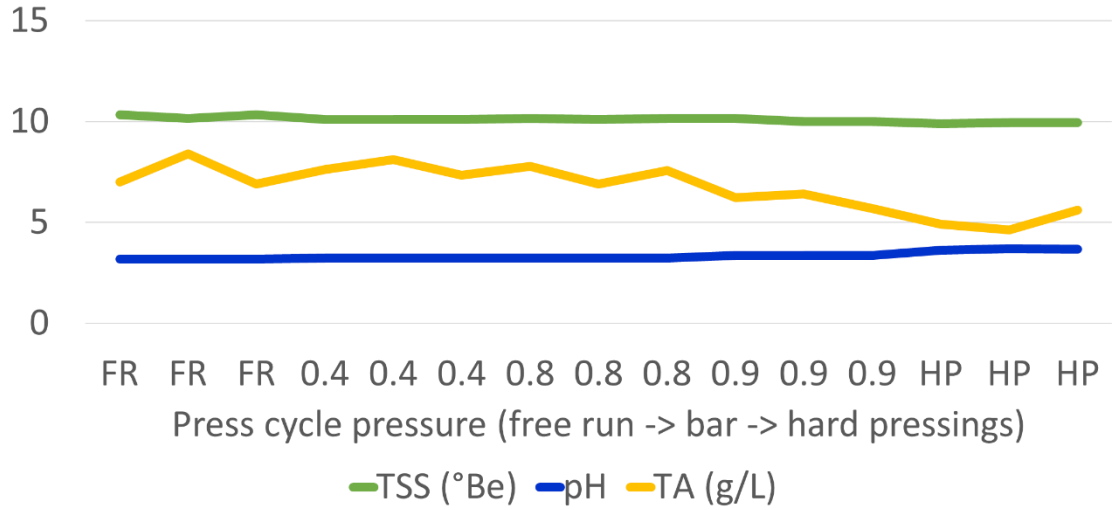


Figure 6-32 Progression of total soluble solids (TSS), pH and titratable acidity (TA) from Pinot noir grapes during press cycle from Longo et al. (2019).

Potassium is the major cation in Pinot noir juice, and concentration increases most rapidly when harder and more rapid pressing commences (Figure 6-33).

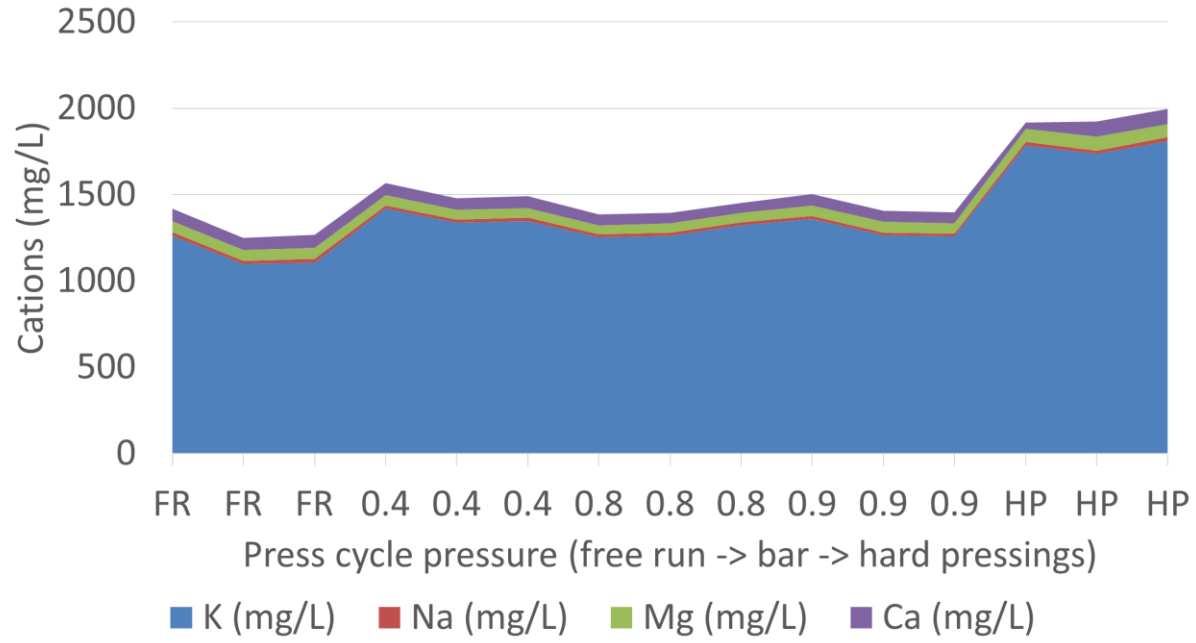


Figure 6-33 Progression of cation extraction from Pinot noir grapes during the press cycle from Longo et al. (2019).

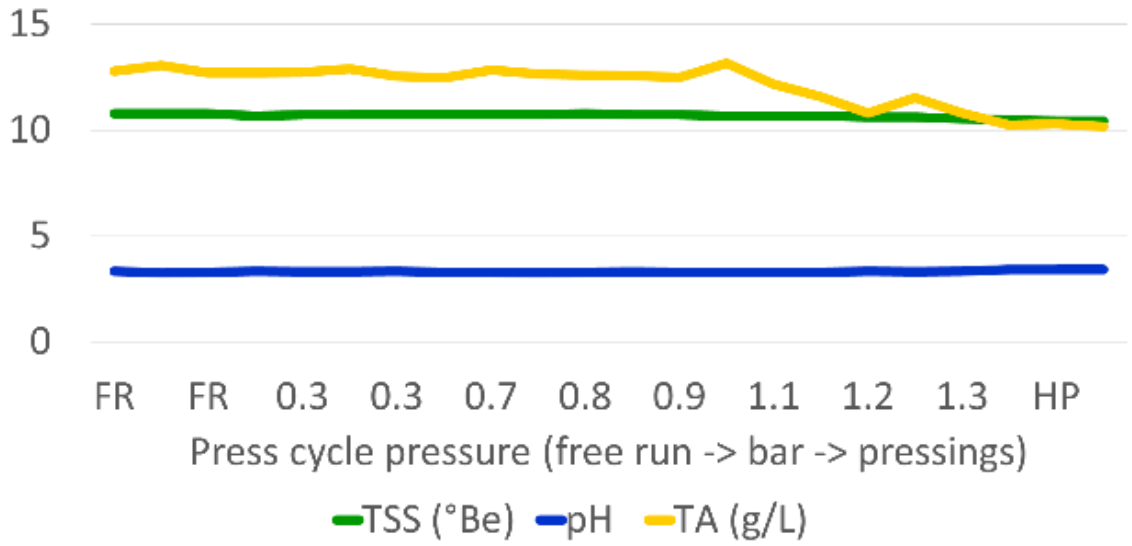


Figure 6-34 Progression of total soluble solids (TSS, pH and titratable acidity (TA) from Chardonnay grapes during press cycle from Longo et al. (2019).

The major organic acid, tartaric, in Chardonnay varies more throughout pressing than malic or citric (Figure 6-35).

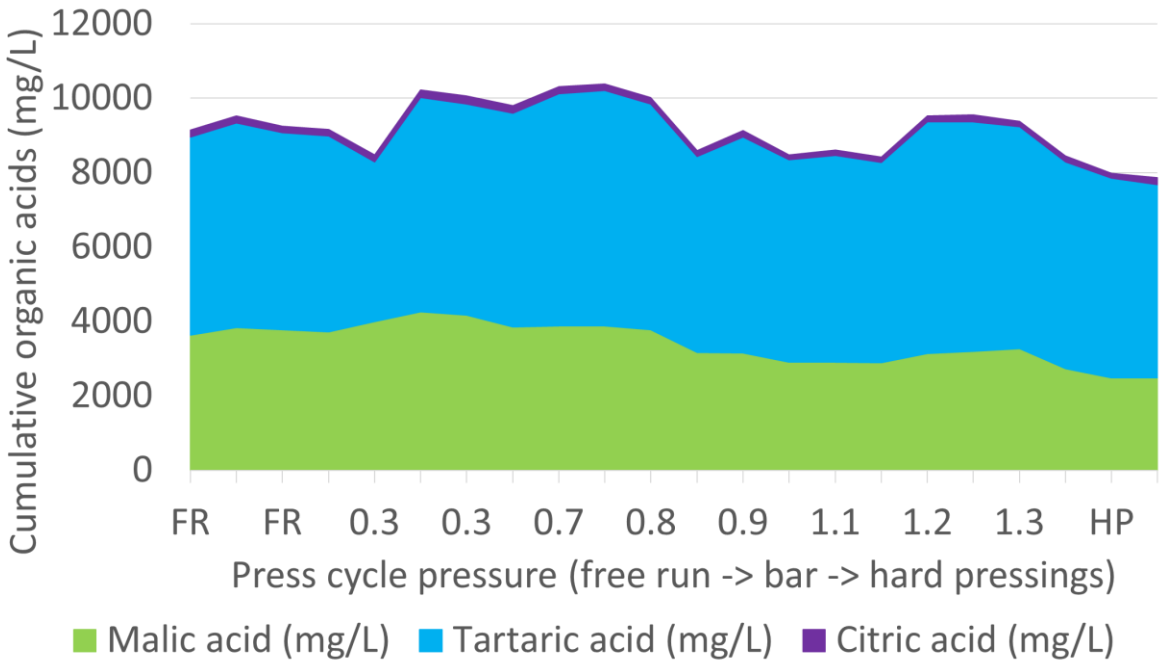


Figure 6-35 Progression of organic acid extraction from Chardonnay grapes during press cycle from Longo et al. (2019).

6.4 Juice provenance

6.4.1 Juice sampling

This aspect of the research was ambitious given the wide range of uncontrolled (and difficult to control for) variables that impacted juice chemistry prior to its arrival at TIA for analysis. The trial was originally designed to enable industry participation, to increase engagement with the process and outcomes. This led to the challenge of many variables, including:

- Vine clone and rootstock (many)
- Regional climate and vineyard micro-climates (many)
- Viticultural practices (many)
- Harvesting method (machine, hand harvest)
- Transportation of fruit from vineyard to press (refrigeration, time in truck)
- Type of press (Bucher, Europress)
- Pressing regime applied (long, short)
- Juice fraction sampled (free-run, cuvee, taille)
- Post-sampling storage (how soon frozen, how long kept frozen, mode of transportation)

As such, the initial year of sampling (2017) provided important opportunities to refine the sampling protocol, but no valid results. In that year, the research team identified challenges with transportation of the samples and learned that samples needed to be transported in dry ice and overnight couriered, rather than transportation by post. Transportation variability and thawing of samples in-transit led to varying levels of oxidation. The quality and completeness of record keeping and sample labelling also varied between participating wineries. For 2017, results were discarded and sampling protocol refined for the following year.

In 2018, three wineries provided samples of Chardonnay and Pinot noir juice. Several samples were removed, however, to manage variables or because there were too few samples for statistical rigour. Eliminated samples were those from: machine harvesting, field blend and taille press fraction. Of the 175 samples received, 70 were excluded, leaving 105 for analysis.

In 2019, a smaller and more carefully controlled pressing and sampling process was used. This eliminated industry participation but allowed standardisation of pressing processes, sampling timepoint (cuvee, taille), sample record keeping and storage. This resulted in 17 Chardonnay and 16 Pinot noir samples, in triplicate, from four regions: Tamar Valley, Pipers Brook, Coal River Valley and North-West Tasmania. These samples were not analysed during the life of the project, but the method described is recommended as a means to reduce extraneous sample-to-sample variability.

6.4.2 Juice analysis 2018

(substantial parts of this section of this report are abridged from McCullough, 2019)

Values from initial standard winery analysis for pH, titratable acidity (TA, in g/L) and total soluble solids (TSS, in °Baumé) for Chardonnay and Pinot noir juice samples from a Bucher press and from a Europress were analysed. Juices were also examined for regional differences in total phenolic and hydroxycinnamate contents (TPC and THC, in au) by UV-Vis absorption at 280 and 320 nm (Iland, 2013). Analysis of variance (ANOVA) was performed on the data. Residuals were normally distributed and there was homogeneity of variance, so Tukey's post hoc test with a 95% confidence interval was applied. There was no statistical difference in these parameters between regions for the juices from the Bucher press (data not shown). Samples from the Europress, however showed regional differences. Table 6-32 shows those parameters that were significantly different. The Europress Pinot noir juices from the Tamar Valley region showed significantly lower average pH than Pipers River juices. Tamar

Valley Chardonnay juices from the Europress were significantly higher in average total phenolics (TPC) and hydroxycinnamates (THC), compared with Chardonnay juices from the Coal River and Pipers River regions.

Table 6-32:
Results of ANOVAs and Tukey's post hoc test with 95% confidence intervals that provided significantly different results by region. All processed by Winery 1 in a Europress. Values followed by the same letter are not statistically significant ($p<0.05$). Test statistics for each category are shown at the bottom

Pinot noir pH		**Chardonnay Total Phenolics (au)**		**Chardonnay Total Hydroxycinnamates (au)**	
Tamar Valley	2.968a	**Tamar Valley**	5.906b	**Tamar Valley**	3.029b
Coal River	3.065ab	**Coal River**	3.392a	**Coal River**	2.010a
Pipers River	3.139b	**Pipers River**	3.683a	**Pipers River**	1.886a
$F_{(2,13)} = 7.31$, $p < 0.010$		$F_{(2,16)} = 20.08$, $p < 0.001$		$F_{(2,16)} = 27.91$, $p < 0.001$	

These finding are not particularly striking in terms of regional differentiation *per se*, however they demonstrate there was regional variation during one vintage in three measurable parameters: pH, TPC and THC. These parameters could contribute to a suit of measures to underpin a 'regional differentiation fingerprinting' technique. A parametric *melange*, if you will, to be cast into light through the deft hand of chemometrics. In terms of wine implications for the observed differences between these parameters, the low pH in the Tamar Valley region Pinot noir juices could have potential for production of more 'astringent' and less 'viscous' wines, and the significantly higher total phenolics in Tamar Valley region Chardonnay juices might contribute greater sensory 'bitterness' (Smith and Waters, 2012) to finished wine.

6.4.3 Juice UV-Visible Spectrophotometry 2018

(substantial parts of this section abridged from McCullough, 2019)

Overall, UV-Visible spectrophotometric analysis coupled with principal component analysis (PCA) showed that one subset of the 2018 samples displayed some regional separation. That subset was handpicked Chardonnay cuvée juices from Winery 1, and the PCA showed some separation between Coal River region juices, and those from the Tamar Valley and Pipers River (Figure 6-37; more detail below). UV-Visible spectrometry and PCA was not successful for strong regional discrimination amongst all other subsets of samples taken during 2018 (see detail below).

Figure 6-36 shows average raw UV-Vis spectral scans of Bucher-pressed Chardonnay from Winery 1 for each of the three regions analysed. It shows broadly similar curves for the three regions, with small differences in the 260-280nm and 300-340nm ranges. Pipers River is lower across these ranges. Raw average scans for the other sets processed at Winery 1 showed similar curves (data not shown).

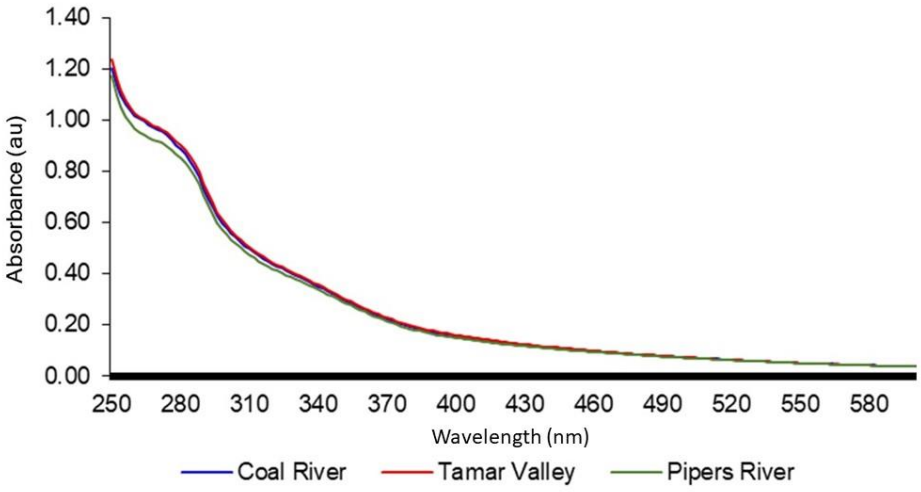


Figure 6-36: Raw UV-Vis spectra of regional averages for handpicked Chardonnay cuvée juice processed by Winery 1 in a Bucher press

In all data sets, spectral scans were taken in the full region 200-600nm. 200-250nm was removed due to quartz cuvette interferences and general noise. 400-600nm data was removed due to lack of influence on analyses.

The PCA plots and loadings plots for the first and second principal components (PCs) obtained relating to Chardonnay and Pinot noir juice sampled by Winery 2 are shown in Figure 6-37 to Figure 6-40. Winery 2 used both a Bucher and a Europress, but did not label samples and which was used is not known.

Figure 6-37 shows the PCA plot for handpicked Chardonnay cuvée juice and displays some regional separation between the Coal River samples in the south and those from the Tamar Valley and Pipers River in the north. This is by both the first PC (the X-axis, explaining 94% of the variation observed) and to a lesser extent the second (the Y-axis, explaining 5% of the variation observed).

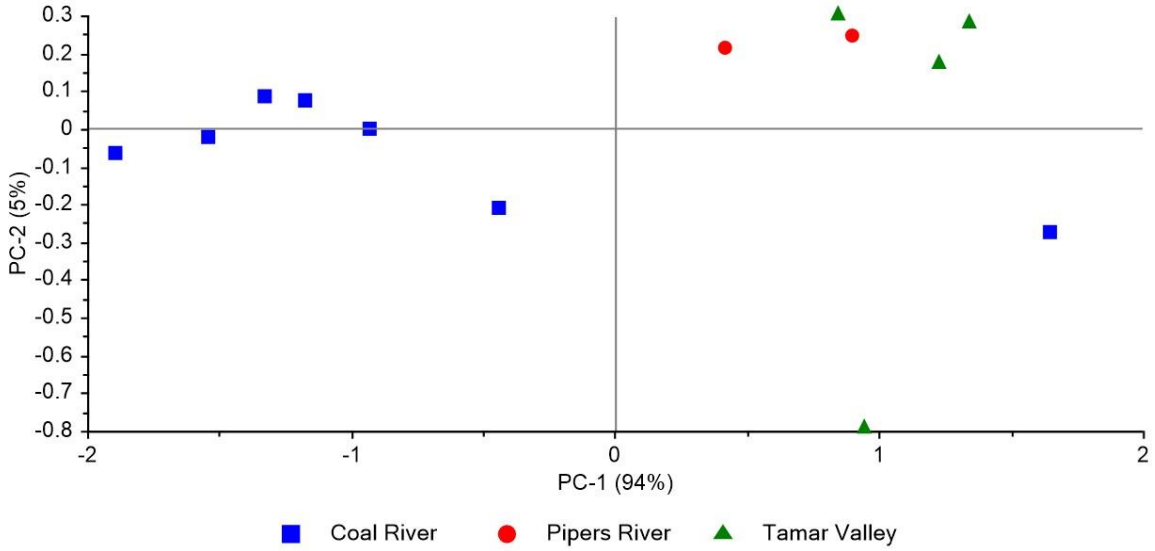


Figure 6-37: PCA score plot of handpicked Chardonnay cuvée juice from Winery 2 delineated by region

The loadings for the Chardonnay PCA plot are displayed in Figure 6.39. These show that both PCs were influenced by a trough in the region 260-280nm, with PC-1 also displaying a peak in the region 320-330nm and PC-2 negatively affected by the same, though with a stronger trough around 320 – 330nm.

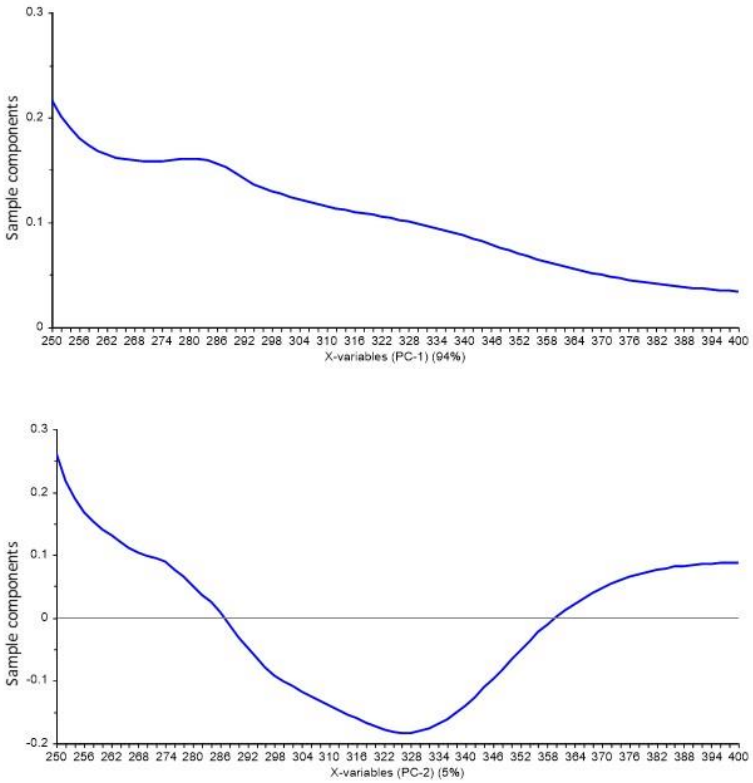


Figure 6-38: PCA loadings plots for PC-1 (top) and PC-2 (bottom) of PCA of handpicked Chardonnay cuvée juice from Winery 2

The PCA plot for Pinot noir juice processed by Winery 2 does not show strong regional separation (Figure 6-39). It is likely that other compositional differences than region are driving the variation, particularly along the first PC. Winery 2's two Adelaide Hills samples were the only juice it used from outside Tasmania for its sparkling wine production and were included in the sample delivery for analysis.

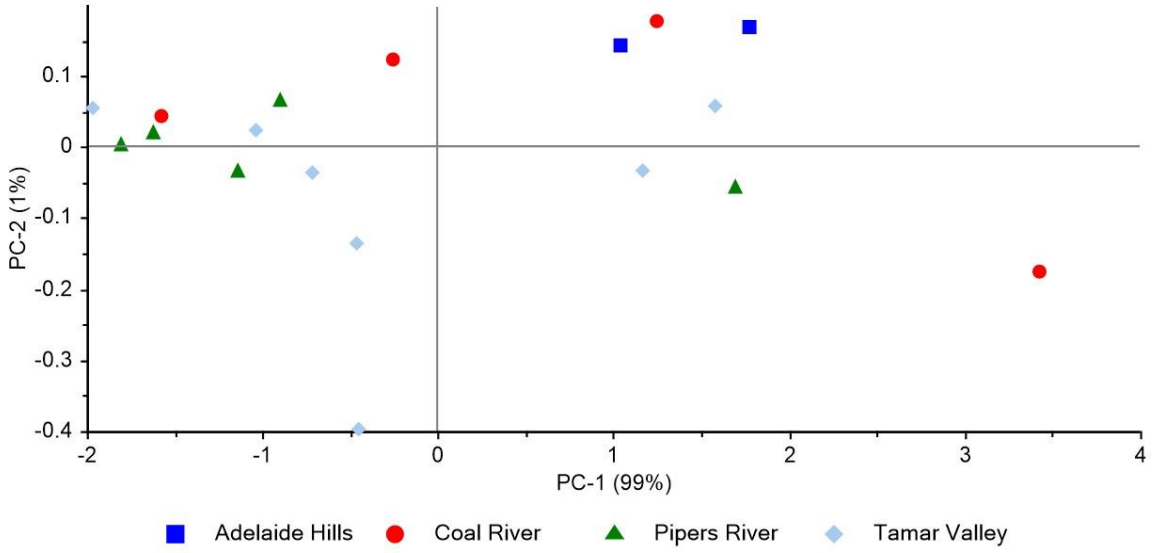


Figure 6-39: **PCA score plot of handpicked Pinot noir cuvée juice from Winery 2 delineated by region**

The loadings for the Pinot noir PCA plot are displayed in Figure 6-40. These show PC-1 was influenced by 260-285nm, while PC-2 was influenced by 260nm. In addition, PC-2 was negatively influenced by 290-330nm.

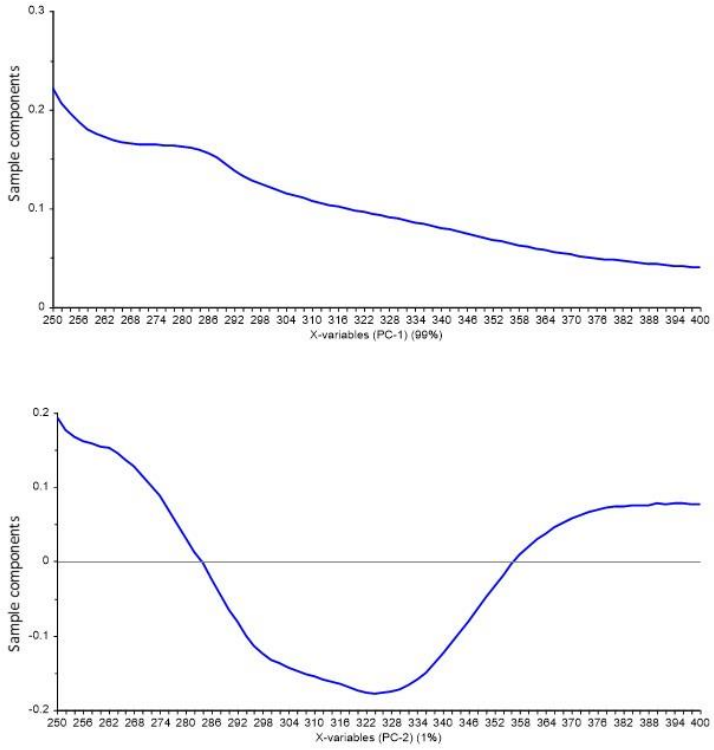


Figure 6-40: **Loadings plots for PC-1 (top) and PC-2 (bottom) of PCA of handpicked Pinot noir cuvée juice from Winery 2**

The PCA plots and loadings plots for the first and second PCs obtained relating to handpicked Chardonnay cuvée juice processed by Winery 1 in a Europress (Figure 6-41 and Figure 6-42) and a Bucher press (Figure 6-43 and Figure 6-44) are shown below. The PCA plot of the Europress fruit does not show robust regional separation particularly given that PC-1 (X-axis, accounted for 99% of observed variation). PC-2 (Y-axis, accounting for <0.5% of observed variation, a tiny amount) potentially shows some differentiation between Tamar Valley and Coal River region juices.

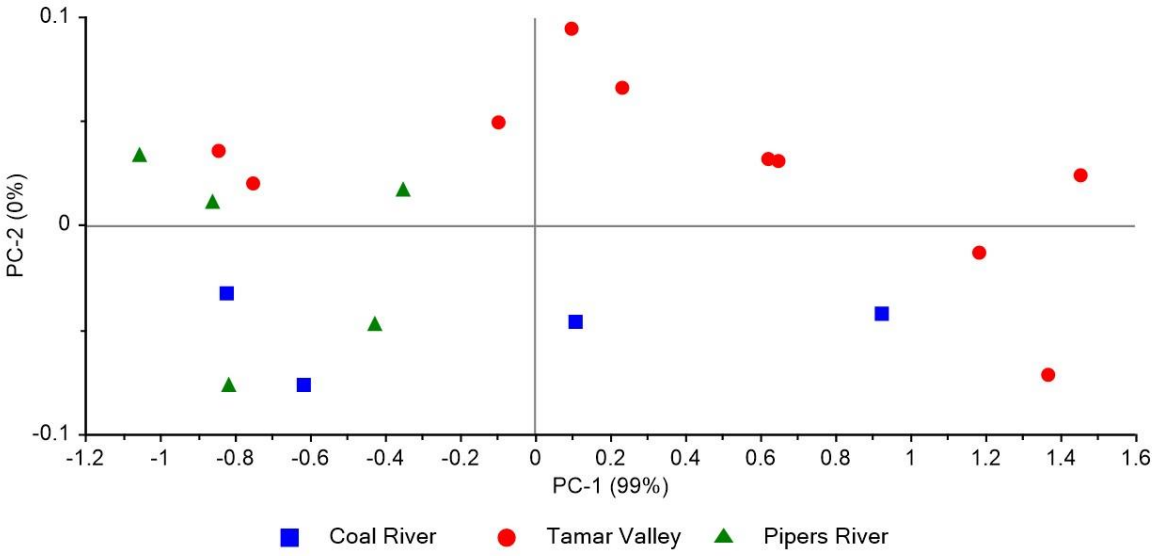


Figure 6-41: **PCA score plot of handpicked Chardonnay cuvée juice from Winery 1, processed in a Europress, delineated by region**

The loadings for the Winery 1 Europress Chardonnay juice are shown in Figure 6-42. These show a shoulder at 280nm for PC-1, while PC-2 is negatively impacted by wavelength. PC-2 appears to be influenced by the wavelengths above 300nm.

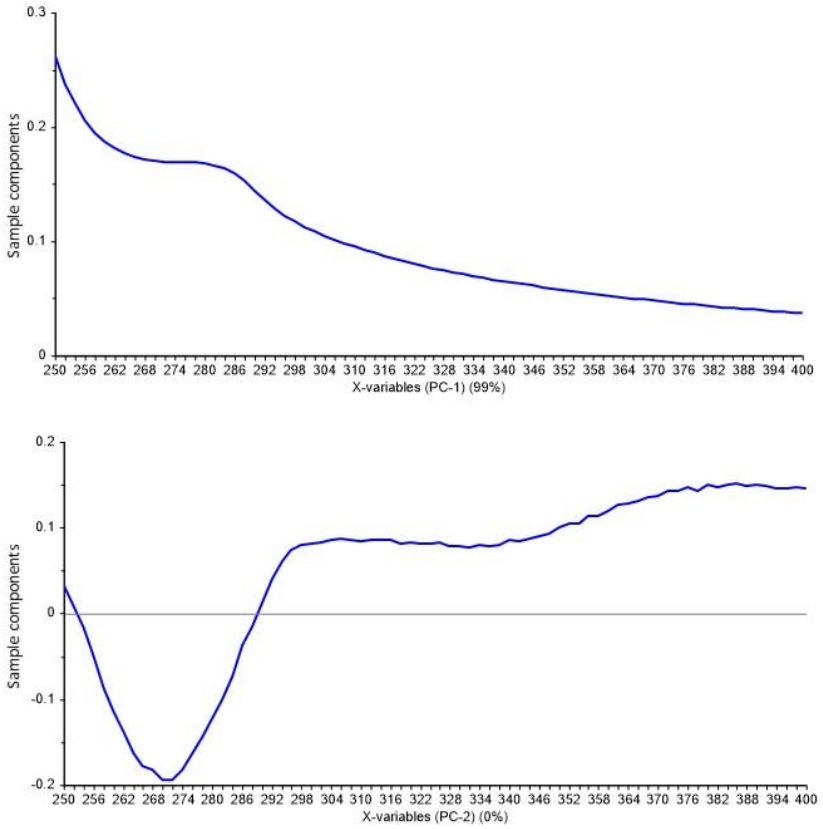


Figure 6-42: **Loadings plots for PC-1 (top) and PC-2 (bottom) of PCA of handpicked Chardonnay cuvée juice from Winery 1, processed in a Europress**

The PCA plot of handpicked Chardonnay cuvée juice processed by Winery 1 in a Bucher press did not show strong regional separation along either the first PC (X-axis, 99% of variation observed) or the second (Y-axis, 1% of variation observed) (Figure 6-43). The loadings plots show influence coming from the wavelengths of 260-280nm for PC-1 and a strong peak at 270nm for PC-2 (Figure 6-44).

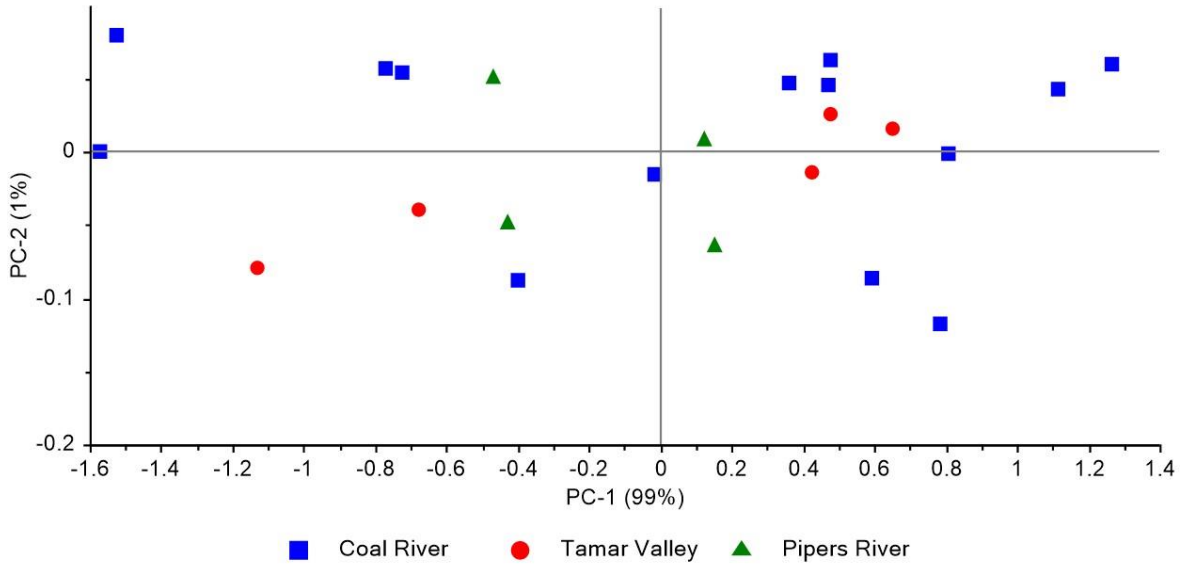


Figure 6-43: **PCA score plot of handpicked Chardonnay cuvée juice from Winery 1, processed in a Bucher press, delineated by region**

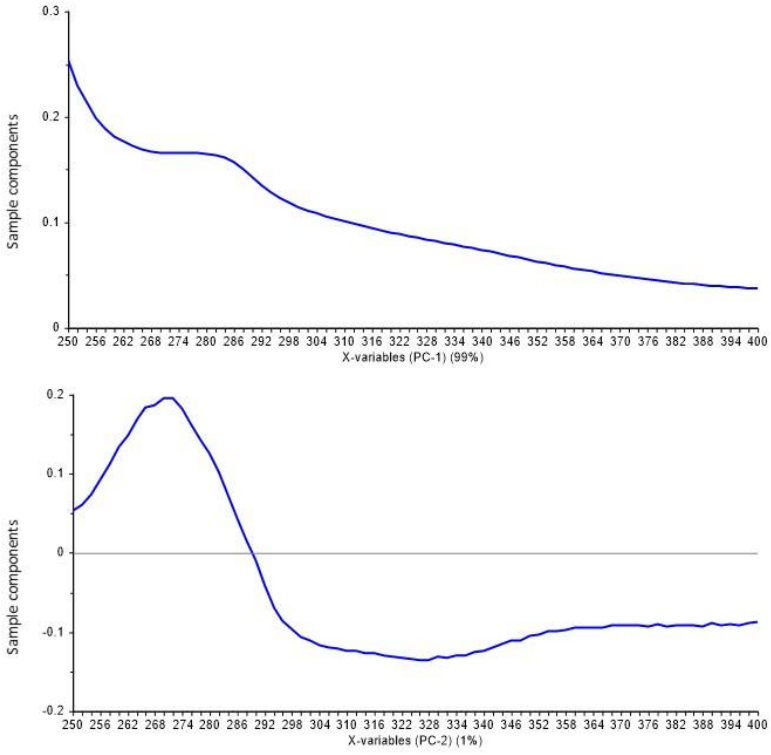


Figure 6-44: **Loadings plots for PC-1 (top) and PC-2 (bottom) of PCA of handpicked Chardonnay cuvée juice from Winery 1, processed in a Europress**

4.4 NIR Analysis

(substantial parts of this section of this report are abridged from McCullouch, 2019)

Overall, many of the subsets of juice subjected to NIR analysis with PCA did not separate or cluster by region. However, the PCA plot of NIR data from the samples from Winery 1 (Chardonnay cuvée juice processed in a Europress) showed regional separation (Figure 6-45; more detail below). These samples sets were delineated by PC-2 (Y-axis, accounting for 25% of variation observed between the samples) which showed the Coal River juices separated from those of the Tamar Valley and Pipers River. There were very small sample sizes and so this finding is tentative, however the loadings plots for PC2 showed a small peak around 1900nm and a short negative trough at 2000nm drove this separation.

Similarly, the handpicked Pinot noir cuvée juices from Winery 2 that had been processed in both Bucher and Europress, showed some separation on PC2 (7%) for Tamar Valley region juices, compared with Coal River and Pipers River region juices (Figure 6-47; more detail below). Again, the loadings analysis suggested that separation on PC2 was associated with a peak at 1900nm. The NIR literature indicates 1900nm is associated with the O–H bond and is related to the water content of a sample (Serva et al , 2019; Cozzolino et al, 2006), however, if this wavelength is associated with regional separation, it may warrant further investigation, as part of an effort to develop potential indicators or target analyses upon which to develop regional discrimination of juices.

Figure 6-45: average raw NIR spectral scans of Bucher-pressed Chardonnay from Winery 1 for each of the three regions analysed.

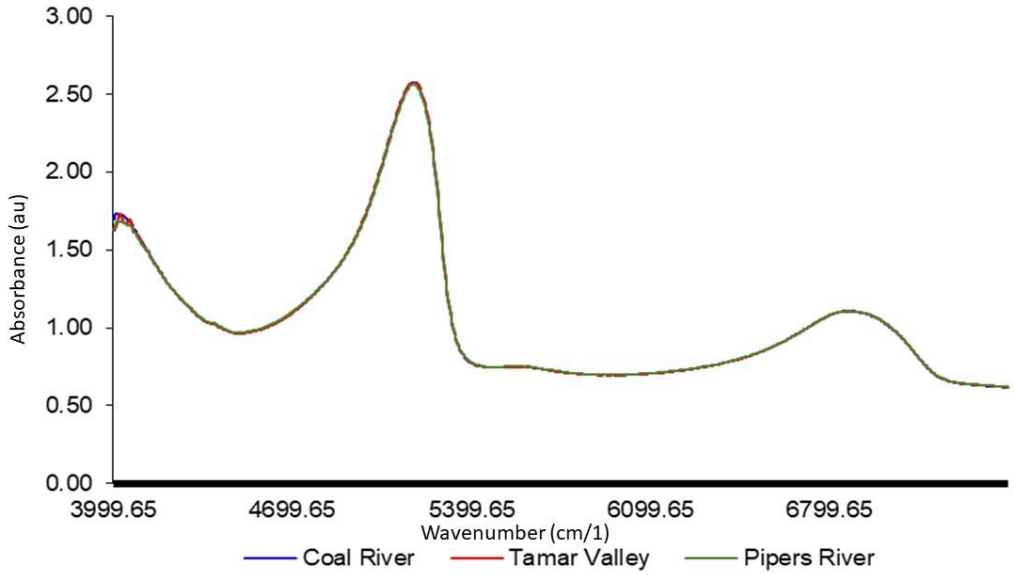


Figure 6-46: Raw NIR spectra of regional averages for handpicked Chardonnay cuvée juice processed by Winery 1 in a Bucher press. Wavenumbers shown correspond to c.2500 – 1330 nm, left to right

The PCA plots and loadings plots for the first and second principal components (PCs) obtained relating to NIR spectral scans of handpicked Pinot noir cuvée juice sampled by Winery 2 are shown in Figure 6-47 . The PCA plot displays some regional separation between Tamar Valley region samples, and the other two regions. While the first PC (the X-axis) explains 85% of the variation observed, the second

(the Y-axis), explaining 7% of the variation observed, accounts for any separation. The loadings plots show that PC-1 was influenced by most spectral regions, in particular around 2100nm (c.4700cm^{-1}), with troughs around 1400 and 1900-2000nm (c.7000 and 5200-5000cm^{-1} respectively). PC-2 was influenced by few regions of the spectra, except for a negative trough in the region of 1950nm and positively around 2100nm (c.5100 and 4700cm^{-1}). Given PC2 was the only one associated with regional differentiation, the wavelengths 1950nm and 2100nm may warrant further examination for developing provenance tools.

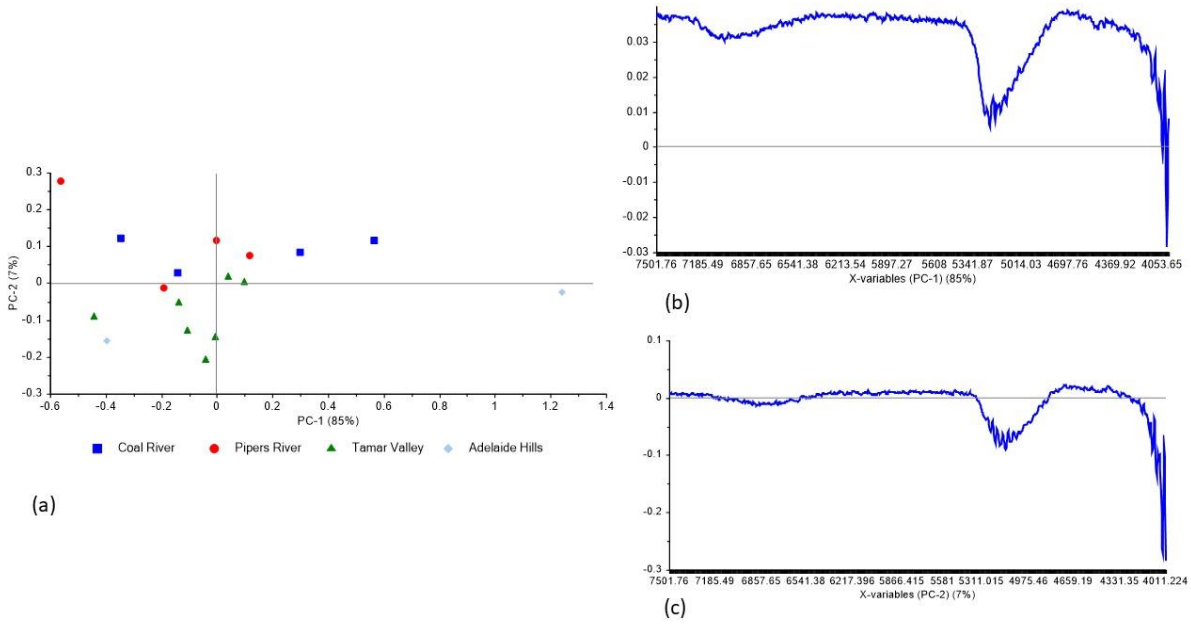


Figure 6-47: PCA score plot (a) and loadings plots for PC-1 (b) and PC-2 (c) of handpicked Pinot noir cuvée juice from Winery 2, processed in both Bucher and Europress, delineated by region. Wavenumbers shown in loadings plots correspond to c.1330 – 2500 nm, left to right

The PCA plot for NIR scans of handpicked Chardonnay cuvée juice, processed by Winery 1 in a Bucher press is shown in Figure 6-48. There is no regional differentiation between samples for this element of the research. The plot displays some concentration of samples from the Tamar Valley and Pipers River, but as with the PCA analysis for the UV-Vis spectra of the same sample set, there is substantial overlapping between samples from the three regions examined. The Coal River samples are spread widely over the plot, but what separation there is, comes from PC-2 (Y-axis) which accounts for 20% of the variation observed. The loadings plots show strong influence on PC-1 from most regions of the spectra, with a slight trough around 1400nm and large negative one around 1900-2000nm (c.7000 and 5200-5000cm^{-1}). PC-2 is again influenced little by most of the spectral regions, but with a slight peak coming at 1900nm (c.5200cm^{-1}).

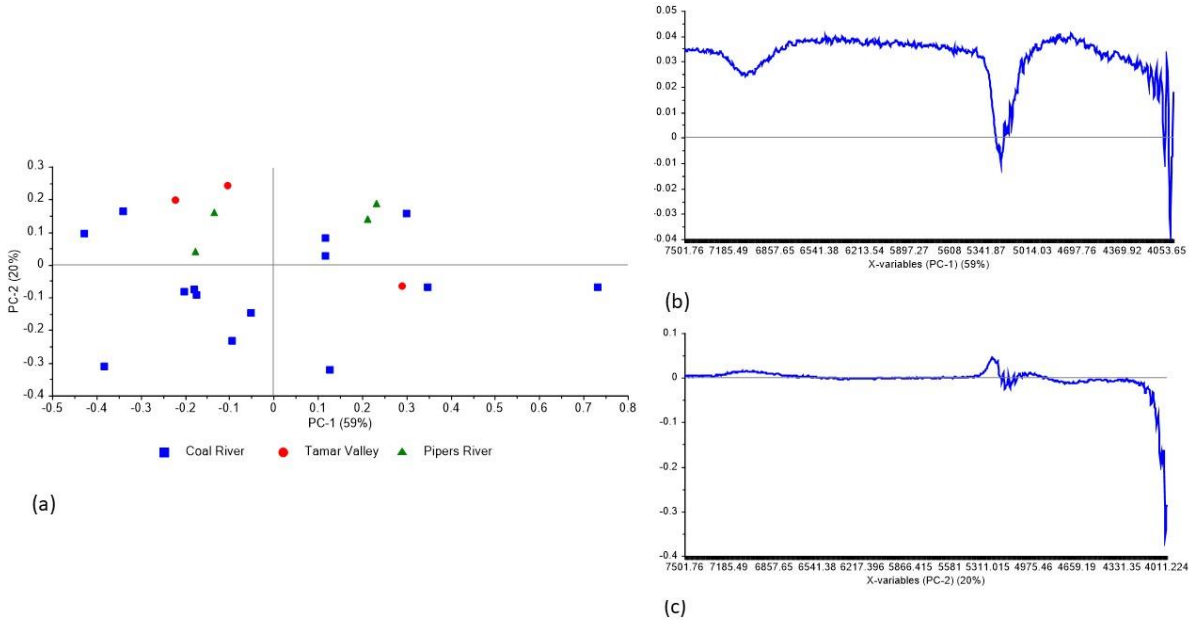


Figure 6-48: PCA score plot (a) and loadings plots for PC-1 (b) and PC-2 (c) of handpicked Chardonnay cuvée juice from Winery 1, processed in a Bucher press, delineated by region. Wavenumbers shown in loadings plots correspond to c.1330 – 2500 nm, left to right

The PCA plot relating to the sample set from Winery 1 of handpicked Chardonnay cuvée juice processed in a Europress is shown in Figure 6-49. As with the PCA of the UV-Vis spectra from the same set, there is some regional separation. However, the regions that are separated most completely are not the same in the analysis of the NIR scans. Regionally delineated by PC-2 (Y-axis, accounting for 25% of variation observed between the samples) the Coal River samples are separate from those of the Tamar Valley and Pipers River. Between the latter two, however, there is overlapping. PC-1 (X-axis), while accounting for more of the observed variation (58%), does not support regional discrimination. The loadings plots show PC-1 is influenced by a large range of the spectrum, peaking particularly in the region of 2100nm, with a trough around 1400nm and another sharp negative one around 1900nm (4700, 7000 and 5200cm^{-1} respectively). PC-2, which accounted for all regional separation, shows little influence, as with other NIR spectral analyses, except for a small peak around 1900nm and a short negative trough at 2000nm (5200 and 5000cm^{-1}).

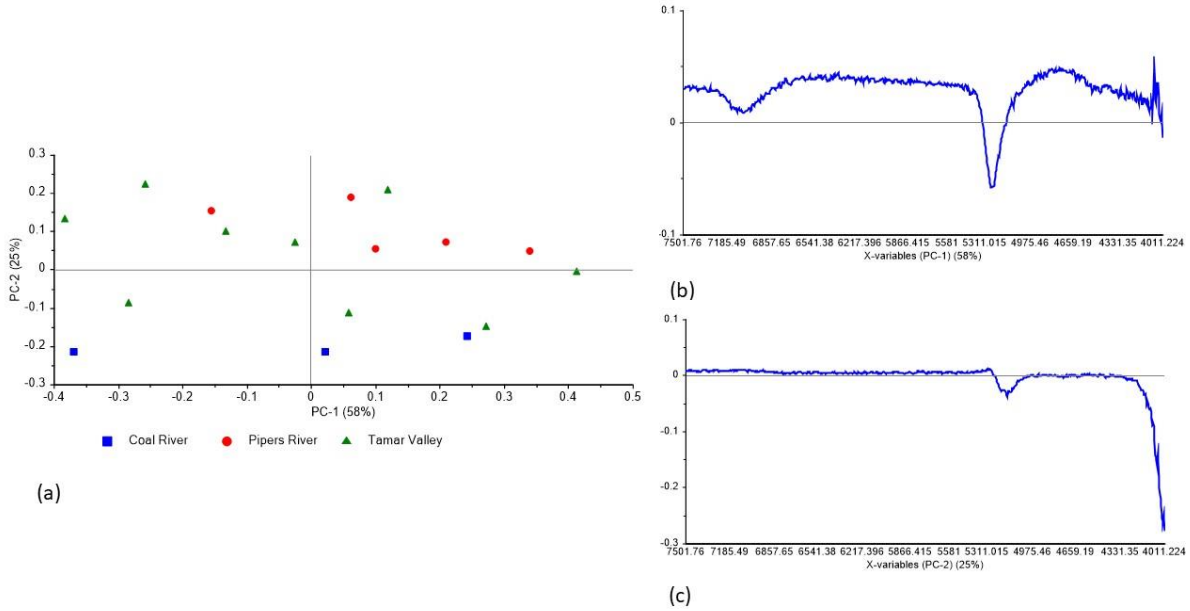


Figure 6-49: PCA score plot (a) and loadings plots for PC-1 (b) and PC-2 (c) of handpicked Chardonnay cuvée juice from Winery 1, processed in a Europress, delineated by region. Wavenumbers shown in loadings plots correspond to c.1330 – 2500 nm, left to right

Based on sampling and analysis over 2017 and 2018, the key learnings from juice provenance research were:

- Due to substantial variability in grape growing, winemaking and sample handling practices, it may be difficult to gather 'industry based' samples for initial construction of a fingerprint model to give reliable evidence of juice provenance;
- Proof-of-concept may require more structured juice sample collection (e.g. replicates of fruit harvested, transported and pressed by standardised laboratory protocol);
- Based on the 2018 results, it is possible that a range of measures may be required to build a model, including basic juice analysis (e.g. average pH, total phenolics (TPC) and hydroxycinnamates (THC) differentiated some regions), UV-Vis spectral analysis (e.g. 260-280nm and 320-330nm differentiated some regions), and NIR (e.g. wavelengths above 1900nm and below 2100nm).
- Any future model would likely need strong and fine-scale discriminative power to overcome juice variation in real industry settings, with highly variable grape cultivation and fruit handling processes.

7 Outcomes and Conclusion

This study produced three important sets of outcomes:

1/ practical ideas for **vineyard managers and sparkling winemakers** to adjust fruit and wine characteristics using some familiar methods (viticulture) and some novel methods (in-line juice assessment, 'hot' cellaring, tirage yeast disruption);

2/ insight into some of the challenges that **climate change** poses for sparkling wine production, with wines from the 'warm, dry' vintage of 2010 showing stronger responses to some of the viticultural interventions than in the 'cool, wet' vintage of 2011; and

3/ information on some of the **impact compounds** that were associated with juices and sparkling wines being either chemically or sensorially distinguishable from each other.

The main conclusions for each of the four parts of the research study were as follows:

7.1 Viticulture

Regardless of viticultural treatment applied, vintage-to-vintage separation of wines was the strongest and most consistent finding. This suggests that long-range weather forecasting may be important when selecting viticultural practices to influence fruit and wine. Where there were chemical impacts associated with viticultural treatments, they tended to be more distinct in the 'warm, dry' vintage of 2010 and were most often associated with Chardonnay rather than Pinot noir (the two varieties examined in this study).

Hydroxycinnamates are low molecular weight phenolics (including caffeic, ferulic, coumaric and caftaric acids). This class of compounds has been linked with positive mouthfeel properties in sparkling wine (eg. viscosity), when compared with other classes of phenolics. There was significant variation in hydroxycinnamate concentration in juices, base wines and sparkling wines, and an association with some of the viticultural treatments. For example, in the warm dry vintage 2010, hydroxycinnamate associated wavelengths drove separation between aged wines from 'high' and 'low' crop load. Spur pruning was associated with higher concentration of hydroxycinnamates which potentially suggests a role for spur pruning to build mouthfeel in flaccid sparkling wines. Hydroxycinnamates have been reported to be produced in response to UV-B exposure of fruit but the leaf removal trial did not reveal differences that support this.

The 'low' crop load wines in each season showed significantly higher concentration of total proteins than 'high' crop load treatment wines. Proteins contribute to the body, sweetness, roundness, and mouthfeel of sparkling wine. Aroma compounds are better retained, astringency reduced and foam properties improved, when proteins are at higher levels. This would suggest that vine nutrient status would be a key consideration for 'high' cropping. Although carefully managed as overly high protein may promote haze formation in wines.

It is possible that the findings of this study offer a further cautionary note on yield restriction. The pattern of esters observed in the 2010 'low' crop load wines suggested those wines were more developed than the 'high' crop load wines that year, and both sets of wines for 2011. For example, ethyl acetate, propanoic acid, and 2-methylpropanol were highest in the 'low' crop load wines from the warm year (2010). These compounds impart ethereal, pungent and rancid, and solvent-like

aromas, respectively; ethyl acetate was present at concentrations above its odour threshold. Climate change projections indicate the sector faces a higher frequency of warm vintages (Remenyi et al., 2019), so yield restriction practices that result in significantly higher ethyl acetate, propanoic acid, and 2-methylpropanol concentrations in wines from warm years, may be detrimental to quality.

In 2010, cane pruned Chardonnay wines showed significantly higher concentration of 2-methylpropanol (wine, solvent, bitter), 3-methybutanol (whisky, malt, burnt), and phenylacetaldehyde (honey, floral) than spur pruned treatment wines. This result may be important as the concentration of phenylacetaldehyde in the cane pruned 2010 Pinot noir wines was two-fold higher than in wines from spur pruned treatment, and well above the odour detection threshold. Phenylacetaldehyde is a cinnamic acid derivative (Ilc et al., 2016). The Chardonnay wines in this trial also demonstrated an interaction between vintage and pruning treatment for hydroxycinnamates and the significant effect observed for phenylacetaldehyde concentration signals the need for closer examination of pruning impacts and flow-on effects, on the expression of hydroxycinnamates and derived compounds in the aged Chardonnay wine matrix.

Polysaccharides have been shown to affect foam stability. Crop load and pruning treatments effected polysaccharide concentration in different ways, and this was highly dependent on vintage conditions. For example, low crop load 2010 wines had higher total polysaccharide than high crop load wines, however in the cool and wet 2011 season the inverse was observed, with high crop load wines recording higher total polysaccharide content. Specifically, the high molecular weight fraction (mannoprotein concentration) was related to crop load, and this fraction has been shown to affect the volatility of wine aroma and flavour compounds (Chalier et al., 2007) and accumulation of phenolic acids, such as p-coumaric acid (Sartor et al., 2019). As a contrast, the cane pruned Chardonnay vines delivered aged sparkling wines with higher medium molecular weight polysaccharides, compared with spur pruned. The medium molecular weight polysaccharides are consistent with arabinoglucan-proteins from grapes (McRae et al., 2017, Bindon et al., 2013a) suggesting cane-pruning may improve foam stability time.

Sensory appraisal of sparkling wines from the viticultural treatments indicated that, after 5.5 years in bottle, few of the viticultural treatments had delivered sensory impacts discernible by an expert panel. This was despite the compositional differences reflected in concentration of some parameters like low molecular weight phenolics, polysaccharides and volatile compounds. In the case of crop load, 'no discernible difference' led to a conclusion that yield restriction was not supported as a means to noticeably improve quality. The sensory panel did, however, register a consistent preference for wines that had been subjected to leaf removal at veraison. This finding could support quality improvement, but further research would be warranted to understand the mechanisms at play.

7.2 Autolysis and autolytic character

This component of the research delivered important findings for winemakers and sparkling wine science. 'Autolytic character' in sparkling wine was demonstrated in the absence of yeast. This suggests that some 'autolytic character' may be the result of the chemical aging of wine. Further, in examining 8 attributes of 'aged character', Chardonnay wines at 24 months bottle age showed 'nutty' and 'honey' characters that were perceived as more intense in wine that had been aged off lees, compared with *tirage* wine. This might suggest that the presence of yeast in Chardonnay wines had stopped, limited or curtailed the development of 'nutty' and 'honey' aromas. These results suggest that yeast might play a mediating, limiting or slowing role in the development of aged characters in Chardonnay wine. This raises the question: Could the yeast additions slow the chemical aging process in wines? It is likely the development of aged character is more complex and is impacted by a range

of factors including: grape- and primary fermentation-derived aroma precursors, oxidative potential of base wines, base wine varietal, chemical aging of wine in bottle, post-bottling yeast-mediated aroma modification (including yeast metabolic by-products, aroma compound adsorption/ desorption, physical breakdown of yeast cells), and metabolites from malolactic fermentation bacteria.

To further interrogate the role of thermodynamics and yeast in wine aroma development, 'hot' cellaring and yeast disruption techniques were applied. Each of these may have application in closer management of sparkling wine quality and efficiency. Overall, the aroma and flavour maturation of traditional method Chardonnay and Pinot noir sparkling wines was manipulated by elevating storage temperature from 15°C to 25°C. More intense maturation associated characters, 'autolytic', 'spicy', 'honey', 'earthy', 'nutty' and 'toasty' were achieved at a relatively young bottle age (24 months). The yeast treatments also had a positive impact on perception of maturation associated characters but to a lesser extent. The benefits of 25°C treatment in terms of precocious 'aged character', however, may be offset by impacts on foaming, compared with control, microwave and enzyme treatment wines:

> '…wines had similar foaming properties, except for the high temperature (25°C) storage treated wines and ultrasound treated wines, in both Chardonnay and Pinot noir. High temperature storage of wine effected the lowest total proteins concentration and an associated decrease in average foam lifetime (foam stability)…' (Gnoinski et al 2021)

It is notable that the sensory results suggested the Chardonnay base wine matrix was less affected by disrupted yeast treatment than the Pinot noir wine matrix. With further development of this method, the results suggest winemakers could potentially select and apply cellaring or a yeast disruption technique associated with the profile they wanted in their finished wines. These ideas are not conclusive, but the results reported suggest development of aged character in sparkling wine might be more precisely managed via addition of disrupted yeast at tirage, or the use of high temperature cellaring.

7.3 BevScan

This study demonstrated that BevScan was able to 'read' and distinguish between bottles of sparkling wine based on the viticultural treatment that had been applied to fruit, pre-harvest. For wines that had treatments applied at tirage, both RF and PLS-DA methods were able to discriminate wines based on bottle age, even when the whole dataset was included in the model. This included data from all eight treatment wines (including control and tirage wines, base wines with and without lees, additional yeast added to the manipulating aged character/autolysis treatment wines, and high cellaring temperature treatment). These BevScan results complement findings from the age or autolysis experiments which documented change to the chemical and sensory characteristics of the wines as they aged (Gnoinski et al. 2021a).

Recalling the 'evolution' of volatile species that was observed for ultrasound and microwave treated Pinot noir, we could speculate that the BevScan results reinforce the idea of wines evolving and that BevScan could be a non-destructive analytical tool for tracking sparkling wine evolution in-bottle.

7.4 Juice phenolics in-line

An objective, real-time method to quantify phenolics was developed to improve consistency and provide data-driven decision-making for more precise grape juice press fractioning. Comparison of three predictive model algorithms showed the best prediction of total phenolics concentrations in press fractions was obtained using 240 and 290 nm in-line in reflectance mode. The prototype

sampling, analysis and line switching tool was developed to the extent of proof-of-concept. The final iteration of the tool was designed to be:

- Portable between wineries, and to take to the press
- Compatible with different types of presses (eg. Bucher and Europress)
- Able to automatically switch tanks when a predetermined juice phenolic threshold has been reached

Automated tank switching on the basis of real-time inline phenolic sensing is an important breakthrough with potential to substantially alter the quality, accuracy and cost-effectiveness of juice pressing for both white and sparkling wine production in Australia, and internationally.

7.5 Juice provenance

Using spectral measures was not demonstrated to be currently effective for juice provenance. This was in part due to the challenge of many juice variables, including:

- Vine clone and rootstock (many)
- Regional climate and vineyard micro-climates (many)
- Viticultural practices (many)
- Harvesting method (machine, hand harvest)
- Transportation of fruit from vineyard to press (refrigeration, time in truck)
- Type of press (Bucher, Europress)
- Pressing regime applied (long, short)
- Juice fraction sampled (free-run, cuvee, taille)
- Post-sampling storage (how soon frozen, how long kept frozen, mode of transportation)

Due to substantial variability in grape growing, winemaking and sample handling practices, it was difficult to gather 'industry based' samples for initial construction of a fingerprint model to give reliable evidence of juice provenance. This study developed and trialled a juice sampling method which might support development of a preliminary model to objectively identify juice provenance.

Based on the 2018 results, it is possible that a range of measures may be required to build such a model, including basic juice analysis (e.g. average pH, total phenolics (TPC) and hydroxycinnamates (THC) differentiated some regions), UV-Vis spectral analysis (e.g. 260-280nm and 320-330nm differentiated some regions), and NIR (e.g. wavelengths above 1900nm and below 2100nm).

8 Recommendations

The wide scope of this study raised many opportunities for future research:

1. Greater attention to use of leaf removal for aroma modification, spur pruning for hydroxycinnamate management, and generally investigating the (disproportionate) response of vines to viticultural practices during warm dry vintages, to support adaptation to climate change.

2. Potentially, precision agriculture approaches could be developed to manage key determinants of fruit quality under high crop load regimes. Particular focus would be on: irrigation (avoiding undue water stress), vine nutrient status (implications for foaming), and pruning and leaf removal options to potentially influence several parameters (bunch size, ripening, spray penetration, humidity in canopy, hydroxycinnamate concentration).

3. Considering the impact of an elevated cellaring temperature of 25°C and alternate yeast treatments (microwave, ultrasound, enzyme) on sparkling wine aged character development, further chemical and sensory studies are warranted. Particular focus would be: optimising the foaming properties of warm cellared wines; the rate and degree of aged character development from applying a wider range of microwave, enzyme and ultrasound yeast regimes. It would also be useful to understand if greater addition of yeast at tirage in the 25°C wines resulted in 'protective' effects for foaming quality (more amino acid residual) or simply resulted in greater production of aldehydes.

4. Proof of concept was generated for several non-destructive assays that could be undertaken in wineries using Bevscan, including assessment of sparkling wine phenolics, provenance and maturation (ready to release to market).

5. Proof of concept was also generated for automating juice sampling and tank switching to improve the efficiency and accuracy of pressing juice for sparkling wine. This work needs a path to market.

9 Appendix 1: Communication

9.1 Journal Papers

(Gnoinski et al., 2021b) - Gnoinski, G. B., Schmidt, S. A., Close, D. C., Goemann, K., Pinfold, T. L. & Kerslake, F. L. 2021b. Novel Methods to Manipulate Autolysis in Sparkling Wine: Effects on Yeast. *Molecules,* 26, 387. **https://doi.org/10.3390/molecules26020387**

(Gnoinski et al., 2021a) - Gnoinski, G. B., Close, D. C., Schmidt, S. A. & Kerslake, F. L. 2021a. Towards Accelerated Autolysis? Dynamics of Phenolics, Proteins, Amino Acids and Lipids in Response to Novel Treatments and during Ageing of Sparkling Wine. *Beverages,* 7, 50. **https://doi.org/10.3390/beverages7030050**

(Sawyer et al., 2021) - Sawyer, S., Longo, R., Solomon, M., Nicolotti, L., Westmore, H., Merry, A., Gnoinski, G., Ylia, A., Dambergs, R. & Kerslake, F. 2021. Autolysis and the duration of ageing on lees independently influence the aroma composition of traditional method sparkling wine. *Australian Journal of Grape and Wine Research,* n/a. **https://doi.org/10.1111/ajgw.12527**

(Longo et al., 2021) - Longo, R., Dambergs, R. G., Westmore, H., Nichols, D. S. & Kerslake, F. L. 2021. A feasibility study on monitoring total phenolic content in sparkling wine press juice fractions using a new in-line system and predictive models. *Food Control,* 123, 106810. **https://doi.org/10.1016/j.foodcont.2019.106810**

(Jones et al., 2018) - Jones, J. E., Kerslake, F. L., Dambergs, R. G. & Close, D. C. 2018. Spur pruning leads to distinctly different phenolics profiles of base sparkling wines than cane pruning. *Vitis,* 57, 103-109. **https://doi.org/10.5073/vitis.2018.57.103-109**

(Kerslake et al., 2018) - Kerslake, F., Longo, R. & Dambergs, R. 2018. Discrimination of Juice Press Fractions for Sparkling Base Wines by a UV-Vis Spectral Phenolic Fingerprint and Chemometrics. *Beverages,* 4, 45. **https://doi.org/10.3390/beverages4020045**

9.2 Conference Papers

Sawyer, S and Longo, R and Solomon, M* and Nicolotti, L* and Westmore, H and Merry, A and Gnoinski, G and Ylia, A* and Dambergs, R and Kerslake, F, "Is it the age or the autolysis? Pulling apart where sparkling wine character comes from", 21 - 24 July, Adelaide, Australia, pp. 11. (2019) [Plenary Presentation] K0023785

Kerslake, FL and Kemp, B and Shakya, S and Dambergs, RG, "The progression of sparkling grape pressing and what comes out when?", 21 - 24 July, Adelaide, Australia, (2019) [Conference Extract] K0023785

Longo, R and Dambergs, RG and Westmore, H and Nichols, D and Kerslake, FL, "A new in-line system to monitor total phenolic content in juice fractions for sparkling wine" , 21 - 24 July, Adelaide, Australia, (2019) [Conference Extract] K0023785

Gnoinski, G and Goemann, K and Pinfold, T and Schmidt, S and Close, D and Kerslake F, "Visualisation of effects on lees from novel methods to induce autolysis in sparkling winemaking", 21 - 24 July, Adelaide, Australia, (2019) [Conference Extract] K0023785

Gnoinski, G and Lima, B and Howell, K and Gonzalez Viejo, C and Fuentes, S and Schmidt, S and Close, D and Kerslake, F, "Influence of traditional and novel winemaking practices on foaming properties of sparkling wines", 21 - 24 July, Adelaide, Australia, (2019) [Conference Extract] K0023785

Longo, R and Dambergs, R and Solomon, M and Nicolotti, L and Ylia, A and Westmore, H and Merry, A and Gnoinski, G and Kerslake, F, "Manipulation of yeast autolysis by physical treatments: effects on the volatile profiles of methode champenoise sparkling wines" 21 - 24 July, Adelaide, Australia, (2019) [Conference Extract] K0023785

Sawyer, S and Longo, R and Solomon, M and Nicolotti, L and Westmore, H and Merry, A and Gnoinski, G and Ylia, A and Dambergs R and Kerslake, F, "Is it age or the autolysis? Pulling apart where sparkling wine character comes from", 21 - 24 July, Adelaide, Australia, (2019) [Conference Extract] K0023785

Kerslake, F and Dambergs, B and Smith, P and Merry, A and Close, D and McRae, J* and Westmore, H, "Crop load for sparkling wines - the high and low debate", Crush 2018: the Grape and Wine Science Symposium, 25-26 September 2018, Adelaide, South Australia, pp. 1-12. (2018) [Plenary Presentation] K0023785

Kerslake, FL and Merry, AM and Jones, JE and McRae, J* and Solomon, M* and Close, DC and Dambergs, RG, "How to achieve your desired sparkling wine style in the vineyard", 2018 Vitinord Scandinavia Program, 31 July - 03 August, Malmö Sweden (2018) [Plenary Presentation] K0023785

Kerslake, F and Dambergs, B and Smith, P and Merry, A and Close, D and McRae, J* and Westmore, H, "Is your hard work in the vineyard making your sparkling wine pop?", Australian Wine Research Institute webinar, Australia, 17 May (2018) [Internal Newsletter] K0023785 **https://www.youtube.com/watch?v=X_i-ZLGV11s&t=7s**

Longo, R and Kerslake, F and Dambergs, B and Westmore, H and Donachie, L, "In-line sensing of grape juice press fractioning by UV-Vis spectroscopy: comparison with a benchtop spectrophotometer cycle", Crush 2018: the Grape and Wine Science Symposium, 25-26 September 2018, Adelaide, South Australia (2018) [Plenary Presentation]

Kerslake, FL, "2010 wines – the results", Crush 2017: the grape and wine science symposium program, 13-14 November 2017, Adelaide, South Australia (2017) [Plenary Presentation] K0023785

Kerslake, FL, 17th AWITC workshop convenor, 'Optimising Australian sparkling wines', 21 - 24 July, Adelaide, Australia, (2019)

Kerslake, FL, 17th AWITC plenary session chair, 'Fresh Science', 21 - 24 July, Adelaide, Australia, (2019)

9.3 Field days, Workshops and Meetings

Kerslake, F and Gnoinski, G and Sawyer, S and Longo, R and Dambergs, R* and Close, D and Solomon, M* and Nicolotti, L* and Ylia, A* and Smith, P* and McRae, J* and Westmore, H and Merry, A, "Manipulating maturation in sparkling wines and 'autolytic character'", Wine Tasmania winemakers symposium (2019) [Keynote Presentation] K0023785

Kerslake, FL and Jones, JE and Dambergs, RG and Smith, PA and Close, DC, "Managing canopies to influence critical chemistry profiles in sparkling Chardonnay wines", School of Land and Food Conference Programme, 13- 15 June 2017, UTAS, Tasmania (2017) [Plenary Presentation] K0023785

Kerslake, FL and Jones, JE and Close, DC and Dambergs, RG and Fryar, N*, "Sparkling wine: production, price, profitability and perception", Ferment 2016, 06-07 June 2016, Hobart, Tasmania (2016) [Plenary Presentation] K0023785

Kerslake, FL and Jones, JE and Close, DC and Dambergs, RG, "Viticultural practices and their effect on sparkling wines", Effervescence: Tasmanian Sparkling Festival 2016, 04 November 2016, Tamar Valley, Tasmania (2016) [Keynote Presentation] K0023785

Kerslake, FL, "2010 wines – the results", Effervescence Technical Day Agenda, 16 November 2017, Tamar Valley, Tasmania (2017) [Keynote Presentation] K0023785

Kerslake, FL and Dambergs, RG and Smith, PA and Merry, AM and Close, DC and McRae, JM and Westmore, HS, "Managing vines to influence critical chemistry profiles in sparkling wines", AWRI Sparkling Wine Symposium Program, 26 June 2018, Melbourne (2018) [Plenary Presentation] K0023785

Longo, R and Kerslake, F and Dambergs, B and Westmore, H and Donachie, L, "In-line sensing of grape juice press fractioning by UV-Vis spectroscopy: comparison with a benchtop spectrophotometer", 2018 Winemakers Symposium: Regionally First and Foremost, 16 October 2018, Tamar Ridge, Tasmania (2018) [Keynote Presentation]

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9.4 Press and newsletter articles

"How Food Works: Part 2", ABC TV Catalyst, aired April 21 2020, **https://www.abc.net.au/catalyst/s21-e02-how-food-works/12157118**

"Unlocking the secrets of sparkling wine" 2019, **https://www.youtube.com/watch?v=mAEqAEpFyrc**

"The wines, they are a-changin'", Ockham's Razor, Beaker Street @ TMAG, Broadcast 21/10/2018, **https://www.abc.net.au/radionational/programs/ockhamsrazor/the-wines-they-are-a-changin/10386424**

French, P. "Tasmanian research project investigates faster sparkling wine maturation" 13 June (2018) **https://www.thedrinksbusiness.com/2018/06/tasmanian-research-project-investigates-faster-sparkling-wine-maturation/**

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"Results from 2010 TIA research on sparkling wine held for Tasmanian producers at Effervescence", The Examiner, Tasmania, 15 November (2016) [Media Interview] **https://www.examiner.com.au/story/4293150/six-year-labour-of-love-ends-in-taste-test-day/**

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"Trio of wine scientists spearhead new national research projects from Tasmania", The Examiner, Tasmania, 7 June (2016) **https://www.examiner.com.au/story/3954705/wine-projects-splash-onto-national-stage/**

"Winemakers and science combine forces in Tasmanian project to find key to better bubbly", The Examiner, Tasmania, 12 June (2018) K0023785

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https://www.wineaustralia.com/news/articles/liberating-winemakers-at-the-press

10 Appendix 2: Staff and Students

10.1 Staff

Project Leader: Dr Fiona Kerslake, Tasmanian Institute of Agriculture, University of Tasmania

Researchers: Dr Robert Dambergs, Wine TQ, National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, Australia

Dr Samantha Sawyer, Tasmanian Institute of Agriculture, University of Tasmania

Dr Angela Merry, Tasmanian Institute of Agriculture, University of Tasmania

Dr Rocco Longo, Tasmanian Institute of Agriculture, University of Tasmania

Dr Roger Latham, Tasmanian Institute of Agriculture, University of Tasmania

Dr Anna Carew, Tasmanian Institute of Agriculture, University of Tasmania

Dr Thomas Rodemann, Central Science Laboratory, University of Tasmania

Dr Karsten Goemann, Central Science Laboratory, University of Tasmania

Professor Dugald Close, Tasmanian Institute of Agriculture, University of Tasmania

Dr Paul Smith, Wine Australia

Dr Jacqui McRae, Australian Wine Research Institute

Dr Luca Nicolotti, Metabolomics Australia

Research assistants: Ms Hanna Westmore, Tasmanian Institute of Agriculture, University of Tasmania

Ms Linda Donachie, Tasmanian Institute of Agriculture, University of Tasmania

Dr Jennifer Lavers, Tasmanian Institute of Agriculture, University of Tasmania

Ms Michele Buntain, Tasmanian Institute of Agriculture, University of Tasmania

Ms Claire Baker, Tasmanian Institute of Agriculture, University of Tasmania

Ms Miquela McGuiness, Tasmanian Institute of Agriculture, University of Tasmania

10.2 Students

Students: Ms Gail Gnoinski, PhD candidate, Tasmanian Institute of Agriculture, University of Tasmania

Mr Hugh McCullough, Masters candidate, Plumpton College, England

Mr Leo Lebanov, PhD candidate, University of Tasmania

11 Appendix 3: Acknowledgements

11.1 Wine Australia

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11.2 Collaborators

Hill-Smith Family Vineyards, Josef Chromy Wines, Apogee Tasmania

Louisa Rose, Jeremy Dineen, Ockie Myburgh, Stewart Byrne, Dr Andre Pirie

Tasters

Wine TQ – Dr Bob Dambergs

AWRI - Dr Jacqui McRae, Mark Solomon, Vicky Amora, Stella Kassara, Laura Brey, Alex Schulkin, Dr Leigh Francis, Dr Dimi Capone, Dr Natoiya Lloyd, Dr Luca Nicolotti, Dr Paul Smith, Louisa Rose.

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Student – Angela Sparrow

Agencies – AWRI, TIA and Wine Tasmania

Manufacturers – Flextank, Croplands

Tasmanian wine industry collaborators - Clover Hill Wines, Frogmore Creek Wines, Jansz Tasmania, Josef Chromy Wines, Meadowbank Wines, Moorilla Wines, Pooley Wines, Tamar Ridge Estates (now Brown Family Wine Group), Tolpuddle Vineyard, Winemaking Tasmania (now Tasmanian Vintners)

11.3 Other collaborators

Red Brick Road Cider

12 Appendix 4: Other Relevant Material….

12.1 Additional Methodology

12.1.1 C_{13}-norisoprenoids - Bound aroma method

The method from Kwasniewski et al. (2010) was adapted for the analysis of bound β-damascenone.

Sample preparation

10mL of wine was adjusted to pH 2 with 2M hydrochloric acid. This was followed by 50uL of 1.2mg/L d_4-β-damascenone internal standard solution. Samples were heated at 100 °C in a heating block for 60 min to facilitate hydrolysis. Immediately after heating, samples were placed in an ice bath to cool. Due to some precipitation during the heating samples were filtered through glass wool and Whatman #1 filter paper prior to SPE analysis.

SPE analysis

Samples were loaded onto SPE cartridges (Agilent, 500mg/6mL LMS) that had been preconditioned with 5mL DCM, 5mL MeOH, 10mL MilliQ water. After sample loading, samples were rinsed with 10mL MilliQ water and dried under vacuum. β-damascenone was eluted with 4mL DCM (a small amount of water was also eluted at this step but it was removed with a pasteur pipette prior to the solvent beind dried). DCM was dried in a TurboVap under nitrogen to a final volume of approximately 300uL.

GCMS analysis

The analysis was performed on an Agilent 6890A gas chromatograph equipped with a Gerstel MPS2 autosampler and coupled to an Agilent 5973N mass selective detector. Instrument control was performed with Agilent G1701EA Revision E.02.02 ChemStation software. The gas chromatograph was fitted with an Agilent DB-5msUI 30m x 0.25mm x 0.25um. Helium (Ultra High Purity) was used as the carrier gas with linear velocity 46 cm/s, flow-rate 1.6 mL/min in constant flow mode. The oven temperature was started at 40 °C, held at this temperature for 2 min then increased to 200 °C at 8 °C/min, finally the temperature was increased to 280 °C at 100 °C/min and held at this temperature for 7.2 min. 2uL of extract was injected into a split/splitless inlet in pulsed splitless mode. The analytes were injected into an Ultra Inert, single taper, glass wool packed liner which was held at 270 °C. The purge flow to the split vent was 8mL/min at 2.1min with the septum purge flow turned off. The mass spectrometer quadrupole temperature was set at 150 °C, the source was set at 230 °C and the transfer line was held at 250 °C. EMV Mode was set to Gain Factor = 1.00 and spectra were recorded in SIM mode. The ions monitored were: *m/z* 73, 179 and 194 for d_4-β-damascenone and *m/z* 69, 175 and 190 for β-damascenone.

12.1.2 C_{13}-norisoprenoids - Free aroma method

Adapted to wine from grapes as performed in Perestrelo et al. (2011).

Sample preparation

7mL of sample was pipetted into a 20mL screw-cap, headspace-SPME vial (Agilent CrossLab). 3mL of saturated NaCl solution was added and vortexed to mix followed by 50uL of 1.2mg/L d_4-β-damascenone internal standard solution.

GCMS analysis

The analysis was performed on an Agilent 6890A gas chromatograph equipped with a Gerstel MPS2 autosampler and coupled to an Agilent 5973N mass selective detector. Instrument control was performed with Agilent G1701EA Revision E.02.02 ChemStation software. The gas chromatograph was fitted with an Agilent DB-5msUI 30m x 0.25mm x 0.25um. Helium (Ultra High Purity) was used as the carrier gas with linear velocity 46 cm/s, flow-rate 1.6 mL/min in constant flow mode. The oven temperature was started at 40 °C, held at this temperature for 2 min then increased to 200 °C at 8 °C/min, finally the temperature was increased to 280 °C at 100 °C/min and held at this temperature for 7.2 min. The vial and its contents were heated to 80 °C for 20 mins in the heater/agitator with the agitator on for 5 secs and off for 2 secs at 500 rpm. A Supelco grey 2cm SPME fibre was exposed to the sample during this heating time through the septum. The fibre was then injected into a

split/splitless inlet in pulsed splitless mode. The analytes were desorbed into a Supelco 0.75mm ID sleeveless SPME liner for 10 min which was held at 200 ?C. The purge flow to the split vent was 8mL/min at 2.1min with the septum purge flow turned off. The mass spectrometer quadrupole temperature was set at 150 ?C, the source was set at 230 ?C and the transfer line was held at 250 ?C. EMV Mode was set to Gain Factor = 1.00 and spectra were recorded in SIM mode. The ions monitored were: *m/z* 73, 179 and 194 for d_4-β-damascenone and *m/z* 69, 175 and 190 for β-damascenone.

This method is quite similar to the free aroma grape method substituting the 7g of grape homogenate for 7mL wine.

12.1.3 Fermentation products and non-targetted volatiles

Fermentation derived volatile profiling

Targeted metabolite profiling of 28 fermentation derived volatile compounds was undertaken on finished bottled wines soon after sensory analysis in the final season (see Table 1 for compound list). Retrospective analysis was not undertaken due to the time elapsed since previous seasons bottlings. Gas chromatography-Mass spectrometry (GS-MS) was undertaken by Metabolomics SA at the Australian Wine Research Institute, Waite Campus Urrbrae. Briefly, SPME GC-MS sample preparations was performed as described by Siebert et al. (2005) with the following modifications.

The gas chromatograph was fitted with a 60m x 0.25mm wax column with 0.25um film thickness. The vial and its contents were heated to 35°C for 5 minutes with agitation. The SPME fibre (polyacrylate) was exposed to the headspace in the sample for 10 minutes and was then desorbed in the injector (splitless mode) for 10 minutes. The injector temperature was set at 260°C. Compounds were identified using rectified internal deuterium labelled in house standards. Oven temperature was started at 35°C, held at this temperature for 2 minutes then increased to 150°C at 55°C/min, and held at this temperature for 2 minutes, then increased to 150°C at 5°C/min, and held for 2 minutes, and finally increased to 230°C and held for 5 minutes. The total run time is 42 minutes.

Raw data from Agilents' ChemStation software (v E.02.02.1431) was converted into MassHunter data files and processed using MassHunter Workstation Software for Quantitative Analysis (v B.04.00). The concentration of analytes in the samples are determined using stable isotope dilution analysis (SIDA) and are reported in ug/L quantities.

Analysis of fermentation products by solid phase microextraction (SPME)/GC-MS analysis (taken from Sawyer et al (in prep.)

Analysis of the fermentation products (esters, carboxylic acids and higher alcohols) was performed according to Siebert, et al. (2005) with some modifications. Briefly, the analysis was performed on an Agilent 7890A (Agilent Technologies Pty. Ltd, Palo Alto, CA, USA) gas chromatograph (GC) equipped with a Gerstel MPS2 multi-purpose sampler and coupled to an Agilent 5975C VL mass selective detector. Instrument control was performed with Agilent ChemStation vE.02.02.1431. The GC was fitted with an Agilent DB-624UI column (30 m × 0.25 mm × 1.4 µm) with helium (Ultra High Purity) as the carrier gas in constant flow mode. The oven temperature was heated to 40°C before being increased to 60°C at 20°C/min and held for 14 min. This was followed by a series of temperature ramps: 1) 70°C at 20°C/min, 2) 80°C at 10°C/min, 3) 150°C at 20°C/min, 4) 210°C at 5°C/min and 5) 260°C at 40°C/min before the temperature was held at 260°C for 3 min. The total run time was 45.5 min.

The 18 wine samples (*n* = 3 treatments × *n* = 3 replicates × *n* = 2 grape varieties) at each timepoint were diluted 1/10 before a sample (1 g) was transferred into buffer solution (9 mL, pH 4.3) in a vial (20 mL) containing salt (2 g). The sample vial was heated to 35°C for 5 min with agitation. The SPME fibre (polyacrylate) was exposed to the sample vial headspace for 15 min before desorption in the injector (splitless mode) for 15 min. The injector temperature was set at 260°C, the mass spectrometer quadrupole temperature was set at 150°C, the source temperature was set at 230°C and the transfer line was held at 260°C. Positive ion electron impact spectra at 70 eV were recorded in SIM and SCAN mode with solvent delay of 4 min.

The raw data from the Agilent ChemStation software was converted and processed using vB.04.00 MassHunter Workstation Software for quantitative analysis. The concentration of analytes in the samples were determined using stable isotope dilution analysis (SIDA).

GCMS Volatile Profiling

Untargeted volatile profiling was performed. Briefly, the analysis was performed on an Agilent 7890A (Agilent Technologies Pty. Ltd, Palo Alto, CA, USA) GC equipped with a Gerstel MPS2 multi-purpose sampler and coupled to an Agilent 5975C VL mass selective detector. Instrument control was performed with Agilent ChemStation vE.02.00. The GC was fitted with a wax column (30 m × 0.18 mm × 0.18 µm) with helium (Ultra High Purity) as the carrier gas in constant flow mode. The oven temperature was heated to 35°C and held for 4 min followed by a series of temperature ramps: 1) 60°C at 4°C/min, 2) 230°C at 8°C/min, and 3) 240°C at 10°C/min before the temperature was held at 240°C for 5 min. The total run time was 37.5 min.

Wine samples (1.5 mL) were weighed before being added to a potassium tartrate buffer solution (3.5 mL, pH 3.2, adjusted with tartaric acid) in a vial (10 mL) containing salt (1 g). The sample vial was heated to 40°C for 10 min with agitation. Headspace (2.5 mL) was sampled with a heated gas tight syringe and injected into a Gerstel PVT (CIS 4) inlet fitted with a Tenax TA inlet liner. Analytes captured on the Tax liner were transferred into the instrument by raising the temperature to 330°C and holding the temperature for 10 min. The mass spectrometer quadrupole temperature was set at 150°C, the source temperature was set at 230°C and the transfer line was held at 250°C. Positive ion electron impact spectra at 70 eV were recorded in SIM and SCAN mode with solvent delay of 3 min.

The raw data files were exported as CDF generic file format before being processed using R (statistical programming environment) v3.3.2 and Bioconductor v2.14 under a Debian Linus 64.bit environment.

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