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# Benchmarking regional and subregional influences on Shiraz fine wines.



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**Project CSU1602-01:**

Benchmarking regional and subregional influences in Shiraz fine wines

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# 3 Abstract

Sensory properties of Shiraz wines from the Barossa Valley, McLaren Vale, Heathcote, Yarra Valley, Canberra District and Hunter Valley have been detailed and compared. Selection of wines utilised a new sensory method, Pivot©Profile. A comprehensive chemical investigation was performed using a range of targeted and untargeted chemical analyses on selected wines and correlated with sensory data to identify compound attributes associated with typicality from the geographical indications (GI) selected. In addition, the project has overlayed key climatic data from the specific region/subregion and vineyard to help explain the chemical and sensory differences reported in the representative wines.

# 4 Executive summary

This project was instigated to investigate multiple questions pertaining to the ability to differentiate fine Australian Shiraz/Syrah wines from regions with high esteem for this grape variety. The project sought to:

- Demonstrate the suitability of a rapid sensory method for characterisation of wine
- Characterise the chemical differences and similarity of fine Shiraz wines from selected regions through targeted and untargeted metabolomic approaches of analysis
- Correlate the chemical and sensory composition of selected wines to the climatic indices associated with the vineyards from which the wines were sourced

The performance of the recently developed rapid sensory descriptive method Pivot© Profile (PP) was assessed with a set of 17 Shiraz/Syrah red wines using two groups; firstly 49 international sommeliers and then 11 Australian winemakers. Premium examples of Australian regional wines were first selected by the authors and other experts familiar with regional styles. The PP results were also compared to results from descriptive analysis (DA) performed by a trained panel. This investigation demonstrated for the first time that PP and DA provide similar insights into the sensory properties of products, and confirmed that PP with expert panelists allows a rapid understanding of the main sensory differences amongst samples.

The sommelier event was well-received and was able to the showcase the quality and diversity of a range of premium Australian Shiraz/Syrah wines. Utilising the sensory method PP and summarising the current investigation exposed an influential group of wine trade professionals to Australian wine research activities and provided an insight into their preferences.

Sets of wines (22 to 28 wines) from six prominent Australian Shiraz producing regions (Barossa Valley, McLaren Vale, Yarra Valley, Heathcote, Canberra District, and the Hunter Valley) were evaluated by groups of local winemakers using the PP method to obtain maps of their sensory characteristics. Three or four wines from each region were then selected using cluster analysis and twenty-two wines evaluated using sensory descriptive analysis. The regional PP assessments provided a sensory fingerprint of the variability of each of the regions studied and identified sensory characteristics that typified the largest groups of wines of each region. The descriptive analysis highlighted sensory characteristics that distinguished the wines from the different regions.

The comparison of PP with DA demonstrated that a rapid assessment of wine using the PP method is both robust and valuable. The PP method has already been used for other Wine Australia projects beyond the current investigation due to the effectiveness and efficacy demonstrated.

The same 22 wines were analysed by 70 chemical measures and 17 site- and season-specific climate indices were determined. From cluster analysis of compositional data wines were well grouped by region of origin. Distinctive chemical fingerprints exist for the regions studied, and the climatic profiles were strongly associated with key compounds influencing sensory differences. Multivariate analyses showed that wines with stalky/cooked vegetal sensory properties had higher cinnamate esters and dimethylsulfide, relating to later budbreak and harvest day. Wines with higher monoterpenes were associated with floral aroma. High radiation

measures were linked to higher tannin, colour density, norisoprenoid compounds and phenylethyl acetate and stronger dark fruit/dried fruit and tannin/colour attributes. High rainfall indices were related to generally low intensity of most sensory attributes and most compositional measures.

The volatile compounds of the chosen wines were also analysed using an untargeted metabolomics approach for gas chromatography time-of-flight mass spectroscopy to provide an overview of the wine volatilome. Several esters identified were found to be important enabling discrimination of wines based upon geographic origin, including ethyl hexanoate, ethyl heptanoate, ethyl dodecanoate, ethyl 2-phenylacetate, ethyl 2-methylbutanoate and ethyl 3- methylbutanoate. These compounds were found to be generally present in higher concentrations in warmer regions, i.e. Hunter Valley, McLaren Vale and Barossa. Ethyl cinnamate and ethyl dihydrocinnamate both showed highest abundance in Yarra Valley wines and least abundance in Hunter Valley wines. The presence of these cinnamates may contribute to differences in sensory attributes based on GI climatic differences, but winemaking practices (e.g. use of whole bunch) was also considered as being important. Overall, the results from the untargeted chemical analyses suggest that the regional compositional differences in varietal wines may be influenced by all processes in the entire wine production chain. However, the chemical basis underlying the regional typicality of Australian Shiraz wines was highlighted and specific volatile compounds that may be associated with a region are identified.

The separation of regional wines using non-volatile compounds was not achieved to same degree as using volatile compounds. However, when the apparent number of compounds in the model was reduced to 26, reasonable regional separation was able to be achieved. A hierarchical cluster analysis confirmed that most wines from the same GIs grouped together. The identification of the non–volatile compounds was not able to be performed to any great extent, however dihydroxybenzoic acid-β-D-glucopiranoside was measured at higher concentration in Hunter Valley wines. An integration of the sensory characteristics, volatile markers and non-volatile chemical markers found through targeted analyses and climatic indices resulted in both regional similarities and distinctions amongst the Shiraz wines. The six regions could be divided into 4 distinct clusters, namely, Canberra District/Yarra Valley, Heathcote, Barossa/McLaren Vale and Hunter Valley. Hunter Valley wines were the most distinct in terms of sensory and compositional characters compared to other regions. A series of heat and water related climate indices modelled for the past 20 years showed distinctive patterns associated with each region, especially for Barossa, Hunter Valley, McLaren Vale and Yarra Valley. The correlations found between climate indices and the overall sensory and chemical composition of wines produced in a region have also been described.

The project was an excellent example of the collaboration between two of Australia's leading wine research organisations, the AWRI and NWGIC. It also enabled Wes Pearson, AWRI Senior Scientist, to successfully complete his PhD candidature as a student of Charles Sturt University. The wonderful support of members of the Wine Australia marketing team, including Andreas Clark, Stuart Barclay, Aaron Ridgway and Mark Davidson is also acknowledged. Their support and enthusiasm enabled findings of this investigation to be presented in both the United States, Hong Kong as well as in Australia to a diverse audience.

# 5 Background

The association of agricultural products to a provenance or specific region (terroir) that imparts a typical and unique sensorial profile is an important concept for providores of high value products. The concept of a terroir is complex and is broadly considered in terms of the interactions between the environment, climate and the social interplay of people, their history and traditional approaches to food production. In the wine/viticulture/food industry several definitions of terroir exist and all acknowledge the importance of a local climate upon the composition of grapes and resulting wine. The Australian wine industry seeks to capture a greater proportion of the world's fine wine market. The branding and marketing of fine wines are frequently associated with a concept of uniqueness, eliciting higher prices by an association of exclusivity. Through knowledge and understand of the typicality of fine Australian wines, winemakers, marketers and sommeliers will have the tools to exploit the influence of terroir in the characterisation of differing wine styles by defining objective compositional measures. Determining the principal influences of macroclimate (and mesoclimate to a lesser extent) on terroir is a challenging conundrum requiring the collective inputs from experts in spatial and climate science, wine production, sensory, analytical and data sciences. Defining objective measures of terroir and provenance will lead to improved understanding of the interplay of climate, geography and people in the production of fine Australian wines.

Terroir is a complex notion, encompassing environmental factors that influence product composition within a specifically defined region and knowledge associated with the production of a food product. Wine style depends principally on grape composition which is largely determined by abiotic factors and vineyard's environmental characteristics. Differences in grape composition pertaining to site arise from vines adapting to different biotic and abiotic environments (Tonietto and Carbonneau, 2004) which in turn influence vine physiology and ultimately berry composition (Deloire *et al.,* 2008). Single markers that define wine style, terroir and regionality are simplistic and identification of an array of markers is required to determine objective and predictive wine sensory features (Schmidtke, 2013). Considerable importance has been placed on rotundone's peppery character in Shiraz wines (Herderich *et al.,* 2012), including environmental, seasonal and within-vineyard (Zhang *et al.,* 2015) variability, although rotundone levels have not yet been used to determine regionality. Untargeted metabolomic approaches that characterise the volatile and non-volatile signatures of grapes (Cramer *et al.,* 2014; Anesi *et al.,* 2015) and wine components (Roullier-Gall *et al.,* 2014) have identified some objective measures of terroir. A concept of terroir is important for producers of fine wines who aim to associate their product with unique geographical areas, associated mesoclimates and landscapes that impact upon wine typicality. Defining a terroir influence for fine wine producers will help enhance the uniqueness of Australian Wines in a global context. Commoditisation of agricultural products arises from global financial pressures to lower inputs, expenses and enhance profitability. The ability for fine wine producers to substantiate claims of uniqueness will reverse this trend; enable product positioning within markets as distinctive and exclusive wines that command premium prices in a global context.

# 6 Project Aims

The planned outputs and performance targets of the project 'Benchmarking regional and subregional influences on Shiraz fine wines' are summarised by;

- Sorting and preference investigation of quintessential Australian and overseas wines conducted by a leading international sommelier group.
- Identify the common and unique sensory features associated with Shiraz/Syrah wine styles from targeted geographical indications (GI). Identify any key wine styles within regions due to factors such as significantly different sub-regional characteristics or winemaking intervention.
- Characterise the climate of the selected sites using climate indices.
- Identify the key links between wine composition, sensory styles and climatic indices in the GI's to be investigated.

These aims were achieved by the completion of the outputs / activities shown in the table below.

## 6.1 Outputs and Activities

| Year 1 | Output | Activities |
|---|---|---|
| a | A selection of fine wines sorted and ranked by region. | Convene expert panels of international sommeliers to sort and rank wines of stylistic importance from the study regions. |
| b | Postdoctoral researcher recruited. | Advertise and recruit a suitable postdoctoral researcher with skills in sensory science and analytical chemistry. |

| Year 2 | Output | Activities |
|---|---|---|
| a | A selection of representative wines from Barossa and McLaren Vale. | Characterise wines according to key wine styles, regional characteristics or winemaking interventions<br>- Barossa and McLaren Vale. |
| b | A selection of representative wines from Hunter Valley. | Characterise wines according to key wine styles, regional characteristics or winemaking interventions<br>- Hunter Valley. |
| c | A selection of representative wines from Canberra. | Characterise wines according to key wine styles, regional characteristics or winemaking interventions<br>- Canberra. |
| d | A selection of representative wines from Yarra Valley and Heathcote. | Characterise wines according to key wine styles, regional characteristics or winemaking interventions<br>-Yarra Valley and Heathcote. |
| e | Dataset of results from sensory descriptive analyses of selected wines.<br>Dataset of wines analysed for targeted compounds and untargeted metabolomics profiling. | Conduct comprehensive sensory descriptive analysis of selected wines from study regions, using a trained panel.<br>Conduct targeted chemical analysis of wines for rotundone, sulfur compounds including DMS, polyfunctional thiols, GG, amino acids, C6 compounds, aldehydes, aliphatic lactones, monoterpenes, norisoprenoids, fermentation volatiles, tannin measures, glycerol, oak volatiles and cineole.<br>Conduct untargeted analysis of wines from sensory descriptive analysis, using GC-qTOF mass spectrometry |
| f | Climatic indices for selected geographical regions. | Conduct GIS spatial analysis of climate (e.g. Huglin index, Fresh/Cool Night Index, rainfall distribution, evaporative demand) for selected geographical regions. |

| Year 3 | Output | Activities |
|---|---|---|
| a | Knowledge on the chemical signature of wines with high correlation to climate. | Correlate climatic indices with targeted/untargeted wine analyses. |
| b | Well-defined chemical profiles of wines from targeted regions with high correlation to sensory descriptive analysis. | Correlate wine composition to sensory descriptive analysis of targeted commercial wines from selected regions. |
| c | Recommendations for benchmarked wine styles associated with climate and/or region. | Determine tangible wine styles associated with climate and/or region. |
| d | Wines analysed for non-volatiles using untargeted metabolomics profiling.<br><br>Key non-volatile features of wines identified for specific regions | Untargeted LC-MS analysis for non-volatile compounds, including polyphenols and tannins, to identify features in wines from defined regions. |

# 7 Method/ Results / Discussion

Five distinct productivity chapters have been produced to describe the five main investigation topics reported. The methodology has been separated into separate chapters to help enhance document flow and readability.

# 8 Chapter 1 - Sommelier tasting and pivot profile investigation for suitability.

This chapter summarises the findings for the following investigations outputs;

- Convene expert panels of international sommeliers to sort and rank wines of stylistic importance from the study regions.
- The sommelier exercise also provided an opportunity to examine the use of a novel method of wine sensory analysis, Pivot© Profile (PP).

## 8.1 Aims and Introduction

In conjunction with the 'World's Top 100 Restaurants' event held in April 2017, Wine Australia coordinated a visit of 49 eminent sommeliers from some of the participating restaurants for an extensive tour of Australian wine regions. The event provided the opportunity to achieve several project aims. Firstly, it allowed the sommelier group to be exposed to the quality and diversity of a range of premium Australian Shiraz/Syrah wines. The event also showcased the use of a relatively new rapid sensory method called Pivot© Profile (PP) and therefore exposed them to the Australian wine research activities.



***Figure 1*** *The authors explaining the Pivot Profile method to a group of international sommeliers, April 2017 in Melbourne, Vic.*

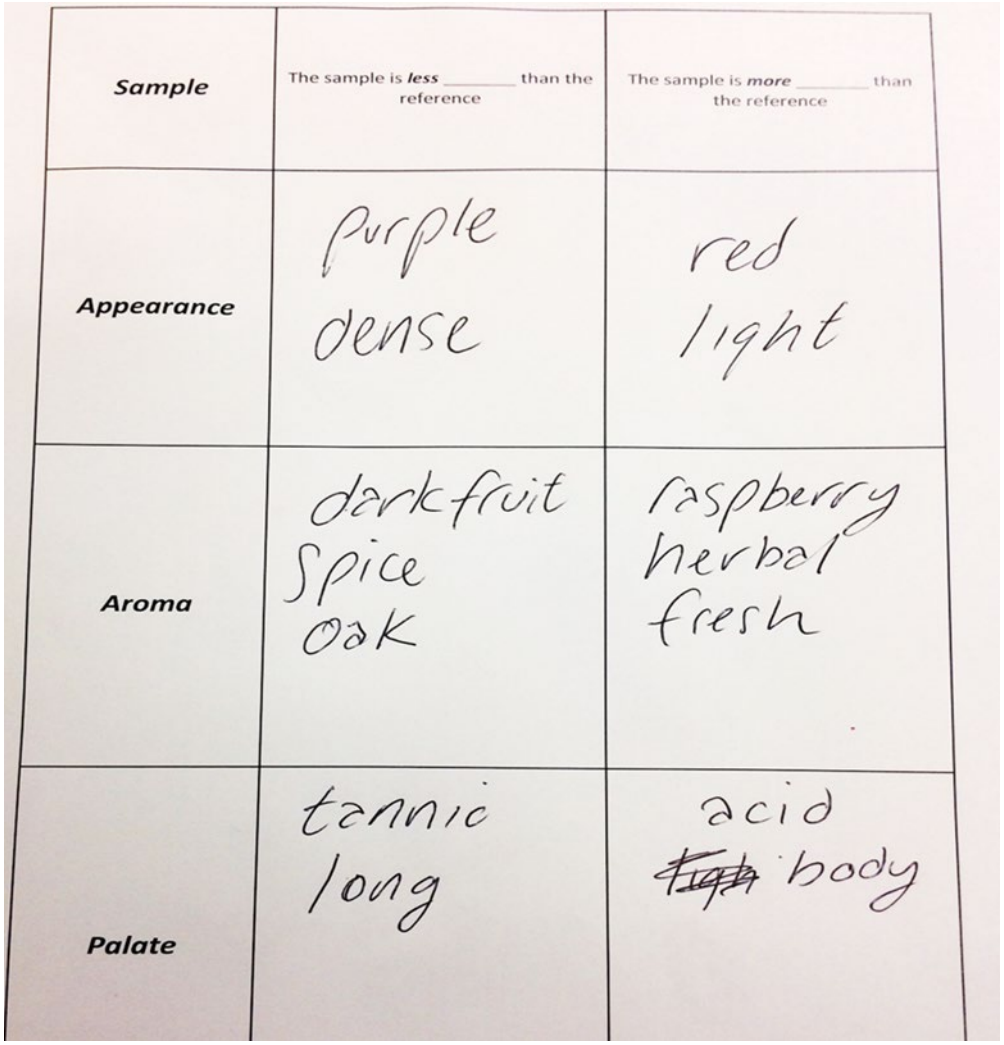
Prior to the tasting, Dr Liz Waters, head of Wine Australia's R&D, outlined the investment and the goals of the project. Max Allen, one of Australia's most influential wine commentators, was invited to participate and help lead the discussion of the wines following the formal tasting session. He summarised Dr Water's introduction as, 'what we want to do is describe the diversity of Shiraz wines in Australia, to describe how different they are. We want to understand what the compounds are in grapes that make those wines different. And we want to understand how the grapevine responds to environmental signals, to the climate, to the soil, to the aspect: how it translates those signals into some physiological response that changes the composition in the berries that you can then taste in the wine. Why? Well apart from natural curiosity, it's really about having knowledge that grape growers and wine producers can use—which levers do they have available that they can play with if they want to optimise the expression of terroir. It's not about having a recipe or mandating how a wine should be grown, it's having more information for decision making.' (Allen, 2017)

A further objective of this tasting exercise was to help validate the PP method's effectiveness to undertake the analysis required for subsequent regional tastings as a part of this Wine Australia investigation. The PP method promised to be a valuable tool for industry tastings and data collection as it is relatively easy to perform. It is also time–effective, as it can be completed in in one session and judges need no training. Previously, only one research group (Thuillier *et al.,* 2015) had previously reported the effectiveness of this method to evaluate wine. This study used the method to analyse only 6 wines and the current study needed to determine if the method was suitable for a study that involved groups of 20 to 25 wines. To test the reliability and validity of the PP method to generate robust sensory characterisations, results were subsequently compared to the much more time consuming, but well-established, quantitative sensory descriptive analysis method. In addition, the PP method was repeated by coordinating a group of Australian winemakers to taste the same wines using the same PP method. Lastly, it was an excellent chance to measure the preferences of an influential international group and compare their impressions of Australian Shiraz to those of the winemakers.

### *8.1.1 Pivot© Profile (PP) Method*

PP is a free description method that can use the well-developed lexicon of professional judges (winemakers, sommeliers, wine writers, wine buyers, etc.) as an asset but also removes the bias that can occur in groups of this type. The judges use a 'pivot' wine as a reference for assessing the samples. Judges assess each coded sample, compare it with the pivot wine, and write descriptors based on how the coded sample differs from the reference. An example of the assessment sheet for one wine is given in **Figure 2**. Judges can use any term to describe the wine; however, the degree modifier 'less' or 'more' is used in conjunction with the descriptor. This allows personal interpretation to describe the sample but forces the judge to fit the term identified into one of two categories (e.g. less sweet, more astringent).

The terms are tabulated by noting whether they were used positively or negatively (*more than the pivot* or *less than the pivot)*. Once all the attributes are counted, the results are summarised in an attributes x wines frequency table. The statistical technique of correspondence analysis (CA) is then performed to obtain a straightforward 'map' of the wines, showing both attributes and products, and allowing a simple way of picturing the degree of sensory differences among the set of wines.



| Sample | The sample is *less* _____ than the reference | The sample is *more* _____ than the reference |
|---|---|---|
| Appearance | purple<br>dense | red<br>light |
| Aroma | dark fruit<br>Spice<br>Oak | raspberry<br>herbal<br>fresh |
| Palate | tannic<br>long | acid<br>~~light~~ body |

***Figure 2****. An example of a completed Pivot© Profile assessment sheet for one wine.*

### *8.1.2 Pivot© Profile (PP) tasting by sommelier group.*

The sommelier Pivot Profile tasting was held in a function room of a major hotel. The 49 assessors (28 male, 21 female) were sommeliers from Australia (12), New Zealand (10), the United Kingdom (8), the United States (7), China (4), Japan (2), Thailand (2), Spain (1), South Korea (1), Singapore (1) and Denmark (1). None of the assessors had previously used the PP method.

Wines for the exercise were selected based on 'icon' status, having a long-standing reputation, including wines deemed to exhibit regional characters. Fourteen current vintage wines were carefully selected from eleven regions: Hunter Valley, Barossa Valley, Eden Valley, McLaren Vale, Adelaide Hills, Yarra Valley, Canberra, Beechworth, Heathcote, Clare Valley, Geelong and Grampians, ranging in retail price from $45 to $250. One wine from New Zealand and two wines from the Northern Rhone Valley in France were also included in the tasting, serving as international benchmarks. The 'Pivot' reference Shiraz wine was selected based on it having varietal characters but lacking distinctively regional characters.

In addition to the PP method, the sommeliers were also asked to rate how much they liked each wine, using a line scale at the bottom of the page, anchored by the terms dislike extremely and like extremely. Following the session, the wines were revealed and discussed.

The PP results can be summarised in a 'map' illustrating the main similarities and differences among the wines (**Figure 3**a). Examining the locations of the wines on Figure 3a, the wines from the warmer regions Barossa, McLaren Vale and Heathcote are plotted to the upper right, while wines from the cooler regions, including Yarra Valley, Adelaide Hills and Canberra, are farthest to the left.

Examining the attributes relating to those wines, the warmer region wines were most associated by the sommeliers with the attributes deep colour, body, fruit, dark fruit, alcohol, intense, and oak. The wines on the left half of the figure have associations with the attributes acid, floral, fresh, red fruit aroma, and herbal, and were described more often with the terms drinkable, elegant, bright, balanced and fresh.

The two Rhone Valley wines had similar sensory properties, and were associated with deep colour, high tannin, and a character the sommeliers referred to as medicinal, dirty, or barnyard.

Overall the results of the Pivot© Profile assessment by the sommeliers provided a valuable insight into their perceptions of Australian Shiraz compared to international benchmarks. The group found the tasting an interesting experience, and found the process of assessing with comparison to a reference wine a straightforward task.




***Figure 3.*** *Pivot profile sensory maps of ultra-premium Shiraz wines for a) a group of 50 international sommeliers, and b) 11 Australian winemakers.*

### *8.1.3 Winemaker's PP tasting*

This tasting was replicated a few weeks later with a group of eleven Australian winemakers, in order to investigate how the background and experience of the expert judges might change the way that wines are characterised, and to examine the preferences and language used by both groups. This tasting was completed at the AWRI, and all aspects of the sommelier tasting were repeated, with identical wines.

When comparing the sommeliers' results to the winemakers' (**Figure 3**b), there are many similarities. Wines from the Barossa, McLaren Vale and Heathcote were again plotted to the upper right, and the associated attributes were similar to those used by the international sommeliers, with the winemakers using the terms; opaque, body, dark fruit, tannin, oak and intensity to describe these wines. A notable difference between the two groups was the positioning of the Beechworth wine, which is much closer in sensory properties to the McLaren Vale wine according to the winemaker group, and the use of the brown and developed terms, which were not used by the sommeliers. The cooler climate wines to the left of **Figure 3**b were described by the terms; floral, red fruit, lifted, vibrant, acid and green. The French wines were characterised by the terms; purple colour, opaque, tannin, VA, reductive and Brett.

Overall the Australian winemakers used somewhat more technical terms and fewer terms related to quality compared to the sommeliers. However it is striking how similar the two maps are, and how similar the terms are generally employed. The two data sets were also compared using a multivariate statistical technique which provides a measure of how similar two data sets are to each other. The similarity coefficient between these two data sets was 0.79, which indicates a high level of similarity.

### *8.1.4 Comparison of the preferences of international Sommeliers and Australian winemakers.*

Another point of comparison between the two groups of assessors was the liking scores for the wines. **Figure 4** shows the relationship between the two groups' liking scores.

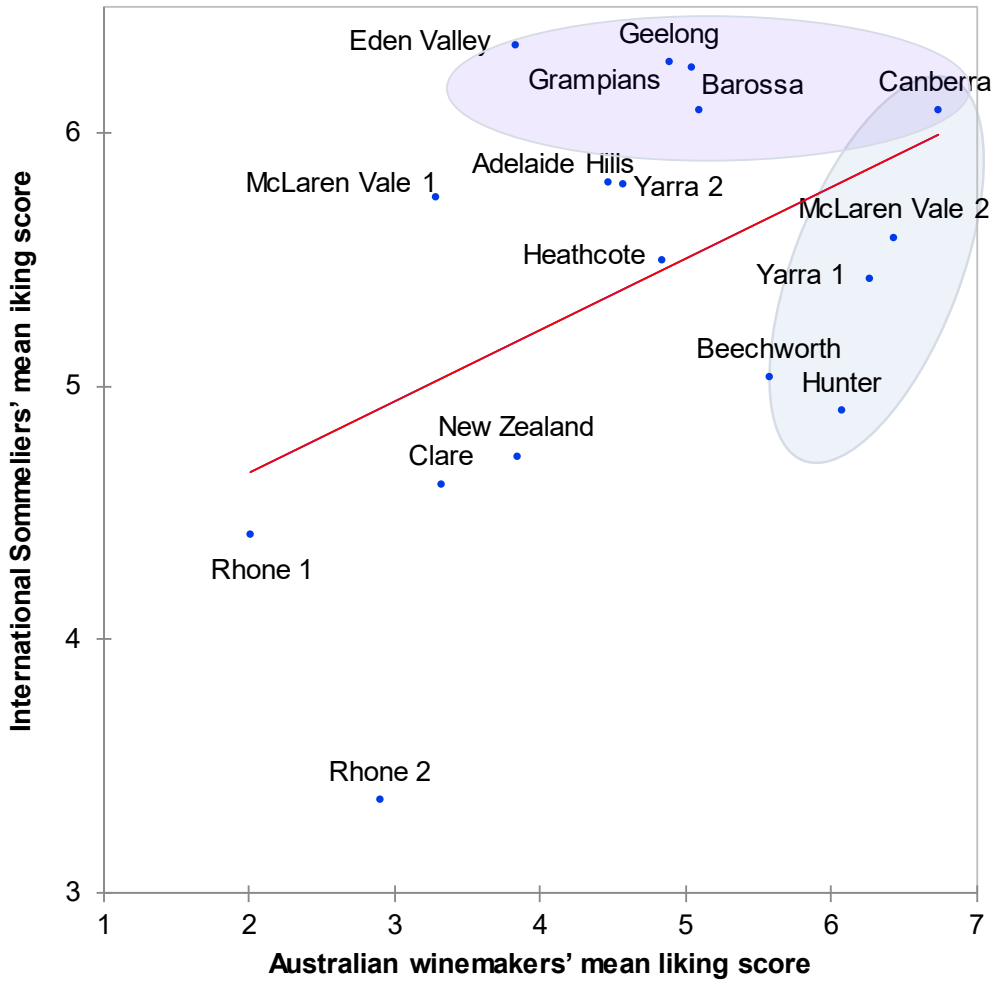



***Figure 4.*** *Relationship between the liking scores for the winemaker group (n=11) and the sommeliers (n=50). The regression line is shown (r=0.467, P=0.059). The wines that were most liked by the winemakers (blue ellipse) and the sommeliers (purple ellipse) are highlighted.*

The sommeliers' most liked wine was the Eden Valley wine, followed by the Geelong and Grampians wines. The least liked wines were Rhone 2 and Rhone 1, followed by the Clare Valley and the New Zealand wines. The winemakers also gave low liking scores for the French wines, this time with Rhone 1 the least liked wine, followed by Rhone 2. McLaren Vale 1 and the Clare Valley wines were the next least liked. Interestingly, the Eden Valley wine was the fifth least liked sample for the winemakers, while was the most liked for the sommeliers. The winemakers' most liked wine was from Canberra, which was also well liked by the sommeliers, followed by McLaren Vale_2 and Yarra_2. Overall there was only a fairly weak correlation between the two group's preference scores.

In assessing in more detail the individual sommeliers liking responses, generally most sommeliers had similar preferences, with good correlation of each judge with the group mean. However, there was also group of judges who more strongly appreciated the relatively lighter, red fruit, herbal, floral wines from Canberra, Adelaide Hills and Yarra Valley.

### *8.1.5 Comparison of PP to conventional sensory analysis.*

A detailed sensory profile on the same wines was completed by the AWRI's trained panel using the descriptive analysis (DA) method. The DA method is the gold standard for sensory profiling, as it produces reliable, detailed and repeatable quantitative sensory data that can be analysed using robust statistical tests. However, the costs involved in recruiting, training and maintaining such a panel are prohibitively expensive for all but the largest wine producers. Therefore, the development of alternative rapid methods offers the potential to generate good sensory data without the costs and time associated with DA.

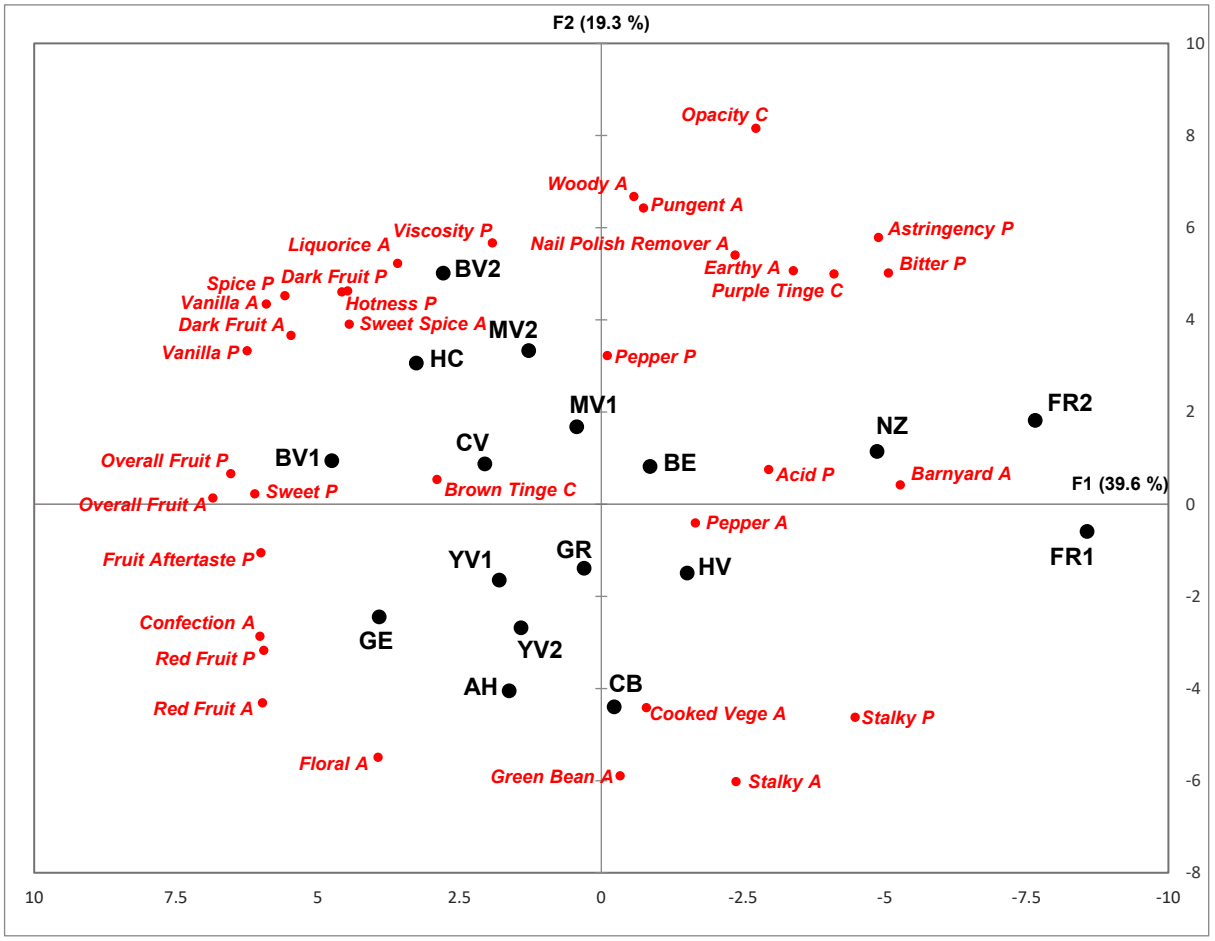



***Figure 5**. PCA scores and loading biplot from the mean values of the descriptive analysis. C; appearance (colour) attributes, A; aroma attributes, P: palate attributes. Wines from the same regions are linked.*

PC1 (39.6% of the explained variance) separated the French samples from Barossa Valley and Heathcote samples, with the wines located to the left of **Figure 5** being rated higher in fruit, confection and vanilla attributes, while the French wines were rated highest in barnyard aroma, as well as astringency, bitterness and stalky. PC2 (19.3% of the variance) primarily shows the variation in colour intensity (opacity) and woody and fruit flavour attributes, compared to 'green' attributes. There was separation of the wines to the left of **Figure 5** along PC2, with those rated higher in dark fruit aroma and flavour located in the top left quadrant, and red fruit characters (red fruit aroma and flavour, confection aroma) located in the bottom left quadrant.

When comparing this characterisation to that from the PP characterizations, the factor 1 scores from the PP biplots relate most closely to PC2 from the descriptive analysis.

Similarly to the PP biplots, sample pairs from the McLaren Vale, Yarra Valley and French regions were located close together, with the Barossa samples again showing some separation with BV_1 being less closely associated with the dark fruit characters. Overall, the pattern of the samples from the DA resembles the PP characterisation with a 90-degree rotation.

A comparison between the sensory profile generated by the AWRI's trained panel and that generated by the winemakers using the PP method was completed using multiple factor analysis. The similarity coefficient between the two data sets was 0.69, indicating that the different methods produced broadly similar results. These results indicate that while the Pivot© Profile is easy and quick to perform, it may not be as comprehensive as conventional descriptive analysis. It is however appropriate to use in certain situations when a less detailed sensory profile is acceptable. Interestingly, PP results showed that some interesting insights can be obtained that are not available with conventional DA, namely descriptions related to overall judgements such as 'drinkable', 'complex', 'round', 'balance', 'fresh' or 'vibrant', which may provide a richer overview of the sample set which is more aligned to both consumers and wine professionals.

#### *8.1.6 Reception of the tasting method*

Max Allen encapsulated a section of the taster's feedbacks and responses. He wrote that it, 'was an unusual but refreshing way to taste wine, and I found it really helped me identify what made one wine different from another'. He also interviewed sommeliers following the tasting and recorded that, 'the experience also introduced some of the somms to a more analytical method of wine evaluation than they experience day to day in their restaurants. 'I'm not used to tasting wine like this,' said James Tuckworth of Il Lido in Perth, Western Australia. 'But I can see how it'd be a good thing to do for the staff, to teach them how to think about wines and how they relate to each other. So often we'll get a customer in who'll say, 'I tasted X and now I want to try something similar but a little more spicy - what can you recommend?'

## **8.2** Conclusion

The recognition that the international sommelier group appreciated premium Australian Shiraz wines of a range of styles, and that the international benchmark wines were not so well liked, provides useful insight into sensory descriptors used and preferences of this influential group. It was notable how diverse were the flavour descriptions for the wines produced from different sites across the country, and that wines from both warmer and cooler climates were equally preferred by the sommeliers. The results highlight the value in studying Shiraz sensory profiles and how they are related to regions and sites, and ultimately the site-specific influences on these characteristics.

The sommelier event was well-received and was able to demonstrate the quality and diversity of a range of premium Australian Shiraz/Syrah wines. Utilising the sensory method PP and summarising the current investigation and exposed an influential group of wine trade professionals to Australian wine research activities.

Overall our experience with the PP method has been a positive one, with the data it yields being both reliable and repeatable. That, combined with the ease of administering the tests and the lack of training needed for judges, leads to the assessment that Pivot© Profile is excellent option when choosing a rapid method to perform sensory analysis. This will allow the investigation team to undertake the next stage of the project investigation, the regional characterisation of Shiraz in six important Geographical Indications (GI) with the use of PP, with confidence.

# 9 Chapter 2 - Regional characterisation of six prominent Australian Shiraz producing regions (Barossa Valley, McLaren Vale, Yarra Valley, Heathcote, Canberra District and the Hunter Valley)

The aim of this work was to assess whether there are region specific sensory differences among commercially produced Shiraz wines sourced from prominent Australian regions, taking into account within region variability, and to characterise the sensory differences of the wines.

This chapter summarises the findings for the following investigations outputs;

- Characterise wines according to key wine styles, regional characteristics or winemaking intervention (Barossa Valley, McLaren Vale, Yarra Valley, Heathcote, Canberra District and the Hunter Valley).
- Conduct comprehensive sensory descriptive analysis of selected wines from study regions, using a trained panel.

## 9.1 Materials and methods

### *9.1.1 Wines*

The wines chosen were all Shiraz/Syrah (no less than 95%) and contained 100% fruit sourced from within the boundaries of the respective region, according to the producers. Wines were all commercially available and sourced through normal commercial channels, with a retail price of between AU$15 and 90. Wines were from either the 2015 or 2016 vintages as these were the current vintages for most producers at the time of the sensory assessment. When feasible, wines from a single site/vineyard were preferentially selected over multi-vineyard blends.

Between 22 and 28 wines were selected from each of six regions (**Table 1**). The regions were selected based on reputation as a premium Shiraz-producing region and on the availability of wines in the marketplace and to include wines from both cooler and warmer climate regions. For four of the six regions, all commercially available wines that met the selection criteria were included in the study. For the two remaining regions (Barossa Valley and McLaren Vale), including of all commercially available wines gave too large a sample size. Therefore, for these two regions, a preliminary sensory assessment was conducted to select wines that represented the diversity of wine styles within each region. The final selection of wines for these two regions was then made considering geographical factors, such as differences in location across the region and altitude, winemaking influences and a spread of retail prices, and with consultation from local winemakers. The regional Pivot (reference) wines were selected from the regional groups of wines. The selection was based on being of sound quality and considered by the authors to have subtle, balanced regional characteristics.

***Table 1.*** *Regions, numbers of wines studied and number of panellists for the regional Pivot Profile sensory assessments.*

| Region | Number of Wines | Number of Panellists |
|---|---|---|
| Barossa Valley, South Australia | 26 | 9 |
| McLaren Vale, South Australia | 28 | 10 |
| Yarra Valley, Victoria | 23 | 9 |
| Heathcote, Victoria | 23 | 12 |
| Canberra, A.C.T /New South Wales | 22 | 14 |
| Hunter Valley, New South Wales | 23 | 12 |

### *9.1.2 Pivot Profile Methodology*

For this study, a relatively large (22 to 28) number of wines from each of the six selected regions (**Table 1**), were initially characterised using a rapid sensory methodology, namely, Pivot Profile (PP) (Thuillier *et al.,* 2015). The purpose of these assessments was: first, to allow an understanding of sensory differences within each region and of the extent of variability and second, to provide data that could be used to select examples of wines from each region that encompass the range of sensory properties to be used in a sensory descriptive analysis study to allow direct comparisons of sensory profiles across regions.

Judges for the PP evaluation of the wines from each region were experienced wine industry professionals from the respective regions, recruited through local wine organisations and personal communication. No training was given, and none of the tasters had previously used the PP method. The tastings were held within each region between May and August 2017 at either a municipal hall or at a winery that had a suitable tasting area.

Between 9 and 14 panellists completed the assessments (**Table 1**). Wines (50 mL) were presented in a randomised order in ISO tasting glasses marked with three-digit codes. Panellists received 100 mL of the Pivot wine, and more was available if requested during the tasting. Data were collected on A4 ballots with spaces for writing more or less than the Pivot for appearance, aroma and palate attributes. Overall, 145 wines were characterised by the PP method, with 22 assessed using both PP and descriptive analysis. Grouping of attributes was completed by one individual to maintain consistency in the assessment grouping like terms. Initially, ballots were transposed into a spreadsheet by sample, and then, attributes were sorted under one general attribute name. For example, terms such as tannin, tannic, drying and astringent were all grouped under the term ‘tannin’. Once grouping was complete, a frequency table was produced for all samples and attributes.

### *9.1.3 Descriptive analysis*

Upon completion of the PP regional tastings 22 wines were selected (**Table 2**) for a sensory quantitative descriptive analysis study performed by the AWRI trained sensory panel.

***Table 2.*** *Details for the wines selected from the sensory descriptive analysis study: codes, origin, vintage, price and alcohol concentration.*

| Region | Wine Code | Sub-Region | Vintage | Price (AUD) | Alc (% v/v) | Single Vineyard? |
|---|---|---|---|---|---|---|
| Barossa Valley | BV1 | Rowland Flat | 2016 | 30 | 14.0 | Y |
| | BV2 | Krondorf | 2015 | 35 | 14.0 | Y |
| | BV3 | Eden Valley | 2015 | 70 | 14.9 | N |
| | BV4 | Eden Valley | 2015 | 35 | 14.7 | Y |
| McLaren Vale | MV1 | Seaview | 2015 | 28 | 14.9 | Y |
| | MV2 | McLaren Vale | 2015 | 28 | 14.2 | Y |
| | MV3 | McLaren Flat | 2016 | 25 | 14.6 | Y |
| | MV4 | Blewitt Springs | 2016 | 29 | 14.3 | Y |
| Heathcote | HC1 | Mount Ida | 2015 | 78 | 15.9 | Y |
| | HC2 | Redesdale/Mia Mia | 2015 | 30 | 14.1 | Y |
| | HC3 | East Mount Camel | 2016 | 30 | 14.4 | Y |
| Hunter Valley | HV1 | Hermitage Rd | 2016 | 35 | 13.7 | Y |
| | HV2 | Polkolbin | 2016 | 65 | 13.8 | Y |
| | HV3 | Polkolbin | 2015 | 50 | 13.7 | Y |
| Canberra | CB1 | Murrumbateman | 2016 | 27 | 14.3 | Y |
| | CB2 | Murrumbateman | 2016 | 36 | 13.9 | N |
| | CB3 | Lake George | 2015 | 45 | 14.6 | Y |
| | CB4 | Majura Valley | 2015 | 34 | 14.0 | Y |
| Yarra Valley | YV1 | Coldstream | 2015 | 92 | 13.0 | Y |
| | YV2 | Healesville | 2015 | 35 | 14.2 | Y |
| | YV3 | Gembrook | 2015 | 40 | 13.7 | Y |
| | YV4 | Dixons Creek | 2015 | 52 | 14.0 | Y |

#### *9.1.4 Data analysis*

Pivot Profile data were prepared and standardised to positive values (Pearson *et al.,* 2020). The data were then analysed using Correspondence Analysis (CA) (XLSTAT, Version 19.6, Addinsoft, Boston, MA, USA) to give a biplot of the wines and the relative frequency of the attributes. Analysis was initially undertaken for appearance, aroma and palate terms individually, and then, another CA analysis was completed for all of the most frequently used attributes (SD > 1.0) together. Agglomerative hierarchical clustering (AHC) using Ward's method was then applied to the raw data matrix to separate the wines into clusters to allow wines that best represented the regional diversity to be included in the descriptive analysis study. Four wines were selected from each of the following regions: Barossa Valley, Canberra, McLaren Vale and the Yarra Valley, together with three wines each from Heathcote and the Hunter Valley.

### **9.2** Results and discussion

#### *9.2.1 Regional Pivot Profile evaluations*

The PP results for each region are shown in **Figure 6**. The data for each region are presented as a CA biplot to show the sensory differences among the wines and the descriptors that are related to the separation. The maps show which wines were associated with specific attributes compared to other wines. The biplots also show the proportion of 'inertia' that can be considered to be related to proportion of variability in the data, with most maps having a value of approximately 40%. This allows assessment of the strongest patterns in the frequency data. Any attributes less than 0.05 from the origin on both axes in **Figure 6** were deleted as these attributes play

a less meaningful role in defining the sensory character of the dimensions. The data were subjected to cluster analysis, and wines from the same cluster were represented by the same colour in the biplots in **Figure 6** and can be considered similar in sensory properties.

The **Barossa Valley** biplot (**Figure 6**a) shows that wines along Factor 1 (F1) were separated based on fruit-related attributes versus jammy/savoury attributes. Interestingly, there were no colour attributes distinguishing the wines. Fruit aroma (A) and Dark fruit A were most associated with wines in the far right of the figure, with other fruit-related attributes, such as Dark fruit Palate (P) and Red fruit P, also associated with these wines. Oak attributes such as Oak A, Smoke A and Spice P, as well as Herbal P, Soft P, Body P, were also used as descriptors for the wines to the right of **Figure 6**a. Wines at the far left of **Figure 6**a were most strongly related to the attributes Mint A, Reduced A, Savoury A and Jammy A. Along Factor 2 (F2), the attributes Fruit A, Tannin P, Green A and Fresh P are opposed to the attributes Body P, Complex A and Intense P.

Three clusters were identified. Cluster 1 consisted of seven wines, and these wines were most associated with the attributes Mint A, Reduced A, Savoury P, Intense P, Complex P, Jammy A, Dry P and Fruit P. The largest, Cluster 2, consisted of 11 wines, associated with Fresh A/P, Green A, Tannin P, Chewy P, Oak P, Acid P, Fruit P, Elegant P, Spice A, Texture P, Length P and Savoury P. Cluster 3 contained eight wines, associated with Dark fruit A and P, Soft P, Spice A/P, Fruit A, Herbal P, Red fruit P, Chocolate A, Smoke A, Oak A and Complex P.

Overall, the Barossa wines can be broadly summarised as falling into three categories: softer, lower tannin wines with high intensity of dark fruit flavour and some oak flavour; wines with jammy characters with mint, savoury or sulfidic characters; and higher tannin, fresh wines with some green notes.

The **McLaren Vale** biplot (**Figure 6**b) shows that Fresh A/P, Purple C and Red fruit A are opposed to Red C, Dense P, Intense A, Oak A/P and Rich P along F1. Wines plotted to the upper half of **Figure 6**b were associated with Oak A/P, Dense P, Complex P and Intense A attributes. Compared to the Barossa results, attributes such as jammy, mint, green or smoky were not used for the McLaren Vale wines, while colour terms and others such as sweet, structural, closed, barnyard and secondary were used. These terms did not have direct equivalents for the Barossa Valley wines.

Three clusters were identified, with the largest, Cluster 1, including 12 wines spread over the top two quadrants (**Figure 6**b), having associations with the attributes Dense P, Oak A/P, Complex A/P, Body P, Tannin P, Intense A and Rich P. Cluster 2 wines were located in the bottom left quad- rant, with these wines described as higher in Red C and were less frequently described by the attributes Red fruit A and Fresh A/P. Cluster 3 samples are all located in the upper right quadrants. The wines in this cluster had a strong association with the attributes related to Fresh A/P and Red fruit A. Overall, the McLaren Vale wines tended to be defined by differences in colour and freshness/red fruit on the aroma and palate, relative to those wines with red colour, greater fruit intensity, tannin, body, complexity and oak character.

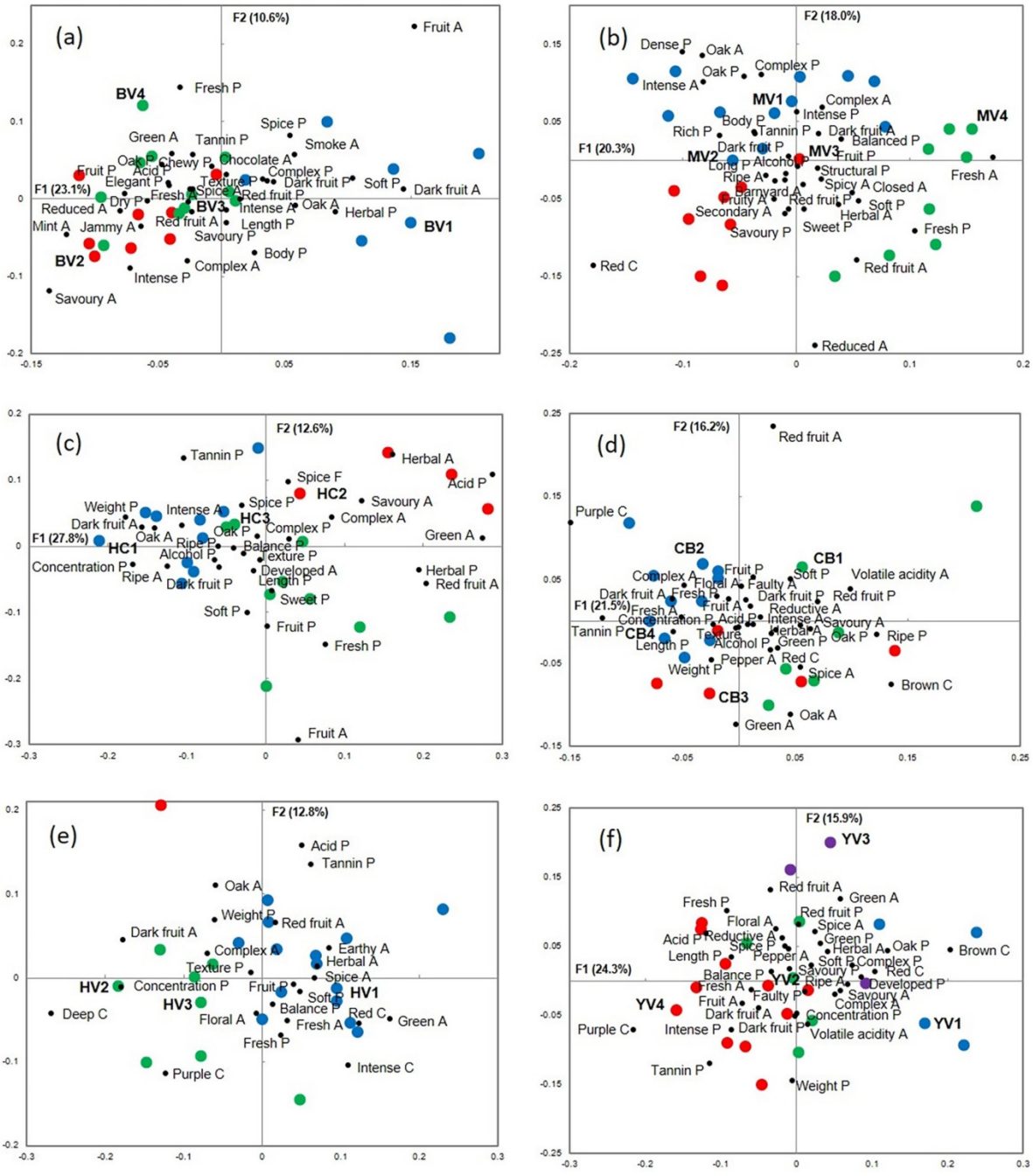



***Figure 6.*** *Correspondence analysis map for Pivot Profile sensory data for wines from (a) Barossa Valley (BV), (b) McLaren Vale (MV), (c) Heathcote (HC), (d) Canberra (CB), (e) Hunter Valley (HV) and (f) Yarra Valley (YV). Clusters are denoted for each region by colour. Samples marked with a code were selected for the multi-regional descriptive analysis. The letter C after an attribute denotes colour; A, aroma; and P, palate.*

For the Heathcote biplot (**Figure 6**c), the major separation of the wines along F1 related to the attributes Dark fruit A/P, Oak A/P, Weight P, Concentration P and Ripe A/P, associated with those wines to the left of **Figure 6**c, as opposed to Red fruit A, Herbal A/P, Green A and Acid P.

Of the three clusters found, the largest Cluster 1 consisted of 10 wines, which were located to the left of **Figure 6**c. These wines were associated with the terms Purple C, Weight P, Dark fruit A/P, Oak A/P, Intense P, Ripe A/P, Concentration P, Alcohol P, Spice A/P and Tannin P. Cluster 2 consisted of only four wines, associated

with the attributes Savoury A, Complex P, Green A, Acid P, Herbal A and Spice P. Cluster 3, with nine wines, was most associated with Soft P, Fruit A/P and Red fruit A.

Overall, the Heathcote wines were separated in a similar manner to the previous warmer climate regions, with major differences in freshness/red fruit comparative to those wines with deeper col- our, high fruit intensity, tannin, body, complexity and oak. The high usage, however, of the terms Herbal A/P and Green A for several wines is different from the Barossa and McLaren Vale characterisations, and many wines were clearly associated with the descriptors ripe and dark fruit.

The Canberra District wines are shown in **Figure 6**d, with the separation along F1 influenced primarily by Purple C, Tannin P and Dark fruit A (negatively loaded on F1) as opposed to Brown C and Ripe P and, to a lesser extent, Red fruit A/P. Interestingly, the term Ripe is associated with the terms Red fruit A/P in this region, but in the other regions, Ripe tended to be associated with Dark fruit A/P. The terms Purple C and Brown C are also important to the separation of the wines.

Overall, Cluster 1 (11 wines) can be summarised as having dark fruit, purple colour and tannin attributes, while Cluster 2 (6 wines) were fresh, peppery, green and herbal. Cluster 3 (5 wines) were oak influenced and ripe.

The Hunter Valley biplot (**Figure 6**e) shows the wines from this region separated along F1 by the terms Deep C, Dark fruit A, Concentration P and Purple C on the left side of **Figure 6**e and Green A, Intense C, Red C, Herbal A, Spice A, Earthy A and Tannin P. Although three clusters were obtained for the Hunter Valley wines, one cluster had only one wine, and can be considered an outlier. This shows the variation in sensory properties for Hunter Valley Shiraz can be considered lower than the other regions studied, with only two major clusters. Cluster 1 consisted of nine wines, and they were associated with the terms Deep and Purple C, Concentration P, Dark fruit A, Complex A, Texture P and Floral A. The one wine in Cluster 3 was strongly associated with Oak A, Acid P and Tannin P. Overall, for the Hunter Valley region, separation of wines tended be based on dark fruit, concentrated wines with deep and purple colour and wines with higher acid and tannin, with earthy, herbal and red fruit characters.

The Yarra Valley biplot (**Figure 6**f) shows that four clusters were identified. The largest Cluster 1 consisted of 11 wines, where almost all were located in the left of **Figure 6**f, associated with the terms Purple C, Tannin P, Fruit A/P, Intense P, Dark fruit A/P, Fresh P, Ripe A and Complex P. Cluster 2 had five wines, associated with the terms Fresh P, Floral A, Pepper A, Savoury P, Soft P, Complex P, Green A and Herbal A. The wines in this cluster were also less frequently described by the attributes Length P, Balance P, Complex P and Fruit A. Cluster 3 had only three wines and was generally associated with the terms Red fruit A/P, Green A/P, Complex P, Red C and Developed A. Cluster 4 had four wines, and these wines were associated with the terms Brown C, Oak A/P, Red C, Savoury A and Developed P.

The sensory characteristics of the Yarra Valley wines can be considered to have higher variability than those of other regions and range from high tannin, high purple colour, concentrated and fruit driven (Cluster 1) to similar

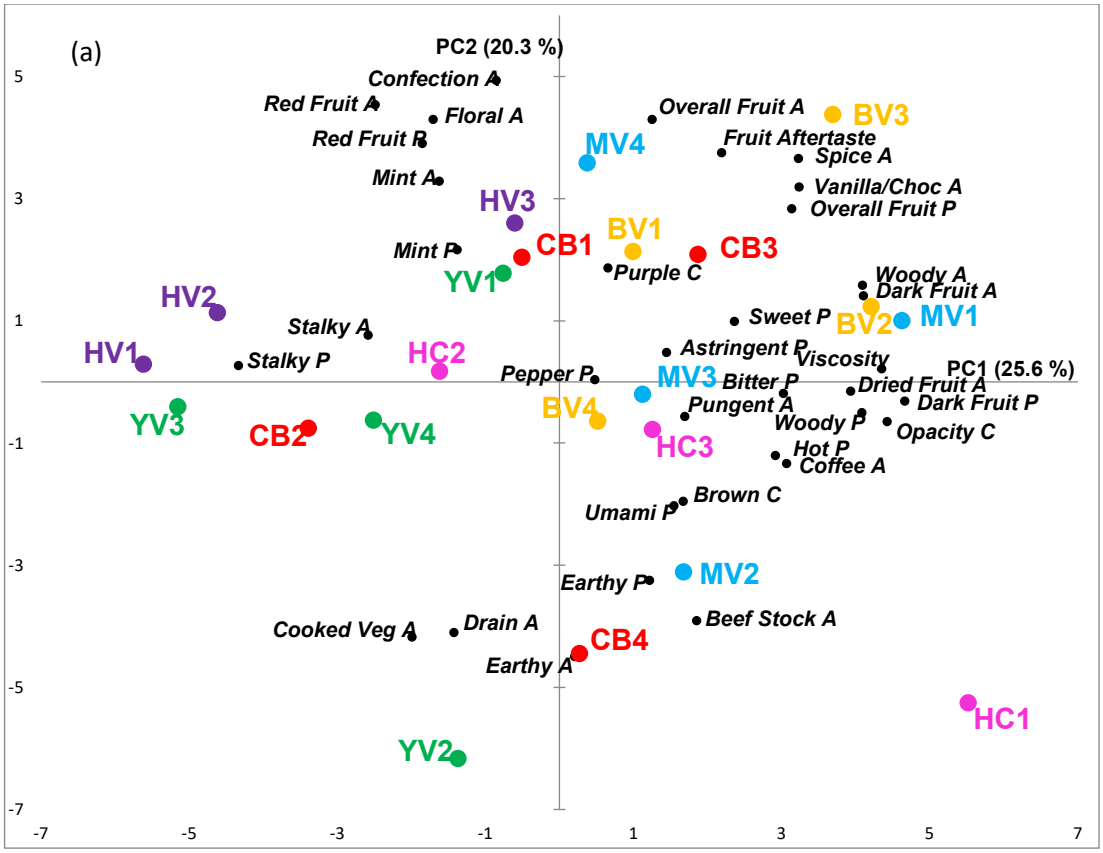
but slightly faulty wines (Cluster 2), to red fruit, green and spicy wines (Cluster 3), to oak driven, brown colour, developed and complex wines (Cluster 4).

Overall, these PP sensory assessments highlighted the range and variability of sensory properties within each region. It has also provided firm indications of the characteristics responsible for regional differences, despite a range of sensory properties represented within each region. The identification of specific styles that might be considered 'typical' is difficult. One possible approach is to examine the largest cluster from each region and assign it as 'regionally typical'. If we assume that each region will have some wines that would be considered outliers from a sensory perspective, resulting from major differences in the production of the wine, it will lie outside the largest cluster. Therefore, examining the sensory characteristics associated with the largest cluster can yield a general profile of what might be typical for the region. This is particularly relevant when examining the biplots of the Canberra and Yarra Valley regions, where there was one cluster that was much larger than the others, and to a lesser extent McLaren Vale, Hunter Valley and Barossa Valley.

### *9.2.2 Descriptive analysis*

To compare wines from the regions in more detail, a subset of three or four wines from each region was carefully selected for study by sensory descriptive analysis (selected samples marked in bold typeface in **Figure 6**a–f). Cluster analysis on the PP data for each region allowed for a choice of wines that can be considered representative of the different sensory profiles found for that region. This approach provides a transparent means of truly evaluating whether wines from different regions may be distinctive. The selection of wines was made primarily by selecting the wine from the cluster analysis that closely matched the centroid of the cluster.

From the sensory descriptive analysis study, the ANOVA showed that all attributes were significantly different among wines ($P < 0.05$) except for Pepper A and Acid P, and therefore were not included in further analyses.



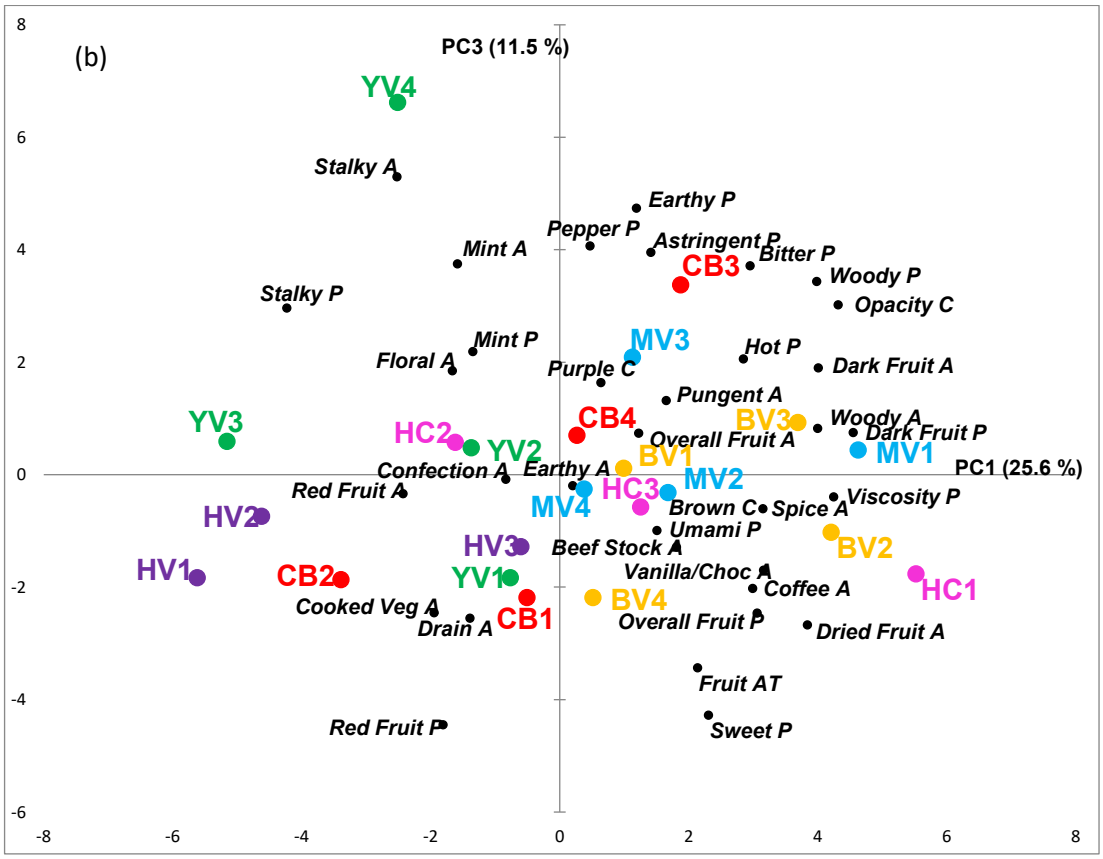




***Figure 7.*** *Biplot of principal components a) 1 and 2 and b) 1 and 3 for the sensory descriptive analysis mean attribute scores (n=12 panellists x 2 replicates) for the 22 wines. Vectors for the sensory attributes and points for the wines are shown, with the wines coded based on region, where BV: Barossa Valley; MV: McLaren Vale; HC: Heathcote; YV: Yarra Valley; HV: Hunter Valley and CB: Canberra.*

**Figure 7** shows the PCA biplots of PC1 and PC2 and also PC1 and PC3. The first two PCs account for 45.9% of the variance, with PC3 explaining a further 11.5% and PC4 (not shown) explaining 8.4%, indicating that the sample set was relatively complex, with several uncorrelated sensory attributes. Separation along PC1 was most strongly influenced by the attributes Woody A and P, Dark fruit A and P, Opacity C, Dried fruit A and Viscosity P, together with Spice A and Vanilla/ Chocolate A, which were highly positively loaded along PC1, with Stalky A and P strongly negatively loaded along PC1. Wines situated in the upper half of **Figure 7a** were rated higher in Confection A, Floral A, Red fruit A and P, as well as Overall fruit A and AT, while those in the lower half of **Figure 7a** were rated higher in Cooked veg A, Drain A, Earthy A and Beef stock A. The separation along PC3 related to the terms Stalky A and Earthy P, together with Pepper P and Astringent P, with wines plotted to the lower half of **Figure 7b** rated lower in these attributes and higher in Red fruit P, Fruit AT and Sweet P.

The separation of the wines revealed a general trend of wines from the cooler regions plotted to the left, while wines from warmer regions such as McLaren Vale, Barossa Valley and Heathcote were to the right, being higher in opacity (colour intensity), viscosity, dark fruit, dried fruit and oak related attributes. These wines were also generally higher in hot, bitter and coffee attributes. Also situated to the left were wines from the Hunter Valley, which has the highest growing degree days (GDD) value of the regions studied and would be considered a warm region. The two vintages studied (2015 and 2016), however, were both wet vintages (Bureau of Meteorology 2020), which likely would have led to early harvests.

The four **Barossa Valley** wines were grouped quite closely together, indicating a similar sensory profile, namely, being high in Opacity C, Dark fruit A/P, Viscosity P, Woody A/P, Dried fruit A, Overall fruit A/P and Fruit AT and rated low in Stalky A/P and intermediate in Red fruit A/P, Floral A and Confection A. All four Barossa Valley wines also had relatively high scores for Sweet P.

Three of the **McLaren Vale** wines were also grouped reasonably tightly with similar sensory properties to those of the Barossa Valley wines. The position of MV2 was more influenced by Earthy A/P and Beef stock A attributes.

The three wines originating from the **Heathcote region** were widely separated mainly along PC1, with the wine HC1 showing a high mean score for the attributes Opacity C, Dark fruit A/P and Dried fruit A, as well as Coffee A, Beef stock A, Umami P and Hot P, with particularly low scores for the attributes Red fruit A/P, Confection A, Floral A and Stalky A/P. The HC2 wine had higher ratings for Red fruit A/P and Stalky A/P.

The four wines from the **Canberra region** were also widely dispersed across **Figure 7**. Interestingly, the two wines situated to the left were from the same subregion (Murrumbateman), with the other two wines from different subregions (Lake George and Majura Valley). The two Murrumbateman wines were both rated highly for the attributes Red fruit A/P and Stalky A/P and low for the attributes positively loaded on PC1, with the CB2 wine notably high in Stalky A/P. The Lake George Canberra wine CB3 was unusually complex, being rated highly for the attributes Brown C, Floral A, Dried fruit A, Opacity C, Viscosity P, Overall fruit A/P, Dark fruit A/P, Spice A, Woody A, Bitter P, Mint A/P and Pepper P. The Majura Valley wine CB4 was rated highly for the attributes Earthy A/P, Cooked veg A, Drain A, Beef stock A, Woody P and Pepper P.

The four **Yarra Valley** wines were separated in **Figure 7** by a similar distance to the Canberra wines, with all wines situated to the left of the figure, indicating low ratings for the attributes heavily positively loaded along PC1 and high ratings for the Stalky A/P attributes. The wine YV1 was located close to the origin, which indicates intermediate ratings. Trends among the Yarra Valley wines include high scores for Stalky A/P for three of the four wines and Mint A/P, Red fruit A/P, Floral A and Pepper P for two of the four wines. Three of the four wines also scored low for the attributes Astringency P and Dried fruit A.

The three **Hunter Valley** wines were all scored highly for the attributes Red fruit A/P; moderately high for Stalky P and Mint A/P; and low for the attributes Dark fruit A/P, Viscosity P, Pepper P and Woody A/P. Of all the regions, the Hunter Valley wines showed the most similarities between the wines studied.

The PCA did not clearly distinguish regions, with no regions being clearly separated and a strong degree of overlap between some regions. This is most probably a result of the selection process from the initial PP assessments. Wines were purposefully selected from each cluster to represent the diversity of all the main characters in each region. Therefore, the wines were deliberately selected to be as varied as possible but still representative of Shiraz from a particular region.

To specifically pinpoint individual attributes that discriminate between regions, a PLS-DA was conducted. A three-factor solution was selected, based on residual validation variance, and the results are shown in **Figure 8**.




***Figure 8.*** *Regression coefficients from a three-factor partial least squares discriminant analysis model generated to relate sensory attributes to regional discrimination for a) Barossa Valley, b) McLaren Vale, c) Heathcote, d) Canberra district, e) Hunter Valley and f) Yarra Valley. Only attributes with regression coefficients greater than +0.01 or less than -0.01 are shown.*

The HV wines were well discriminated from the other regions ($R^2$ calibration for the model 0.61, $R^2$ validation 0.21), with Purple C positively related to the discrimination, and the relative absence of Brown C, Dark fruit A, Pepper P, Woody P, Bitter P and Hot P, as well as most other attributes (as indicated by the negative regression coefficients), being important to classify wines from this region from those of the other five regions. Wines from Canberra were also moderately well discriminated ($R^2$ calibration 0.43, $R^2$ validation 0.03), with **Figure 8**f showing that higher Brown C, Stalky A, Pepper P, Mint P, Bitter P and lower Purple C contributed the most in separating Canberra wines from the other regions. Yarra Valley wines were predicted to a similar degree ($R^2$ calibration 0.39, v $R^2$ validation 0.03), with the most positively related attributes being Brown C, Stalky A/P, Pepper P and Cooked veg A and the most negatively related being Purple C, Vanilla/Choc A, Spice A, Sweet P, Viscosity P, Astringent P and Fruit AT. The Barossa Valley classification was less clear ($R^2$calibration 0.29, $R^2$validation 0.02), with two Barossa wines not predicted well by the model (BV1 and BV4). The strongest positively related attributes for the Barossa classification were Vanilla/Choc A, Spice A, Woody A, Overall fruit P, Sweet P, Viscosity P and Fruit AT, with the negatively associated attributes being Cooked veg A, Beef stock A, Earthy P and Stalky P. McLaren Vale wines were classified poorly ($R^2$ calibration 0.20, $R^2$ validation not calculable). The attributes Opacity C, Purple C, Vanilla/Choc A and Astringent P were positively associated with the MV discrimination, while negative attributes included Brown C, Stalky A/P and Mint P. Finally, the Heathcote region was predicted slightly poorer than that of Barossa Valley ($R^2$ calibration 0.25, $R^2$ validation not calculable), with only one wine well classified (HC1), although no wines from other regions were misclassified as Heathcote. Opacity C and Purple C, Beef stock A, Earthy P, Umami P and Astringent P were positively associated with Heathcote regional discrimination, while Red fruit A/P, Floral A, Stalky A and Mint P were most strongly negatively related. As a second step in the analysis, the PLS-DA model was then used to predict each wine's region based on its sensory characteristics. Using this model, only CB2 and HC2 were misclassified (both as Yarra Valley wines). These results from the PLS-DA provide some reinforcing evidence regarding the key sensory attributes and their relative contributions that were distinctive for each region's wines, with several attributes found to be unique for a specific region: with Mint P for Canberra; Cooked veg A for Yarra Valley; Overall fruit P, Sweet P and Viscosity P for Barossa Valley; and Beef stock A, Earthy P, Umami P for Heathcote. McLaren Vale did not have a unique positive or negative attribute, while the Hunter Valley region's model included the negatively associated distinctive attribute Dark fruit A. The PLS-DA models, as noted above, were not highly robust, and a larger number of wines from each region would be required to be characterised to allow reliable predictive ability for unknown samples, with higher $R^2$ validation values.

The descriptive panel described some interesting attributes that were linked to the region of origin. The term Beef stock A was rated high in wines from Heathcote but was also noted in some Canberra and Yarra Valley wines. The Heathcote mean for this attribute, however, is nearly double that of the next highest regional mean. The origin of this attribute is unclear. Mint as an attribute is better understood, often used synonymously with the term eucalypt (Robinson *et al.,* 2011, 2012), which can be associated with Australian red wines in a global context. The origin of these sensory characters can often be attributed to nearby eucalypt trees (Capone *et al.,* 2012). In the present study, this attribute was moderately associated with Canberra wines. In the PP tastings, the term earthy was used only by the winemakers assessing Hunter Valley wines and was associated with herbal and green terms; however, it was used by the DA panel to describe several other wines, and the earthy aroma

was correlated with sulfide-related terms Drain A and Cooked veg A. The attribute Pepper has often been associated with Australian Shiraz from cooler regions (Parker *et al.,* 2007, Siebert *et al.,* 2008). Two of the four wines from both the Canberra and Yarra Valley regions had higher scores for this attribute, which might be expected as they would be considered the cooler of the regions included in the study. The term Coffee A was found to be significant across all wines, $P < 0.1$; however, upon examination of the mean scores, the five highest scoring wines came from five different regions. Regarding the 'green' attributes indicated by winemakers in the PP assessment of each region's wines, the DA results provided evidence that the wines of some regions were more likely to have a 'stalky' green character, while others were described as cooked veg. 'Cooked veg' has been previously linked to the sulfur compound dimethyl sulfide, which may be in turn related to nitrogen management in both the vineyard and winery through urea or diammonium phosphate use (Ugliano *et al.,* 2008). In contrast, stalky has been recently linked to the concentration of 3-isobutyl-2-methoxypyrazine in Shiraz wines made with rachis included in the fermentation (Capone *et al.,* 2018), for example, in whole bunch fermentation. The volatile and non-volatile compounds that relate to the sensory properties found in this study will be discussed in the next chapter of this report.

The effects of winemaking decisions must be considered when discussing regionality. There are some production decisions in Australia that may be considered 'cultural', meaning the technique has a history of usage in the region and is found in many wines produced therein. An example of this is the inclusion of small amounts of Viognier in Shiraz wines produced in Canberra. Another example is the inclusion of whole bunches in Shiraz ferments in the Yarra Valley. Harvest ripeness or the use of a large proportion of new oak barrels are also good examples of these cultural influences. From both the PP and the DA data, warmer regions in the study tended to have less variable sensory properties than the cooler climate regions. Whether this is related to grapes harvested at a lower ripeness level being more sensitive to the effects of site or simply greater variability in climate requires further investigation.

Comparing the results of the present study with the findings of a similar investigation of Shiraz wines by Johnson *et al.,* (2013), there are some similarities, with the earlier study also indicating McLaren Vale wines were higher in astringency; a Heathcote wine was high in savoury; Barossa Valley wines were high in dark fruit attributes and lower in astringency; Canberra wines were quite variable; and Hunter Valley wines were relatively low in many attributes. The results of the current study therefore both reinforce and expand on the results of the earlier work.

As with earlier regional studies involving commercial wines, the limitations of this study include an inability to disentangle site-specific effects from viticultural and winemaking practices. Thus, some differences observed are likely to be because of winemaking practices, as discussed above, while others can relate to viticultural practices, such as the use of rootstocks, clone, vine density, cover crops, the use of mulch, irrigation decisions, fruit exposure and crop level, among others. Thus, the regional differences indicated encompass the term terroir in its broadest sense, involving the physical environment in which the vines grow and the management practices because of human factors.

The sensory attributes that were highlighted as distinctive can provide winemakers and viticulturists with targets for production decisions to attempt to potentially optimise these attributes. It should be noted that segments of consumers will react strongly to some attributes, such as astringency, sulfidic flavours or green characters (Francis and Williamson, 2015), so a degree of caution would be recommended.

## 9.3 Conclusion

This study provides a detailed insight into the range of sensory differences found in Australian Shiraz wines that relate to the region of origin. The approach of utilising PP assessments of 145 wines provided a robust means of selecting the wines for the DA comparative study, as well as assessing the extent of variability of sensory properties within each of the regions studied.

While there was a degree of overlap in sensory properties of wines from the different regions, the study showed that there were region-specific variations among the wines, with certain attributes identified that can be considered distinctive for some regions, while for others, it is the overall pattern of multiple sensory properties that makes those wines unique.

The study has shown the flavour diversity of Australian Shiraz wines and the extent to which differences in sensory properties can be ascribed to region of origin. The insights from this work will assist winemakers, marketers, wine trade and consumers to understand which sensory attributes can be expected from a wine sourced from the regions studied. The work will provide sensory attribute targets for individual producers to optimise so as to align with regional styles if desired. The approach followed using the PP method combined with DA can be applied in other wine regions and countries and also with other products to simplify the collection of complex sensory data.

# 10 Chapter 3 - Regionality in Australian Shiraz: compositional and climate measures that relate to key sensory attributes

This section of the investigation aimed to characterise the chemical and climate profiles of wines from six important Australian regions and determine how they relate to sensory differences. This chapter summarises the findings for the following investigations outputs;

- Correlate climatic indices with targeted wine analyses.
- Conduct GIS spatial analysis of climate (e.g. Huglin index, Fresh/Cool Night Index, rainfall distribution, evaporative demand) for selected geographical regions.
- Correlate wine composition to sensory descriptive analysis of targeted commercial wines from selected regions.
- Determine tangible wine styles associated with climate and/or region.

## **10.1** Materials and Methods

### *10.1.1 Wines and Sensory/Chemical Analyses*

The wines studied were as detailed in the previous chapter. Briefly, 22 commercially produced Shiraz wines from six different regions were selected from sets of between 20 to 30 wines from each region, using the results from the Pivot© Profile rapid sensory method. The regions selected were Barossa Valley (BV), McLaren Vale (MV), Canberra District (CB), Yarra Valley (YV), Heathcote (HC) and Hunter Valley (HV). Three or four wines per region were chosen for further study, representing the range of sensory properties of wines from each region, from the 2015 or 2016 vintages. The wines were profiled using quantitative sensory descriptive analysis. Details of the methods and results for the descriptive analysis are also detailed in the previous chapter.

Comprehensive chemical analyses of the wines were conducted including basic chemical measures, yeast derived volatiles, monoterpenes, oak volatiles, sulfur compounds, norisoprenoids and non-volatiles. All measures differed significantly across the 22 wines except cis-2-hexenol and butyl acetate, and consequently these two compounds were excluded from further analysis. An ANOVA with region as a factor was performed on all the chemical data and showed that 18 of the 70 measures were significantly different ($P < 0.05$) across regions, while another 8 showed a trend ($P < 0.1$).

Mean values of the regions for all chemical measures can be found in **Table 4** and **Table 5**.

### *10.1.2 Viticultural Climate Metrics*

Climatological typicity at the vineyard site is the base point for phenological and viticulturally important climate metrics. For each year at each site, climate metrics described in **Table 3** were calculated, and then the average and standard deviation of each of the climate metrics at each site were calculated for the 20-year period.

***Table 3.*** *Viticultural climate metrics calculated for each vineyard site of the study wines.*

| Metric Code | Metric Description |
|---|---|
| GDD | Total growing degree days from 1 September to 31 May for 20-yearperiod up to 30 June 2019 |
| GDDph | Growing degree days (phenological), total GDD between estimated budbreak and estimated maturity |
| Budbreak day | Estimated budbreak, days after 30 June |
| Harvest day | Estimated maturity (harvest) date, days after 30 June |
| GSTt | Growing season temperature (traditional), i.e. average temperature between 1 Oct and 30 Apr |
| GSTp | Growing season temperature (phenological), i.e. average temperature between estimated budbreak and estimated maturity |
| CNIt | Cool night index (traditional), i.e. average minimum temperature of March |
| CNIp | Cool night index (phenological), i.e. average minimum temperature of 15 days preceding estimated maturity |
| RPTp | Ripening period temperature (phenological), i.e. average temperature of 30 days preceding estimated maturity |
| RainGS | Total rain during growing season, mm |
| RainRP | Total rain during ripening period, mm |
| EvapGS | Total evaporation during growing season, mm |
| EvapRP | Total evaporation during ripening period, mm |
| RadnRP | Average solar radiation during ripening period, $MJ/m^2$ |
| VPRP | Average vapour pressure during ripening period, hPa |
| RHmaxTRP | Average daily relative humidity at the day's maximum temperatureduring ripening period, % |
| RHminTRP | Average daily relative humidity at the day's minimum temperature during ripening period, % |

Growing season is the period between estimated budbreak and estimated maturity. Ripening period is the period from 30 days prior to estimated maturity date to maturity date. Note that cool night index was calculated using a 15-day period preceding estimated harvest date, as preliminary analyses indicated this produced a stronger predictor than if using a 30-day period.

## **10.2** Results and discussion

***Table 4.*** *Mean non-volatile chemical compositional values for wines from the six regions (± standard deviation). P values from ANOVA with region as a factor and Fishers' least significant difference (LSD) values(P = 0.1) are also shown.*

| Measure | Barossa Valley (n=4) | McLaren Vale (n=4) | Heathcote (n=3) | Hunter Valley (n=3) | Canberra District (n=4) | Yarra Valley (n=4) | *P* | LSD |
|---|---|---|---|---|---|---|---|---|
| Alcohol (% v/v) | 14.4±0.6 | 14.6±0.2 | 14.8±0.9 | 13.8±0 | 14.3±0.3 | 13.7±0.4 | 0.055† | 0.70 |
| pH | 3.68±0.07 | 3.59±0.05 | 3.68±0.04 | 3.48±0.03 | 3.83±0.16 | 3.58±0.11 | **0.003** | 0.14 |
| Free $SO_2$ (mg/L) | 12±5 | 13±4 | 11±4 | 17±4 | 22±7 | 15±4 | 0.13 | – |
| Total $SO_2$ (mg/L) | 42±11 | 49±14 | 51±20 | 64±0 | 72±23 | 60±22 | 0.31 | – |
| Titratable acidity (g/L) | 5.73±0.56 | 6.23±0.35 | 5.73±0.29 | 6.50±0.40 | 5.68±0.38 | 5.98±0.17 | 0.067† | 0.57 |
| Volatile acidity (mg/L) | 0.65±0.09 | 0.68±0.12 | 0.66±0.09 | 0.66±0.07 | 0.70±0.08 | 0.57±0.13 | 0.60 | – |
| % Pigmented tannin | 21.5±4.1 | 24.3±4.1 | 25.7±9.8 | 23.8±8.7 | 16.8±4.0 | 21.5±2.1 | 0.38 | – |
| Chemical age | 0.50±0.06 | 0.53±0.04 | 0.54±0.10 | 0.51±0.09 | 0.46±0.06 | 0.51±0.03 | 0.62 | – |
| Colour density | 11.1±1.3 | 12.4±1.9 | 12.2±2.7 | 8.3±0.4 | 8.8±1.3 | 10.3±1.6 | **0.019** | 2.4 |
| Free anthocyanins (mg/L) | 191±54 | 183±35 | 176±66 | 134±51 | 207±43 | 181±36 | 0.532 | – |
| Hue | 0.81±0.06 | 0.78±0.04 | 0.74±0.03 | 0.73±0.05 | 0.74±0.03 | 0.76±0.03 | 0.163 | – |
| Pigmented tannin | 3.08±0.40 | 3.71±0.67 | 3.91±1.58 | 2.46±0.42 | 2.36±0.51 | 2.99±0.45 | 0.066† | 1.1 |
| Total phenolics | 54.2±5.6 | 58.6±8.7 | 59.3±8.4 | 54.0±2.0 | 50.8±8.6 | 56.0±11.7 | 0.745 | – |
| Total pigment | 14.7±2.9 | 15.3±1.9 | 15.3±2.8 | 10.8±1.9 | 14.3±1.9 | 14.0±2.3 | 0.211 | – |
| Total tannin (g/L) | 1.60±0.28 | 1.85±0.44 | 1.87±0.48 | 1.54±0.14 | 1.40±0.39 | 1.64±0.66 | 0.681 | – |

† indicates the parameter was significant by region $P < 0.1$.

***Table 5**. Mean volatile chemical compositional data for wines from the regions (± standard deviation). Concentration as µg/L unless otherwise noted. P values from ANOVA with region as a factor and LSD values (P = 0.1) are also shown. Aroma detection threshold data are provided for the volatile compounds in model wine unless otherwise noted.*

| Compound | Barossa Valley | McLaren Vale | Heathcote | Hunter Valley | Canberra District | Yarra Valley | Aroma Threshold | *P* | LSD |
|---|---|---|---|---|---|---|---|---|---|
| 4-Ethylguaiacol | 2.5±5.0 | 18.7±37.3 | nd | 18.0±3.5 | nd | nd | 33[4] | 0.383 | – |
| 4-Ethylphenol | 14±26 | 155±82 | 16±27 | 9±8 | 3±6 | 23±36 | 440[2] | 0.522 | – |
| 4-Methylguaiacol | 6.7±5.0 | 2.8±1.7 | 5.2±1.6 | 5.0±1.7 | 4.0±0.8 | 3.0±2.0 | 30[11] | 0.327 | – |
| 5-Methylfurfural | 29.8±11.7 | 24.4±32.8 | 60.0±46.3 | 92.1±60.1 | 52.7±34.7 | 21.2±29.5 | 20000[1] | 0.161 | – |
| *cis*-Oak lactone | 159±65 | 130±84.1 | 115±56.4 | 119±45 | 122±36 | 102±78 | 140[6] (wine) | 0.878 | – |
| Eugenol | 23±8 | 16±11 | 19±8 | 23±6 | 16±5 | 12±14 | 6[4] | 0.63 | – |
| Furfural | 228±41 | 198±62 | 172±14 | 310±97 | 358±83 | 147±26 | 14000[4] | **0.001** | 90 |
| Guaiacol | 27±5 | 21±5 | 20±5 | 28±8 | 28±2 | 20±5 | 9.5[4] | 0.115 | – |
| *trans*-Oak lactone | 102±47 | 40±28 | 85±42 | 77±13 | 72±26 | 74±69 | 20[6] (wine) | 0.509 | – |
| Vanillin | 171±24 | 122±70 | 144±17 | 117±53 | 184±49 | 95±65 | 200[3] | 0.543 | – |
| Dimethyl sulfide | 103±37 | 69±20 | 74±19 | 44±5 | 94±48 | 211±132 | 10[3] | **0.036** | 96 |
| Hydrogen sulfide | 2.6±0.2 | 2.3±0.1 | 2.9±0.4 | 2.5±0.3 | 2.6±0.2 | 3.4±1.3 | 0.08[14] | 0.202 | – |
| Methanethiol | 2.8±0.8 | 1.9±0.3 | 2.4±0.2 | 2.0±0.3 | 3.4±0.6 | 2.4±0.4 | 0.3[16] | **0.006** | 0.75 |
| Methyl thioacetate | 6.9±1.6 | 6.9±4.1 | 7.4±1.0 | 2.0±3.8 | 5.9±5.8 | 4.1±4.7 | 50[12] | 0.54 | – |
| Carbon disulfide | 7.5±5.3 | 2.8±0.5 | 8.0±5.7 | 2.9±0.4 | 5.9±5.1 | 10.3±9.0 | 38[14] | 0.397 | – |

| Compound | Barossa Valley | McLaren Vale | Heathcote | Hunter Valley | Canberra District | Yarra Valley | Aroma Threshold | *P* | LSD |
|---|---|---|---|---|---|---|---|---|---|
| Ethyl propanoate | 276±47 | 253±40 | 199±26 | 264±170 | 186±24 | 188±33 | 10000[17] (beer) | **0.004** | 50.8 |
| Ethyl isobutyrate | 175±26 | 305±33 | 275±68 | 328±191 | 165±56 | 202±5 | 15[3] | **0.047** | 117 |
| Propyl acetate | 35.5±9.9 | 26.0±9.6 | 18.2±3.3 | 32.4±9.2 | 30.2±18.9 | 20.1±4.7 | 54[10] | 0.272 | – |
| Isobutyl acetate | 69.5±7.2 | 74.6±13.7 | 87.5±22.0 | 71.6±30.7 | 89.0±22.8 | 68.1±20.9 | 1600[19] (beer) | 0.585 | – |
| Ethyl butyrate | 261±66 | 189±11 | 206±21 | 237±68 | 169±29 | 222±58 | 20[3] | 0.15 | – |
| Ethyl 2-methylbutyrate | 34.2±9.4 | 63.3±12.3 | 39.0±12.2 | 68.7±50.4 | 24.9±13.9 | 47.5±10.6 | 1[3] | 0.084† | 31.4 |
| Ethyl 3-methylbutyrate | 49.5±11.9 | 80.8±7.5 | 54.4±16.7 | 80.8±52.3 | 34.3±18.4 | 61.8±12.4 | 3[3] | 0.079† | 33.7 |
| 3-Methylbutyl acetate | 603±88 | 587±51 | 470±98 | 504±122 | 497±122 | 493±80 | 30[3] | 0.323 | – |
| Ethyl hexanoate | 439±52 | 314±47 | 364±78 | 424±120 | 300±32 | 298±14 | 5[3] | **0.015** | 91 |
| Hexyl acetate | 7.6±5.6 | 6.1±1.9 | 3.4±0.8 | 6.3±2.5 | 3.6±2.0 | 2.6±1.1 | 2400[18] (wine) | 0.168 | – |
| *cis-3*-Hexenyl acetate | 0.32±0.08 | 0.35±0.08 | 0.19±0.06 | 0.35±0.06 | 0.33±0.33 | 0.18±0.03 | – | 0.464 | – |
| Ethyl octanoate | 426±42 | 320±38 | 369±87 | 450±168 | 319±75 | 390±88 | 2[3] | 0.277 | – |
| Ethyl decanoate | 96.7±20.4 | 73.8±13.8 | 86.7±16.6 | 179.4±83.6 | 89.9±23.1 | 75.1±24.4 | 200[4] | **0.014** | 52.7 |

| Compound | Barossa Valley | McLaren Vale | Heathcote | Hunter Valley | Canberra District | Yarra Valley | Aroma Threshold | *P* | LSD |
|---|---|---|---|---|---|---|---|---|---|
| Ethyl phenylacetate | 3.6±0.9 | 6.4±2.2 | 3.5±0.5 | 5.9±4.0 | 3.8±1.0 | 3.9±1.2 | 650[10] (mango wine) | 0.203 | – |
| Phenylethyl acetate | 38.7±3.4 | 52.2±14.5 | 32.7±7.4 | 24.0±9.3 | 36.4±10.5 | 37.1±16.0 | 250[3] | 0.090† | 17.0 |
| Ethyl dodecanoate | 7.8±4.2 | 3.9±1.5 | 7. 7±5.8 | 8.9±3.3 | 10.9±5.0 | 4.5±1.1 | 400[23] (water) | 0.144 | – |
| Ethyl dihydrocinnamate | 0.78±0.17 | 0.54±0.23 | 0.91±0.19 | 0.39±0.07 | 1.00±0.24 | 1.64±0.81 | 1.6[4] | **0.008** | 0.59 |
| Ethyl cinnamate | 2.3±0.8 | 1.2±0.1 | 3.1±1.6 | 1.4±0.4 | 2.8±0.9 | 7.8±4.8 | 1.1[4] | **0.008** | 3.3 |
| *trans*-2-Hexenol | 35.9±11.4 | 21.6±4.4 | 34.2±7.7 | 29.5±18.4 | 45.6±10.6 | 27.7±18.0 | 600[9] | 0.196 | – |
| *cis*-3-Hexenol | 208±78 | 222±45 | 198±116 | 271±117 | 283±210 | 151±44 | 400[4] | 0.655 | – |
| *trans*-3-Hexenol | 49.6±17.3 | 50.0±11.0 | 40.7±4.6 | 39.7±13.3 | 46.3±11.3 | 25.7±10.9 | 400[3] | 0.106 | – |
| α-Terpinene | 0.133±0.012 | 0.105±0.021 | 0.098±0.016 | 0.105±0.023 | 0.113±0.032 | 0.114±0.034 | 400[15] | 0.506 | – |
| γ-Terpinene | 0.076±0.003 | 0.057±0.011 | 0.060±0.010 | 0.065±0.021 | 0.079±0.021 | 0.062±0.025 | 200[15] | 0.425 | – |
| Terpinolene | 0.241±0.049 | 0.169±0.024 | 0.171±0.062 | 0.209±0.003 | 0.287±0.086 | 0.176±0.063 | – | 0.055† | 0.084 |
| Linalool | 11.1±7.1 | 7.8±1.1 | 8.3±4.2 | 5.6±2.3 | 18.4±7.7 | 7.6±3.9 | 25[4] | **0.043** | 7.7 |
| 4-Terpineol | 0.60±0.20 | 0.81±0.21 | 0.22±0.03 | 0.49±0.08 | 0.25±0.09 | 0.43±0.15 | 0.34[5] | **<0.001** | 0.22 |
| α-Terpineol | 1.24±0.21 | 1.21±0.14 | 0.85±0.20 | 1.30±0.27 | 1.14±0.32 | 1.12±0.16 | 250[4] | 0.213 | – |
| Citronellol | 4.7±1.7 | 4.0±1.7 | 4.5±1.5 | 2.6±1.3 | 8.3±1.7 | 4.3±3.0 | 100[3] | **0.024** | 2.9 |

| Compound | Barossa Valley | McLaren Vale | Heathcote | Hunter Valley | Canberra District | Yarra Valley | Aroma Threshold | *P* | LSD |
|---|---|---|---|---|---|---|---|---|---|
| *trans*-Geraniol | 5.6±4.4 | 3.8±0.7 | 3.9±2.6 | 4.0±1.1 | 16.2±9.9 | 3.3±0.7 | 30[1] | **0.012** | 7.23 |
| *trans*-Geranylacetone | 0.064±0.022 | 0.075±0.031 | 0.072±0.020 | 0.067±0.010 | 0.069±0.027 | 0.070±0.013 | – | 0.987 | – |
| 1,4-Cineole | 0.047±0.011 | 0.060±0.012 | 0.032±0.007 | 0.058±0.009 | 0.018±0.002 | 0.045±0.010 | 0.63[7] | **<0.001** | 0.014 |
| Eucalyptol | 3.6±2.2 | 1.7±0.5 | 3.8±3.9 | 1.0±1.0 | 2.6±1.1 | 1.0±1.3 | 1.1[8] | 0.251 | – |
| γ-Nonalactone | 17.6±5.4 | 21.1±6.7 | 10.0±0.8 | 12.9±2.8 | 12.6±5.3 | 8.4±2.7 | 76[20] | **0.013** | 6.9 |
| Rotundone (ng/L) | 5.0±1.7 | 4.7±1.0 | 2.9±0.9 | 7.0±1.4 | 9.4±4.5 | 18.3±23.1 | 16[21] (wine) | 0.383 | – |
| α-Ionone | 0.020±0.003 | 0.021±0.003 | 0.017±0.002 | 0.015±0.004 | 0.017±0.003 | 0.015±0.003 | 15.3[22] | 0.076† | 0.005 |
| β-Damascenone | 1.48±0.29 | 1.51±0.30 | 1.23±0.48 | 0.95±0.57 | 1.24±0.51 | 0.73±0.16 | 0.05[3] | 0.087† | 0.59 |
| β-Ionone | 0.089±0.028 | 0.103±0.037 | 0.095±0.002 | 0.043±0.009 | 0.095±0.032 | 0.053±0.021 | 0.09[4] | **0.033** | 0.039 |
| Isobutyl methoxypyrazine (ng/L) | nd | nd | nd | nd | nd | 1.25±2.50 | 2[13] | 0.521 | – |

Thresholds denoted with superscript numbers reference the following: 1 – Gambetta *et al.,* (2014), 2 – Chatonnet *et al.,* (1992), 3 – Guth (1997), 4 – Ferriera *et al.,* (2000), 5 – Francis and Newton (2005), 6 – Brown *et al.,* (2006), 7 – Antalick *et al.,* (2015), 8 – Saliba *et al.,* (2009), 9 – Zalacain *et al.,* (2007), 10 – Pino and Queris (2011), 11 – Boidron *et al.,* (1988), 12 – Ye *et al.,* (2016), 13 – Maga (1990), 14 – Siebert *et al.,* (2009), 15 – Kim and Park (2015), 16 – Solomon *et al.,* (2010), 17 – Harrison and Collins (1968), 18 – Simpson (1979), 19 – Engan (1972), 20 – Nakamura *et al.,* (1988), 21 – Wood *et al.,* (2008), 22 – Etievant (1991), 24 – Boonbumrung *et al.,* (2001).
Compounds significant $P < 0.05$ by region are in bold font.
† indicates the compound was significant by region $P < 0.1$.
nd: not detected. Limits of quantitation for nd compounds: IBMP = 5ng/L, 4- Ethylguaiacol = 10µg/L

### *10.2.1 Region of origin differences in chemical composition*

The wines from the Hunter region (HV) had the lowest pH values, with McLaren Vale (MV) wines also relatively low, while Canberra District (CB), Barossa Valley (BV) and Heathcote (HC) wines all had higher pH values compared to the overall group mean. As expected, these pH values correlate negatively with titratable acidity. Both the HV and Yarra Valley (YV) wines had the lowest alcohol concentration, with both regions having wines with alcohol values below the overall group mean (14.25%), while MV, BV and HC were above the overall group mean, although standard deviation shows a larger range for BV and HC.

Non-volatile analysis provided measures relating to colour/pigment, phenolics and tannin. These variables were generally not significantly different across regions, although colour density and pigmented tannin differed, with the MV and HC regions having higher values for these measures, and the CB region notably lower. The HV wines also have values for this group of analyses that tend to be lower than the rest of the regions, with CB and HV samples significantly different from the MV and HC samples for both measures.

The volatile compounds 4-methylguaiacol, 5-methylfurfural, cis-oak lactone, trans-oak lactone, eugenol, guaiacol and vanillin are indicative of the influences of winemaking choice, with the origin of these compounds found in the oak barrels used in production (Boidron *et al.,* 1988). Use of oak barrels varies from producer to producer, but also region to region. The compounds cis- and trans-oak lactone, eugenol, guaiacol and vanillin were present near or above their sensory detection threshold in many of the wines studied, although were not found to be significantly different by region. However, three of the four BV wines had higher concentrations of these compounds, suggesting a possible trend of greater new oak barrel use in this region.

The concentration of dimethyl sulfide (DMS, significant by region $P < 0.05$) was well above the reported sensory threshold for all wines, with three of the YV samples showing the highest values for this compound, which is implicated in blackcurrant or vegetal aroma (Van Leeuwen *et al.,* 2020). Methanethiol was also significantly different among regions, with CB higher in this generally undesirable rubber/sewage-like compound.

The ester component of the analysis was comprehensive, with 19 compounds quantified. Significant regional differences were observed for the yeast derived esters ethyl propanoate, ethyl isobutyrate, ethyl hexanoate and ethyl decanoate, as well as ethyl cinnamate and ethyl dihydrocinnamate, while there was a trend for differences in ethyl 2- and 3-methylbutyrate and phenylethyl acetate. The BV wines were higher in ethyl propanoate, and ethyl hexanoate, and lower in ethyl isobutyrate and ethyl 2- and 3- methylbutyrate. MV wines were also higher in ethyl propanoate and ethyl isobutyrate as well as ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, and phenylethyl acetate. These two regions tended to have contrasting ester profiles. The CB and YV regions had generally lower values for most ester compounds. However, the YV wines had higher concentrations of ethyl dihydrocinnamate and ethyl cinnamate (both significant by region $P < 0.05$), with mean values above the reported sensory threshold. The HV and MV samples were also notably low for these compounds.

Of the three C6 hexenol derivatives that were measured, there were no major regional differences and the concentration of each was well below the reported aroma detection threshold. Consequently, it is unlikely that these compounds have a strong influence on the regional sensory characters of these wines.

Both MV and BV were found to have higher values of γ-nonalactone (significant by region $P < 0.05$), while HC and YV trended to lower scores. However, all samples were below aroma threshold values. This compound has been implicated in dried fruit aroma (Pons *et al.,* 2008). Dried fruit aroma has also been linked to the compounds 3-methyl-2,4-nonanedione, massoia lactone, furaneol and homofuraneol (Allamy *et al.,* 2018) among others, but as none of these compounds were measured in this study confirmation of these findings was not possible. 1,4-Cineole was also significant $P < 0.05$ and was higher in MV and HV samples, and lower in HC and CB, but still well below the sensory detection threshold.

The analysis of monoterpenes consisted of eight compounds, with five of the eight significant by region $P < 0.1$. The CB wines had generally higher values for these floral, citrus-like compounds, while HC and YV had lower values. MV also tended to be lower in these compounds, with the exception of 4-terpineol, where the three of the four wines had very high values compared to the rest of the sample set. CB had higher concentrations of the compounds γ-terpinene, terpinolene, linalool, citronellol and trans-geraniol, although all were below the aroma detection threshold.

The concentration of rotundone, the potent 'black pepper' sesquiterpene, in the sample set was generally low, with no significant regional difference, however YV3 showed a value more than six times the group mean. One other YV wine and two CB wines also had moderately high rotundone concentration, which has been reported to be related to cooler growing season temperatures and solar radiation (van Leeuwen *et al.,* 2020).

Measured grape-derived norisoprenoids involved in fruity aroma (β-damascenone, α- and β-ionone) were found to be all lower in the wines from YV and HV. The lower mean β-ionone levels in the YV and HV wines were significantly below the reported sensory detection level, whilst none of the α- ionone levels were above the sensory detection threshold.

There was only one wine that had detectable 3-isobutyl-2-methoxypyrazine (IBMP), the highly potent 'green pepper' compound, a YV wine with 5 ng/L. While Shiraz berries do not have detectable IBMP, recent reports have indicated that bunch rachis (stem) inclusion in fermentation can have appreciable influence on this compound (Day *et al.,* 2020).

In order to investigate the regional differences, PCA was completed on the chemical data for the significant compounds by region ($P < 0.1$). The mean sensory attribute rating data for each wine from was then overlaid as supplementary data. The results for these significant compounds were also then subjected to cluster analysis. The biplot for PC1 and PC2 and the cluster analysis dendrogram are shown in **Figure 9a and b**.

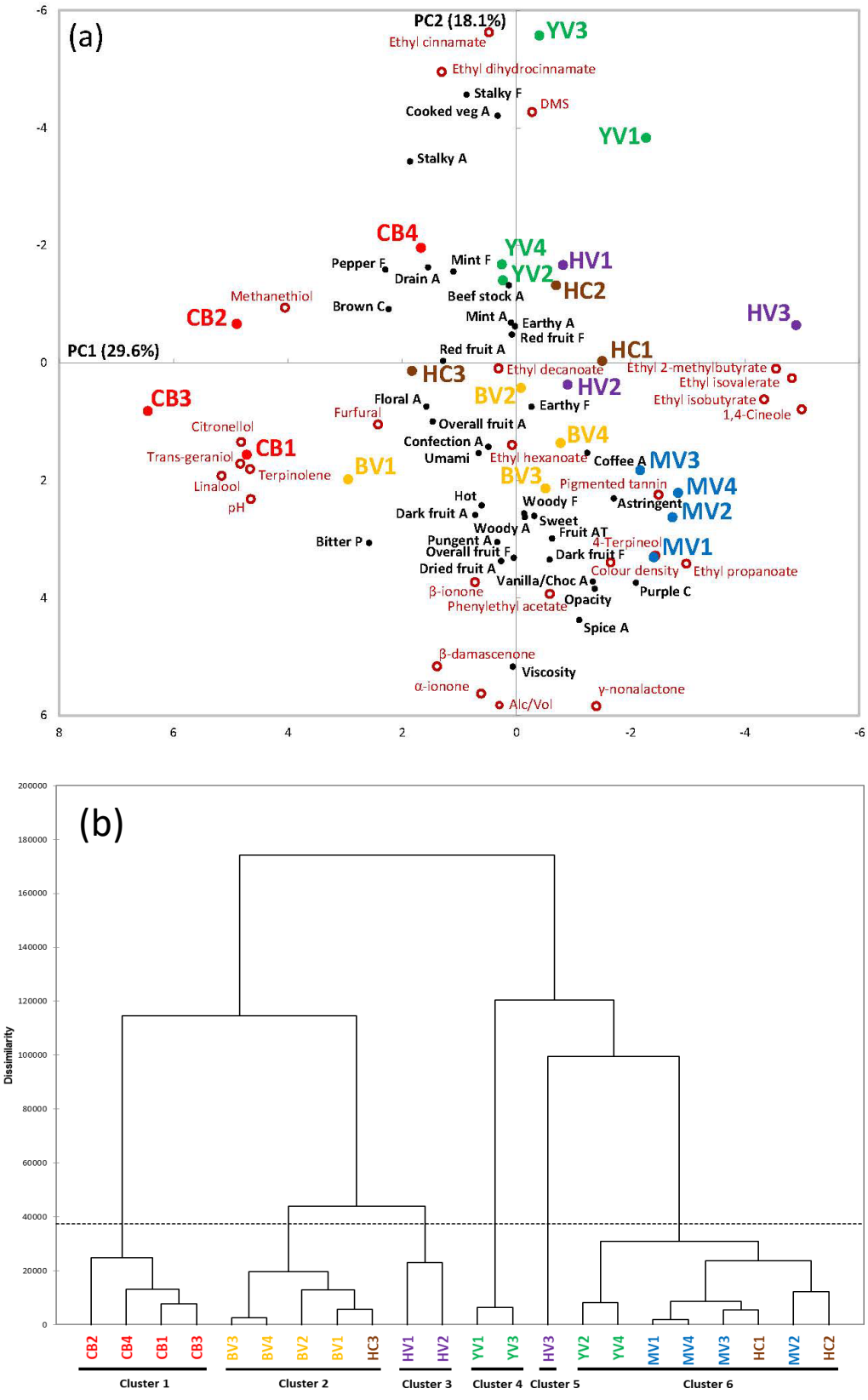



***Figure 9 a and b.*** *(a) Biplot of principal components 1 and 2 of the chemical measures that significantly differed across the six regions (P < 0.1) for the 22 wines, with sensory attribute rating mean scores included as supplementary information. Components 1 and 2 explained 29.6% and 18.1% of the variance respectively. Chemical measures are in open circles, with sensory attributes in black text. Wines are colour coded based on region (BV: Barossa Valley; MV: McLaren Vale; HC: Heathcote; YV: Yarra Valley; HV: Hunter Valley and CB: Canberra). (b) Agglomerative HierarchicalClustering (AHC) of*

*the same chemical measures. Clusters denoted by black bars and samples are colour coded by region.*

The PCA biplot in **Figure 9a** explained 47.7% of the variance and highlights the compounds that differed significantly across the regions. The wines from each of the regions are in the main closely grouped. The CB samples, and especially CB1, CB2 and CB3 show higher overall concentration of the monoterpene compounds and methanethiol, as well as higher pH, being well separated from wines of the other regions, plotted to the left of **Figure 9a**. The sensory attributes brown colour, pepper flavour, drain aroma, floral and red fruit aroma were related to the positioning of the CB wines. The YV wines as noted above had higher concentration of ethyl dihydrocinnamate, ethyl cinnamate, and DMS, especially YV1 and YV3, which were relatively low in alcohol and in norisoprenoid concentration. The YV wines were also rated highly in stalky and cooked vegetal attributes. These characters could be related to whole bunch inclusion during primary ferment – a practice that anecdotally tends to be employed by YV winemakers and has been shown to increase ethyl cinnamate concentrations in wines (Casassa *et al.,* 2021). The MV wines were well separated from the other wines and the most tightly grouped region, with higher colour density and pigmented tannin values, as well as being higher in multiple esters, norisoprenoids, 4-terpineol, 1,4-cineole and γ-nonalactone.

The BV wines were also closely grouped and had similar but somewhat lower concentration of the same compounds that related to the MV region wines. The sensory attributes that were most linked to the compounds of the MV and BV wines included viscosity, opacity (colour intensity), hot, purple colour, astringency, dark fruit, dried fruit, sweet, woody, spice and to a lesser extent bitterness. Higher concentrations of norisoprenoids have been reported to increase fruity aromas and flavours, and have been shown to be at higher concentration in wines made from vines exposed to increased solar radiation (Mendes-Pinto, 2009). The HV wines were notably high in ethyl 2-methyl butyrate, ethyl 3-methylbutyrate, ethyl isobutyrate and 1,4 cineole, but no sensory attributes were positively related to these compounds. PC3 (13.8% of the variance, not shown) separated the HV wines from the other regions, showing higher concentration for the esters, ethyl decanoate, ethyl propanoate and ethyl hexanoate, and these were linked to the sensory attributes red fruit A and F, and confection A. PC3 also highlighted the low values for pigmented tannin and colour density in the HV wines. The HC wines were positioned to the centre of **Figure 9a** being generally intermediate in all compounds measured and were not linked to specific sensory attributes.

Agglomerative hierarchal clustering was also completed on the wines using the chemical compounds found to be significant by region (**Figure 9b**). The AHC results highlight the same trends seen in the PCA biplot, with all the CB, BV and MV wines clustered together. The wine HC3 was included in the same cluster as the four BV samples, while the other two HC samples are found in the cluster containing the four MV samples. This cluster also contains two YV wines (YV2 and YV4). Sample HV3 was found to be unique in the clustering, while the HV1 and HV2 samples were also found to be unlike any of the other samples and were clustered by themselves. YV samples YV1 and YV3 were also found to be sufficiently dissimilar to any of the other samples and were clustered separately.

### *10.2.2 Climate Data*

The climate data involved multiple detailed indices to describe the characteristics of the regions. Some parameters, such as rainfall and growing degree days (GDD), are well known for broadly distinguishing viticultural regions. The multiple climatic indices for each specific site utilised in the present study provided insight into more subtle differences between the regions. The use of wines from two vintages also allowed some seasonal variations to be investigated.

**Figure 10** shows a principal component analysis of the climate data, to visualise the characteristics of the 22 vineyards. This biplot illustrates the relationships amongst the climatic indices and also displays differences and similarities between each vineyard site and their most defining climatic indices, with 73.2% of the variance explained with the first two factors.

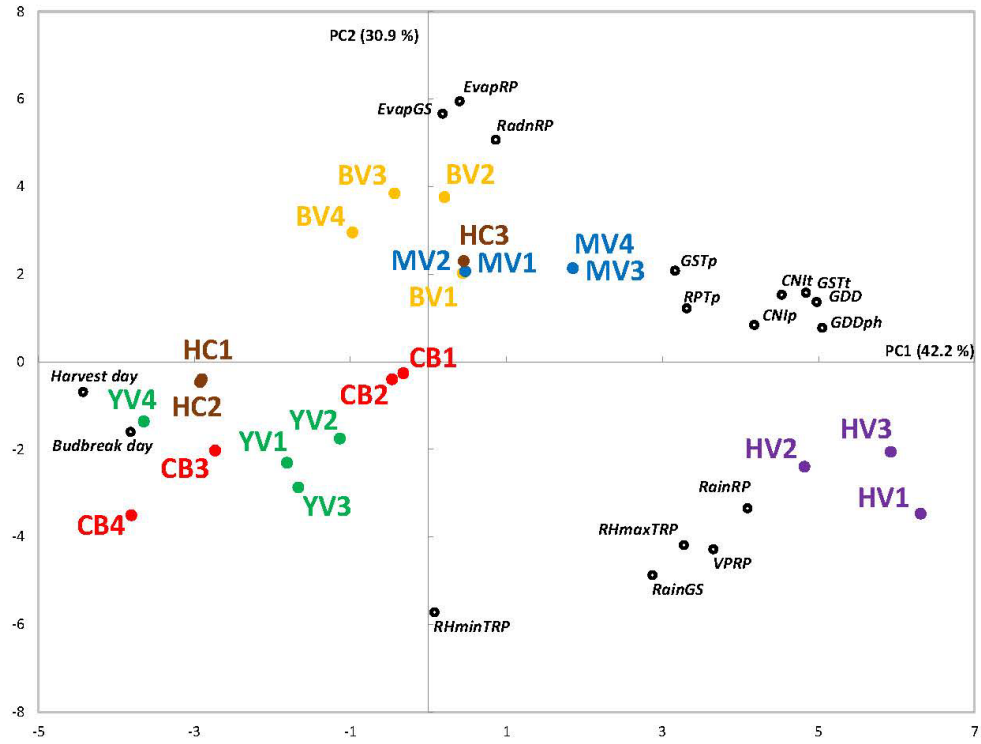



***Figure 10.*** *Biplot of principal components 1 and 2 of the climate indices for the sites of the 22 winesfrom six different Australian regions, explaining 73.1% of the variance. Factor 3 (not shown) explained a further 11.5%.*

The largest separation of the vineyards was along PC1, with temperature related indices GDD, GDDph and GSTt highly positively correlated and responsible for most of the separation of vineyardsalong PC1, together with the negatively correlated metrics harvest day and budbreak day. The HV vineyards, plotted to the far right of **Figure 9** were tightly grouped and had highest values for GDD, GDDph and GSTt, and also with high rainfall and related measures RainRP, VPRP, RainGS, and RHmaxTRP, with low Harvest Day and Budbreak day values. Conversely the YV and CB wines wereto the far left of **Figure 9**, with high Harvest Day and Budbreak Day metrics. Vineyards from these regions were not so tightly grouped, with CB1 and CB2 clearly separated from CB3 and CB4. This shows some of the climate variation within the CB region, as CB1 and CB2 were both

from the Murrumbateman subregion, while CB3 and CB4 were located approximately 30km to the east at Lake George. YV4 is approximately 40 km from the other YV vineyards and the separation along PC1 again demonstrates later Harvest Day and Budbreak day values.

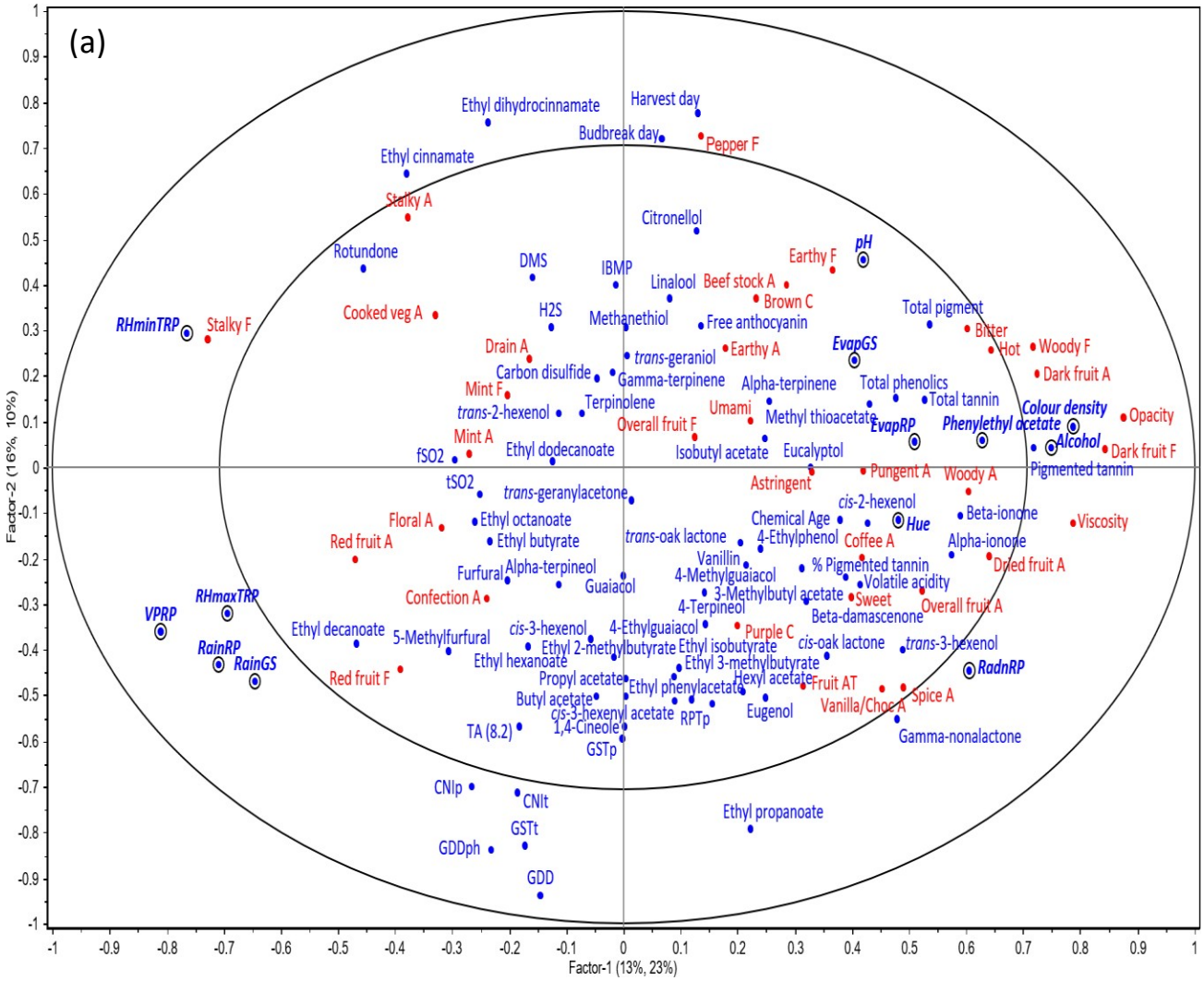
The two HC samples HC1 and HC2 were positioned close to the YV and CB sites, while HC3 was situated close to the MV and BV vineyards. This highlights the climatic diversity in the HC region. Italso follows the same pattern as the clustering of significant chemical compounds of wines from the BV and the HC3 sites into one cluster in the AHC analysis (**Figure 9b**) as described above. BV and MV lie towards the middle of PC1, with temperature related measures higher than for CB and YV, while not as high as the HV vineyards, and the MV wines slightly higher than those of BV. Factor 2 separates these vineyards with high values for solar radiation and evaporation measures (EvapRP, EvapGS and RadnRP), and low rainfall measures. The rainfall values for BV seasons. and shows little differentiation between MV when comparing both GST designations. The vineyards from the Hunter Valley have the most distinctly different rainfall and associated indices. 2015 and 2016 were high rainfall seasons in the Hunter Valley. Even if the HV vineyard which had the lowest rainfall is considered, it is still more than double that of the majority of other samples. The high vapour pressure (VP) and low solar radiation (Radn) figures also reflect the higherrainfall in the HV.

When examining the standard deviations from the 20-year means of the climate data, there are a few trends worth highlighting. The indicator Harvest Day has a negative standard deviationfrom the mean for all samples in the set, meaning that in 2015 and 2016, every site had a harvest day (estimated) earlier than the 20-year mean. Heathcote appears to be the most affected, with two of the three sites -2.0 standard deviations from the mean, and the third -1.8. GSTp also has all positive standard deviations away from the 20-year mean for all sites but one, signifying an increase in growing season temperatures in all regions. The measures GDDph and RadnRP also confirm this trend, with positive standard deviations from the mean in 20 of the 22 sites. This agrees with Australian Bureau of Meteorology data that indicates that September, October, and November (Spring designation) in 2014 and 2015 were the 3$^{rd}$ and 1$^{st}$ hottest springs over a 20-year period for South Eastern Australia (Bureau of Meteorology, 2020). Similarly, rainfall totals were the 3$^{rd}$ and 2$^{nd}$ lowest recorded.

### *10.2.3 Relating sensory attributes to chemical composition and climate measures*

To help determine the chemical compounds that relate to specific sensory attributes in the wines, andespecially those sensory attributes that related to regional specific differences among the wines, the statistical methodology PLS-R was utilised. This incorporated all sensory attributes in one model, including all chemical compounds measured that differed among the 22 wines, rather than simply those that were significantly different between regions. The climate indices were also included, to investigate potential causative links related to the vineyard site.

From the PLS-R it was found that four factors were sufficient, based on the size of the residual validation variance and assessment of $R^2$ validation (also known as $Q^2$) values. **Figure 11** shows the biplot of factors 1 and 2 of the PLS, which provides a visualisation of the main relationships in the data sets. Regression coefficients from the analysis were used below to assess the associations of the measures to the sensory attributes.


(a)
Factor-2 (16%, 10%)
Factor-1 (13%, 23%)
Ethyl dihydrocinnamate
Harvest day
Budbreak day
Pepper F
Ethyl cinnamate
Stalky A
Citronellol
Rotundone
DMS
IBMP
Earthy F
pH
Linalool
Beef stock A
Brown C
Cooked veg A
H2S
Methanethiol
Free anthocyanin
Total pigment
RHminTRP
Stalky F
Bitter
Hot
Woody F
Drain A
Earthy A
EvapGS
trans-geraniol
Dark fruit A
Carbon disulfide
Gamma-terpinene
Alpha-terpinene
Total phenolics
Mint F
Terpinolene
Umami
Total tannin
Colour density
Opacity
trans-2-hexenol
Methyl thioacetate
EvapRP
Phenylethyl acetate
Overall fruit F
Mint A
Alcohol
Dark fruit F
fSO2
Ethyl dodecanoate
Isobutyl acetate
Eucalyptol
Pigmented tannin
tSO2
Pungent A
Woody A
Astringent
trans-geranylacetone
cis-2-hexenol
Floral A
Ethyl octanoate
Chemical Age
Hue
Beta-ionone
Viscosity
Red fruit A
Ethyl butyrate
trans-oak lactone
4-Ethylphenol
Coffee A
Alpha-ionone
Vanillin
Furfural
Alpha-terpineol
4-Methylguaiacol
% Pigmented tannin
Dried fruit A
Guaiacol
Volatile acidity
RHmaxTRP
Confection A
3-Methylbutyl acetate
Sweet
Overall fruit A
VPRP
4-Terpineol
Beta-damascenone
Ethyl decanoate
5-Methylfurfural
cis-3-hexenol
4-Ethylguaiacol
Purple C
RainRP
Ethyl 2-methylbutyrate
Ethyl isobutyrate
cis-oak lactone
trans-3-hexenol
RainGS
Ethyl hexanoate
Ethyl 3-methylbutyrate
RadnRP
Red fruit F
Propyl acetate
Hexyl acetate
Fruit AT
Ethyl phenylacetate
Spice A
Butyl acetate
Vanilla/Choc A
cis-3-hexenyl acetate
RPTp
Eugenol
TA (8.2)
1,4-Cineole
Gamma-nonalactone
GSTp
CNIp
CNIt
Ethyl propanoate
GSTt
GDDph
GDD

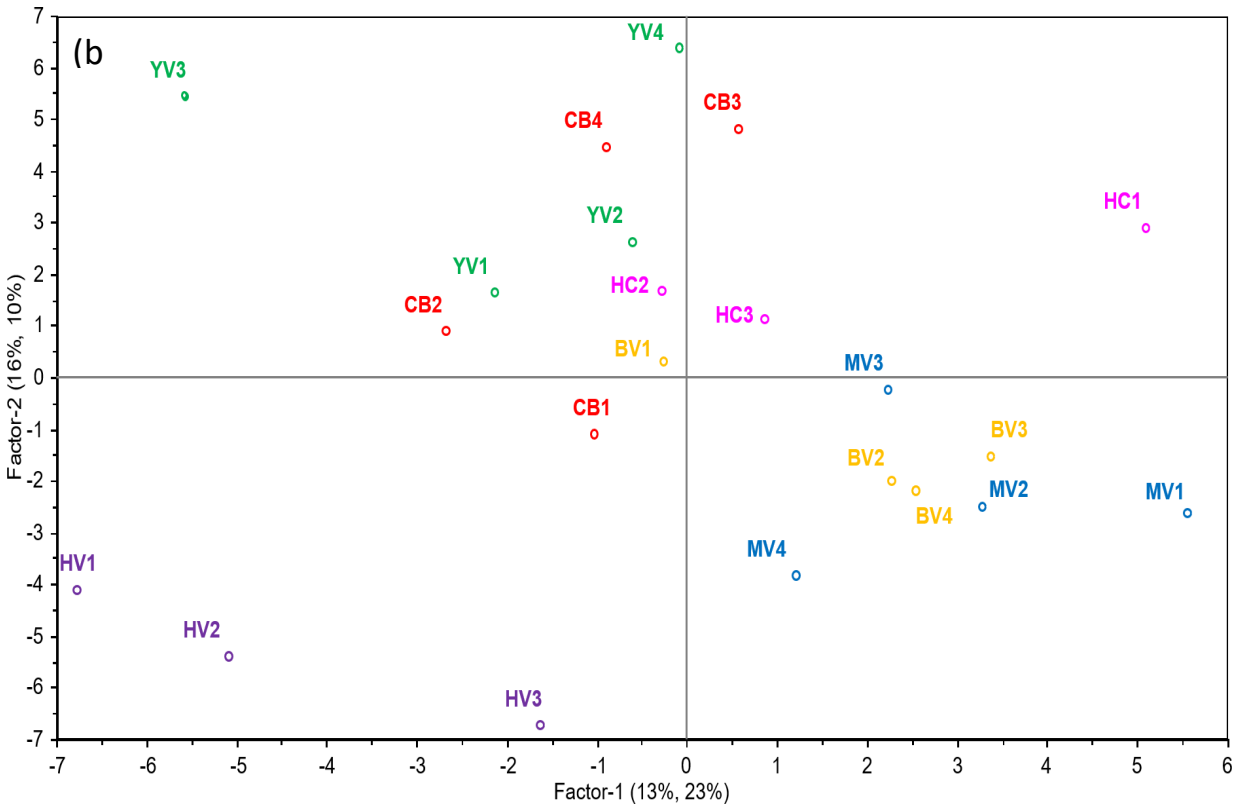



***Figure 11 a and b.*** *Factors 1 and 2 of the PLS-R: (a) loadings and (b) wine scores from a four-factor model for sensory attributes (y variables, red text) and chemical compounds and climate measures (xvariables, blue text). Measures in bold italics with black circles surrounding the dots signify significant variables as indicated by the uncertainty test. The proportion of the X-variance explained by the factors is denoted by the first value in parentheses and the proportion of Y-variance by the second value. Samples scores shows wines coloured by region (BV: Barossa Valley; MV: McLaren Vale; HC: Heathcote; YV: Yarra Valley; HV: Hunter Valley and CB: Canberra District).*

The first four factors of the PLS-R analysis modelled 48% of the variance of the sensory data. Those sensory attributes that were well modelled are those situated within the two ellipses, which represent 50% and 100% of the explained variance respectively. The x-variables that relate significantly to the sensory data are highlighted in **Figure 11a**, although other measures are also likely to be important, notably those with strong loadings and situated close to specific sensory attributes.

The stalky aroma and flavour attributes were moderately well modelled ($R^2$ calibration predicted vs measured values of 0.57 and 0.67 respectively). Those wines with higher ratings for stalky aroma and flavour, mainly YV and CB wines, were situated to the upper left quadrant of **Figure 11**. The stalky attributes were positively associated with the climate index RHminTRP, as well as late Bud Break and Harvest day, and the compounds ethyl cinnamate, ethyl dihydrocinnamate and rotundone. They were negatively associated with the solar radiation index RadnRP, and γ-nonalactone, ethyl propanoate and the oak related compounds cis-oak lactone and eugenol. IBMP was also indicated as contributing to these stalky attributes, even though IBMP was only found in one wine (YV4, 5 ng/L).

This wine also had the highest concentration for the two cinnamate esters. The pepper flavour attribute, highest for two YV and two CB wines, was also relatively well modelled ($R^2$ calibration 0.61), and was also positively associated with ethyl cinnamate, ethyl dihydrocinnamate and rotundone, together with harvest day and budbreak day, citronellol, IBMP and pH. Conversely, rotundone had negative associations with the group of climate indices at the lower section of **Figure 11a**, including GDD, GDDph, GSTt, GSTp, CNIt and CNIp. The indication of late budbreak day and harvest day leading to higher rotundone levels is of interest, providing guidance for potential means of enhancing the level of this potent compound through vineyard establishment or management decisions.

The wines with strong colour, high woody flavour, high dark fruit aroma and flavour, dried fruit aroma, high viscosity, hotness and bitterness, to the right of **Figure 11**, were predominantly BV and MV wines, along with the one HV wine, HC1. Each of these attributes were reasonably well modelled ($R^2$ calibration values of 0.48-0.72), with largely the same x-variables positively related, notably colour density, pigmented tannin, total tannin, pH, total pigment, alcohol, phenylethyl acetate, cis-2- hexenol, γ-nonalactone, β-damascenone, α- and β-ionone, with the climate indices RadnRP, EvapRP and EvapGS also significantly positively associated with these attributes and RainGS, RainRP, VPRP and RHmaxTRP strongly negatively associated. The relationship between RadnRP and β- damascenone and α-ionone agrees with previous research that has correlated solar radiation with higher production of norisoprenoids (Mendes-Pinto 2009). Factor four (not shown) highlighted dried fruit aroma and its relationship with β-ionone and EvapRP. γ-Nonalactone has previously been implicated in dried fruit aroma (Pons *et al.,* 2008) and a positive association with the attribute can been seen in **Figure 11a**. The oak related compounds cis-oak lactone and eugenol were stronger contributors to woody and spice attributes as would be expected (Boidron *et al.,* 1988). Astringency was reasonably modelled ($R^2$ calibration value of 0.51), with a positive association for total tannin and total phenolics.

The red fruit attributes, which were relatively high for the HV wines, were reasonably well modelled by the x-variables ($R^2$ calibration values of 0.66 and 0.56). The compounds 5-methyl furfural (caramel-like) and furfural (nutty) were positively related to these attributes, together with propyl acetate, ethyl decanoate, cis 3-hexenol and cis 3-hexenyl acetate, with colour density, pigmented tannin, tannin, phenylethyl acetate and alcohol negatively associated. The climate indices Rain RP, Rain GS, RHmaxTRP and VPRP were all positively related to the red fruit attributes. In addition, GDD and GDDph were also associated with the red fruit attributes.

Floral aroma, which was a feature of the CB wines, was not particularly well modelled ($R^2$ calibration 0.49), although there was some evidence that the monoterpenes α-terpineol, terpinolene and linalool were contributing to this aroma (SI Figure 1a).

Sweetness ($R^2$ calibration 0.42) was positively related to a number of 'sweet' smelling volatiles, including cis- and trans-oak lactone, vanillin, and α- and β-ionone.

The HC wines were rated highly in dark fruit and opacity attributes, as well as coffee and beef stock aroma, attributes which were distinctive for this region. The two latter attributes were not well modelled by the chemical

compounds or the climate metrics measured in this study. HC1 had the highest alcohol percentage of all wines, signifying harvest at a higher level of ripeness than the other HC wines. The important influence of alcohol on multiple wine sensory characteristics is well documented (Gawel *et al.,* 2007; Runnebaum *et al.,* 2011; King *et al.,* 2013). It is difficult to discern if these are regional characters due to climate or other vineyard specific metrics or alternatively products of winemaking decisions.

Overall, for the wines and vintages studied, it was apparent that the climate indices played a large role in separating the wines, with most of the indices relating to the sensory variation.

## **10.3** Conclusion

This study has highlighted that many of the sensory attributes important to Shiraz, and especially to specific regionally distinctive sensory attributes, could be related to a relatively small set of chemical compounds. Floral attributes of Canberra District wines were linked to monoterpenes; stalky and cooked vegetal characters of Yarra Valley wines to two cinnamate compounds plus DMS; pepper to rotundone; dark fruit and dried fruit attributes of Barossa Valley and McLaren Vale to several potent fruity norisoprenoids and phenylethyl acetate; and red fruit characters of Hunter Valley wines to 5- methylfurfural and furfural and several esters. Other important sensory attributes such as colour, astringency and viscosity were also well associated with straightforward non-volatile measures.

The chemical compounds identified can be used as targets in viticultural or winemaking studies or as key markers for mapping spatial variation in vineyards, within or between regions, such has been done with rotundone (Bramley *et al.,* 2017). The inclusion in the study of detailed site- and season- specific climatic indices, including phenological measures, gave great insight into likely causative influences on wine chemical composition and hence sensory properties.

Understanding regional characteristics in Australian Shiraz wines, whether chemical, climatic or sensory characteristics, will lead to a more complete appreciation of what it means for a wine to come from a particular place. Evaluating the comprehensive chemical profile of a large, diverse and representative sample set of wines from specific regions allowed understanding of the important compositional characteristics of wines from a particular region and also the implications of those characteristics on the resulting sensory profile. These parameters can help regional wine producers identify and potentially enhance sensory characteristics identified as regionally specific, and better understand the climate influences that promote them.

# 11 Chapter 4 - Investigation of the regional typicality of Australian Shiraz wines using untargeted metabolomics.

This chapter summarises the findings for the following investigations outputs;

- Conduct untargeted analysis of wines from sensory descriptive analysis, using GC-qTOF mass spectrometry.
- Determine tangible wine styles associated with region.

## 11.1 Introduction

The previous output chapter (Chapter 3 - Regionality in Australian Shiraz: compositional and climate measures that relate to key sensoryattributes) reported the concentrations of 70 volatile compounds that have been regarded as having important sensory characters and routinely targeted in wine chemistry research. However, wine is a complex mixture of compounds with more than 800 volatile metabolites having been reported (Robinson *et al.,* 2014). The compounds that are most important in differentiating GI may not be those bearing the higher sensory importance. In fact, one review noted that out of the 141 grape and wine volatile compounds commonly reported by untargeted analyses, only 35 were considered of having high odour impact (Ilc *et al.,* 2016). Instead, the compounds that showed the most variation between GIs may be metabolites of specific processes in the wine production, which can reflect on the unique choice in viticultural and/or oenological practices of a GI. In addition, flavour research has shown that the overall wine aroma is a concerted action of many compounds (Ferreira *et al.,* 2016), targeting a small number of compounds may miss some of the relationships and interactions between different volatile compounds that may contribute to regional wine styles. As we have no a priori knowledge of what compounds and their interactions may reflect the regional differences, we need to adopt a comprehensive and holistic approach, such as an untargeted metabolomics method, to study the wine volatilome.

Furthermore, in targeted and quantitated research of wine volatile, the downstream data processing usually involves univariate significant test (Student's t test or ANOVA) to select compounds that are significantly different between treatments (usually at $p < 0.05$). Such compounds are then subjected to an unsupervised analysis method, typically principal component analysis (PCA) or cluster analysis, to discover the latent structure in the compositional data that can reflect certain biological or production-related variations among samples. However, since PCA seeks to find the largest variations in the overall structure of the dataset, the boundaries between predefined sample groups may not be apparent and often overlapping (Enot et al. 2008). In comparison, supervised classification methods take into consideration the class information and seek to extract the latent pattern in data that best discriminate between classes, and are superior in addressing multiclass problem than unsupervised methods (Enot *et al.,* 2008).

For the two reasons outlined above, in addition to the targeted chemical analysis reported in the previous chapter, examining the overall Shiraz wine volatilome can provide additional information in discerning regional differences. In this part of the project we employed an untargeted metabolomics approach in analysing the volatilome of the same Shiraz wines selected and reported in Chapter 3, with three objectives:

- Investigate if patterns could be drawn from the overall Shiraz volatilome that can discern wines based on their GI.
- Investigate if regional differences can be explained by a relatively small number of volatile compounds.
- Establish a workflow that can be applied to future untargeted metabolomics work

## **11.2** Method

Shiraz wines (22 wines in triplicate) were subjected to a previously published solid phase extraction protocol that was optimised for extracting minor and trace volatile compounds from wine (Lopez *et al.,* 2002). The extracts were analysed on GC-QTOF MS operated in scan mode. A pooled quality assurance sample (QA) was created by mixing equal volume of each wine, and was used to monitor data quality. The raw MS data was imported into R environment for data extraction and modelling. 321 putative compounds were extracted from the raw MS data. The intensity of each compound was normalised and scaled for statistical analysis. A PCA was performed as an initial inspection of data quality as well as unbiased sample grouping.

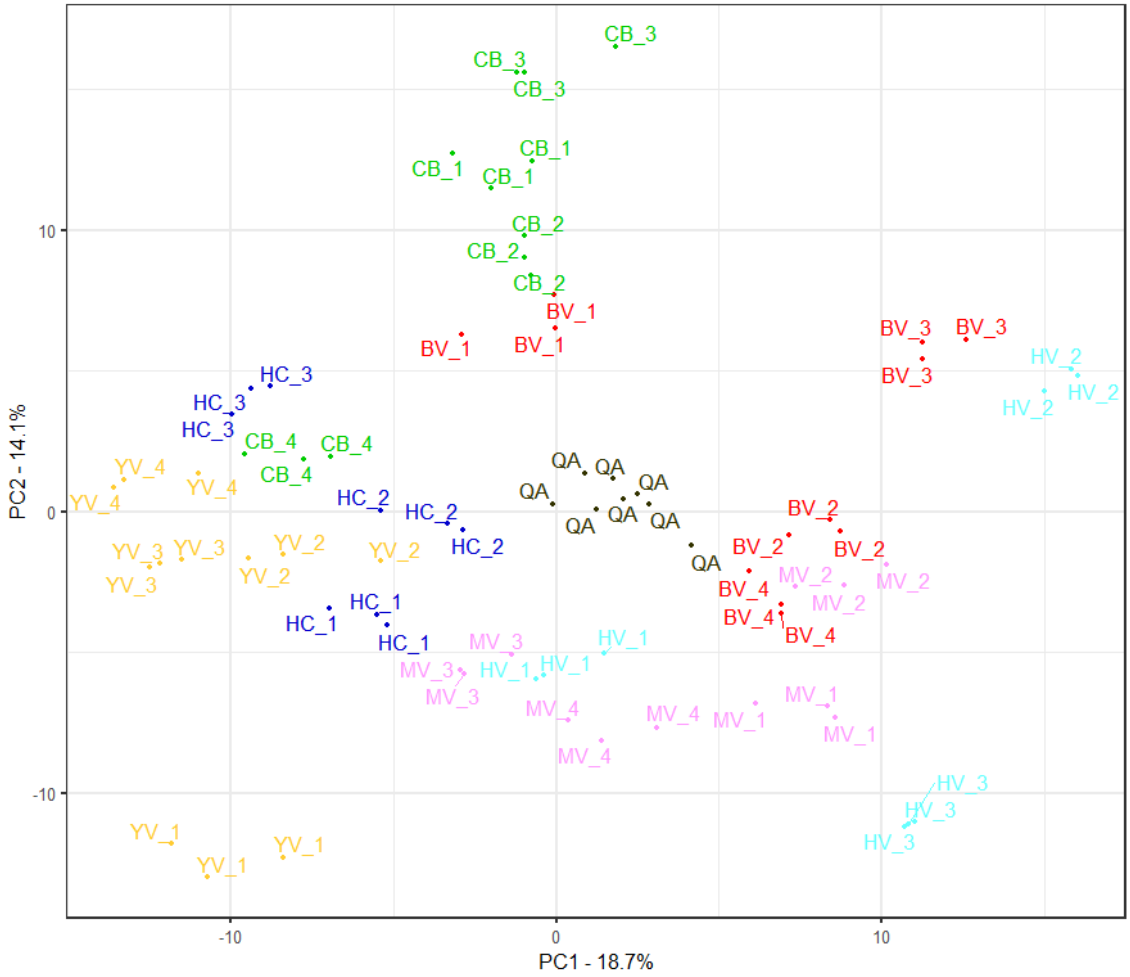



***Figure 12.*** *PCA including all wine and QA samples using 321 putative compounds. The sample with the same code corresponds to the independent bottles of the same wine from the GIs: Barossa Valley (🔴), Canberra (🟢), Heathcote (🔵), Hunter Valley (🔵), McLaren Vale (🟣), Yarra Valley (🟡) and quality assurance sample (⚫).*

A classification model based on the random forests algorithm (Breiman, 2001) was subsequently built using all 66 samples and 321 putative compounds, which could achieve a 100% separation of all wines according to their GIs. In order to discover the most important volatile compounds for regional separation, the number of compounds involved in the model was sequentially reduced following a published protocol (Han *et al.,* 2016), until the overall classification accuracy of the model was compromised. The remaining putative compounds were considered to be the most important in driving regional differences. It was determined that 80 putative compounds were sufficient to discriminate all samples correctly according to GI, and they were regarded as markers for regionality and subjected to identification in order to explore their possible formation pathways and sensory importance in wine.

## **11.3** Results and Discussion

### *11.3.1 Objective 1: Shiraz wine volatilome showed clear regional distinctions*

PCA of the whole sample set showed that QA samples clustered tightly in the centre of the distribution of samples (**Figure 12**), which indicated good data quality as QA is a mixture of all wines, and thus should be the

centre point of all samples. Furthermore, all replicates of each wine clustered closely with each other on the PCA plot, proving good reproducibility with the current sample preparation approach. However, discrete and separate clusters based on GI were not evident in the first two dimensions (accounting for 32.8% of total variance). Therefore, supervised learning methods Random Forest was subsequently applied to detect patterns associated with GI.

Compared to PCA, the RF based classification model could clearly separate each region, which can be visualised using multi-dimensional scaling (MSD), with the first two dimensions accounting for 50% total variations in the sample set (**Figure 13**). The first dimension clearly separates Barossa Valley and McLaren Vale wines from those made in Yarra Valley and Canberra District. Hunter Valley and Heathcote wines are positioned between these two divisions. Hunter Valley wines overlap with Barossa Valley wines whereas Heathcote wines appear to have commonality with Canberra District wines along this dimension. The second dimension separates samples from between Barossa Valley and McLaren Vale, as well as between Yarra Valley and Canberra District.

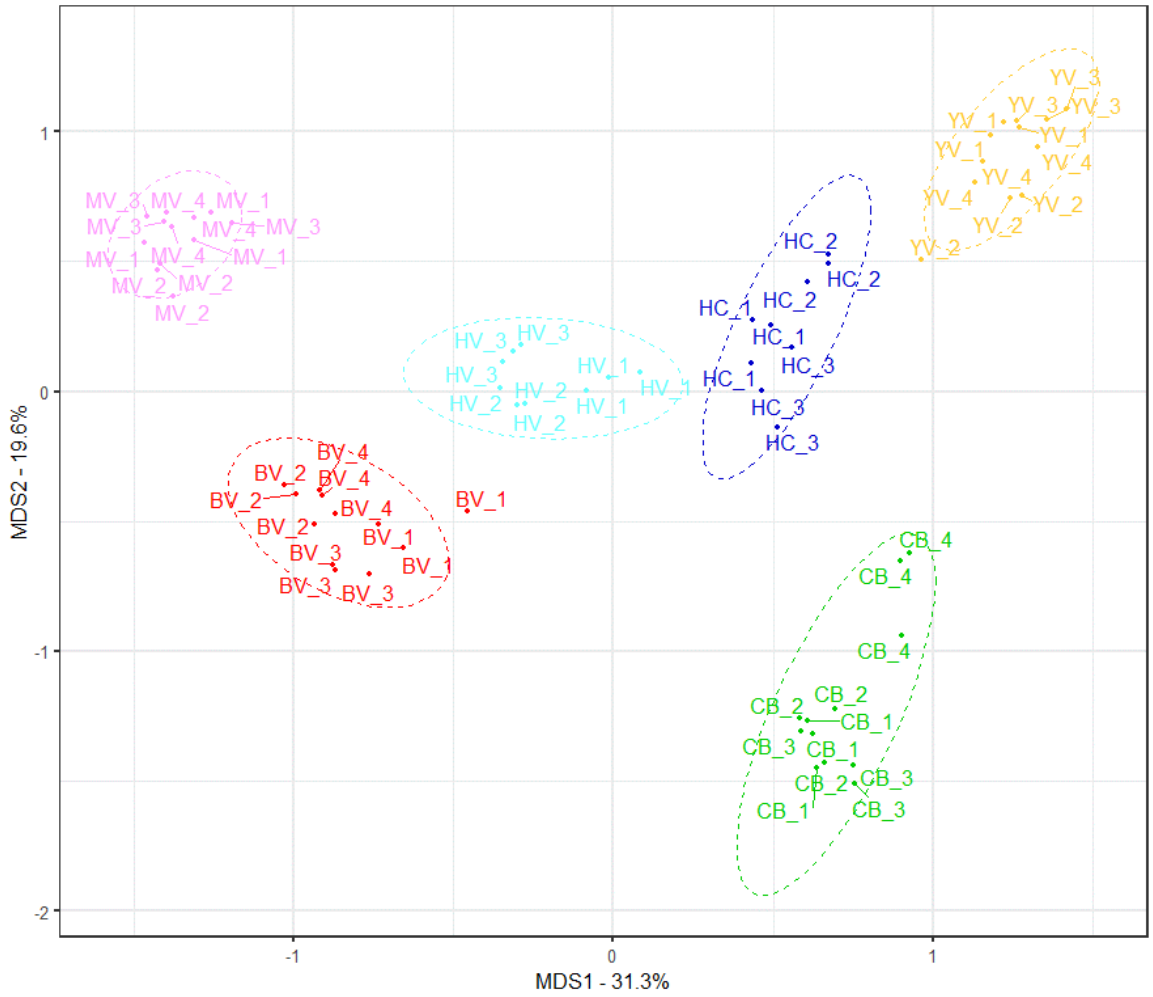



***Figure 13.*** *The multi-dimensional scaling plot based on the distance matrix generated by random forests model. The sample with the same code corresponds to the independent bottles of the same wine from the GIs: Barossa Valley (●), Canberra (●). Heathcote (●), Hunter Valley (●), McLaren Vale (●), Yarra Valley (●). The dotted lines represent the 95% confidence intervals based on multivariate t-distribution.*

Based on this successful initial model, we then sought to select compounds that are most characteristic of a GI. By step-wise eliminating the less important predictors, we were able to reduce the number of putative compounds from 321 to only 80, without compromising the 100% separation achieved in the original model.

Out of the 80 putative compounds, 45 could be either unequivocally or tentatively identified (**Table 5**). One of the limitations of the current work is not being able to provide unequivocal identification for a number of compounds that showed regional distinction. Although GC-EI-MS libraries such as NIST contains spectra for a great number of compounds, and a reasonably good match for each feature detected in the current study, it was challenging to find analytical standards for all the matched compounds. Furthermore, many of these compounds have no reported RI on a GC column phase similar to the one used in the current study. Therefore, a number of compounds that showed distinctive regional differences were not identified.

For the compounds that could be identified, some have been previously identified in grape and wine. They showed clear and distinctive patterns between regions. Canberra wines were distinctively higher in *cis*-pyranoid linalool oxide, benzyl alcohol, benzyl acetate, 2-methylhexanoic acid and ethyl 4-acetoxybutanoate. Wines from Yarra Valley and Heathcote seem to be characterised by relatively low abundances of compounds such as *p*-cresol, 1-octen-3-ol, *trans*-3-hexen-1-ol, and γ-nonalactone, while these compounds were present in higher amounts in wines from Barossa, McLaren Vale and Hunter Valley. According to the cluster analysis, Heathcote and Yarra Valley wines were similar in volatile composition. Hunter Valley wines had a distinctly higher relative concentration for 1-octanol, methyl salicylate, manool oxide, phenol, and ethyl vanillate.

***Table 6.*** *Mean and standard deviation (in parentheses) of relative concentrations of the selected volatile compounds in each GI, sorted in descending order of their importance to the RF model.*

| | Barossa | Canberra District | Heathcote | Hunter Valley | McLaren Vale | Yarra Valley |
|---|---|---|---|---|---|---|
| 3-Ethoxy-1-propanol | 0.033 (0.006) | 0.05 (0.062) | 0.008 (0.001) | 0.039 (0.016) | 0.025 (0.015) | 0.017 (0.006) |
| Benzenebutanal | 0.372 (0.055) | 0.753 (0.53) | 0.345 (0.268) | 1.669 (1.354) | 0.732 (0.071) | 0.141 (0.063) |
| 4-Heptanol | 0.019 (0.005) | 0.015 (0.008) | 0.038 (0.01) | 0.012 (0.002) | 0.018 (0.004) | 0.011 (0.002) |
| Isobutyric acid | 0.309 (0.045) | 0.582 (0.12) | 0.635 (0.11) | 0.601 (0.341) | 0.634 (0.096) | 0.399 (0.074) |
| Phenol | 0.054 (0.008) | 0.048 (0.01) | 0.037 (0.008) | 0.575 (0.583) | 0.043 (0.007) | 0.047 (0.007) |
| Ethyl furan-2-carboxylate | 0.252 (0.057) | 0.175 (0.04) | 0.209 (0.033) | 0.632 (0.114) | 0.245 (0.028) | 0.311 (0.115) |
| Ethyl vanillate | 1.524 (0.541) | 1.808 (0.495) | 1.075 (0.084) | 5.119 (0.691) | 1.721 (0.44) | 1.095 (0.442) |
| Ethyl *(Z)*-2-butenoate | 0.046 (0.006) | 0.036 (0.004) | 0.042 (0.003) | 0.042 (0.016) | 0.028 (0.004) | 0.049 (0.01) |
| Methyl salicylate | 0.063 (0.041) | 0.185 (0.13) | 0.038 (0.023) | 0.59 (0.463) | 0.024 (0.005) | 0.041 (0.012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3-Allylcyclohexene | 0.018 (0.005) | 0.008 (0.002) | 0.007 (0.002) | 0.013 (0.009) | 0.015 (0.004) | 0.004 (0.001) |
| Manool oxide | <0.001 (<0.001) | <0.001 (<0.001) | <0.001 (<0.001) | 0.003 (0.001) | <0.001 (<0.001) | 0.001 (<0.001) |
| Ethyl cinnamate | 0.028 (0.012) | 0.035 (0.012) | 0.041 (0.021) | 0.019 (0.008) | 0.018 (0.005) | 0.125 (0.078) |
| *p*-Cresol | 0.027 (0.006) | 0.028 (0.015) | 0.02 (0.001) | 0.022 (0.003) | 0.019 (0.001) | 0.018 (0.004) |
| Furfural | 0.737 (0.187) | 1.055 (0.316) | 0.536 (0.076) | 1.129 (0.366) | 0.607 (0.188) | 0.394 (0.188) |
| Ethyl 3-methylbutanoate | 0.269 (0.061) | 0.187 (0.082) | 0.303 (0.096) | 0.438 (0.271) | 0.449 (0.027) | 0.34 (0.08) |
| Ethyl 2-phenylacetate | 0.238 (0.049) | 0.248 (0.068) | 0.239 (0.03) | 0.36 (0.2) | 0.392 (0.069) | 0.255 (0.034) |
| Terpinen-4-ol | 0.006 (0.002) | 0.003 (0.001) | 0.002 (<0.001) | 0.005 (0.001) | 0.007 (0.002) | 0.004 (0.001) |
| Ethyl 2-methylbutanoate | 0.237 (0.052) | 0.172 (0.076) | 0.269 (0.082) | 0.495 (0.349) | 0.463 (0.092) | 0.335 (0.079) |
| Diethyl malate | 1.365 (0.857) | 0.692 (0.261) | 2.692 (2.385) | 4.63 (2.93) | 3.148 (0.546) | 1.72 (0.308) |
| Benzyl alcohol | 0.051 (0.014) | 0.082 (0.02) | 0.036 (0.01) | 0.039 (0.009) | 0.036 (0.007) | 0.043 (0.015) |
| Ethyl salicylate | 0.005 (0.003) | 0.012 (0.005) | 0.006 (0.004) | 0.007 (0.003) | 0.003 (<0.001) | 0.02 (0.013) |
| Ethyl dihydrocinnamate | 0.025 (0.006) | 0.034 (0.007) | 0.028 (0.006) | 0.013 (0.003) | 0.018 (0.006) | 0.053 (0.027) |
| 1-Octanol | 0.093 (0.02) | 0.08 (0.011) | 0.068 (0.016) | 0.227 (0.041) | 0.068 (0.006) | 0.116 (0.054) |
| 1,3-Diphenylpropan-2-ol | 0.29 (0.065) | 0.336 (0.052) | 0.308 (0.031) | 0.427 (0.209) | 0.531 (0.073) | 0.316 (0.107) |
| 3,5,5-Trimethyl-2-hexene | 0.145 (0.046) | 0.102 (0.015) | 0.08 (0.019) | 0.118 (0.023) | 0.148 (0.033) | 0.071 (0.01) |
| 1-Octen-3-ol | 0.033 (0.009) | 0.023 (0.007) | 0.017 (0.005) | 0.029 (0.009) | 0.024 (0.005) | 0.012 (0.002) |
| 4-(Ethoxymethyl)phenol | 0.076 (0.016) | 0.091 (0.022) | 0.072 (0.008) | 0.066 (0.016) | 0.068 (0.009) | 0.045 (0.011) |
| (+)-Diethyl L-tartrate | 0.029 (0.019) | 0.022 (0.012) | 0.016 (0.007) | 0.114 (0.069) | 0.029 (0.01) | 0.102 (0.144) |
| Acetylfuran | 0.026 (0.01) | 0.022 (0.01) | 0.018 (0.005) | 0.022 (0.012) | 0.018 (0.003) | 0.011 (0.002) |
| TDN | 0.057 (0.011) | 0.024 (0.005) | 0.03 (0.007) | 0.091 (0.042) | 0.053 (0.004) | 0.045 (0.015) |
| *N,N*-Dibutylformamide | 0.041 (0.028) | 0.035 (0.003) | 0.018 (0.003) | 0.041 (0.018) | 0.07 (0.061) | 0.059 (0.038) |
| Ethyl 4-acetoxybutanoate | 0.06 (0.018) | 0.099 (0.038) | 0.075 (0.031) | 0.025 (0.004) | 0.054 (0.004) | 0.045 (0.02) |
| γ-Nonalactone | 0.247 (0.078) | 0.162 (0.071) | 0.128 (0.021) | 0.16 (0.036) | 0.271 (0.066) | 0.098 (0.015) |
| Ethyl dodecanoate | 0.004 (0.001) | 0.016 (0.015) | 0.002 (0.002) | 0.03 (0.028) | 0.006 (0.003) | 0.002 (<0.001) |
| Butyl ethyl succinate | 0.594 (0.111) | 0.288 (0.122) | 0.29 (0.06) | 0.347 (0.017) | 0.411 (0.173) | 0.317 (0.177) |
| *cis*-Pyranoid linalool oxide | 0.047 (0.017) | 0.056 (0.027) | 0.032 (0.01) | 0.022 (0.008) | 0.025 (0.006) | 0.054 (0.023) |
| γ-Undecalactone | 1.978 (0.47) | 1.726 (0.432) | 1.729 (0.418) | 3.255 (0.158) | 2.447 (0.425) | 2.227 (0.449) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ethyl hexanoate | 1.987 (0.245) | 1.344 (0.136) | 1.692 (0.377) | 1.849 (0.442) | 1.353 (0.174) | 1.337 (0.071) |
| Ethyl 2-hydroxycaproate | 1.557 (0.226) | 1.175 (0.2) | 1.695 (0.184) | 1.906 (0.984) | 2.498 (0.486) | 1.947 (0.796) |
| 2-Methylhexanoic acid | 0.216 (0.066) | 0.404 (0.312) | 0.286 (0.106) | 0.146 (0.024) | 0.201 (0.022) | 0.2 (0.056) |
| Dihydroactinidiolide | 0.08 (0.016) | 0.059 (0.016) | 0.055 (0.009) | 0.056 (0.015) | 0.078 (0.017) | 0.035 (0.007) |
| Benzyl acetate | 0.006 (0.001) | 0.007 (0.001) | 0.004 (0.001) | 0.004 (0.001) | 0.005 (0.001) | 0.005 (0.002) |
| *trans*-3-hexen-1-ol | 0.339 (0.137) | 0.316 (0.082) | 0.229 (0.035) | 0.253 (0.071) | 0.31 (0.075) | 0.162 (0.056) |
| Cyclamal | 0.003 (<0.001) | 0.001 (<0.001) | 0.001 (<0.001) | 0.002 (<0.001) | 0.002 (<0.001) | 0.001 (<0.001) |
| Ethyl heptanoate | 0.009 (0.003) | 0.007 (0.002) | 0.006 (0.001) | 0.006 (0.001) | 0.01 (0.003) | 0.005 (0.001) |

Grape-derived volatile compounds have been demonstrated to be good biomarkers to differentiate varietal wines between different regions and vineyards (Schmidtke *et al.,* 2020, Šuklje *et al.,* 2019). These compounds include aliphatic alcohols, benzenoids, and terpenoids, which mostly exist in grapes as glycoconjugated forms. They are transformed into free volatile forms during vinification and storage through enzymatic hydrolysis or acid catalysis.

Free terpenoids are important odorants in wine. Several terpenoids were found to contribute to the current RF model, including three monoterpenes (*cis*-pyranoid linalool oxide, terpinen-4-ol, and dihydroactinidiolide), one diterpene (manool oxide), as well as two C13-norisoprenoids (1,1,6,-trimethyl-1,2-dihydronapthalene (TDN) and cyclamal) (**Table 5**). Manool oxide had the highest contribution to the RF model while the cyclamal had the lowest contribution. Monoterpenes and diterpenes are synthesised through the methylerythritol phosphate pathway, which can be slightly affected by the growing conditions. They are mostly present in grapes in odourless glycosylated and polyhydroxylated forms, and the concentration of their free volatile form in wine is highly dependent on the winemaking procedure, i.e. extraction from grapes and transformation during fermentation and storage (Mateo and Jiménez 2000). In the current study, relative concentrations of selected monoterpenes and diterpenes showed different pattern across regions: *cis*-pyranoid linalool oxide was found to have higher relative concentrations in wines Canberra and Yarra Valley, while other terpenes were present in higher amounts in wines from Barossa, McLaren Vale and Hunter Valley. In contrast to mono- and di-terpenes, C13-norisoprenoids are bio-degradation products of carotenoids in grapes. It has been reported that grapes from warmer climates have higher concentration of carotenoids, and sun exposure significantly increases the precursor degradation (Black *et al.,* 2015). Thus, it is not surprising that C13-norisoprenoids are widely reported as bio-markers for wines produced in warmer climate (Gambetta *et al.,* 2017; Šuklje *et al.,* 2019). In the current study, the highest relative concentrations of TDN were found in wines from Hunter Valley, followed by wines from Barossa Valley and McLaren Vale (**Table 5**), the three warmest regions judged by their historical average growing season temperature.

Benzenoids are another group of compounds that are derived from precursors in grapes. Of the compounds identified in this study, 3 benzenoids have previously been reported in wine. Benzyl alcohol and its acetate ester were found to be at higher relative concentrations in Canberra wines, consistent with other reports that these compounds are more abundant in grape and wine from cooler regions (Gambetta *et al.,* 2017, Šuklje *et al.,* 2019). Another benzenoid, methyl salicylate was found at significantly higher levels in Hunter Valley wines compared to all other regions. Concentration of methyl salicylate was purportedly attributable to several glycosidic precursors from grapes in a study of Italian grape varieties (Carlin *et al.,* 2019). Although methyl salicylate is not a potent volatile compound and has not been subjected to frequent studies in wine sensory, a recent study has shown that when present at its sub-threshold concentrations, it was likely to contribute to overall fruity aroma in cherry wines (Niu *et al.,* 2019).

A large proportion of selected compounds were fermentation-derived esters. Among these were three fatty acid ethyl esters, i.e. ethyl 2-methylbutanoate, ethyl 3-methylbutanoate and ethyl hexanoate, which have been shown to be major contributors to fruity, especially red- and blackberry, aromas in red wine (Escudero *et al.,* 2007). They were found to be generally present in higher concentrations in Hunter Valley and McLaren Vale wines (**Table 5**). Ethyl cinnamate and ethyl dihydrocinnamate were both selected by the RF model, and exhibited the same pattern, i.e. both showed highest abundance in Yarra Valley wines and least abundance in Hunter Valley wines. This is consistent with the targeted quantification results reported in Chapter 3. Both cinnamates could impart sweet-caramel note in red wine (Escudero *et al.,* 2007), and were shown to be elevated by pre-fermentation cold soak and whole cluster inclusion in fermentation (Casassa *et al.,* 2021). Their prominence in the Yarra Valley Shiraz could be indicative of particular winemaking techniques favoured by the producers in this region.

Another group of compounds included in the RF model was related to wine storage and ageing. The majority of premium Australian Shiraz wines are aged in oak barrels from several months up to two years. During this time, aside from pH-dependent hydrolytic reactions that modify existing compounds such as esters and glycosides, new compounds are also formed or extracted from the oak (Jarauta *et al.,* 2005). Two compounds selected by the RF model, furfural and γ-Nonalactone, are attributed to extraction from oak (Jarauta *et al.,* 2005). However, more characteristic oak-derived compounds, such as *cis*- and *trans*- whiskylactone and vanillin, although were identified in the wines, were not selected by the RF model, preventing inference on the regional practice in using oak barrel/products in Shiraz wine production. Ethyl vanillate is another compound that has been reported to increase with ageing, from either oak extraction or hydrolysis of glycosidic precursors (Jarauta *et al.,* 2005). Hunter Valley wines had a much higher relative concentrations of ethyl vanillate compared to wines from other regions.

The volatile compounds selected by the RF model arise from multiple sources, i.e. grapes, fermentation, and ageing. This is not surprising, considering that many natural and artificial factors are involved in the wine production, all significantly contributing to the regional typicality of Shiraz wines. Some of these compounds have well known sensory impact in wine, while others were scarcely studied or reported. Previous untargeted analyses using GC × GC – QTOFMS usually report 100 to 300 volatile compounds that show significant

differences among regions, many of which have unknown impact on wine sensory (Barbará *et al.,* 2020, Carlin *et al.,* 2016, Robinson *et al.,* 2011, Šuklje *et al.,* 2019, Welke *et al.,* 2012). Therefore, although some volatile compounds were frequently reported in untargeted studies as showing variations between wines subjected to different treatments, their origins and influences on wine sensory are still relatively unknown. One such compound, for example, is Ethyl furan-2-carboxylate, which was one of the top contributors to our RF model due to its substantially higher concentration in Hunter Valley wines. A recent untargeted study also identified it as one of the most important volatile compounds in differentiating Brazilian Syrah wines made from early harvested grapes compared to from normal and late harvest (Barbará *et al.,* 2020). However, the sensory impact and its formation pathway in wine is relatively unknown. Further work, including gas chromatography-olfactometry and aroma reconstitution studies (Escudero *et al.,* 2007, Mayr *et al.,* 2014), may help to ascertain the influence of the less studied compounds to the sensory profile of individual wines and the overall regional typicality.

## **11.4** Conclusions

The 22 commercial Shiraz wines, despite their complex nature, showed distinctive regional chemical composition, within which some volatile components were identified. The volatile profiling showed that regional typicality is a multi-faceted concept and encompasses volatiles derived from grapes, vinification process and storage, which are in turn affected by natural elements and human interventions. A relatively small number of compounds was able to classify the studied wines according to their GI, seemingly indicating that a predictive model may be possible for classifying regional wine styles.

### *11.4.1 Objective 2: Establishing an untargeted metabolomics workflow in wine analysis*

A second objective of this project is to establish a workflow that can be applied to future untargeted metabolomics study for wine chemistry. Unlike targeted analysis where limit of detection, limit of quantification, recovery and repeatability need to be validated before the absolute concentration of the metabolites could be quantified, there is no such rigid and standardised validation criteria for untargeted analysis. Furthermore, rather than pre-defining a single ion fragment per compound to monitor on MS in targeted analysis, untargeted analysis simultaneously monitor all ion fragments generated by MS, and thus over 10,000 ion peaks can be generated in a single run, adding to the complexity of ensuring data quality. Thus a workflow needs to be defined for untargeted analysis protocol that uses experimental design to eliminate systematic errors so that these analytical noises are not modelled by the statistical analysis and misinterpreted as patterns in metabolites associated with the research question. Through our work in benchmarking the Shiraz wine volatilome, we established an untargeted metabolomics workflow at NWGIC that can be applied to future research in this area (**Figure 14**). The full description of this workflow, as well as more details on Shiraz wine volatilome is published in Australian Journal of Grape and Wine Research (Li *et al.,* 2021, https://doi.org/10.1111/ajgw.12493)

In the next chapter, we'll demonstrate the use of this workflow in untargeted analysis of non-volatile compounds in the Shiraz wines.

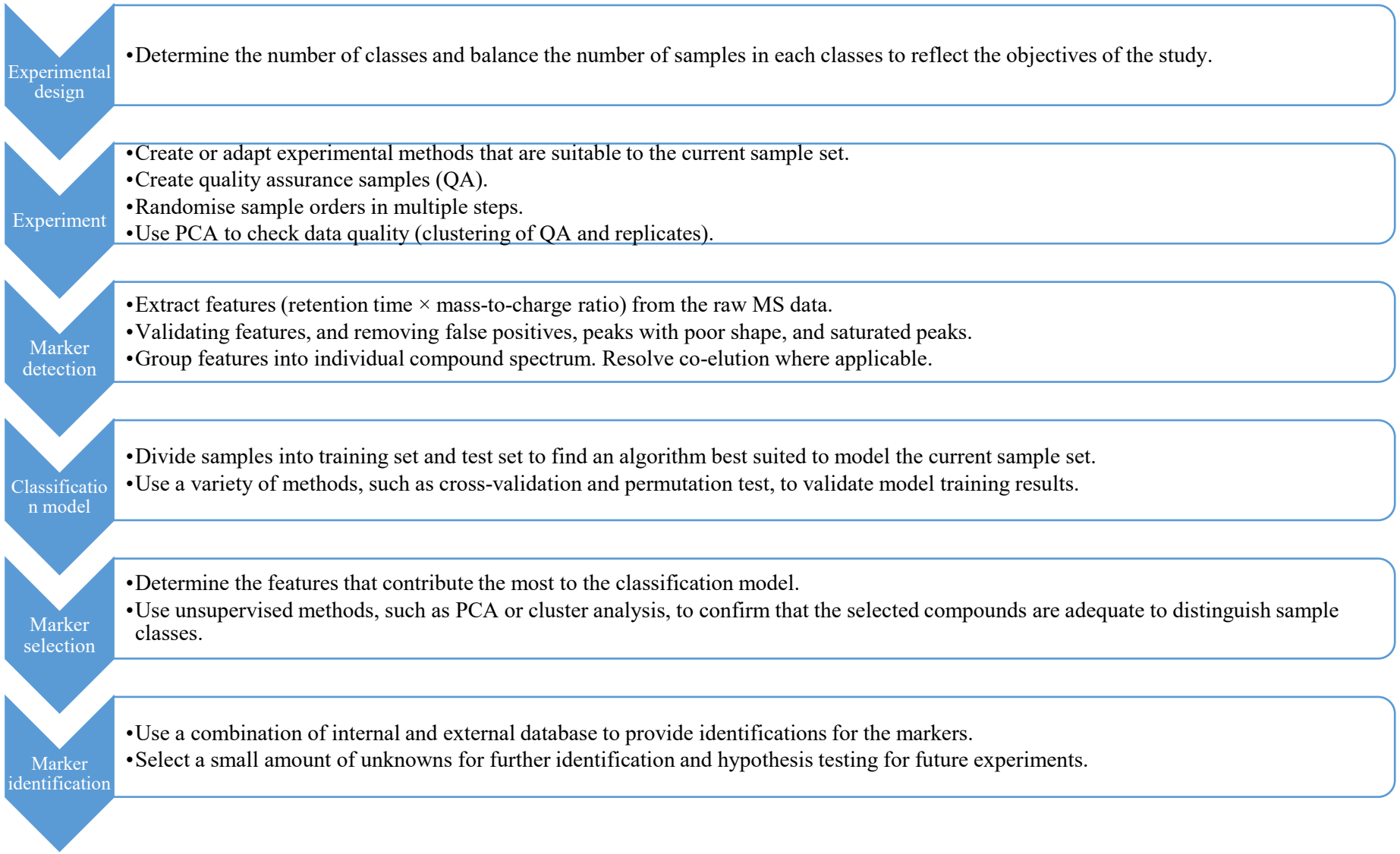



***Figure 14.*** *Workflow established through the current project.*

# 12 Chapter 5 - Untargeted LC-MS analysis for non-volatile compounds.

This chapter is divided into two sections. The first summarises the findings for the investigation output - Untargeted LC-MS analysis for non-volatile compounds, including polyphenols and tannins, to identify features in wines from defined regions.

The second section reports on key findings to;

- Correlate climatic indices with untargeted wine analyses.
- Determine tangible wine styles associated with climate and/or region (to complement wine style associated with targeted chemical analyses reported in Chapter 3).

## **12.1** Untargeted metabolomics study of non-volatile compounds in Shiraz wines

### *12.1.1 Background*

In the previous chapter, we reported an untargeted metabolomics profiling of the volatile composition of the Shiraz wines. To complement this result, untargeted analyses were also conducted on the non-volatile components of the same wines, using an ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS). Compared to GC-MS-based metabolomics, LC-MS is a more widely used tool in the field of metabolomics. Its application is also rapidly developing in grape and wine research in the past decade, usually as a tool for hypothesis generating and new compound discovery (Pinu, 2018). It has been applied to studying the role of oxygen during white wine storage (Arapitsas *et al.,* 2016), exploring the different metabolite profile generated by MLF simulating or inhibiting yeast strains (Liu *et al.,* 2016), and profiling stilbenes in Raboso Piave and Primitivo grapes (Flamini *et al.,* 2013), to name a few. Recently it was applied to discriminate 11 monovarietal red wines from 12 regions, in order to establish a database of metabolomics fingerprint associated with distinctive Italian terroirs (Arapitsas *et al.,* 2020). In this chapter, we report a similar albeit more preliminary research into the non-volatile metabolites of Australian Shiraz wines, using the workflow presented in the previous chapter. The main objective was to examine if a relatively small number of non-volatile metabolites could be found that separate Shiraz wines according to their regions, similar to what we demonstrated for volatile metabolites.

### *12.1.2 Materials and Methods*

Two bottles were used as replicates for each of the 22 Shiraz wines. The LC-MS untargeted analysis of wines was conducted following a published and validated protocol (Arapitsas and Mattivi, 2018). Briefly, wine was opened under $N_2$ atmosphere and 1 mL of wine was diluted with 1 mL of milliQ water. *o*-coumaric acid was added as an internal standard to a final concentration of 10 mg/L. The wine was filtered through 0.2 μm PTFE filters and analysed on an Agilent 7200 UPLC-Q-TOF-MS. 8 μL of sample was injected onto a C18 column

used for reverse phase LC and the chromatographic conditions were identical to those reported in the protocol. The Q-TOF-MS was operated in negative electron spray ionisation mode, with all parameters identical to those reported in Han *et al.,* 2014.

The processing of raw MS data files and the subsequent statistical modelling were performed following the workflow reported in Chapter 4. After visually validating chromatogram, 852 putative compounds were found. A PCA based on these figures was performed (**Figure 15**) to check data quality. The distribution of samples and QAs were similar to that reported for GC-Q-TOF-MS results. Most QA samples were clustered tightly at the centre of the distribution, with one slightly deviated from the cluster. The duplicates of each one was within proximity to each other. However, cluster based on GI was not conclusive: wines from Barossa, Hunter Valley and McLaren Vale seemed to group closely with other wines within the GI, while those from Canberra District, Heathcote and Yarra Valley spread across the PCA plot, indicating variability within the region. This preliminary data was then subjected to supervised modelling technique to reveal patterns in the non-volatile metabolites that showed regional typicality.

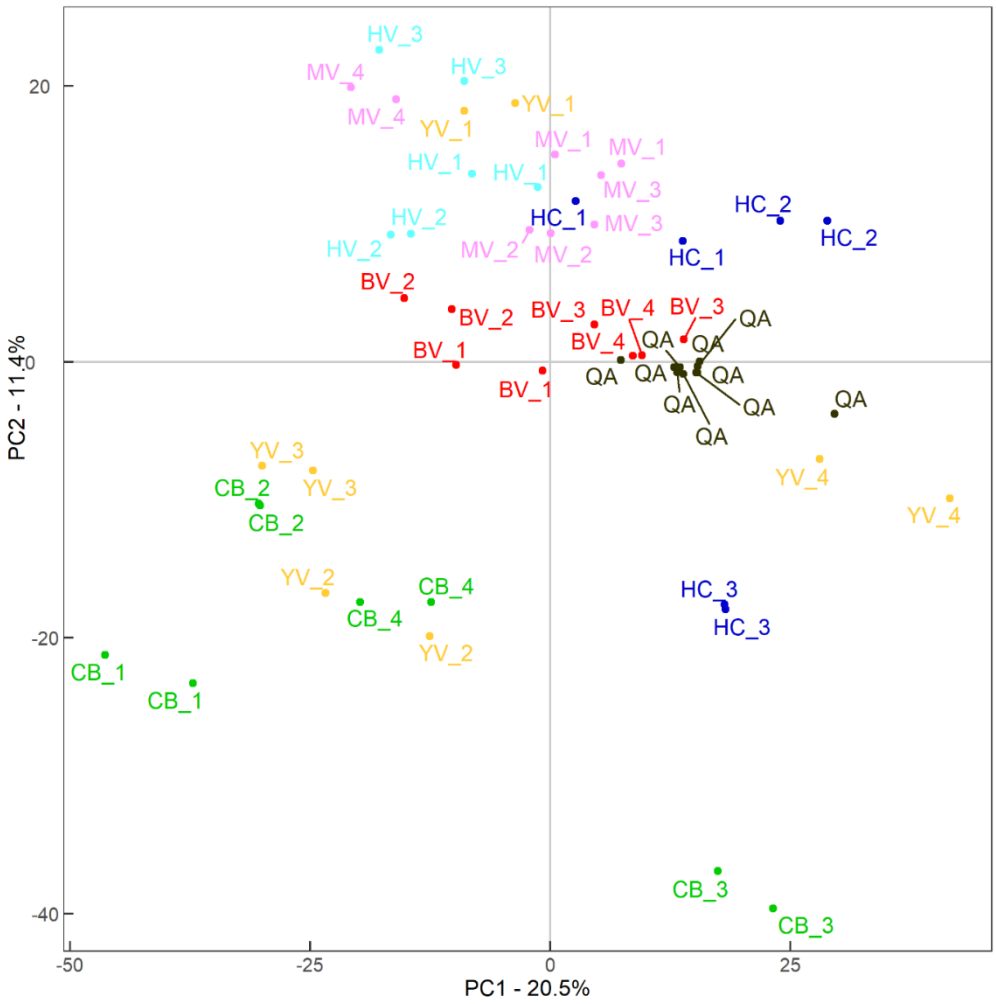



***Figure 15.*** *PCA including all wine and QA samples using 852 putative compounds. The sample with the same code corresponds to the independent bottles of the same wine from the GIs: Barossa Valley (●), Canberra (●), Heathcote (●), Hunter Valley (●), McLaren Vale (●), Yarra Valley (●) and quality assurance sample (●).*

### *12.1.3 Results and Discussion*

The separation of wines based on GI using non-volatile compounds was not as clear as that using volatile compounds (**Figure 16 A and B**). The first three dimensions of MDS (accounting for 47.1% total variance) showed some overlapping between regions, especially between Heathcote and Barossa Valley. Following the workflow reported in Chapter 4, the less important compounds contributing to the RF model were sequentially removed. Reducing the number of putative compounds in the model from 852 to just 26 seemed to improve separation between regions in the first three dimensions of the MDS, which accounted for 57.2% of total variations (**Figure 16 C and D**). The 95% confidence intervals of Barossa and Heathcote wines no longer overlapped in this new RF model. In addition, reducing the number of compounds seemed to also reduce the variance within a region (i.e. the spread of the wines from the same region on the MDS plot), especially for Hunter Valley and Yarra Valley. A hierarchical cluster analysis was performed using the 26 selected compounds and visualised in a heatmap (**Figure 17**). The cluster analysis confirmed that most wines from the same GI are grouped together, except for Barossa wines. Two Barossa wines were considered to be similar to McLaren Vale wines, while the other two shared more similarity with Heathcote wines.

One of the major limitations of LC-MS based untargeted metabolomics is compound identification. To provide unequivocal identification, the feature of interest needs to match 2 or more orthogonal properties of an authentic chemical standard (Arapitsas *et al.,* 2016). Putative annotation can be achieved based on matching to public databases and literature data. Q-TOF-MS provides accurate mass measurement for metabolites, which can be used as one of the properties for identification. However, although various large database, such as METLIN Mass Spectral Database (Scripps Center for Metabolomics), is searchable using accurate mass, it is almost impossible to ascertain if any of the compounds with the same accurate mass (< 5 ppm difference) is indeed the correct identification. However, matching unknowns to databases that only contain metabolites that have been positively identified in grape and wine samples increase the likelihood of correct identification. Several grape and wine untargeted metabolomics studies using LC-Q-TOF-MS have published large lists of metabolites identified in their samples (Arapitsas *et al.,* 2020, Flamini *et al.,* 2013, 2015), from which 260 metabolites identified under negative ESI were collated into our in-house database. The theoretical mass for $[M-H]^-$ ions of these metabolites were calculated and searched against the 26 putative compounds selected by the RF model. Four hits were found for mass difference less than 5 ppm (**Table 6**), which comprised of two phenolic acid glycosides and two flavonoids. In particular, dihydroxybenzoic acid-β-D-glucopiranoside appear to be present in significantly higher concentration in Hunter Valley wines compared to all other GIs. Unfortunately no polyphenolic compounds were able to be identified.

A potential approach to overcome the challenge of identification, including the recognition of polyphenols and tannins, would be to construct an in-house library for specific biological pathways or metabolite classes of interest. For example, in a recent study that used LC-Q-TOF based untargeted metabolomics to study the regionality of Italian red wines (Arapitsas *et al.,* 2020), the authors were able to select 2356 distinctive putative compounds that showed significant differences in relative concentrations across the regions using supervised modelling technique, but could not identify the majority of them. Therefore, they used their in-house database

that contained confirmed metabolites from their previous oenological studies and found 131 of these metabolites in the Italian red wines. Although not every metabolite showed significant regional differences, general patterns could be recognised in compounds belonging to specific groups, such as flavonoids, N-containing compounds (amino acids and peptides) and monomeric and oligomeric flavanols. However, to reach this level of identification, a long-term research project would be required to create purpose built databases that contain compounds whose identities are confirmed with analytical standards and/or MS/MS experiments. The Italian database described above, or the one compiled for New Zealand Sauvignon Blanc juice and wine over the years (Pinu *et al.,* 2019) are examples of the size and complexity of the databases required.

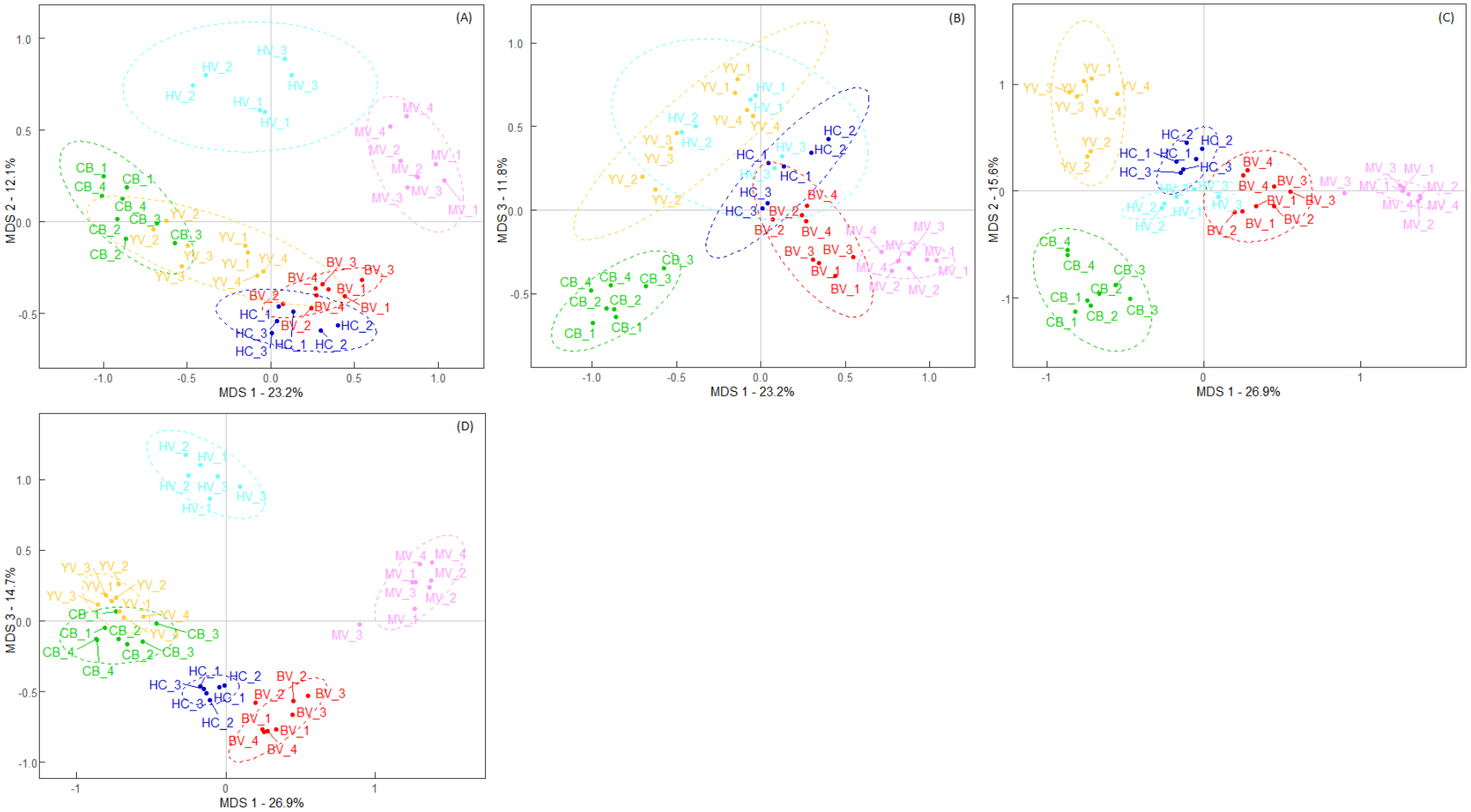



***Figure 16.*** *The multi-dimensional scaling plot based on the distance matrix generated by random forests model. (A) and (B) include the first three dimensions for the RF model built on 852 putative compounds, while (C) and (D) include the first three dimensions for the RF model built on 26 putative compounds. The sample with the same code corresponds to the independent bottles of the same wine from the GIs: Barossa Valley (●), Canberra (●), Heathcote (●), Hunter Valley (●), McLaren Vale (●), Yarra Valley (●). The dotted lines represent the 95% confidence intervals based on multivariate t-distribution.*

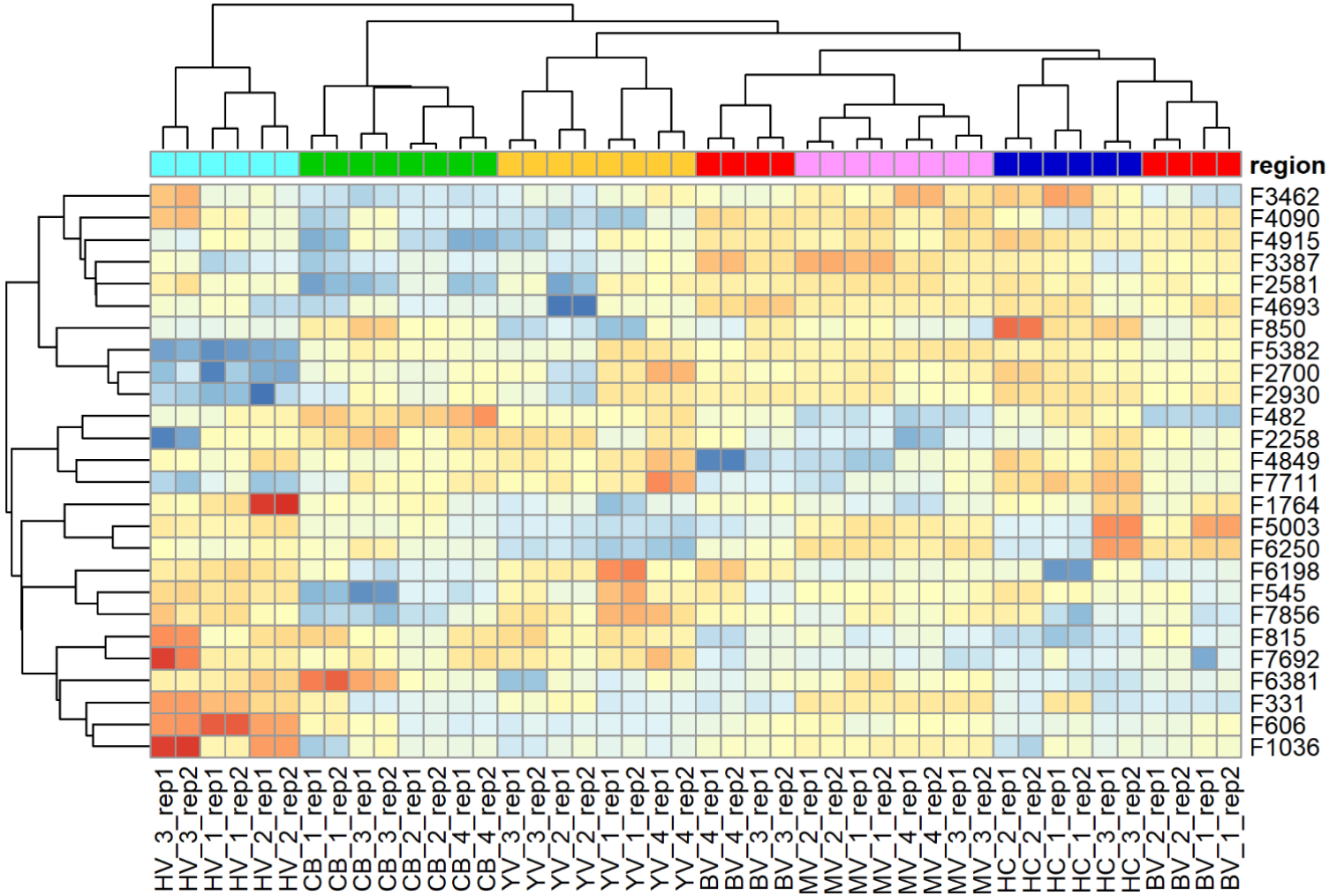



***Figure 17.*** *Heatmap using the selected putative compounds from RF model. Rep1 and rep2 represent 2 independent bottles of the same wine from the GIs: Barossa Valley (■), Canberra (■). Heathcote (■), Hunter Valley (■), McLaren Vale (■), and Yarra Valley (■). The putative compounds clustered were coded as F and a numeral.*

***Table 7.*** *Four non-volatile compounds tentatively identified by comparing accurate mass found using Q-TOF-MS with literature.*

| Putative compound | measured m/z | tentative identification | chemical formula | theoretical m/z | m/z error (ppm) | group | Reference[a] | m/z in this spectrum |
|---|---|---|---|---|---|---|---|---|
| F331 | 315.0718 | dihydroxybenzoic acid-β-D-glucopiranoside | C13H16O9 | 315.0716 | 0.63 | phenolic acid | 2 | 109.0286, **153.0192**, 154.0166, 315.0718 |
| F2581 | 341.0880 | caffeic acid-β-D-glucopyranoside | C15H18O9 | 341.0873 | 2.05 | phenolic acid | 2 | 341.0880 |
| F1764 | 287.0564 | dihydrokaempferol | C15H12O6 | 287.0561 | 1.05 | flavonoid | 1 | **287.0564**, 288.0599 |
| F4915 | 493.0627 | myricetin-3-*o*-glucuronide | C21H18O14 | 493.0624 | 0.61 | flavonoid | 1, 2, 3 | **493.0627**, 494.0663 |

[a]Metabolites were reported in 1. Arapitsas *et al.,* 2020; 2. Flamini *et al.,* 2013; and 3. Falmini *et al.,* 2015.

## 12.2 Section 2: Exploring the modelled climate indices for each vineyard site and exploring the regional differences using climate, sensory and untargeted metabolomics analyses.

### *12.2.1 Identifying patterns in climatic indices based on GI*

In this study, we took a novel approach to estimating phenology that combines cultural practice with climatic observations. Using heat accumulation data and actual harvest date for Shiraz at each vineyard site for the period from 1 July 1999 to 20 June 2019, we estimated the budbreak date and maturity date for each site of each year. We define the growing season as the time between budbreak and maturity and the ripening period as 30 days prior to maturity date. Therefore, at each vineyard site, maximum and minimum temperatures, rainfall, evaporation, radiation, and vapour pressure (at 9 am) were calculated for the growing season and maturity period for a 20-year period (**Table 3**). Each climate index was centred and scaled, and a correlation matrix was calculated (data not shown). Some highly correlated relationships ($r > 0.8$) were identified, in particular between the same indices calculated for the growing season and ripening period. Thus, only the indices calculated for the growing season were retained. Most of growing degree days (GDD) and growing season mean temperature (GST) indices were also highly correlated, and thus most growing season temperature indices were removed. After removing these highly correlated relationships, nine indices remained for the subsequent modelling (**Table 7**). The indices were roughly categorised into measuring either heat or water. The former included growing season degree days, growing season and ripening period mean temperature, cool night index, radiation and evaporation, while the latter included total rainfall as well as humidity measurements.

***Table 8.*** *Climate indices used in the modelling*

| Climate index | Type | Explanation |
|---|---|---|
| GSTp | Heat | Growing season temperature (phenological), i.e. average temperature between estimated budbreak and estimated maturity (˚C) |
| CNIp | Heat | Cool night index (phenological), i.e. average minimum temperature of 15 days preceding estimated maturity (˚C) |
| RPTp | Heat | Ripening period temperature (phenological), i.e. average temperature of 30 days preceding estimated maturity (˚C) |
| GDDph | Heat | Growing degree days (phenological), total GDD between estimated budbreak and estimated maturity. |
| RainGS | Water | Total rain during growing season, mm |
| EvapGS | Heat | Total evaporation during growing season, mm |
| RadnGS | Heat | Average solar radiation during growing season, MJ/m^2 |
| RHmaxTGS | Water | Average daily relative humidity at the day's maximum temperature during growing season, % |
| RHminTGS | Water | Average daily relative humidity at the day's minimum temperature during growing season, % |

A hierarchical cluster analysis was performed using the selected climate indices for the vineyard sites (**Figure 18**). Both indices and vineyards were clustered using the complete linkage method based on Euclidean distance. Cluster analysis revealed clear separation between heat and water related indices. Each vineyard generally

showed similar pattern for the same type of climate indices. Regional differences can be distinguished using climate indices, especially for Barossa, Hunter Valley, McLaren Vale and Yarra Valley. Hunter Valley showed very high GDD and GST, as well as very high rainfall and humidity measurements; however, solar radiation and evaporation were low for these vineyards. Both McLaren Vale and Barossa Valley vineyards were characterised by moderate GDD and GST terms, but low rainfall and humidity. Yarra Valley vineyards had low GDD and GST and moderate rainfall and humidity. The Canberra vineyards formed two clusters, each containing two vineyards. CB_1 and CB_2 clustered together and had slightly higher heat-related measurements and evaporation than CB3_3 and CB_4. HC_3 was separated from the other two Heathcote vineyards, primarily because of its higher heat-related measurements.

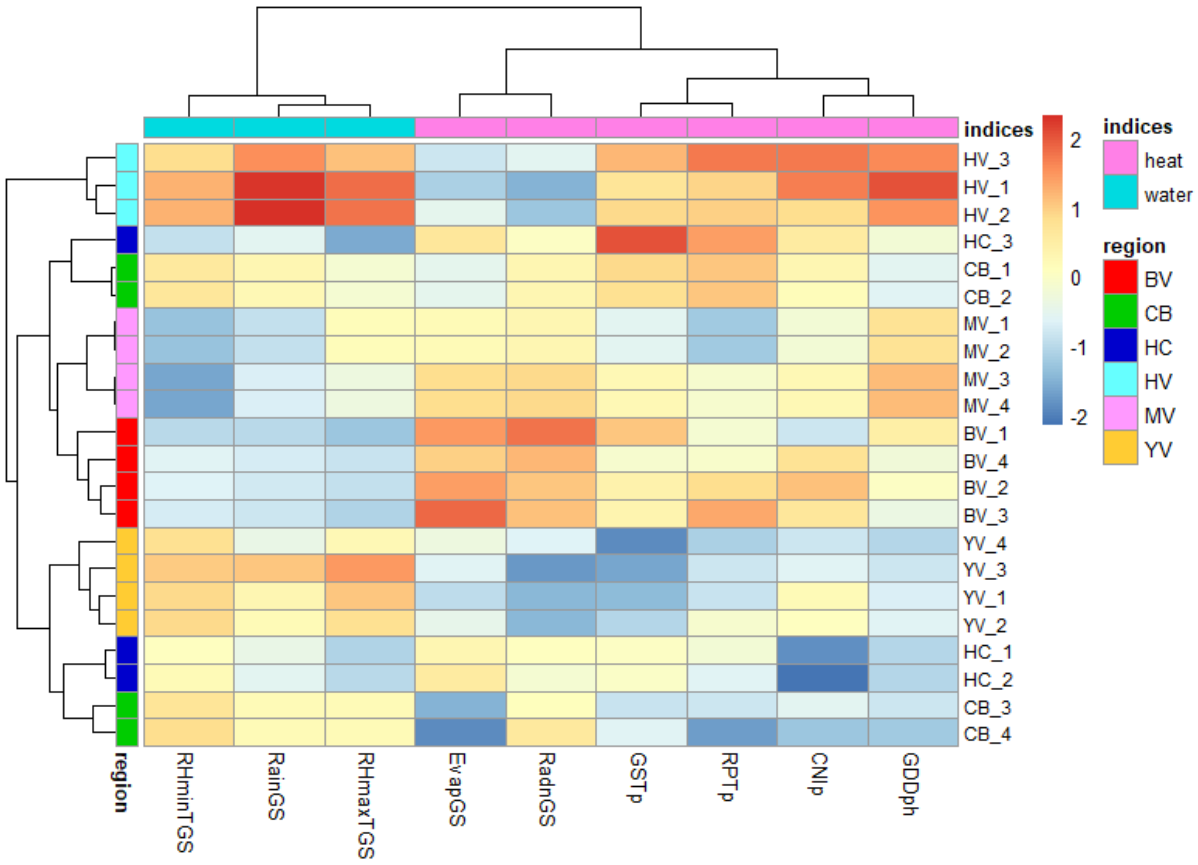



***Figure 18.*** *Heatmap using the selected climate indices. Each row is a separate vineyard from the GIs: Barossa Valley (■), Canberra (■). Heathcote (■), Hunter Valley (■), McLaren Vale (■), and Yarra Valley (■).*

### 12.2.1. Exploring the regional differences using multiple factor analysis

Overall, to explore the regional differences of Australian Shiraz wines, three types of measurements were performed, namely, descriptive sensory analysis, untargeted volatile compound analysis, and untargeted non-volatile compound analysis. Furthermore, climate indices were calculated for each vineyard site to reveal the possible correlation between regional climate patterns and wine sensory and compositional characteristics. To explore how each of the four types of data contribute to regional differences, a multiple factor analysis (MFA)

was performed, using the wine chemistry and sensory quantitative data blocks as active groups to find the common dimensionalities between the wines. GI and climate data were regarded as supplementary variables and projected into the subspaces defined by the active groups, in order to examine the relationship between climate indices and wine characteristics, as well as similarity and differences between GIs based on the overall wine profile. Only the variables that showed regional differences were used in the MFA, including the sensory characters that are significantly different across the regions determined by ANOVA, as well as regional volatile and non-volatile markers chosen by the random forests models (reported in the previous chapters). Overall, 144 variables were involved in the MFA model, with 80 volatile compounds, 26 non-volatile compounds, 29 sensory descriptions and nine climate indices.

The first two dimensions of MFA explains 42.8% total variability in the sample set (**Figure 19**). Non-volatile compounds had the highest contribution on both the first and the second dimensions, while sensory descriptions had moderate contributions on both dimension (**Figure 19** A). Volatile compounds had a high contribution on the first dimension and a moderate contribution on the second dimension. GI was explained well on both dimensions, while the climate indices were more related to the second dimension than the first dimension (**Figure 19**A).

MFA plot revealed distinctive clusters based on GI (**Figure 19** B). Notably, MFA is an unsupervised method, and therefore the clustering reflects the unbiased grouping between samples based on the chemical and sensory variables. Three distinctive clusters were separated on the first dimension, namely Yarra Valley/Canberra, Heathcote, and McLaren Vale/Barossa. The Hunter Valley wines had a wide spread on the first dimension, indicating variability among the wines in the variables that had high contributions to this dimension. However, the second dimension showed evident separation between Hunter Valley and the rest of the GIs. The Yarra Valley/Canberra and McLaren Vale/Barossa clusters were not separated by the second dimension, while Heathcote was more negatively correlated with this dimension, although large variability could be observed within this GI.

The top 30% variables that contributed to each of the first two dimensions of MFA were selected and visualised (**Figure 20**). A range of the volatile, non-volatile and sensory variables were chosen. Hunter Valley wines were characterised with higher red fruit characters, and were higher in a range of volatile and non-volatile compounds including phenol, manool oxide, 1-octanol, ethyl furan-2-carboxylate, γ-undecalactone, ethyl vanillate, dihydorxybenzoid acid-b-D-glucopyranoside and dihydrokaempferol. Directly opposite on this dimension were Heathcote wines and they were characterised with more dried fruit, dark fruit, beef stock, woody flavour, beef stock and bitter characters, and had relative higher concentrations of a range of unknown non-volatile compounds. McLaren Vale and Barossa wines were rated higher in vanilla/chocolate aromas and purple colour, and also had relatively higher concentrations of dihydroactinidiolide, γ-nonalactone, 3-allylcyclohexene and caffeic acid b-D-glucopyranoside. Directly opposite to these wines on the first dimension are Canberra and Yarra Valley wines, which were rated higher in cooked vegetable and drain aromas and had higher relative concentration of ethyl salicylate.

When climate indices were projected into the first two dimensions of the MFA scores plot (**Figure 21**), total rain during growing season (RainGS), phenological growing degree days (GDDph), average daily relative humidity at the day's maximum temperature (RHmaxTGS), and phenological cool night index (CNIp) were highly positively correlated with the second dimension. This was probably driven by their relatively higher values in Hunter Valley vineyards (**Figure 18**), reflecting the hot and humid climate characteristics in the Hunter Valley vineyards examined by the current project. Climate factors did not correlate well with the first dimension of MFA, except for moderate positive correlations of growing season mean temperature and ripening period mean temperature, possibly due to their relatively higher values in Barossa and McLaren Vale vineyards.

A range of unknown compounds, both from volatile and non-volatile analyses, were shown to be highly contributable to MFA. One of the significant limitations of the current study is the inability to identify a large proportion of the unknown volatile compounds and the majority of the unknown non-volatile compounds. More in-depth studies are needed to ascertain the molecular structures of the unknown compounds. Given the time consuming nature of the process, it can only be applied to a very small set of the most important unknown markers associated with the regional differences.

## **12.3** Conclusions

By integrating the sensory characteristics, volatile markers and non-volatile chemical markers discovered through untargeted analysis, regional similarities and distinctions could be found among Shiraz wines. The six regions examined in the current project, could be divided into 4 clusters, namely, Canberra District/Yarra Valley, Heathcote, Barossa/McLaren Vale and Hunter Valley. Hunter Valley wines were seemingly more distinctive in its sensory and compositional characters compared to other regions. A series of heat and water related climate indices modelled for the past 20 years showed distinctive patterns associated with each region, especially for Barossa, Hunter Valley, McLaren Vale and Yarra Valley. The correlations found between climate indices and the overall sensory and chemical composition of wines produced in a region were described.

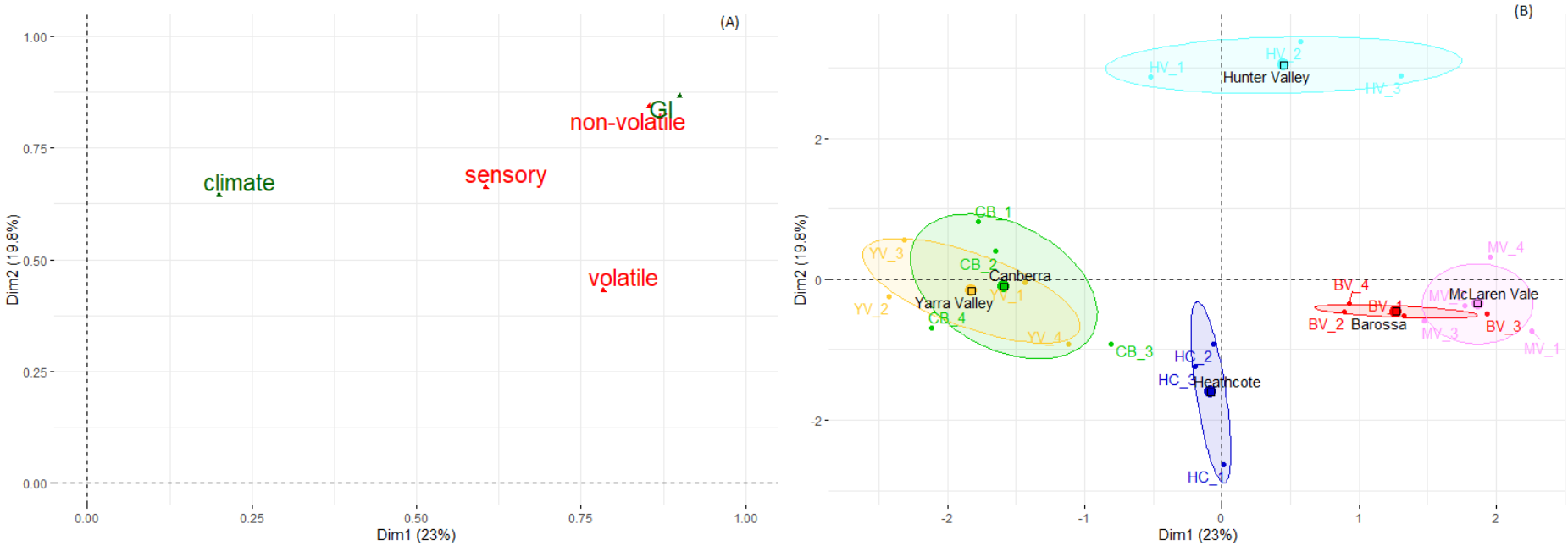



***Figure 19.*** *Multiple factor analysis of overall wine characteristics. (A) The contribution to the MFA by the three active groups of variables, i.e. sensory descriptors, volatile markers and non-volatile markers. Climate and GI were projected into the MFA dimensions as supplementary data. (B) Distribution of individual wines on MFA scores plot. The different colours represented GIs: Barossa Valley (■), Canberra (■), Heathcote (■), Hunter Valley (■), McLaren Vale (■), and Yarra Valley (■). Coloured circles represent the 95% confidence intervals. The mean point of each cluster was also shown.*

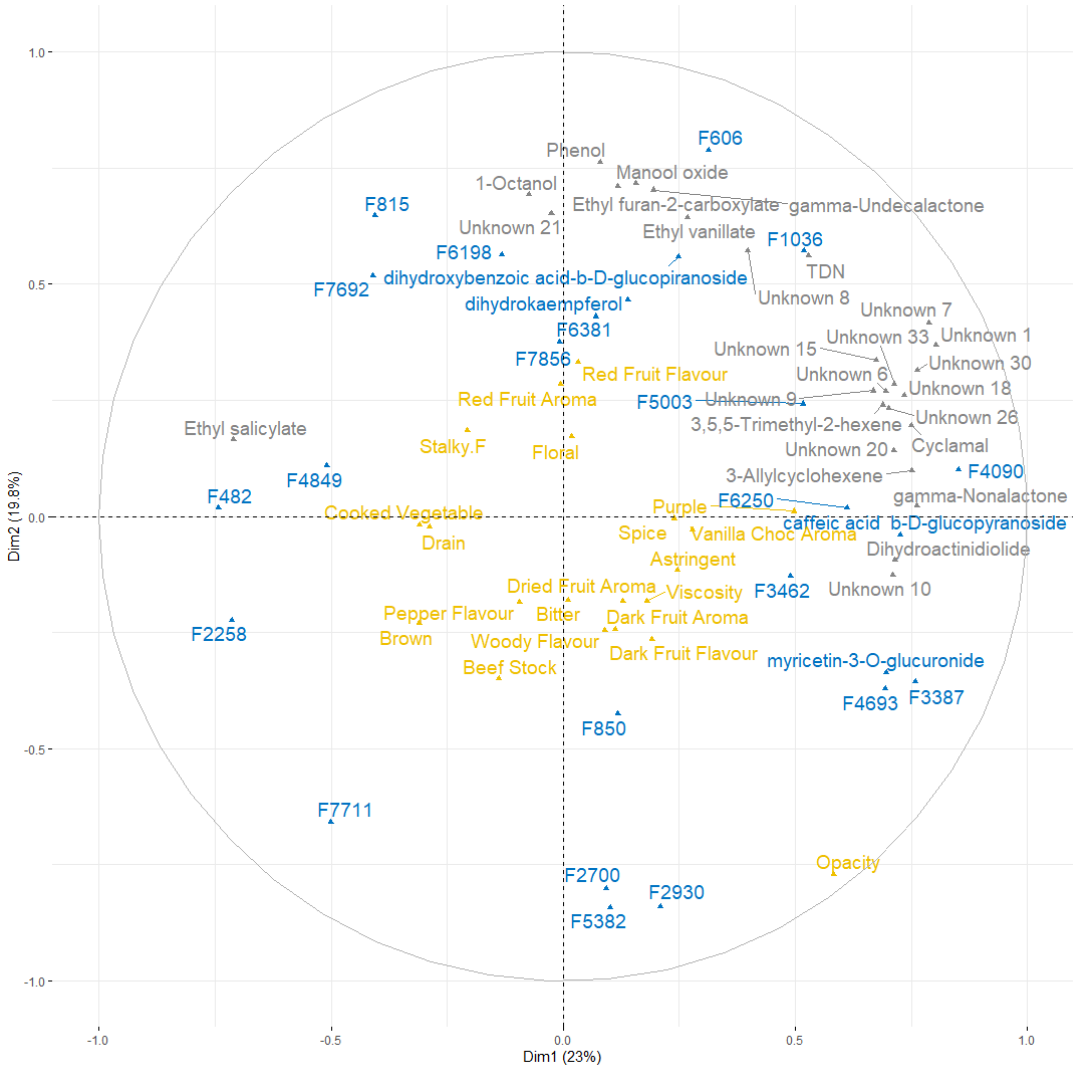



***Figure 20.*** *Active variables that were among the top 30% highest contribution to the first two dimensions of the multiple factor analysis. The different colours indicate different groups of active variables, with yellow denoting the sensory variables, blue denoting the non-volatile compounds and grey denoting the volatile compounds.*

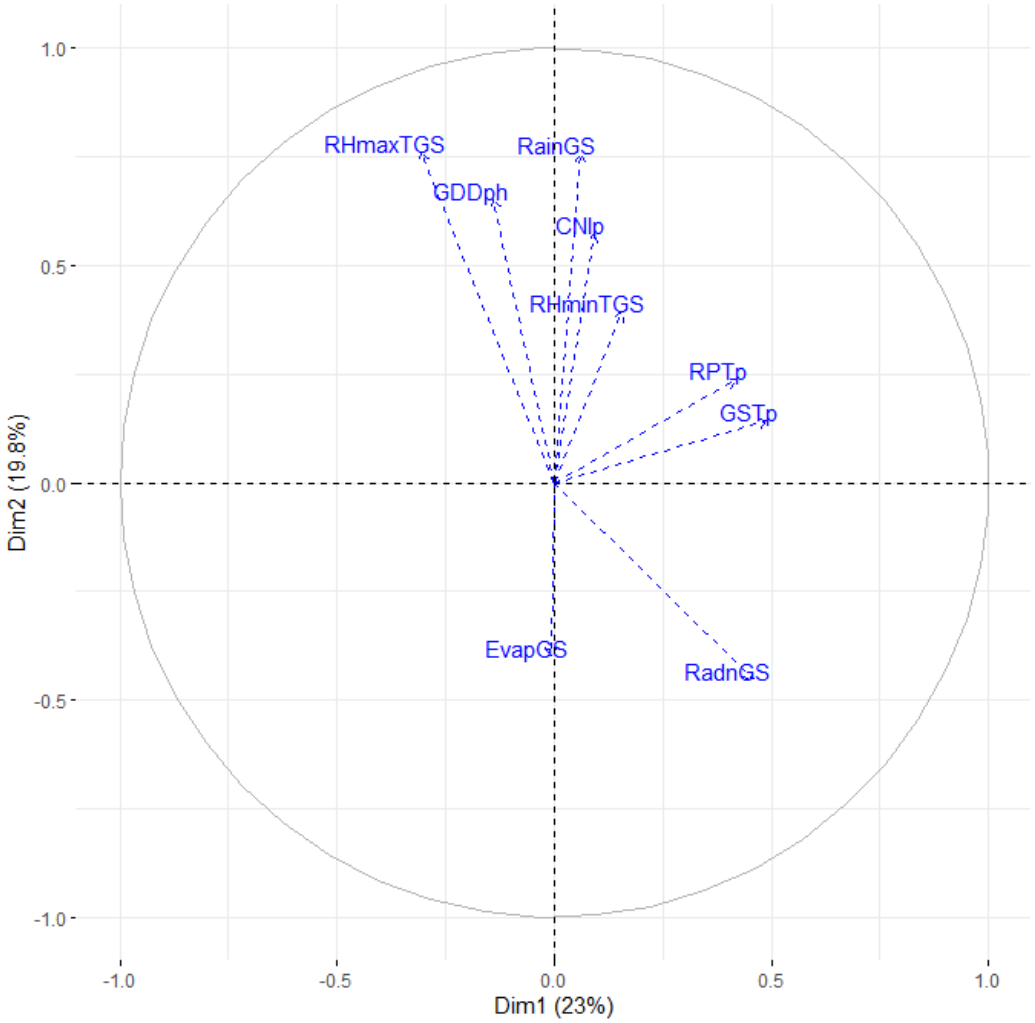



***Figure 21.*** *Climate variables projected into the first two dimensions of the multiple factor analysis as supplementary variables. The definitions of the variables are reported in* ***Table 7****.*

# 13 Conclusions / Outcomes

The sommelier tasting event was appreciated with excellent feedback from attendees and press. The quality and diversity of Australian Shiraz was demonstrated and introduced an influential group of wine professionals to the range of provenance-related research activities supported by Wine Australia.

The investigation of the Pivot© Profile (PP) method demonstrated that it produced consistent results with similar outcomes to that of the gold standard technique routinely used for sensory analysis, sensory descriptive analysis (DA). Subsequent use of the PP method in regional tastings confirmed it to be a suitable alternative in sensory studied in situations where time and cost preclude the use of DA.

This work has given detailed quantitative data on the sensory properties associated with each of the 6 important Shiraz-producing regions, Barossa Valley, McLaren Vale, Heathcote, Yarra Valley, Hunter Valley and the Canberra district. Distinctive, region-specific sensory characteristics have been detailed in chapter 2 of this report. This project also examined the correlations between the sensory properties of Australian Shiraz wines, their chemical profiles and the climatic regions from which the grapes were sourced. Examples of key associations described in chapter 3 include the Canberra wines being rated highly for floral characteristics and being high in monoterpene compounds. Yarra Valley wines were high in cinnamate compounds, which related to stalky characters while the Barossa Valley wines and McLaren Vale wines had higher dark fruit characters which were linked to compounds including damascenone, decalactone and ionones.

The approach of applying multiple site- and season-specific climate measures and relating these to chemical composition and the characteristic sensory attributes of regional Australian Shiraz wines can help producers better understand and influence the effect of place on their wines. The sensory fingerprints that differentiate one region from another will benefit both producers and the trade to appreciate what can be expected from different regions and allow targeting of production decisions to enhance distinctive sensory attributes and improved communication between marketers and consumers. The information can also be utilised help position the Shiraz wines in international markets. Grape growers, winemakers and wine brands can use this information to maximise the expression of terroir in wine to help secure a sustainable future for the Australian wine sector.

An untargeted metabolomic approach was also developed in order to provide an overview of the wine volatilome. Overall, the Shiraz wines studied showed distinctive regional chemical composition, with several volatile components identified as important discriminators. Esters including ethyl hexanoate, ethyl heptanoate, ethyl dodecanoate, ethyl 2-phenylacetate, ethyl 2-methylbutanoate and ethyl 3-methylbutanoate were found to be important enabling discrimination of wines based upon geographic origin. These compounds were found to be generally present in higher concentrations in the warmer regions studied. The cinnamate compounds were again found in highest concentration in the Yarra Valley. In this study a relatively small number of compounds were able to classify the wines according to their GI, indicating that a similar predictive model could be utilised for future classification studies. The difficulty in identifying most non-volatiles, including polyphenolics, was discussed and future research directions proposed.

Correlation of the sensory characteristics, untargeted volatile and non-volatile chemical compounds and climatic indices was also explored. Regional similarities and distinctions amongst the Shiraz wines was found, and the six regions could be divided into 4 distinct clusters, namely, Canberra District/Yarra Valley, Heathcote, Barossa/McLaren Vale and Hunter Valley. Hunter Valley wines were the most distinct in terms of sensory and compositional characters compared to other regions. A series of heat and water related climate indices modelled for the past 20 years showed distinctive patterns associated with each region, especially for Barossa, Hunter Valley, McLaren Vale and Yarra Valley. The correlations found between climate indices and the overall sensory and chemical composition of wines produced in a region have also been described.

# 14 Recommendations

The Pivot© Profile (PP) method was validated for quantitative sensory analyses for reasonably large sets of wines. As financial expenditures must to be carefully considered in all sensory studies, other research groups and in-house industry teams should use the method with confidence. Using the PP method as a platform to demonstrate the quality and diversity of Australian Shiraz has been regarded as a success at both international and national levels. Similar educational exercises with other varieties could be implemented to help market Australian wines. The study has also provided a template for future researchers to examine regionality, either with Shiraz or any other variety.

The sensory fingerprints that differentiate one region from another will benefit both producers and the trade to appreciate what can be expected from different regions and allow targeting of production decisions to enhance distinctive sensory attributes and improved communication between marketers and consumers. The information can also be utilised help position the Shiraz wines in international markets. Defining terroir influence will allow Australian fine wine producers to substantiate uniqueness claims to command premium prices in a global context. This project has accomplished for Shiraz and shown it is possible for other regions and varieties.

Future research could more closely investigate specific GIs and possible sub-regions within any GI chosen for investigation. Parameters that include vine differences as well as fruit and final wine characteristics could be monitored and identified for a number of sites in a particular GI. These could be related to environmental conditions by correlating with key climatic data and taking into consideration other important factors including; soil characteristics (e.g. soil moisture) as well as vine differences including age, clones and rootstocks. Management practices could also be investigated to help determine the potential to optimise the terroir of a particular site.

# 15 Appendix 1: Communication

## 15.1 Refereed Journals

Pearson, W., Schmidtke, L., Francis, I. L., & Blackman, J. W. (2020). An investigation of the Pivot© Profile sensory analysis method using wine experts: Comparison with descriptive analysis and results from two expert panels. *Food Quality and Preference*, ***83*****,** 103858.

Pearson, W., Schmidtke, L. M., Francis, I. L., Carr, B. T., & Blackman, J. W. (2020). Characterising inter-and intra-regional variation in sensory profiles of Australian Shiraz wines from six regions. *Australian Journal of Grape and Wine Research*, **26**(4), 372-384.

Li, S., Blackman, J.W. & Schmidtke, L.M. (2021). Exploring the regional typicality of Australian Shiraz wines using untargeted metabolomics. *Australian Journal of Grape and Wine Research*, DOI: 10.1111/ajgw.12493 (In Production).

Pearson, W., Schmidtke, L. M., Francis, I. L., Hall. A., Li, S. & Blackman, J. W. (2021). Regionality in Australian Shiraz: compositional and climate measures that relate to key sensory attributes. *Australian Journal of Grape and Wine Research*, AJGWR-20-107.R1 (Accepted 21/02/2021)

## 15.2 Conference Proceedings

Pearson, W., Schmidtke, L., Francis, L., Li, S., Blackman, J., & Carr, T. (2019). Understanding the role of regionality in Shiraz–sensory and chemical profiles of Shiraz wines from six different regions. In *17th Australian Wine Industry Technical Conference Adelaide, South Australia 21–24 July 2019* (p. 75).

## 15.3 Posters

Li, S., Schmidtke, L., Hall, A., Pearson, W., Francis, L., Vrhovsek, U. & Blackman, J. (2019). Unraveling regional typicality of Australian premium Shiraz through an untargeted metabolomics approach. In *17th Australian Wine Industry Technical Conference (AWITC 2019)*.

Li, S., Schmidtke, L., Blackman, J., Vrhovsek, U., & Carlin, S. (2019). Volatile compound finger print of Shiraz wine from 6 Australian GI. In *17th Australian Wine Industry Technical Conference (AWITC 2019)*.

Schmidtke, L., Li, S., Vrhovsek, U., Carlin, S., & Blackman, J. (2019). Unravelling regional typicality of Australian premium Shiraz through an untargeted metabolomics approach. In *Oeno (d'Oenoligie de Bordeaux) 2019/IVAS (In VIno ANalytica Scientia) 2019*.

## 15.4 Industry Journals

Pearson, W., Schmidtke, L., Francis, L., & Blackman, J. (2018). Provenance, preference and pivot: Exploring premium Shiraz with international sommeliers and Australian winemakers using a new rapid sensory method. *Wine and Viticulture Journal*, *33*(4), 35-38.

## 15.5 Seminars

Benchmarking Australian Shiraz terroir Wine Australia 50 world sommeliers event, Australian Shiraz regional tasting, Melbourne (03/02/2017) Pearson, W., Blackman, J. Schmidtke, L & Francis, I.L.

Benchmark Shiraz Tasting, Aussie Wine Week in New York (20/09/2017) Pearson, W., Blackman, J. & Davidson, M.

Benchmarking Australian Shiraz: a sensory exercise. VinExpo, Hong Kong. (30/05/2018) Pearson, W., Blackman, J. & Davidson, M.

Benchmarking Australian Shiraz Workshop. 17th Australian Wine Industry Technical Conference (AWITC 2019) 21/07/2019 Blackman, J., Hall, A., Pearson, W. &, Li, S.

Benchmarking Australian Shiraz Australia Decanted Lake Tahoe, United States. 10/07/2018 Blackman, J., Pearson, W. & Davidson, M.

Australasian Seminar - Masters of Wine Training course 18/11/2018 Blackman, J. & Pearson, W., Adelaide.

Regional typicality of Australian Shiraz Free Online Seminar presented by NWGIC 23/10/20 John Blackman & Sijing Li

Unravelling regional typicality of Australian premium Shiraz wines through an untargeted metabolomics approach. 13th International terroir conference Virtual Congress Adelaide (18/11/2020) Sijing Li

# 16 Appendix 3: References


Allamy, L., Darriet, P., Pons, A. (2018) Molecular interpretation of dried-fruit aromas in Merlot and Cabernet Sauvignon musts and young wines: Impact of over-ripening. *Food Chemistry* **206**, 245-253.

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# 17 Appendix 4:

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