Winegrowing Futures

Final report

Theme 5

Matching wine composition to consumers





The National Wine and Grape Industry Centre is a research centre within Charles Sturt University in alliance with the Department of Primary Industries NSW and the NSW Wine Industry Association

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THEME 5 Matching wine composition to consumers

Abstract

The outcomes of theme 5 fall broadly within three categories. Firstly, the project built capacity in the consumer-sensory, experimental winemaking and chemical analysis domains at the NWGIC. Secondly, the project developed new methods within those same areas. Finally, the project identified wine styles that appeal to consumers, how they can be developed and possibly even how they could be marketed.

Summary

Research in this report has shown that a substantial sensory profile can be produced for young Hunter Valley Semillon (HVS) which would be suited for immediate consumption. Furthermore, one style was identified that possessed both early drinking appeal and an apparent aging potential. Further research is needed to discover the winemaking and viticultural practices that lead to each of the four styles defined, as well as consumer preference research to determine whether all styles appeal to consumers.

In addition fresh fruit characters and acid-sugar balance were found to be important drivers of preference for HVS. To a lesser extent, developed characters were important to a specialised segment of the market. An avenue for Hunter Valley Semillon to become even more approachable to consumers, with a new focus on an early drinking style, was discussed. It is hoped that quintessential HVS, "one of Australia's great gifts to the world of wine", will become more popular with consumers in the long term, as they are introduced to the early drinking style and graduate to other styles.

The influence of the level of critical tasting and industry experience on sweet preference for consumer categories, reported here, has not been previously reported in the scientific literature. This investigation demonstrated that each consumer category based on knowledge, experience and involvement in the wine industry, preferred different levels of residual sugar and sweetness in HVS. Experienced consumers preferred wines with less added glucose than did the novice group and significant differences existed at high glucose additions (32.0 g L^{-1}). Research confirmed the necessity to control for level of experience within a general consumer group, not just between consumers and 'experts'. Results suggest that a higher than typical residual sugar level may increase consumer acceptance of HVS for novice and expert consumers alike, but may also alter winemaker's acceptance of specific styles of this wine. The use of paired comparison tests also enabled a quantitative basis for winemakers to produce HVS with a range of residual sugars appropriate to the acceptance of the consumer. By choosing test wines that were not typically associated with overt sweetness, the most likely conditions to find differences between 'experts', 'novices' and winemaker consumers have been fulfilled. Having established differences do exist between specific consumer groups for sweet preference in HSV, future research is now required to extend this investigation to other wine styles, and broader consumer segments such as specific export markets of importance to the wine industry.

The effect of three years bottle age on HVS has been investigated using sensory descriptive analysis. Different HVS wine styles have been shown to develop at different rates. The ability of many HVS wines to retain their original primary fruit character whilst also developing desirable aged attributes has also been demonstrated.

The ranking of perceived aroma compounds and the relative potency of aroma compounds as extracted from Semillon wine was investigated. Of the compounds identified, those stemming from yeast-fermentation processes would appear the most common group of compounds contributing to the aroma of the extracts. A range of unknown compounds were found to be of high aroma potency and are potential targets for future identification and recombination experiments.

Research led to an improved understanding of the role of carbohydrate and nutrient reserves during grape maturation under different environmental conditions, particularly with regard to fruit and wine composition. The understanding of different vineyard management practices on critical aroma and flavour compounds assists in optimizing grape production. Different vineyard management practices altered nutrient and carbohydrate reserves and ultimately vine performance. Understanding the impact of these

management practices on fruit N compounds assists in optimising grape and wine production. Post-harvest N additions generated different sensory attributes in wine compared with those wines made from different N-status plants. This information suggests further benefits from optimisation of N management in the vineyard. A complete amino acid profile of berry and wine samples was analysed to investigate more detailed nitrogen storage and mobilisation processes during berry ripening and fermentation. The analysis of this data is currently in process. Further research in yeast nutrient requirements is still necessary.

The effect of crop removal on Riverina Semillon was investigated. There was only a small impact on the final aroma profile of Semillon wines with 33% and, even, 50% bunch removal at pea size (EL stage 31). Any differences were too small for trained panellists to describe and, thus, probably too small for the average wine consumer to differentiate. Thus, reducing crop load of Semillon in the Riverina below what is normal seems to have little effects on wine flavour profile. The flavour components still need to be analysed chemically in the grape and in the wine at different stages of ageing to understand their synthesis and degradation. The vineyard itself seems to be more important than the actual crop removal. The wine of vineyard C showed a relatively strong change when the fruit was removed; on the other hand, no effect could be observed on the wines from vineyard Y. As with sites, the effect of season was more important for wine aroma profile than the fruit removal.

With regard to HVS, despite there being large differences between site characteristics and, even vineyard management, the experimental wines were only perceived to differ slightly. Differences were too small to be detected by the tasting panel. However, it is possible that some differences may have been masked by the winemaking protocol used. Furthermore, the season had a stronger effect on the final aroma profile than any of the site characteristics. Thus, it seems that being able to produce wines fitting into in the styles defined by this project is not limited by vineyard site characterisations or, indeed, many aspects of vine management.

Furthermore, research in this theme:

- Showed regional wine shows have the potential to be a vehicle to collect cost-effective and reliable consumer preference data.
- Developed a link between taste preference and personality type that offers a solution to improve on traditional market segmentation approaches to consumer preference studies.
- Validated a small scale wine making protocol for white wine.

Background

Site-specific practices and Semillon wine composition at different price points

There exists some scientific evidence that supports the relationship between yield and grape and wine composition (Chapman *et al*. 2004), but other work shows that it is not significant (Keller *et al*. 2005). Through pruning and fruit or shoot removal we can manipulate vine cropping levels. These different crop regulation methods can have varying levels of accuracy in targeting desired yields, different effects on vine physiology and fruit composition, and finally different economic consequences (some will be more expensive than others). This area has been a subject of some study (Clingeleffer 2002), however there is still a lot of uncertainty in the wine industry in this respect, and furthermore there are few scientific publications on this topic relevant to Australia. Vine cropping levels will also tend to vary with site and season.

With the sharp drop in grape prices, there is naturally increasing interest among grape growers to maximise yields while maintaining quality. This is especially relevant for the bulk and popular premium wine production in the Riverina. What consequences will these maximised yields have on fruit composition and wine attributes, and how will they affect vine physiology and short- and long-term carbohydrate partitioning (itself affecting vine health, productivity and longevity, Lakso 2006)—warrants an investigation. There are significant financial benefits for growers in knowing the maximum sustainable cropping level of their Semillon vines that will have no appreciable negative effects.

Adding to the complexity of the issue, the behaviour of vines under different yield levels is widely perceived to be affected by vineyard site (and season). This has not been sufficiently scientifically tested, not under Australian conditions.

A related issue is the investigation of the method for crop regulation that will give the best accuracy, but that will require lowest inputs, and at the same time cause no negative effects on vine physiology (including long-term effects). This has not been investigated in any great detail, but there is some

evidence that points to the relevance of such research (Peter Clingeleffer *pers. comm*.). These issues are of a pressing financial interest for grape growers that are struggling to obtain positive returns from their vineyards.

Different vineyard sites impose different environmental conditions on vines that, even when in a close physical proximity, can sometimes be quite distinct (Tesic *et al*. 2002). The effect of site on Semillon ripening and eventually on wine attributes, at different price point quality categories, needs to be examined. In addition, sites can exhibit a varying degree of interaction with seasonal conditions—vintage season will therefore be another important factor studied in this theme. This is particularly relevant for the production of super premium, noble rot-affected dessert wines from Semillon, for which the Riverina is increasingly becoming renowned. The environmental conditions that favour occurrence of noble rot, as well as viticultural practices that can increase the chances of this happening (G Barbeau *pers. comm*.) have not been investigated at all in Australia. This information can have great financial benefits for the producers of Semillon dessert wines.

Semillon wines from the Hunter Valley have international recognition as wines of outstanding quality. Aged Hunter Semillon is an icon wine with, for some styles, a marked capacity for longevity. Although one previous AWRI study examined flavour precursors in Semillon, no work has been reported so far that would attempt to relate viticultural practices and site-specific environmental conditions (meso-, micro-climate and soil type) on the development of these precursors and subsequently on flavour profiles of these ultra premium and icon wines. This knowledge would clearly have significant economic consequences for the producers of such wines in the Hunter Valley, and can also contribute to the more successful marketing by facilitating the development of regional and sub-regional 'signature' wines.

This theme seeks to address this limitation in industry knowledge of Semillon by examining factors that affect the flavour and aroma compounds. Field sites will be established in the Hunter Valley and in Riverina. At the Riverina sites treatments imposing different crop will be applied. Berry and wine samples will be examined using advanced chromatographic techniques and the resulting flavour/aroma profiles will be related to viticultural management practices.

The impact of vine reserves and crop load on grape and wine composition in Semillon vines

Vine reserves vary during the growing season, the multitude of these changes depend on nutrient uptake and assimilate production, but also on demand implemented by the vines for growth and development. Grapes are a major sink for both sugars and nutrients and therefore can depend on these reserves. It has been shown that timing of nutrient supply and irrigation management can have a considerable impact on grape composition (Holzapfel *et al*. 2003; Wade *et al*. 2004). The nutrient acquired after harvest can impact on vine nutrient status in the following season and have the potential to be carried over to the fruit in the form of higher amino N in the must (Holzapfel *et al*. 2006*b*), such timing of N applications are reflected in wine quality (Treeby *et al*. 2000). Altered nutrient and carbohydrate reserves accumulated during the post-harvest period (Holzapfel and Smith 2005) can impact on vine productivity and basic grape composition (Smith and Holzapfel 2003; Holzapfel *et al*. 2006*a*). These findings suggest that vine reserves can contribute to grape composition directly, but also indirectly through altered crop loads. Stress on the vine or nutrient and irrigation management might give the accumulated reserves a critical role during grape maturation, influencing berry composition and ultimately wine quality.

Site-specific practices and Semillon wine composition at different price points

Three Semillon field sites will be established in both the Riverina and Hunter Valley. The sites will be carefully selected according to different potential for yield and fruit/wine composition. Site conditions will be monitored by soil analysis, soil moisture measurements, on-site weather station, and the use of sensors to determine microclimatic conditions. There will be four complete seasons of data collected in this trial. The first season will be used for site selection and refinement of methodology and vinification protocols. Bunch removal treatments will be applied on replicated plots of twenty vines to provide three distinctly different cropping levels (low, medium and high). This set-up will provide enough grapes for small-lot winemaking even in the low yielding treatment. All other important factors, such as pruning, irrigation and nutrition, will be uniform across the trial sites within a region as per

standard industry practice. A replicated trial with potted Semillon vines will also be conducted at the NWGIC to separate effects of certain viticultural stresses (including nematode infestation) and their interactions.

At a planning meeting (26 April, 2006), the Hunter Valley Winemakers expressed a desire to have more information on consumer response to Hunter Valley Semillon. In order to obtain knowledge of consumer predilection to particular Hunter Semillon styles produced the following methodology would be followed for each wine:

- Consumer preference assessment
- Sensory profiling
- Chemical profiling

Viticulture trials will overlap each wine style being produced.

Berry and vine samples will be collected for analyses throughout the season and at harvest the trial plots will be hand picked and yield and yield components determined in both theme components. Berry samples will be analysed by GCxGC-MS as described below.

The small lot winemaking facility at the NWGIC in Wagga Wagga will be used to prepare wines from the Riverina trial on a 100 kg scale. The Hunter Valley wines will be made by a contractor in that region. Larger scale winemaking will be used for the Hunter Valley trial to allow increased resolution in characterising produced wines, especially their sensory evaluation. This will allow pinpointing site effects on sensory attributes, which are of particular relevance in the Hunter Valley, a region already perceived to have distinct differences between vineyard sites. Given the high value of the product in this region, these differences can translate into a significant different financial result.

Vinification protocols will be developed during 2006–07. Samples will be taken during and after the ferment for subsequent chromatographic analysis. Berries and wines will be analysed by GCxGC-MS for flavour and aroma compounds. Emphasis will be placed on using Solid Phase Micro Extraction to allow direct analysis without the need for sample preparation as this will minimise the loss of flavour and aroma compounds. LC-MS and LC-NMR will be used to determine non-volatile flavour compounds. Based on the chromatographic analyses, fingerprint profiles of the flavour and aroma components will be produced.

During 2007–08, the ongoing training of the tasting panel for this experiment will begin. The training will concentrate on flavours that are previously identified as relevant for the Hunter and Riverina Semillon wines in the background flavour research to be conducted in 2005–06.

All data will be analysed using Partial Least Square (PLS). This powerful statistical analysis is capable of uncovering relationships between different categories of data within large data sets that will be collected during the experiment. Collected data will be used to determine the most appropriate environmental and viticultural strategies needed to produce the best quality grapes and wines. Additionally, these data will be correlated with vine performance data (especially starch and soluble sugars) to produce a more complete picture of the intrinsic chemistry and quality determining components of Semillon grapes that impact on the quality of Semillon wines.

Aims

The aims of this theme were to

1. Clarify the link between vineyard management, vine physiology, fruit composition and wine composition in Semillon
2. Examine the link between wine composition and sensory attributes assessed by scientific methodology on the one hand with consumer preference on the other
3. Determine interaction of site-specific practices and the production of "super premium" category Semillon wines in the Hunter region
4. Enhance understanding of carbohydrate partitioning short- and long-term in differentially cropped Semillon (including maximum attainable yields) aimed at the "popular premium" wine category in the Riverina
5. Investigate the importance of carbohydrate and nutrient reserves on grape composition and the potential impact on wine quality
6. Investigate thinning methods, such as machine crop thinning and its effect on vine physiology and health in the Riverina
7. Use advanced chromatographic techniques (GCxGC-MS, LC-MS, LC-NMR) for aroma and flavour profile development in Semillon
8. Understand Semillon vine resource distribution during grape maturation on grape composition under different environmental conditions and crop level.

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5.5.5	What do consumers think about Semillon?	2
5.5.6	Winemaking Consistency	4 and 5
5.5.7	Wine Chemistry Analysis of Semillon Flavour Compounds	7
5.5.8	Grape and wine composition in Semillon influenced by vine resources under different environmental conditions	4, 5 and 8
5.5.9	Vineyard management and wine styles in the Riverina and Hunter Valley.	1, 3, 6 and 8

Experiments

A substantial part of Theme 5 was method development. The Theme has developed new methods to chemically analyse Semillon, a new robust method to make experimental white wine, and developed procedures to determine key wine styles and consumer preference.

The sensory and consumer work was broken into five sections:

1. Sensory characterisation of HVS, including definition of styles
2. Consumer preference for styles
3. Consumer acceptance for acid-sweet balance
4. Sensorial effects of aging
5. Marketing possibilities for HVS.

Rather than separately report method for each, and then results/discussion, we have presented each of the 5 sections in full below. We then outline the development of an experimental winemaking approach, including the results that have validated the approach. Following that, we present the methodology section of the chemistry work, followed by results/discussion in the following section.

Experiment 5.1
Characterisation of Hunter Valley Semillon

Materials and methods

The Hunter Valley is a premium wine region on the east coast of Australia. The two principle viticultural areas are the Lower Hunter, centred around Cessnock and the Upper Hunter, which encompasses regions adjacent to Denman, Singleton and Muswellbrook. The Lower Hunter produces the majority of the finest Hunter Valley Semillon (HVS) wines. Cessnock lies at 32°54'S, 151°21'E and most vineyards are approximately 200 m above sea level. Climatic features include a 23.0–24.9°C mean January temperature and high growing season rainfall including common precipitation during harvest (Gladstones 2004), initially suggest the environmental features are not suited to premium wine production (Halliday 1998). However cooling afternoon sea breezes and accompanying cloud cover aid in temperature moderation. Furthermore, warm nights encourage physiological ripeness rather than simply sugar accumulation, and quality wine, in particular Semillon and Shiraz, is produced (Gladstones 1992).

Hunter Valley Semillon (HVS) is regarded as an iconic style within the Australian wine industry and has an enviable, possibly unsurpassed, wine show record. Youthful HVS has the reputation of being austere and requires aging to provide substantial flavour (Robinson 2006). Henderson (2001) writes that it is dull before becoming wonderfully rich and succulent with age. Ryan (1992) defines HVS as light, grassy/herbaceous, before aging to develop rich honeyed, toasty and nutty characteristics. Clarke and Spurrier (2001) describe HVS as lean with an acid backbone before developing a more substantial palate with concentrated developed aromas and flavours with aging. The complexity of flavour that can develop in aged HVS has led to the description of the style as being "one of Australia's great gifts to the world of wine" (Robinson 2008). These types of descriptions would appear to indicate that a number of HVS categories exist.

Sensory descriptive analysis has been used to quantitatively characterise the sensory attributes of varieties from specific regions. Descriptive analyses of varieties including Pinot Noir (Guinard and Cliff 1987) Cabernet Sauvignon (Heymann and Noble 1987), Zinfandel (Noble and Shannon 1987) Chardonel (Mirarefi *et al.* 2004) and Seyval Blanc

(Andrews 1990) have been published. While a study of four Semillons from Turkey have been included in a sensory descriptive paper (Elmacı *et al*. 2007), a characterisation of possible styles of Semillon in any particular region has not been previously undertaken.

This study aims to investigate the range of profiles, or styles, typically found for HVS. This will validate whether bottle age is important to flavour development and whether effectively two styles exist; further, whether the wine is always austere when young, or whether substantial flavour profiles exist to warrant definition of additional styles.

The Hunter Valley Vineyard Association (HVVA) was requested to construct an extensive list of wines which they believed would represent the gamut of HVS flavours. The HVVA is an organisation that is composed of respected winemakers and viticulturists with vast experience in the production of HVS. The HVVA conducted a seminar with 70 participants in which each of the 32 nominated wines were discussed in an open forum. All wines were 100% HVS. The descriptors discussed were collated and a revised list of wines that contained as diverse as possible styles of wine was constructed. Particular focus concerned wines that had distinctly recognisable characteristics. Sixteen different wines from this inventory were requested from participating wineries and were subsequently donated. The wines used in the study along with respective vintage are listed in *Table 5.1*.

Chemical analysis

Table 5.1 also contains key wine chemical measures such as sugars and pH. The glucose and fructose assays were performed using a D-Glucose/ D-Fructose enzymatic kit from Boehringer (Mannheim, Germany). The pH and titratable acidity (TA) were performed using a Cyberscan 510 pH meter. The malic acid assay was measured using a L-Malic enzymatic kit from Boehringer (Mannheim, Germany). The alcohol was measured using the Anton-Paar Alcolyser DMA 450 Density meter.

Sensory Descriptive Analysis (DA)

The panel was composed of six females and nine males, 21 to 45 years of age. The panellists were selected to participate on the basis of interest and availability. All had previous wine tasting experience and the majority also had been involved with prior descriptive analysis panels that had also used the Compusense™ computer programme.

Table 5.1 Wine code, vintage and key chemical measures for each of the sixteen HVS wines selected for the descriptive analysis.

	Vintage	Glucose (g/L)	Fructose (g/L)	Titratable acid (g/L)	pH	Alcohol (% v/v)
Wine 1	2006	0.61	5.93	6.7	3.25	10.48
Wine 2	2006	0.93	0.97	7.3	3.05	10.71
Wine 3	2006	1.55	2.11	6.9	2.97	10.64
Wine 4	2005	0.13	1.25	6.8	3.08	11.87
Wine 5	2006	0.23	3.39	6.9	2.93	10.68
Wine 6	2005	1.41	1.56	7.7	3.09	10.13
Wine 7	2004	0.05	0.16	6.5	3.24	11.57
Wine 8	2002	0.32	0.31	6.5	3.15	10.32
Wine 9	1996	0.43	0.78	6.2	3.09	10.50
Wine 10	1998	0.24	2.04	7.5	2.89	10.37
Wine 11	2001	0.16	0.27	8.2	2.92	11.33
Wine 12	2006	0.13	0.00	6.3	3.09	10.43
Wine 13	2002	0.17	0.03	7.5	2.89	10.12
Wine 14	2003	1.49	1.73	7.1	3.16	11.37
Wine15	2002	0.49	3.48	7.6	2.89	10.09
Wine 16	2006	0.00	0.08	7.7	2.84	10.64

Training: The training period consisted of eighteen group meetings over a nine week period week in May–July 2007. The first purpose of these sessions was to develop a list of aroma descriptors that would describe and differentiate the 16 wines. During the first two meetings, panellists were asked to describe the wines before them, without consulting reference aroma standards. At both sessions two young and two old wines were provided and panellists were asked to list up to six of the most dominant aromas in each wine. Results were then tabulated on a white-board which provided immediate feedback to panellists. They were then instructed to sample the wines again to see if the descriptors provided a means of labelling that enable recognition. Thirty-seven different descriptors were obtained from the first two sessions and are listed on *Table 5.2*.

The next three sessions each had 12 or 13 descriptors and an accompanying reference standard. Panellists were asked whether or not they could detect each attribute in each of four wines. They were also asked to evaluate the 37 different aroma standards and determine how those standards should be modified and the possibility of additional standards to be introduced. Terms that were detected by less than three of the judges were eliminated.

As the training sessions continued, the list of descriptors was gradually refined such that a smaller number of descriptors were included in the final testing. The inclusion criteria followed the international standard ISO 11035:94 (ISO 1994), where consideration was given to the relevance to HV Semillon, the discrimination between samples afforded and the panel's ability to simply detect and easily know each descriptor. This was achieved by evaluating three or four wines in duplicate for specific attributes characteristics on different days, performing statistical analyses and providing feedback to panellists.

At the conclusion of the training period, thirteen attributes were selected and their corresponding standards agreed upon by consensus. The list of attributes and composition of the matching standards are presented on *Table 5.3*. Additionally, sweetness and acidity reference standards were presented so that rating of these tastes could be practised. Rating of acidity and sweetness were practised in a similar manner as that described for aromas. Finally, the panellists conducted two training sessions in to familiarise themselves with the Compusense® program used to collect the results.

Table 5.2 Exhaustive initial list of descriptors for Hunter Valley Semillon.

lime	lemon	nutmeg	leafy
asparagus	honey	toast	straw
caramel	hay	vegemite	vanilla
kerosene	pea	dusty	apricot
nut	grapefruit	coconut	citrus
toast	lychee	peach	confectionary
melon	butterscotch	butter	bread
citrus	capsicum	caramel	pineapple
honey	grass	oak	floral

Formal Judging: The 16 wines were evaluated in triplicate in 12 tasting sessions held over a two week period. The Compusense™ programme generated random three digit identification numbers for each wine at each session. This number was transcribed on to appropriate standard (ISO 1997) tasting glasses. The order of presentation of the wines was determined using a randomised complete block design with three replications performed by each panellist for each wine. Four wines were tasted in each tasting

Table 5.3 List of attributes and composition of standards.

Term	**Composition of reference standard***
Asparagus	½ teaspoon of juice from canned asparagus
Confectionary	chopped green and red Allen's ™ frog-shaped jelly confectionery
Floral	2 rose petals soaked for 10 minutes and removed
Grapefruit	1 teaspoon of fresh grapefruit juice and small piece of rind soaked for 5 minutes and removed
Grass	¼ cup of fresh grass soaked for 10 minutes
Hay/straw	¼ cup horse chaff soaked in wine for 15 minutes
Honey	2 teaspoons of honey
Kerosene	0.001 µL kerosene
Lemon / lime	½ teaspoon each of fresh lemon and lime and small piece of each rind soaked for 5 minutes and removed
Lychee	3 teaspoons of juice from canned lychee
Orange marmalade	3 teaspoons stirred thoroughly
Toast	scrapings from 1 piece of white toast

* In 50 mL of 50:50 distilled water:base wine

session. All evaluations were conducted under white fluorescent lights in individual tasting booths at room temperature of 22±1°C. Wine samples were served at 13±2°C.

At the beginning of the tasting session, panellists were asked to sniff each of the aroma standards. The panellists then rated each of the attributes using an unstructured line scale (ISO 2003) from 'Low' to 'High'. Panellists were requested to sniff water regularly to help alleviate fatigue as well as rinsing their mouth with filtered water and waiting for 30 seconds between samples. Results were entered directly into the Compusense™ programme via computers in each tasting booth.

Results and discussion

A two-factor PCA solution was accepted since two factors had eigenvalues above two, each accounted for a substantial proportion of the variance and characterised a key conceptual dimension in HVS. Factor one was characterised by 'bottle age', accounting for 72.6% of the variance and factor two characterised by an 'acid-sweet' dimension and accounted for 9.9% of variance. In total, the two-factor solution accounted for 82.5% of variance, representing good explanation of the dataset.

A factor plot is shown at *Figure 5.1*, with descriptors only. It shows that bottle age contrasted wines high in toast, honey, orange marmalade and kerosene to those wines higher in floral, confectionary, lemon/ lime, grassy and pineapple characters. The toast, honey and orange marmalade characteristics in particular and to a lesser extent a kerosene character have commonly been associated with HVS wines with some bottle aging (ISO 2003, Iland and Gago 2002). These characters are often termed 'developed' characteristics.

Figure 5.1 overlays the 16 wines on the two-factor solution and illustrates that the wines with 'developed' characters are the wines of 5–11 years of age. The wines driven by floral, confectionary, grapefruit and grassy characters are 1 year old at the time of analysis.

The second principal component or 'acid-sweet dimension' is chiefly influenced by acidity with opposite characters of floral, confectionary, pineapple, honey and orange marmalade. This dimension is not simply acid-sugar, but a more complex acid-sweet, where sweet is associated with characters such as floral and honey. Chemical analysis of the wines suggested that overall sugar was not a major differentiator, suggesting that principle component one is not simply an acid-sugar dichotomy. The relative sweetness of fructose is considered to be greater than that of glucose (Stone and Oliver 1969) and some wines had fructose levels above 3 g L^{-1}. Fabian and Blum (Fabian and Blum 1943) report that the sensitivity to fructose is 3.5 g L^{-1} such that some wines may elicit a higher perception of relative sweetness. Wines 1, 5 and 15 were highest in fructose and amongst the highest in overall sugar, yet were well separated by this second principal component by a factor which is not readily apparent.

As well as illustrating the relationship amongst the various descriptive terms, the PCA also displays the proximity of each wine with respect to both each other and their defining characteristics (see *Figure 5.2*).

Wines with similar characteristics were determined from the PCA and combined with the use of mean intensity ratings; wines were then classified into one of four styles. The sensory profile of each style is represented on *Figure 5.3*. In this figure, the centre represents low attribute intensity, with increasing intensity towards the perimeter.

Style 1: Only one wine was located in this region of the PCA. Wine 1 was from the 2006 vintage and had the highest average rating for confectionary, floral, lemon/ lime and pineapple aroma. The highest residual sugar was also clearly apparent to the tasting panel as this wine had a high intensity rating for sweetness. Wine 1 also had the highest pH of all samples and one of the lowest titratable acidity (TA) measurements. The perception of acidity is a complex phenomenon, influenced by sugar and acid levels to name just two. The sensory panel rated this wine style as having the lowest perceived acidity level, probably a direct reflection above. This is an unusual style for HVS, which probably explains its isolated location on the PCA. Wine 1 also had the lowest intensity rating averages for kerosene, toast and orange marmalade attributes. Produced in order to maintain a relatively high sweetness and lower acidity, Style 1 has relatively overt fruit characters and would appear to be suitable for early consumption.

Style 2: All wines in Style 2 are from either from the 2005 or 2006 vintages. Wines in this group have a lower perceived sweetness and higher perceived acidity than Style 1. The lemon/ lime, lychee, floral, pineapple and confectionary characters are also lower but more intense than in Styles 3 and 4. The grapefruit aroma is higher in Style 2 than for any other styles. The higher acidity and lower sweetness indicates these wines are made with future bottle development anticipated. At

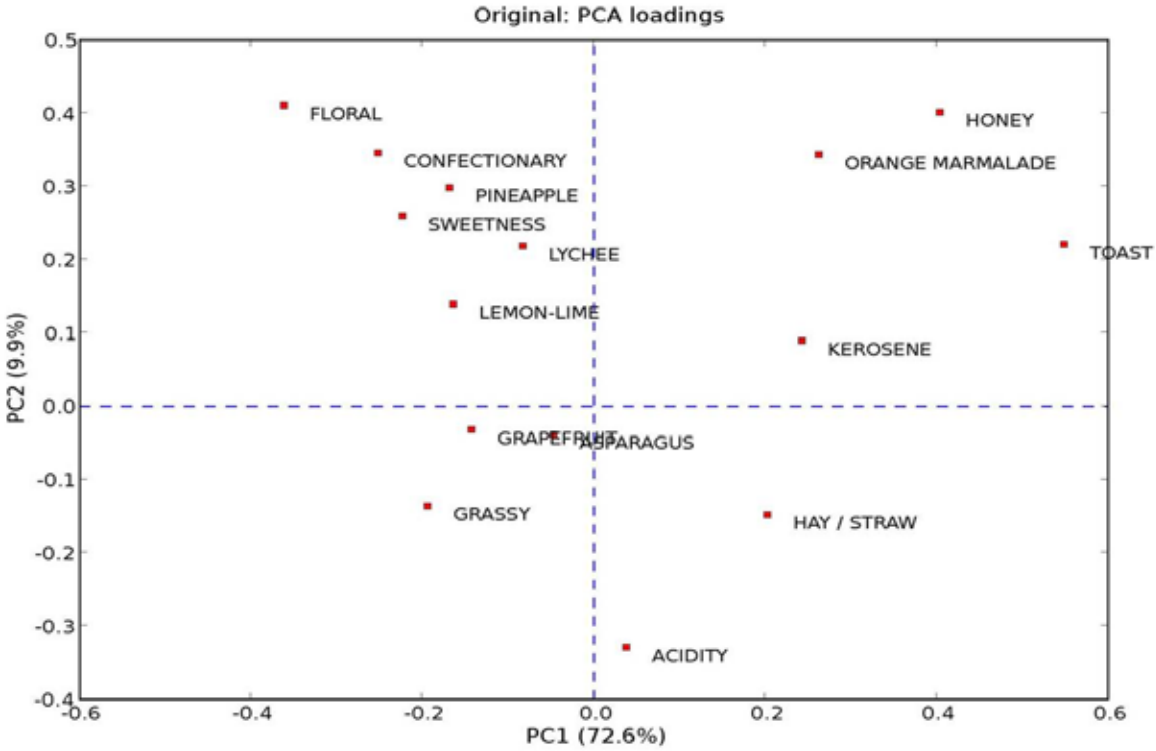


Figure 5.1 PCA factor plot for attributes, where PC1 is 'bottle age' and PC2 is 'acid-sweet'.

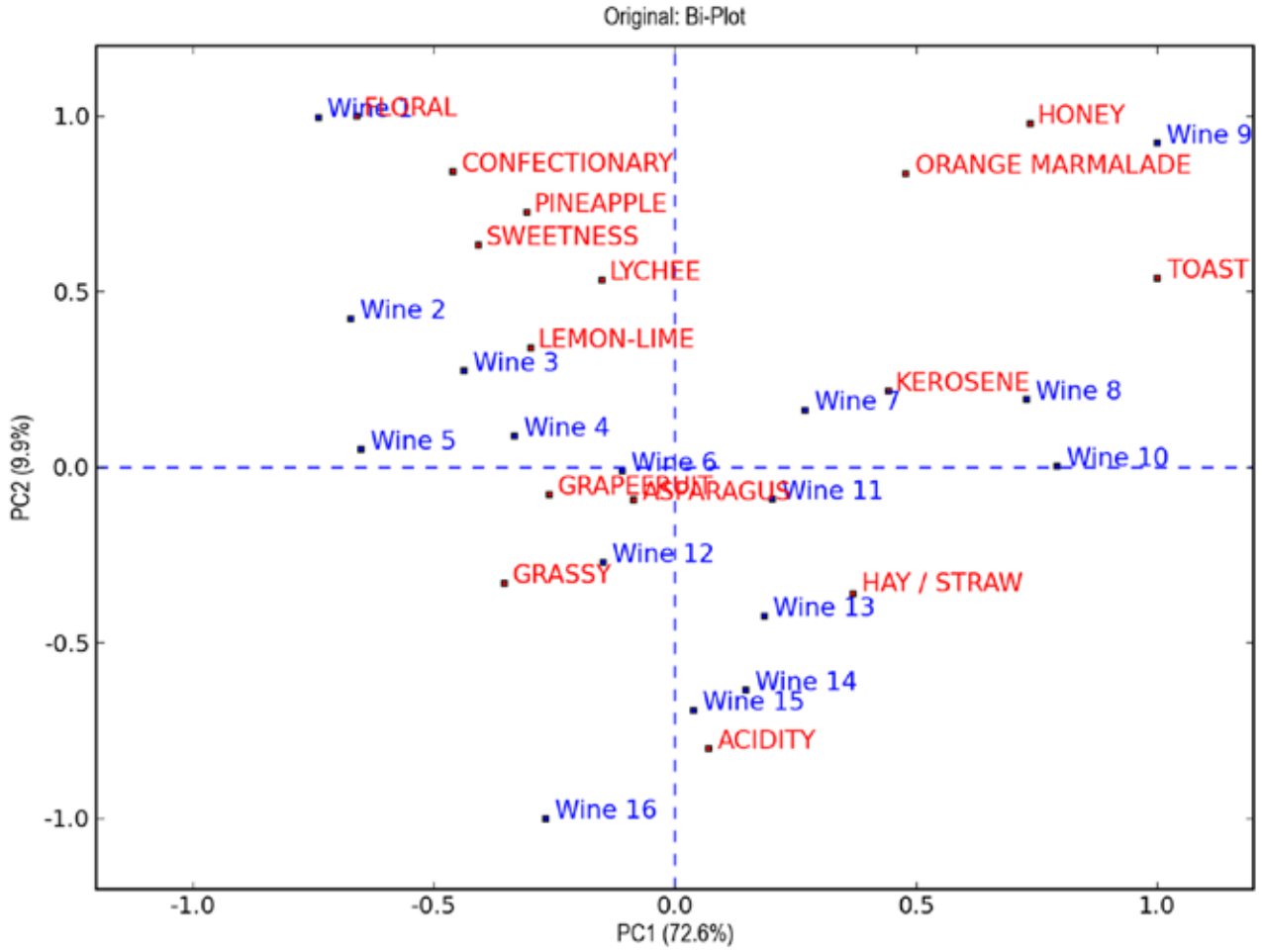


Figure 5.2 PCA factor plot for attributes with wines overlayed. PC1 is 'bottle age' and PC2 is 'acid-sweet'.

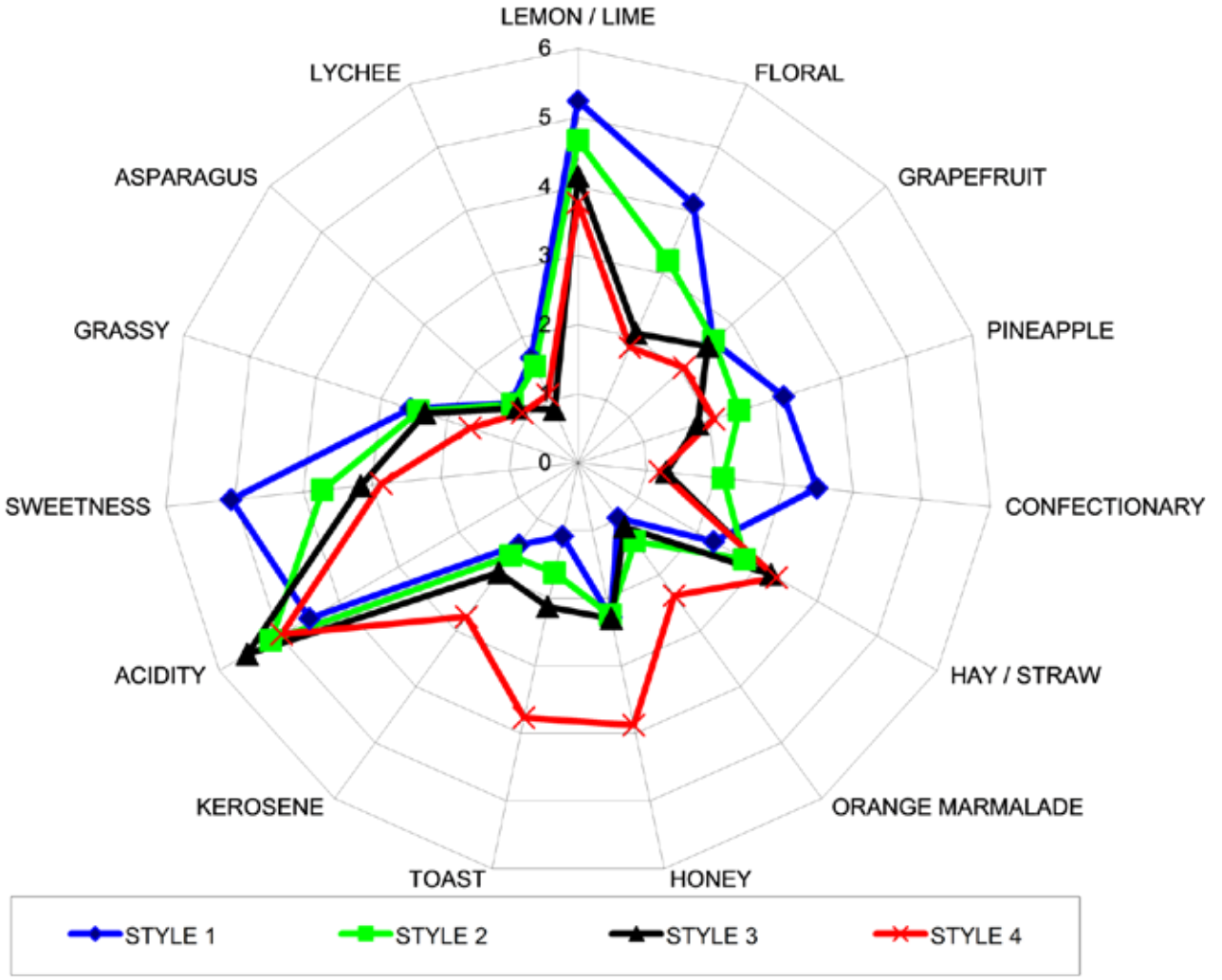


Figure 5.3 Mean attribute intensity of wines in each of Styles 1, 2, 3 and 4.

the time of testing though, developed characters of honey, toast, orange marmalade and kerosene were relatively low. The relatively high intensity of fresh fruit characteristics also would appear to make these wines an attractive early drinking proposition (as for Style 1) with the potential for positive development with further bottle age.

Style 3: The wines which are located in this section of the PCA had the highest perceived acidity of all styles. The wines from this category had the lowest pineapple and lychee aroma of all wine styles and the intensities of floral, confectionary, grapefruit, asparagus, grassy and lemon-lime aromas are lower than were found in Styles 1 and 2. *Figure 5.3* illustrates that Style 3 is also relatively low in perceived sweetness. Acid levels are important for bottle aging potential, such that this style seems to be intended for aging; it is likely that these wines will transition to Style 4 after several years.

Style 4: All wines in this section of the PCA had a minimum age of three years. The three oldest wines in the study; Wine 11 from 2001, Wine 10 from 1998 and Wine 9 from 1996 are located in this region of the PCA. Wines in Style 4 were found to have the highest intensity of developed characters—toast, honey, orange marmalade and kerosene. The highest hay/straw character average was also obtained by this cluster of wines. Not surprisingly the oldest wine, Wine 9, was found to have the highest honey, toast and orange marmalade characters. Interestingly, a 2002 vintage wine, Wine 8, had the second highest honey and orange marmalade intensity as well as the most intense kerosene aroma.

Style 4 wines are lower in fruit characters (floral, confectionary, grapefruit and lemon/ lime) and grassy character in comparison to Styles 1, 2 and 3. Nevertheless, it is apparent the lemon/lime aroma is still present in this Semillon style. Similarly, the average acidity intensity was only marginally lower than found in Styles 2 and 3 and higher than Style 1. The lowest perception of sweetness was also observed in Style 4. This style aligns most with the colloquial description of HVS–developed characters along with maintaining lemon-lime characteristics and fresh acidity (Ryan 1992, Bulleid 2005).

Experiment 5.2
Consumer preference of Hunter Valley Semillon

Materials and methods

Four unique styles of Hunter Valley Semillon (HVS) have been identified (Blackman and Saliba 2009). This study aimed to determine the consumer taste-preference for each style. The next phase of the work was to determine the level of consumer interest in each style, to further assist wine businesses to balance their wine portfolio. By establishing demand for each style, businesses can be strategic about how much wine is produced in each category.

A total of 303 adults were recruited from food and wine events staged in Sydney and Wagga Wagga, NSW. Participants indicated preferences for each of the six wines and also answered a range of socio-demographic questions. An analysis of variance (ANOVA) revealed no significant differences in preference between the two locations, so data were combined for further analyses. The mean age of participants was 39.9 years (SD=15.1), with a minimum age of 18 and maximum of 78 years.

In addition to the preference ratings for each of six wines, participants answered purchase intent and answers to a range of questions about HVS which were used to derive the marketing plan reported separately.

Results and discussion

ANOVA (not shown) showed that Style 1 was significantly preferred to the other styles. The predilection for this style was also clearly demonstrated by all internal preference maps (*Figure 5.4A, B* and *C*).

Style 1 had more sweetness and a lower acidity than the other styles. It also had the highest confectionary attribute and was amongst the highest for lemon/lime, pineapple and floral. Style 2 wines were characterised by lemon/lime crispness and were preferred by frequent drinkers. Style 4 wines, those with more developed characters, were preferred by more experienced consumers. Style 3, defined by taut acidity, rated slightly higher than Style 4 in mean preference (*Figure 5.6*). However no definable segment was found to like Style 3. These results are summarised by *Figure 5.5*.

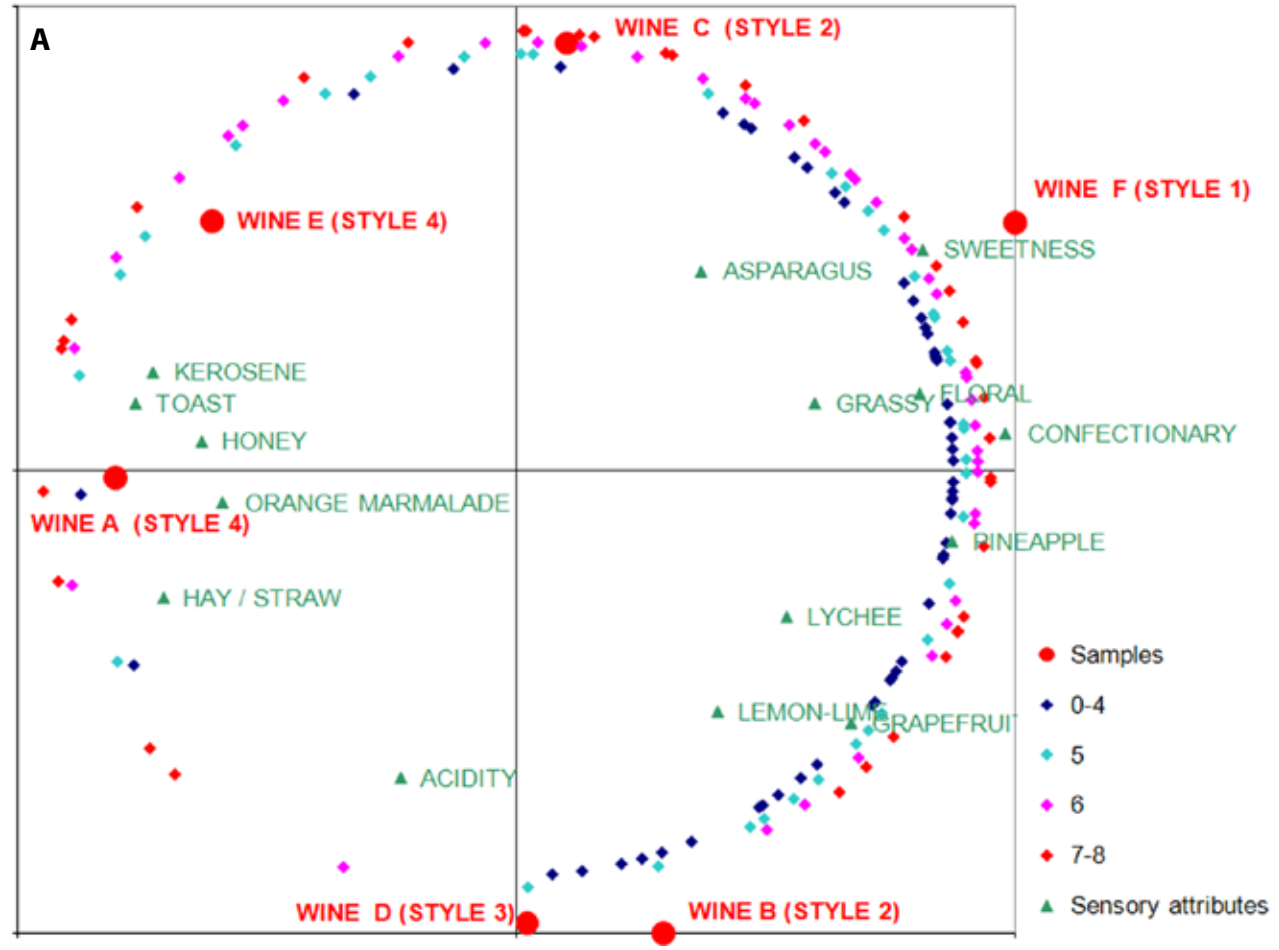
To a lesser extent, developed characters associated with Style 4 were important to a niche group, and future work in the project will examine the sensory changes associated with moderate bottle ageing. The issue of acid-sugar balance was thought to be implicated with level of experience. As such, a preference study was conducted using three groups: winemakers from the Hunter Valley region with experience with HVS; an experienced wine drinking consumer group; and a novice group.

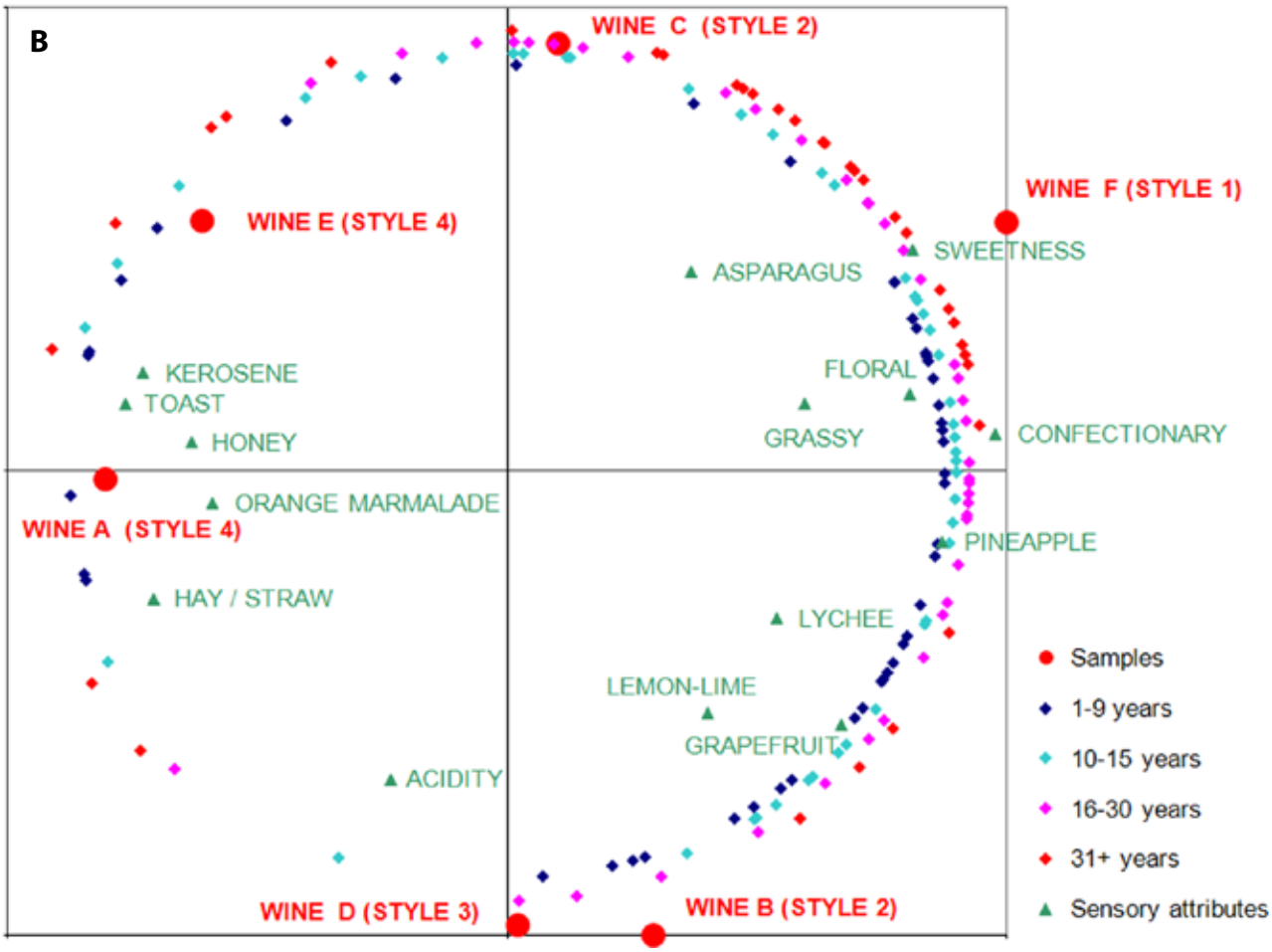
A summarised pictorial of the results of the internal preference mapping can be seen in *Figure 5.5*. Style 2 was preferred by frequent drinkers, presumably as a good match with food at the dinner table. Style 4 was preferred by more experienced and consumers who self-reported as 'knowledgeable', making it a good target for that niche group. Style 3 did not have any particular group that preferred it more than another style, however, it is noteworthy that it is sometimes considered the precursor to Style 4 after aging.

Implications for winemaking: The internal preference mapping also reveals that none of the groups who disliked the Style 1 wine, leading to the conclusion that Style 1 is an ideal wine to satisfy a large range of consumers. The 'one size fits all' approach may not be ideal in a wine context, however, it is useful for a region to have a style that has a broad range of interest amongst consumers.

As all four styles are important to the Hunter Valley and will continue to be produced, the possibility of using Style 1 wines as an introduction to HVS has been suggested. This may enable HVS to become more popular with consumers in the long term, as they are introduced to this early drinking style and subsequently graduate to other styles. Style 1 has been discussed in detail in earlier sections, possessing moderate sugar and acid levels with fresh fruit aromas, could be vinified by picking fruit which is slightly riper than in the case of Style 2. This would allow a reduction of acidity, known to be found challenging to novice consumers (Blackman, Saliba and Schmidtke 2010). Halting the fermentation when some residual sugar remained and a possible short time on fine yeast lees could help produce the "softer" Style 1. Style 3 wines, slightly austere with noticeable acidity, are sometimes considered the precursor to Style 4 after aging. As Style 4 is wine style of undisputed importance, earlier picked batches must also be considered but the avoidance of green characters appears necessary.

Implications for viticulture: Grapegrowers in the Hunter Valley have had generations striving for the right balance for Semillon, and with an enviable wine show record, who would argue that they have it wrong? Anecdotally, vineyards with alluvial


A
WINE C (STYLE 2)
WINE F (STYLE 1)
WINE E (STYLE 4)
SWEETNESS
ASPARAGUS
KEROSENE
TOAST
HONEY
GRASSY
FLORAL
CONFECTIONARY
ORANGE MARMALADE
WINE A (STYLE 4)
PINEAPPLE
HAY / STRAW
LYCHEE
LEMON-LIME
GRAPEFRUIT
ACIDITY
WINE D (STYLE 3)
WINE B (STYLE 2)
Samples
0-4
5
6
7-8
Sensory attributes


B
WINE C (STYLE 2)
WINE F (STYLE 1)
WINE E (STYLE 4)
SWEETNESS
ASPARAGUS
KEROSENE
TOAST
HONEY
FLORAL
GRASSY
CONFECTIONARY
ORANGE MARMALADE
WINE A (STYLE 4)
PINEAPPLE
HAY / STRAW
LYCHEE
LEMON-LIME
GRAPEFRUIT
ACIDITY
WINE D (STYLE 3)
WINE B (STYLE 2)
Samples
1-9 years
10-15 years
16-30 years
31+ years
Sensory attributes

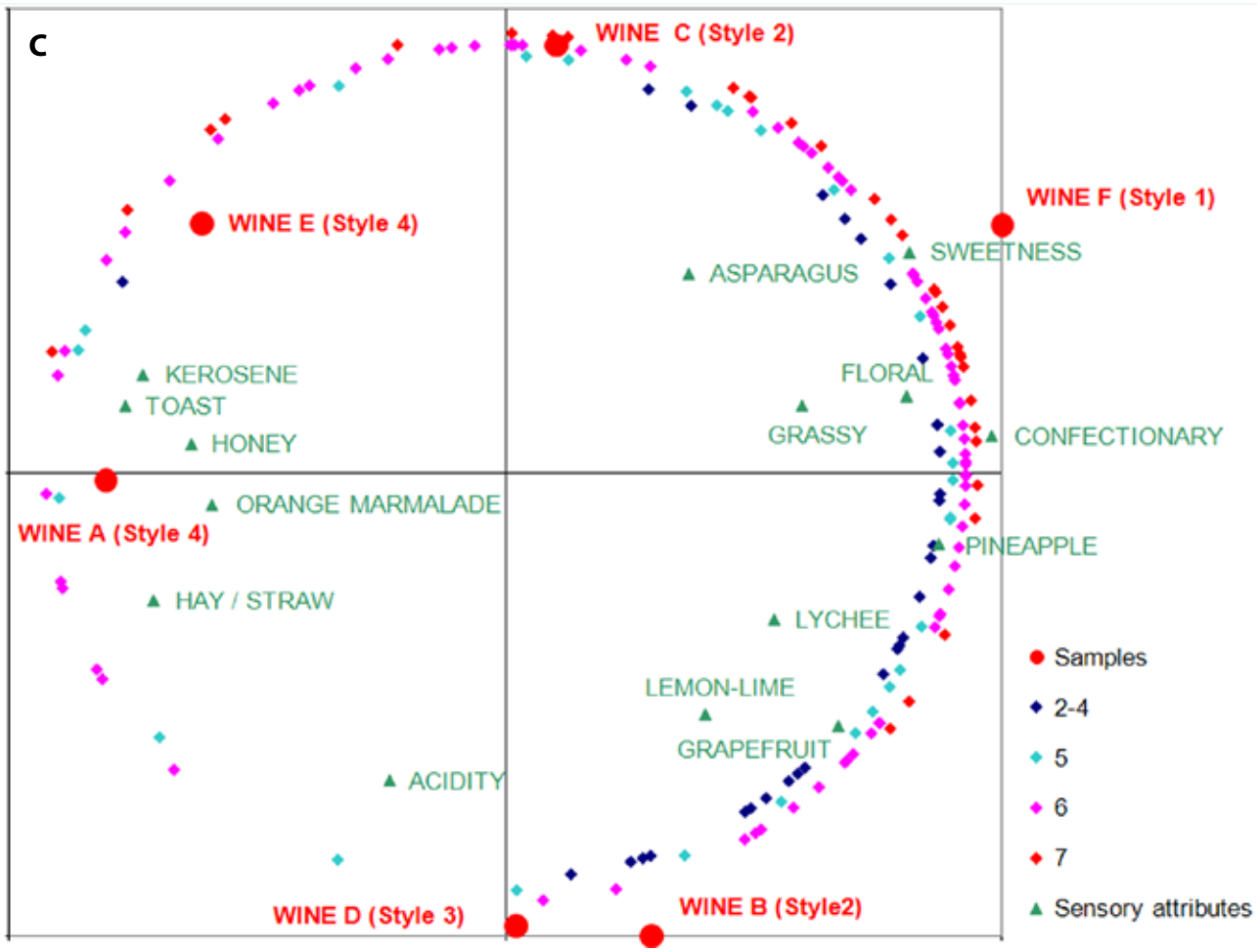


Figure 5.4 Internal preference maps of HVS wines. A: self-reporting of wine knowledge; and B: years wine has been consumed.

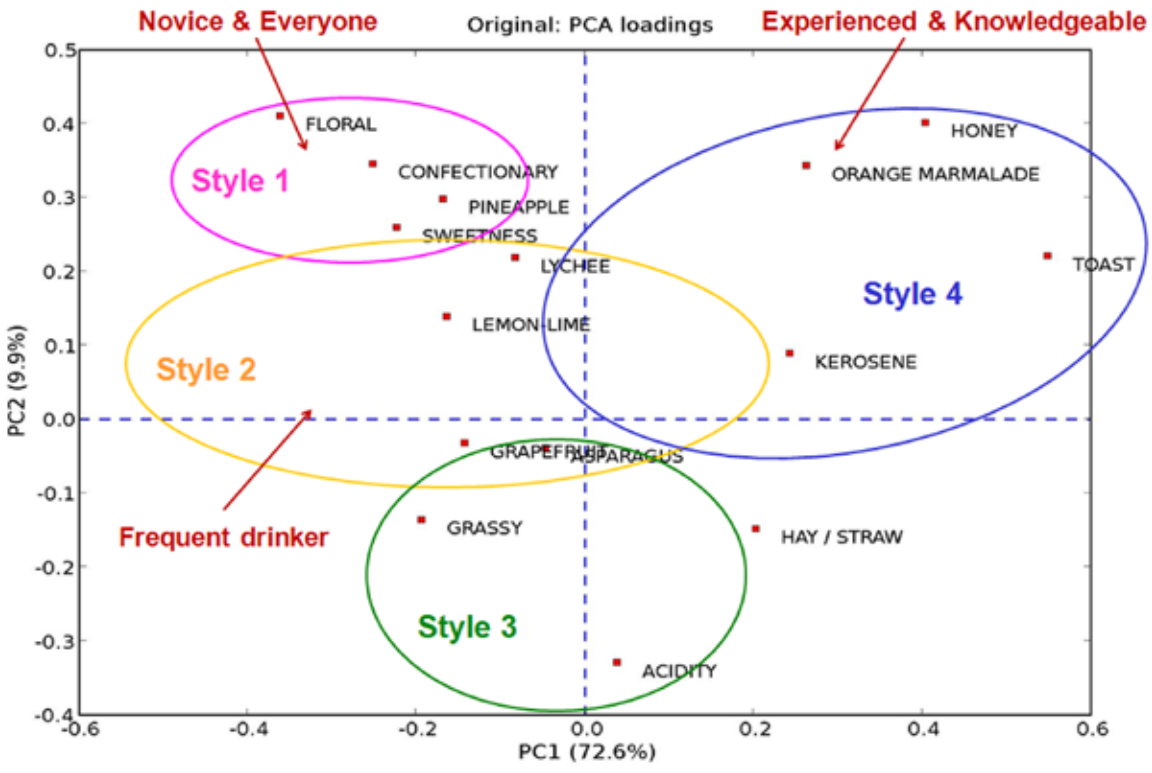


Figure 5.5 Factor plot illustrating sensory attributes, style boundaries and consumer segments showing a preference for each style.

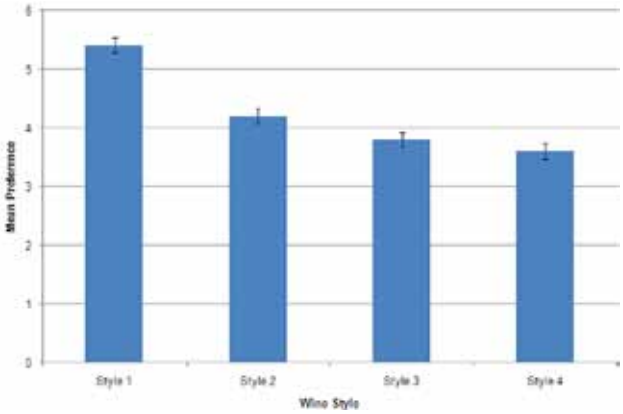


Figure 5.6 Mean and standard error for preference for each style.

sandy loam soils and underlying limestone are the foundation of the Hunter's success with HVS. Other common features of the premium vineyards include traditional Polkolbin single wire trellising, cane pruning, non-irrigated or limited irrigation and limited yields (4–10 t ha^{-1}). As Semillon is a thin skinned variety which is easily damaged, hand picking is often employed to help retain freshness. The NWGIC project has also collected a range of data to help understand the influence of vineyard site and viticultural practise. This data includes:

- Soil composition data at selected vineyard
- Climatic conditions such as temperature, rainfall and humidity
- Grapevine growth information including leaf areas, pruning weights, shoot lengths bunch numbers/ weight and yields.

Small-lot winemaking has also been performed at these sites (successfully in 2009 and 2010 and without great reward in the difficult 2008 vintage). The project will report the correlation of this data when the complex statistical analysis is completed.

Experiment 5.3
Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines

Materials and methods

The consumer preference study of Hunter Valley Semillon (HVS) (Experiment 5.2) indicted that sweetness and/or residual sugar/acidity balance are an important characteristic in consumer predilection for different styles of HVS. Consequently, a study to determine the preference for different residual levels was an obvious research topic. To maximise research value, two groups consumers, novices and experience were recruited. The sweetness acceptance of Hunter Valley winemakers was also tested in order to determine if a difference existed between the groups.

Anecdotal claims about consumer preference for sweetness abound, yet there is limited empirical research on this topic. Research that has been reported is equivocal, because key constructs, that of a 'consumer,' 'experience' and 'sweetness,' have not been consistently applied. Early research found that American consumers had a preference for sweet rather than dry wines (Filipello, Berg, Hinreiner and Webb 1955). The relationship between sweetness and consumer preference for Moselle wine determined that a residual sugar level of between 10 and 24 g L^{-1} was favoured (Kielhöfer 1955). However a potential confound was that high quality examples of this wine, often late-harvest and/or botrytised, by nature have associated sweetness. Furthermore, high titratable acidity levels in Moselle wines have typically required residual sugar to mask the natural sourness (Kielhöfer, 1955).

More recently it has been reported that Chinese consumers prefer a moderate level of sweetness in Australian red wines (Francis, Osidacz and Robichaud 2009). Lattey, Bramley, Francis, Herderich and Pretorius (2007) reported that the sweetest wine was the most preferred when investigating Australian consumer preference for a range of Shiraz and Cabernet Sauvignon wines. While this research indicates consumers prefer sweet rather than dry wine, the exact level of residual sugar in the product that drives preference is less clear. It is therefore important to establish how much is too much residual sugar, and whether moderate levels are preferred over low levels for specific wine styles and consumer segments.

In a study investigating different sweeteners for Seyval Blanc and Elvira wine, a linear relationship between residual sugar and preference was found using five experienced tasters (Duitschaever, Buteau and Ashton 1980). This research indicated an increasing preference for Seyval Blanc with levels up to 25 g L^{-1} residual sugar and 37 g L^{-1} for the Elvira varietal wine. Higher residual sugar levels were not tested. This implies that more residual sugar is always better than less (although there is logically a point at which this is implausible).

Experienced judges (i.e. 'experts') may also find sweetness more appealing in some styles; Amerine and Ough (1967) concluded that a group of experienced judges preferred Rosé wines with a residual sugar level of 10–20 g L^{-1} rather than drier wines. In new world wine-producing countries such as the United States, where the study was undertaken, Rosé is a wine style often associated with sweetness. It is therefore likely that off-dry examples would not automatically be considered out of style. It is unclear whether similar results would be found for experienced wine judges using a style not associated with some sweetness. Further, Amerine *et al*. (1967) found that their data followed a bimodal distribution, where some judges preferred drier wines and some preferred sweeter wines. The younger judges preferred sweeter wines whereas the older judges preferred dry wines. This suggests that level of experience may be important in the determination of sweet taste preference in wine.

Previous consumer research on sweet preference in wine has not specifically investigated the interaction between preference and level of experience. The work that has been done on sweet preference in wine has either not controlled for level of experience (Kielhöfer *et al*. 1955; Filipello *et al*. 1955; Duitschaever *et al*. 1980) or has not systematically manipulated the level of sugar (Francis *et al*. 2009; Lattey *et al*. 2007). The manipulation of sweetness can be achieved by spiking and using well-established consumer sensory testing methods; defining experience however is more challenging. For instance, a consumer who reports 25 years of wine drinking experience may not have the same level of knowledge or involvement in wine than someone with far fewer years of experience. Conversely, objective wine knowledge, as measured by Johnson and Bruwer (2003), does not take into account the exposure associated with higher consumption volume and greater periods of time drinking wine.

Solomon (1990) used two consumer segments to investigate wine related communication, one with industry involvement termed 'experts', and one with no industry experience, termed 'novices'. More recent attempts to segment consumers according to wine knowledge have generated a third 'intermediate' group (Melcher and Schooler 1996; Parr, Heatherbell and White 2002). Attempts to quantify wine knowledge in order to segment consumers using scales has also been made, however, limitations to this approach are evident. Hughson and Boakes (2001) created a multiple choice scale to measure wine knowledge in an Australian sample (Australian Wine Knowledge Questionnaire). The questionnaire has constructs that measure knowledge and consumption frequency, but not a consumer's level of involvement or years of experience in the wine industry. Attitude and expectation are clearly mediated by past exposure and are important in determining preference, something that has been established for some time in the social psychology literature (Ajzen and Fishbein 1980) and has been established more recently in a food context (Yeomans, Chambers, Blumenthal and Blake 2008). The scale has been validated as a tool to discriminate between 'expert' and 'novice' groups, but may not be appropriate to segment for an intermediate group.

Hunter Valley Semillon (HVS) is regarded as an iconic style within the Australian wine industry (Robinson 1999). It is much-lauded by wine professionals and has an enviable wine show record in both Australian and International wine shows. Most HVS is produced in a dry style, with a residual sugar level typically 0–3 g L^{-1}. Four distinct HVS styles have been identified (Blackman and Saliba 2009) and preliminary consumer testing indicated that the style with the highest residual sugar was the most preferred. Consumer preference for HVS wines has not previously been reported. Additionally, the interaction between level of experience and sweet preference in white wine is a novel contribution to this field of research. The purpose of this investigation is to determine the sweet preference of HVS for three distinct categories of consumers based upon level of knowledge, drinking experience, consumption habits and industry involvement.

This investigation adopted a three-category consumer model: novice (consumer), experienced (consumer) and winemaker; with category definitions based on a combination of self reported consumption, wine knowledge, years of experience drinking wine

and involvement in the wine industry, consistent with previous research (Melcher and Schooler 1996).

Respondents were sought until 25 of each of the 'experienced' and 'novice' were recruited using movie ticket vouchers as inducements. Respondents were defined on the basis of their self-reported consumption and wine knowledge. A novice was defined as being a wine consumer for less than ten years and who did not self-report as "knowledgeable". An experienced consumer was defined as being a wine consumer for more than ten years, drinking at least several times a week and self-reported as "interested" or "knowledgeable". Respondents who reported as being wine consumers for less than ten years but also as "knowledgeable" were also classified as experienced consumers. The third category was comprised of winemakers currently working in the Hunter Valley.

Wine

In order to determine the preferred sweetness level for HVS, wines were spiked with varying levels of glucose. As acid is known to interact with perceived sweetness (Pangborn, Ough and Chrisp 1964; Noordeloos and Nagel 1972), two wines with varying levels of residual sugar (RS) and titratable acidity (TA) were chosen. Two Semillon wines were selected as base wines, with the following chemical parameters: Wine A–pH 3.04, TA 7.67 g L^{-1} and RS 3.3 g L^{-1}; Wine B–pH 3.22, TA 6.80 g L^{-1} and RS 8.2 g L^{-1}.

Testing procedure

Different levels of glucose and fructose, the sugars most commonly found in wine, were tested in a pilot study to determine the most suitable addition rates. Additions of fructose, at dosage rates considerably lower than those tested for glucose, were found to influence the acid/sweetness balance of the wine too far towards sweetness. Grape juice concentrate was viewed as unsatisfactory due to its' propensity to impart a range of flavours to wine. The glucose addition rates which were determined for use did not affect the acid/sweetness balance of the wines or change other sensory attributes of the wines.

Paired comparison tests were conducted in accordance with the method described by Prescott, Norris, Kunst and Kim (2005). Respondents were asked to compare the base wine (control) to the adulterated wines at each glucose addition level (2.0, 4.0, 8.0, 16.0 and 32. g L^{-1} glucose) and indicate their preference on a scoresheet. Base wine position (left or right) was randomised. To prevent palate fatigue and sample carry over, paired samples were presented with sugar levels in adulterated wines in increasing concentration; respondents were instructed to expectorate, rinse their mouth with water and wait 1 minute between samples.

Evaluations were conducted between 10 am to noon in an air-conditioned (22±2°C) room equipped with white fluorescent lighting, white benches and height-adjustable chairs, to provide a comfortable environment purposely designed for teaching wine sensory evaluation. Thirty millilitres of each wine was served at 13±2°C in ISO 9000 XL-5 standard tasting glasses labelled with randomised 3-digit numbers. As two different wines were being studied, the testing required the participants to perform the tasting on two different days.

Statistical analysis

The Statistical Package for the Social sciences (SPSS) was used to perform statistical analysis of the data. A Kruskal Wallis test was performed to determine if the preference for each glucose addition differed between each of the three experience categories. Data for both wines was combined for these analyses.

Results and discussion

Figures 5.7 to *5.9* illustrate the number of respondents who preferred the glucose spiked wine at each of the additional glucose concentrations for consumer categories 'novice', 'experienced' and 'wine makers' respectively. Using the 0.05 confidence level for paired comparison tests (n=25, Roessler *et al.* 1956) the maximum level of glucose that could be added and still preferred by the novice group in comparison to the base wine was therefore determined to be 16.0 g L^{-1} for Wine A and 4.0 g L^{-1} for the Wine B. This infers that novices preferred Wine A with up to an additional 16.0 g L^{-1}, and Wine B sweetened with 4.0 g L^{-1} of glucose, compared to the corresponding base wines.

'Experienced' consumers preferred Wine A with up to an additional 13.5 g L^{-1}, and wine B sweetened with an additional 2.0–4.0 g L^{-1} of glucose compared to the respective base wines. Additionally, fewer of the 'experienced' group preferred the wines sweetened with the 16.0 and 32.0 g L^{-1} glucose additions than the novice group. 'Winemakers' preferred both Wine A and Wine B (in comparison to respective base wines) with up to only an additional 2.0 g L^{-1} of additional glucose.

Paired comparison tests have previously been utilised to identify a consumer rejection threshold (CRT) for

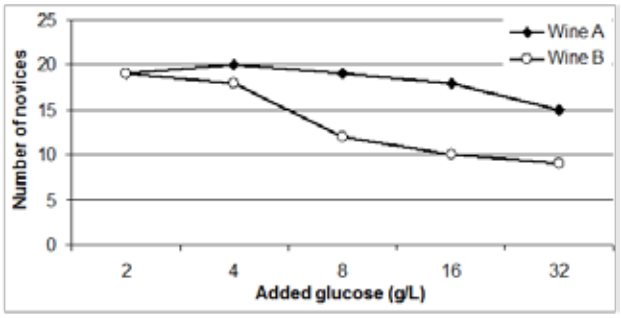


Figure 5.7 Number of novices who preferred the spiked sample in preference to the control for each glucose level for both wines. Dotted line (number of judgements=18) represents minimum agreeing judgements necessary to establish preference using α=0.05 for paired comparison tests (N=25).

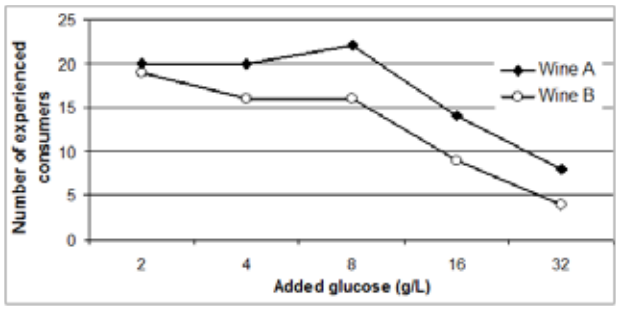


Figure 5.8 Number of experienced consumers who preferred the spiked sample in preference to the control for each glucose level for both wines. Dotted line (number of judgements=18) represents minimum agreeing judgements necessary to establish preference using α=0.05 for paired comparison tests (N=25).

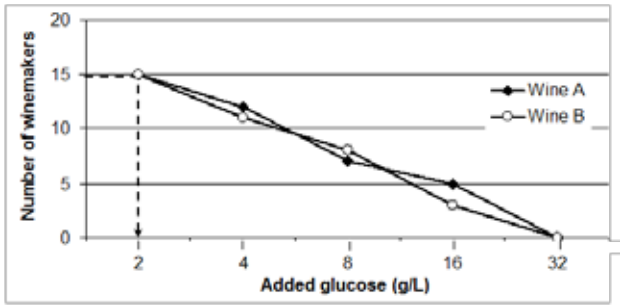


Figure 5.9 Number of winemakers who preferred the spiked sample in preference to the control for each glucose level for both wines. Dotted line (number of judgements=15) represents minimum agreeing judgements necessary to establish preference using α=0.05 for paired comparison tests (N=20).

a wine taint (Prescott *et al.* 2005). Saliba *et al.* (2009) also utilised this approach for determining a CRT for cineole, a compound reported to impart positive flavour at low levels and negative flavour at high levels. The determination of accepted sugar concentration in wines, indicated by the maximum level of glucose in adulterated wines which were preferred to the base wine, is an extension of the paired comparison test. This study has defined a quantitative approach for the determination of residual sugar concentrations for specific wines, within which winemakers can aim to more readily satisfy consumer acceptance, thereby allowing decisions for residual sweetness in a wine to be objectively based.

The Kruskal Wallis analyses reveal substantial differences in sweet preference across the three groups, and in particular the comparisons between winemakers and the two consumer groups. Specifically, the analysis revealed that there was a significant difference for the 32.0 g L^{-1} glucose addition between all three experience categories. Novice and experienced consumer groups had a significantly different preference for the 8.0 and 16.0 g L^{-1} glucose addition compared to the winemaker group. The novice category also had a preference for the 4.0 g L^{-1} glucose addition when compared to the winemaker group. *Figures 5.7* to *5.9* indicate that consumers in all three categories (novices, experienced and winemakers) preferred both wines when 2.0 g L^{-1} glucose was added. Early studies have indicated a consumer predilection for sweeter wines (Filipello *et al.* 1955; Kielhöfer 1955; Duitschaever *et al.* 1980) although this work was conducted with wine styles associated with overt sweetness and without specifically investigating level of consumer experience. HVS is almost always produced with little residual sugar, possibly creating an expectation by those with considerable experience of HVS whereby sweet examples might be judged as 'out of style'. Our results suggest that preference for sweetness over rides this effect, as all groups preferred an addition of glucose at a rate of 2.0 g L^{-1} to level of moderate sweetness.

Winemakers preferred a lower level of glucose added to base wines than both consumer groups. Although the authors of this study are aware of anecdotal claims of inexperienced consumers preferring wine with higher levels of residual sugar, no scientific literature has previously validated this notion. The present investigation has established differences in sweet preference between all three groups, but in particular, large differences between the consumer groups and winemakers. Given the level of involvement and consumption frequency associated with winemaking, it was expected that the winemaker group would differ substantially from the consumer groups. The differences reported between all three groups suggests that the influence of experience is negatively

correlated with preference, whereby some experience causes a reduction in sweet preference and additional experience reduces it even further.

HVS was chosen as the test wine twofold: firstly, because it is not normally associated with overt sweetness; secondly because it performs well at wine shows but does not experience commensurate sales performance. The latter phenomenon may be substantially explained by our results. Winemakers largely rely on their own preference when producing a wine and it is clear that winemakers in the current study preferred drier wines that consumers. This is likely to lead to the production of wines that are preferred by other winemakers (i.e. perform well in wine shows) but are not necessarily liked or well accepted by consumers. Our results suggest that higher levels of residual sugar would lead to HVS wines that are more broadly accepted by consumers, especially those with low levels of wine knowledge and tasting experience, thereby significantly increasing the potential market for these wines. It may therefore, be commercially sensible for a sweeter style of HSV to be added to the portfolio of wines for wineries commercially dependent on various styles of Hunter Semillon.

In this study the participants were informed that the wine was a commercially available Australian wine but were not provided with information such as variety, brand or vintage. By choosing wines not normally associated with overt sweetness, the most favourable conditions to enable differences between 'experts' and consumers in sweet preference of the wines have been fulfilled. It is possible that some of the more experienced consumers and a greater number of the winemakers, were able to correctly identify the wine variety and style due to their previous exposure to this wine. Hedonic preference is influenced by a consumer's expectation of the product (Yeomans *et al.* 2008), thus prior experience may have influenced winemakers predilection for wines with lower glucose additions given the typicality of HVS. Factors such as peer pressure, expert recommendations and previous experience have also been shown to contribute to the total consumer expectation (Saba *et al.* 1998; Tuorila *et al.* 1994; Lesschaeve and Noble 2005). It is difficult to address this methodological limitation, since the use of experienced consumers is naturally associated with higher levels of knowledge and product awareness.

The effect of learned liking could explain the main effect demonstrated in the current experiment –that level of experience is inversely related to sweetness preference. Wine is an acidic beverage and white wines also commonly have a degree of bitterness due to phenolic content. Bitter and sour tastes have traditionally been typified as unpleasant (Steiner 1977; Anliker *et al.* 1991). Even short-term exposure has increased preference of bitter foods and beverages (Mattes 1994; Stein *et al.* 2003). The acidity level in the wine style studied was also a possible significant contributing factor. It has been previously demonstrated that increasing acidity has a strong masking effect on the perception of sweetness in white wine (Pangborn *et al.* 1964; Noordeloos *et al.* 1972) and that acidity raise a subject's sugar threshold (Berg *et al.* 1955). Kielhöfer (1955) has also previously demonstrated the effect of acidity on sweetness in high acid Moselle wines. The findings that a lower sugar addition was preferred in the wine with less acidity and an already higher amount of residual sugar, is also indicative of the previously reported interrelationship of sweetness and acidity.

Furthermore, sensory expertise has previously been found to influence liking ratings in red wines (Frøst and Noble 2002). In an experiment examining sweet taste preference and personality type, Saliba *et al.* (2009) purported that the unique combination of alcohol and acid in wine would cause new consumers to seek sweetness to mask these components. It may be that respondents in the current study demonstrated this effect, such that sweetness acted to mask these basic tastes for less experienced consumers.

Some conflicting evidence has been presented regarding the impact of constituents other than sugar upon the perceived sweetness of wines. Noble and Bursick (1984) reported that increased glycerol levels resulted in an increased perception of sweetness and viscosity in white wines. Higher ethanol concentrations are also considered to impart a degree of sweetness (Zamora *et al.* 2006). However sensory studies investigating concentrations of glycerol and ethanol typically found in wine, has showed that neither affected sweetness uniformly (Gawel *et al.* 2007). It has also been demonstrated that the phenomena of cognitive association leads to a wine's fruit flavour and aroma eliciting the perception of sweetness (Prescott 1999). Further studies investigating the sweet preference of white wines could therefore make use of wines with differing fruit aroma intensities as well as variations in glycerol and ethanol concentrations.

Experiment 5.4
Sensory characterisation of bottle age using Hunter Valley Semillon Wine

Materials and methods

The sensory characteristics of wine change as they age. Typically flavours and aroma derived from the fruit and fermentation decrease whilst aged or developed characters become more prominent. Sensory descriptive analysis (DA) has been used extensively to quantitatively characterise a range of varieties from specific regions (Heymann and Noble 1987; Vilanova and Vilarino 2006; Vilanova 2006; de la Presa-Owens and Noble 1995; Noble and Shannon 1987; Guinard and Cliff 1987; Andrews *et al*. 1990; Reynolds *et al*. 1994; Noble *et al*. 1984; Douglas *et al*. 2001; Mirarefi *et al*. 2004). Although chemical changes due to aging (and more commonly oxidation) have been studied at length, the change in sensory characteristics due to bottle aging has been investigated to a lesser degree. The purpose of the published sensory studies that involve aging include; the influence of different storage factors such as temperature (Marais 1986; Marais and Pool 1980; de la Presa-Owens and Noble 1997; Recamales *et al*. 2011; Sivertsen *et al*. 2001); bottle closures (Perez-Coello *et al*. 2003; Mas *et al*. 2002; Skouroumounis *et al*. 2005; Godden *et al*. 2001); and the effect of ascorbic acid (Iñaki Etaio *et al*. 2009).

A few studies have investigated the organoleptic evolution of the wine without altering the storage conditions (Etievant *et al*. 1989; Gonzalez-Vinas *et al*. 1998; Chisholm *et al*. 1995; Oliveira *et al*. 2008; Iland and Gago 1995). White wine aging studies have been primarily concerned with quantifying the loss of fresh fruity characters such as citrus, tropical fruit and floral characters as well as the increase of undesirable aged characters such as straw, and green, asparagus or capsicum aromas (Recamales 2011; Gonzalez-Vinas *et al*. 1998; Chisholm *et al*. 1995; Oliveira *et al*. 2008). This research has been primarily undertaken in order to make predictions of appropriate maximum storage time in bottles in order to help maximise consumer acceptance. Even research on Riesling, a wine known for it's ability to develop desirable characters such as honey and toast (Iland and Gago 1995), has primarily been centred upon the development of undesirable levels of petrol or kerosene characters (Marais *et al*. 1992; Winterhalter 1991; Winterhalter *et al*. 1990).

Hunter Valley Semillon is an iconic Australian wine. Previous research has determined four unique styles, within which, bottle age is a key factor (Blackman and Saliba 2009). Consumer studies have indicated that substantial preference differences exist for the styles and therefore it is important to investigate the effect of bottle storage (Blackman *et al*. 2011). Moreover this research indicates that aged Semillon style is favoured by particular consumer groups and this research enables the evolvement of positive characters to be investigated. The retention of fresh fruit characters in quintessential aged Semillon (Blackman and Saliba 2009) will also be further examined. Although Semillon was used in a bottle closure trial, the investigation utilised a single wine made from Clare Valley fruit (Skouroumounis *et al*. 2005). The purpose of this study is to investigate the aging effect on a range of different Hunter Valley wines, which have previously been identified as being significantly different. This research enables the investigation how the different styles transition after a three year period of storage.

As the 2010 tasting and training followed a very similar procedure to that of the initial sensory descriptive analysis in 2007 described in Blackman and Saliba (2009), the method will be briefly outlined.

Seventy experienced winemakers and viticulturists from the Hunter Valley region participated in a forum to select sixteen representative wines that displayed distinctly recognisable characteristics. Wines, from the 1996 to 2006 vintages (*Table 5.4*) were subsequently donated.

Sensory Descriptive Analysis (DA)

The panellists were selected to participate on the basis of interest and availability. In 2007, the panel was composed of six females and nine males, 21 to 45 years of age. In 2010, 16 panellists contributed to the judging, the majority of which were involved in the 2007 tasting. All panellists had previously been members of descriptive analysis panels and used the Compusense™ computer programme.

The inclusion criteria for the 15 attributes in 2007 followed the international standard ISO 11035:94 (ISO 1994). Consideration focussed on panellist's ability to detect, recognise and rate each descriptor on a consistent basis. This was achieved by evaluating three or four wines in duplicate for specific attributes characteristics on different days, performing statistical analyses and providing feedback to panellists.

Table 5.4 Wine code, vintage and key chemical measures for each of the sixteen HVS wines selected for the descriptive analysis. Style has been taken from Blackman and Saliba (2009).

	Vintage	Style (2007)	Glucose (g/L)	Fructose (g/L)	Titratable acid (g/L)	pH	Alcohol (% v/v)
Wine 1	2002	4	0.32	0.31	6.5	3.15	10.32
Wine 2	2006	2	1.55	2.11	6.9	2.97	10.64
Wine 3	2006	2	0.13	0	6.3	3.09	10.43
Wine 4	2001	4	0.16	0.27	8.2	2.92	11.33
Wine 5	1998	4	0.24	2.04	7.5	2.89	10.37
Wine 6	2004	4	0.05	0.16	6.5	3.24	11.57
Wine 7	2006	2	0.93	0.97	7.3	3.05	10.71
Wine 8	1996	4	0.43	0.78	6.2	3.09	10.50
Wine 9	2006	2	0.23	3.39	6.9	2.93	10.68
Wine 10	2006	3	0.00	0.08	7.7	2.84	10.64
Wine 11	2002	3	0.49	3.48	7.6	2.89	10.09
Wine 12	2003	3	1.49	1.73	7.1	3.16	11.37
Wine 13	2005	2	0.13	1.25	6.8	3.08	11.87
Wine 14	2002	4	0.17	0.03	7.5	2.89	10.12
Wine15	2005	2	1.41	1.56	7.7	3.09	10.13
Wine 16	2006	1	0.61	5.93	6.7	3.25	10.48

The same 15 descriptors used to differentiate the wines in the first DA were also used in 2010. This was done as the array of vintages described in 2007 provided a diverse range of characteristics, including those which had already evolved with bottle aging. During the course of the training, panellists were also asked to rate any additional attributes they detected. No further attributes were deemed necessary. Use of the same descriptors facilitated training and allowed direct comparisons between the two DAs.

The list of attributes and composition of reference standards are presented on *Table 5.5*. Additionally, sweetness and acidity reference standards were presented so that rating of these tastes could be practised. Rating of acidity and sweetness were practised in a similar manner as that described for aromas.

Formal Judging: In both 2007 and 2010, the 16 wines were evaluated in triplicate in 12 tasting sessions held over a two week period. The Compusense™ programme generated random three digit identification numbers for each wine at each session. This number was transcribed on to appropriate standard (ISO 1997) tasting glasses. The order of presentation of the wines was determined using a randomised complete block design with three replications performed by each panellist for each wine. Four wines were tasted in each tasting session. All evaluations were conducted under white fluorescent lights in individual tasting booths at room temperature of 22±1°C. Wine samples were served at 13±2°C.

At the beginning of the tasting session, panellists were asked to sniff each of the aroma standards. The panellists then rated each of the attributes using an unstructured line scale (ISO 2003) from 'Low' to 'High'. Panellists were requested to sniff water regularly to help alleviate fatigue as well as rinsing their mouth with filtered water and waiting for 30 seconds

Table 5.5 List of attributes and composition of standards.

Term	Composition of reference standard*
Asparagus	½ teaspoon juice from canned asparagus.
Confectionary	chopped green and red Allen's™ frog-shaped jelly confectionery
Floral	2 rose petals soaked for 10 minutes and removed
Grapefruit	1 teaspoon fresh grapefruit juice and small piece of rind soaked for 5 minutes and removed
Grass	¼ cup fresh grass soaked for 10 minutes
Hay/straw	¼ cup horse chaff soaked in wine for 15 minutes
Honey	2 teaspoons of honey
Kerosene	0.001 µL kerosene
Lemon/lime	½ teaspoon each, fresh lemon and lime juice and small piece of each rind soaked for 5 minutes and removed
Lychee	3 teaspoons of juice from canned lychee
Orange marmalade	3 teaspoons stirred thoroughly
Toast	scrapings from 1 piece of white toast

* in 50 mL of 50:50 distilled water:base wine.

between samples. Results were entered directly into the Compusense™ programme via computers in each tasting booth.

Statistical Analysis

An analysis of variance (data not shown) of the descriptive analysis data for each attribute was performed. For all attributes except for asparagus, the sample wines were a significant source of variation. Although asparagus is included in the table of means/ multi comparison study table, it has not been included in further statistical analyses.

Results and discussion

The individual change in attributes can be seen in *Table 5.6* (mean of attributes with significant differences) and almost all of the almost all of the developed characters (orange marmalade, honey, toast and kerosene) have increased, many significantly, in the 2010 tasting as compared to the 2007.

Principal component analysis (PCA) (*Figure 5.10*) was performed in order to compare the characteristics of each wine with all other wines. The correlation matrix was used to generate a two-factor solution. A two-factor solution was accepted as it accounted for 72% of the total variation, passed the conditions of the scree test and interpretability (Lawless and Heymann 1998).

Factor 1 accounted for 57.1% of the variance, with the variable loadings having the greatest common meaning. The first component separated the samples

Table 5.6 Means of all attributes for both 2007 and 2010 tastings. *, ** and *** indicate significance at p<0.05, p<0.01 and p<0.001 respectively

	Mean Intensity		p	Mean Intensity		p	Mean Intensity		p	Mean Intensity		p
Attribute	**1A**	**1B**		**2A**	**2B**		**3A**	**3B**		**4A**	**4B**	
Lemon/lime	3.7	3.3	-	4.4	3.8	-	4.5	3.9	-	4.1	3.2	**
Floral	1.6	1.5	-	3.6	1.9	***	2.6	1.9	-	2.2	1.4	*
Grapefruit	2.2	1.9	-	2.5	2.4	-	2.4	2.4	-	2.2	1.9	-
Pineapple	1.6	1.8	-	2.3	1.5	*	2.2	1.7	-	2.3	1.6	*
Confectionary	1.0	0.9	-	2.6	1.4	***	1.8	1.3	-	1.3	1.1	-
Hay/straw	3.9	2.3	***	2.9	2.6	-	2.8	2.9	-	3.0	2.5	-
Grassy	1.7	0.8	**	2.5	1.4	**	2.3	1.7	-	1.8	1.1	**
Asparagus	1.4	1.0	-	1.4	1.1	-	1.6	1.1	-	0.9	0.7	-
Lychee	1.0	1.2	-	1.8	1.6	-	1.3	1.3	-	1.0	1.3	-
Orange marmalade	2.7	3.6	*	1.4	1.7	-	1.5	1.6	-	2.1	2.5	-
Honey	4.5	6.0	***	2.4	3.2	*	2.6	2.8	-	3.6	4.0	-
Toast	4.2	4.5	-	1.3	2.7	***	2.3	2.5	-	2.7	4.1	***
Kerosene	3.7	3.0	*	1.7	2.4	*	1.7	2.4	*	2.6	2.6	-
Acidity	5.1	4.6	-	4.8	5.3	-	5.0	5.0	-	5.3	5.6	-
Sweetness	2.5	3.8	***	3.9	3.1	**	3.3	3.3	-	2.9	3.0	-

Attribute	**5A**	**5B**		**6A**	**6B**		**7A**	**7B**		**8A**	**8B**	
Lemon/lime	3.2	2.8	-	3.7	3.4	-	5.4	3.5	<0.0001	3.7	3.2	-
Floral	1.5	1.4	-	1.8	1.6	-	4.2	1.6	<0.0001	1.7	1.5	-
Grapefruit	1.7	2.0	-	2.2	2.1	-	2.8	2.4	-	1.7	1.7	-
Pineapple	1.2	1.8	-	2.4	1.8	-	3.3	1.7	<0.0001	1.9	1.7	-
Confectionary	1.1	1.0	-	1.6	0.9	0.0313	2.5	1.3	0.0001	0.8	0.8	-
Hay/straw	3.8	3.0	*	3.0	2.5	-	2.2	2.3	-	3.8	3.0	0.0360
Grassy	1.3	1.0	-	1.6	1.4	-	2.6	2.0	-	1.3	0.9	-
Asparagus	1.1	0.8	-	1.2	1.0	-	1.4	1.2	-	0.8	0.7	-
Lychee	0.9	1.1	-	1.1	1.3	-	2.1	1.4	0.0060	1.2	1.3	-
Orange marmalade	2.2	3.1	**	2.5	2.6	-	1.5	1.7	-	3.6	4.1	-
Honey	4.1	5.5	***	3.3	4.0	-	1.8	2.5	<0.0001	5.5	5.8	-
Toast	5.3	5.3	-	3.2	3.7	-	1.2	2.1	0.0174	5.5	5.6	-
Kerosene	2.8	2.5	-	2.5	2.7	-	1.3	2.2	0.0036	3.1	3.1	-
Acidity	5.2	5.5	-	4.3	4.9	-	5.4	5.3	-	4.9	4.9	-
Sweetness	2.8	3.6	**	2.9	3.4	0.0372	3.9	3.7	-	2.7	3.4	0.0142

according to relative intensity of confectionary, pineapple, floral, lemon/lime, floral and grassy attributes, as opposed to orange marmalade, honey, toast and to a lesser extent hay/straw. The fresh fruit characters are typical of the youngest wines from both the 2007 and the 2010 tastings. The reduction in fresh fruit intensity is in strong agreement with previous white wine aging studies (Chisholm *et al*. 1995; Oliveira *et al*. 2008; Iland and Gago 1995; Marais *et al*. 1992). The wines more characterised by developed characters were the older wines used in the study and is also consistent with the initial characterisation where bottle age accounted for a large proportion of the variance (Blackman and Saliba 2009).

More interestingly / uniquely the relative individual change of each wine due to three years bottle storage is demonstrated in this dimension. For all 16 wine samples, the wine tested in 2010 has moved from the fresh fruit character towards the developed characters. The amount of change can be quantified graphically by charting the difference in PCA Dimension 1 values for each wine from 2007 and 2010 (*Figure 5.11*) as described by Cocchi *et al* (2005), where the change in first component describes the evolution of the wines after three years storage.

Each comparison indicates that all samples moved in the same direction—the fresh fruit characters becoming less indicative and/or the developed characters becoming more dominant. The larger the difference between first component scores, illustrates a greater relative change towards a wine characterised more by developed characters and less by the

Table 5.6 continued

	Mean Intensity		p	Mean Intensity		p	Mean Intensity		p	Mean Intensity		p
Attribute	**9A**	**9B**		**10A**	**10B**		**11A**	**11B**		**12A**	**12B**	
Lemon/lime	4.5	3.7	0.0071	4.2	3.6	-	4.2	3.2	0.0018	3.9	3.5	-
Floral	3.9	1.8	<0.0001	2.4	1.5	0.0086	2.0	1.8	-	1.8	1.5	-
Grapefruit	2.8	2.6	-	2.9	2.2	-	2.3	2.1	-	2.4	2.2	-
Pineapple	3.2	2	0.0007	2.2	1.4	0.0311	1.9	1.7	-	1.5	1.4	-
Confectionary	2.8	1.1	<0.0001	1.6	0.9	0.0321	1.2	1.1	-	0.9	0.8	-
Hay/straw	2.5	2.3	-	3.1	2.6	-	3.3	2.4	0.0167	3.3	3.0	-
Grassy	3.1	1.7	0.0001	2.5	1.6	0.0033	2.2	1.4	-	2.4	1.6	0.0282
Asparagus	1.2	0.9	-	1.1	1.1	-	1.1	0.8	-	1.5	1.3	-
Lychee	1.4	1.3	-	0.9	1.0	-	0.9	1.4	0.0469	0.6	1.3	0.0166
Orange marmalade	1.1	1.6	-	0.8	1.3	-	1.1	1.9	-	1.6	2.3	0.0473
Honey	1.8	2.6	0.0230	1.6	2.9	0.0003	2.8	3.5	-	2.6	3.4	0.0194
Toast	1.1	2.7	<0.0001	1.6	2.5	0.0105	2.2	3.5	0.0002	2.8	3.1	-
Kerosene	1.2	2.1	0.0071	1.8	2.4	-	1.8	2.7	0.0077	2.4	2.4	-
Acidity	5.7	4.7	0.0009	5.8	5.7	-	5.7	5.1	-	5.0	5.0	-
Sweetness	3.9	4.2	-	3.1	3.0	-	3.0	3.7	0.0150	3.4	3.3	-

Attribute	**13A**	**13B**		**14A**	**14B**		**15A**	**15B**		**16A**	**16B**	
Lemon/lime	4.4	3.4	0.0011	3.6	3.2	-	4.5	3.8	0.0258	5.2	4.2	0.0007
Floral	2.2	1.6	-	1.9	1.9	-	2.8	1.8	0.0062	4.1	2.4	<0.0001
Grapefruit	2.6	2.3	-	2.2	2.1	-	2.5	2.1	-	2.6	2.4	-
Pineapple	2.1	1.3	0.0182	1.9	1.3	-	2.0	1.7	-	3.2	1.7	<0.0001
Confectionary	1.5	1.1	-	1.2	1.5	-	1.7	1.3	-	3.5	1.9	<0.0001
Hay/straw	3.2	2.4	0.0241	3.1	3.0	-	2.8	2.9	-	2.2	2.8	-
Grassy	2.3	1.9	-	1.8	1.4	-	2.2	1.6	-	2.5	1.4	0.0014
Asparagus	1.1	1.5	-	1.0	0.9	-	1.2	0.8	-	1.3	0.8	-
Lychee	1.0	1.2	-	0.9	1.3	-	1.2	1.4	-	1.6	1.3	-
Orange marmalade	1.3	1.8	-	1.4	2.5	0.0027	2.0	2.1	-	1.0	1.7	-
Honey	2.2	3.0	0.0255	2.8	3.5	-	2.7	3.4	0.0422	2.3	3.0	0.0412
Toast	2.0	3.1	0.0039	3.7	3.7	-	2.2	3.3	0.0019	1.1	2.4	0.0003
Kerosene	2.1	2.2	-	2.3	2.6	-	2.2	2.3	-	1.5	2.0	-
Acidity	5.0	5.4	-	5.6	5.5	-	4.8	5.3	-	4.5	4.2	-
Sweetness	3.7	3.3	-	3.1	3.2	-	3.7	3.0	0.0219	5.1	5.6	-

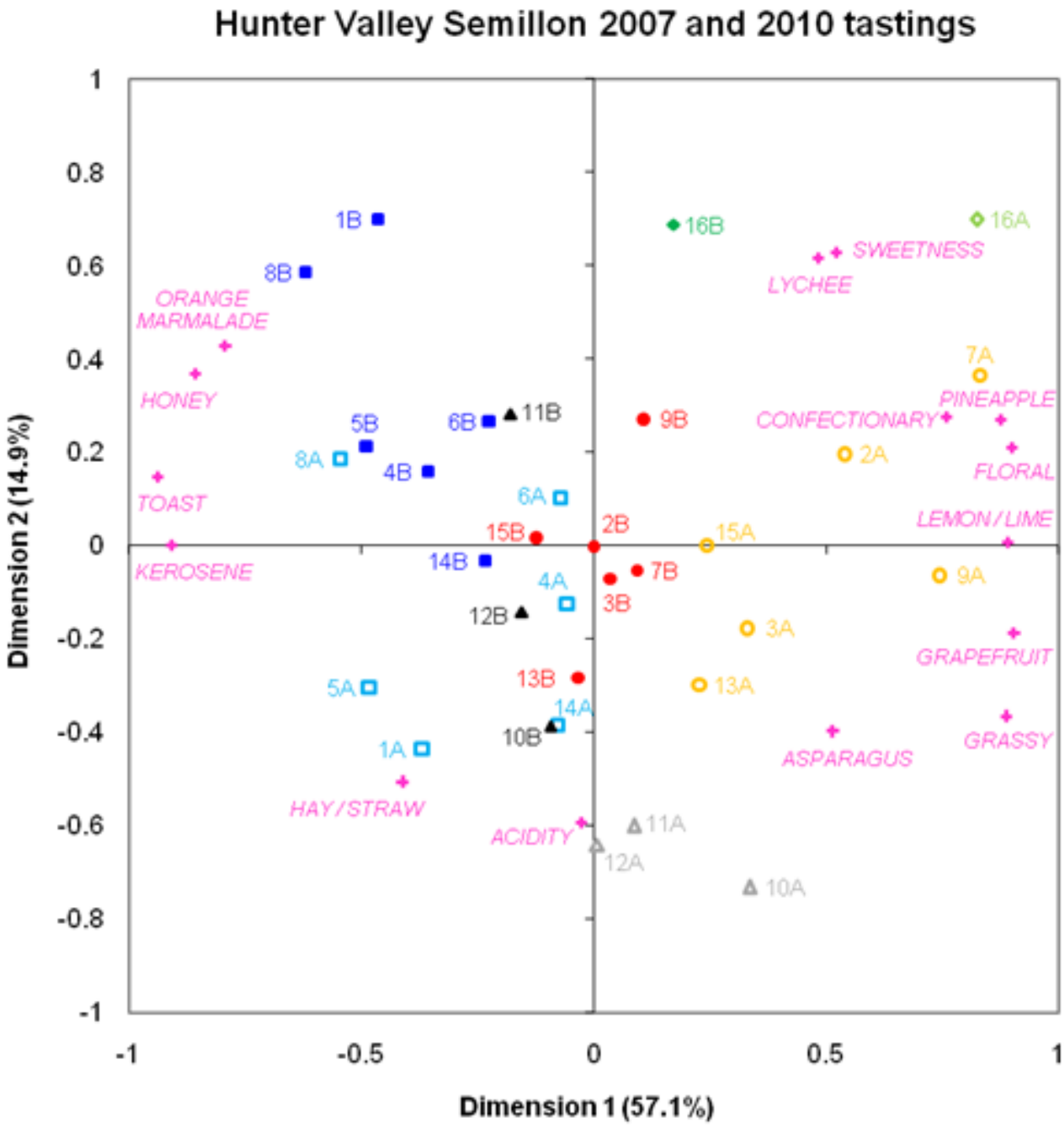


Figure 5.10 PCA biplot of Hunter Valley Semillon wine (1–16) tastings in 2007 and 2010. Descriptors (+) are shown in pink; A: hollow light coloured symbols indicate 2007 tastings; B: filled dark coloured symbols indicate 2010 tastings; ◊ and ♦: wine style 1; ○ and ● : wine style 2; △ and ▲: wine style 3; □ and ■: wine style 4.

confectionary, pineapple, floral, lemon/lime, floral and grassy attributes.

The relative magnitude of changed appears to be affected in two ways. The most evident factor is that the youngest wines have evolved by the greatest amount. The seven wines which changed most were, in order, Wine 7, Wine 16, Wine 9, Wine 2, Wine 10, Wine 15 and Wine 3, and all were from the latest vintage, 2006. Wine 15, from the 2005 vintage was the only exception. *Figure 5.12* shows the change in attributes for Wine 16, showing clearly the significant changes in many of the attributes. The wines which changed least were the oldest wines used in the study, Wines 5 and 8 from 1998 and 1996 respectively. It is apparent that the already lower intensity of fresh fruit character in older wines diminishes more slowly whilst the developed aroma increases relatively little. *Figure 5.13* illustrates the marginal (and non significant) decrease in fresh fruit characters (typically found in quintessential aged HVS) and the small increase in the developed characters for 1996 wine, Wine 8.

The different styles of Semillon identified in 2007 also appear to have evolved at dissimilar rates. In

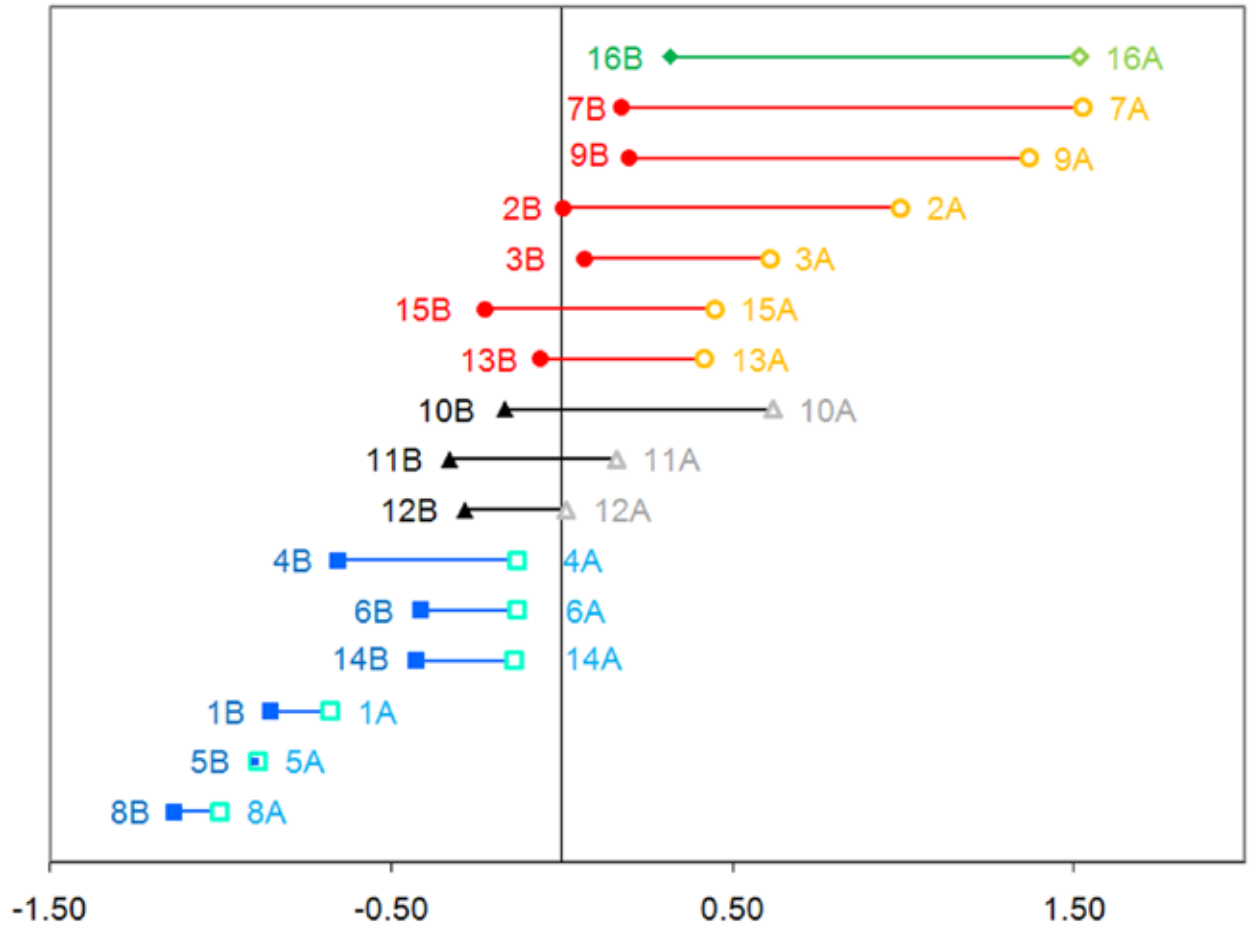


Figure 5.11 Difference across dimension 1 in PCA biplot of Hunter Valley Semillon wine (1–16) tastings in 2007 and 2010 (*Figure 5.9*). A: hollow light coloured symbols indicate 2007 tastings; B: filled dark coloured symbols indicate 2010 tastings; ◊ and ♦: wine style 1; ○ and ● : wine style 2; △ and ▲: wine style 3; □ and ■: wine style 4.

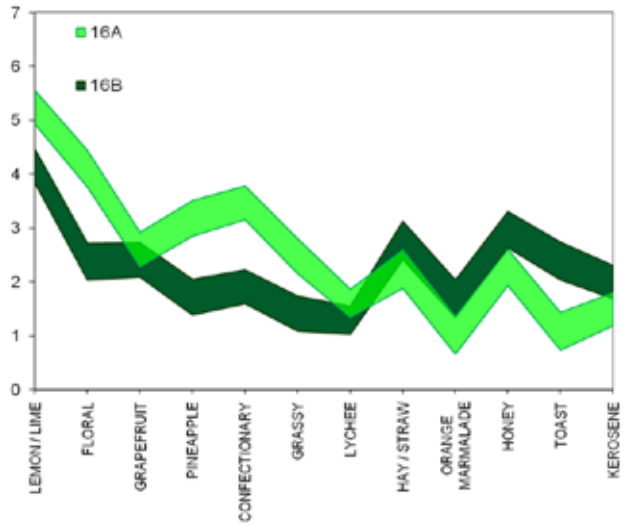


Figure 5.12 Tape graphs of HVS wine (Wine16) tastings in 2007 and 2010. A: light coloured tape indicate 2007 tastings; B: dark coloured tape indicate 2010 tastings. The tape is centred on the sample mean score and the width is +/- one half of Fisher's LSD.

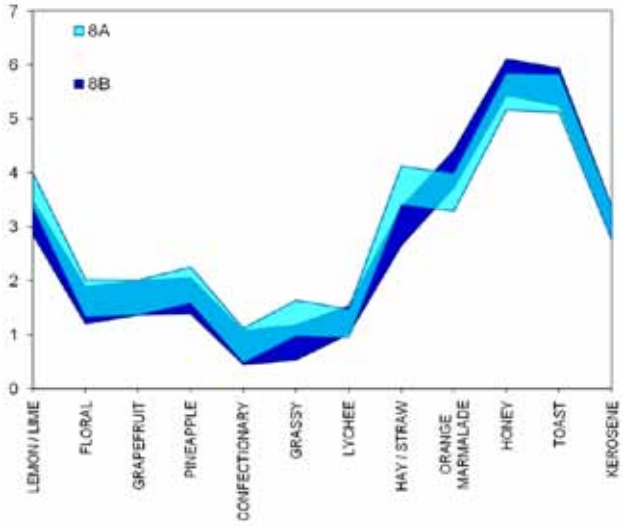


Figure 5.13 Tape graphs of HVS wine (Wine 8) tastings in 2007 and 2010. A: light coloured tape indicate 2007 tastings; B: dark coloured tape indicate 2010 tastings. The tape is centred on the sample mean score and the width is +/- one half of Fisher's LSD.

general, wine Styles 1 and 2 have developed to the greatest extent in relative terms. Wine Style 3, wines characterised by taut acidity and slight grassiness, have developed to a lesser degree. Whilst the vintage of the wines also explains this finding, the different composition of the wines is also having an effect. For example, Wine 10 moved a lesser amount than most other 2006 vintage wines. Possible reasons include a lower acidity (typical of those in wine Style 3) or due to the fact that it did not contain prominent fresh fruit characters in the 2007 tasting.

Although Wine 3, is a Style 1 wine, it did not move as much as some of its' 2006 vintage Style 1 counterparts. This wine is has an anecdotal history of longevity (the 1996 Wine 8 is the same label), and the less obvious change is not fully rationalised by its' moderate acidity (pH 3.09). The subtle decrease in fresh fruit characters and slight increase in developed characters is illustrated on *Figure 5.14*. Wine production influences, such as producing wine with lower levels of oxidisable phenolic compounds or higher levels of preservatives such as sulphur dioxide or ascorbic acid, could also be responsible for the different rates of development.

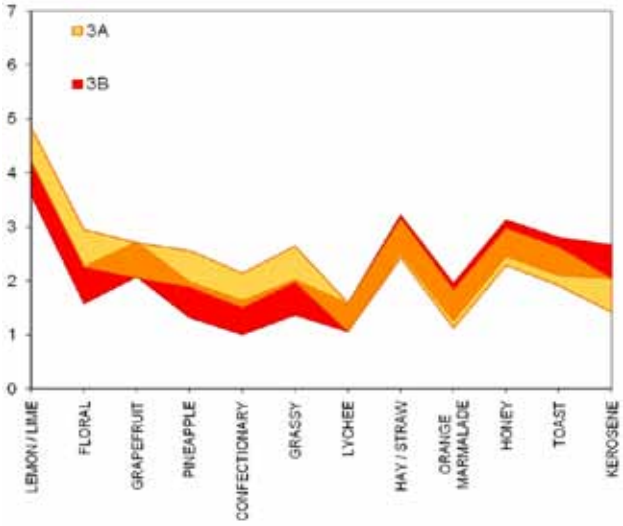


Figure 5.14 Tape graphs of HVS wine (Wine3) tastings in 2007 and 2010. A: light coloured tape indicate 2007 tastings; B: dark coloured tape indicate 2010 tastings. The tape is centred on the sample mean score and the width is +/- one half of Fisher's LSD.

The changes in sensory characteristics for the 2002 vintage Wine 1 (*Figure 5.15*) are also noteworthy. The perceived decrease in kerosene character is one of the few instances when a developed character was found to decrease between the tasting in 2007 and 2010, and the only case of a significant reduction of a developed

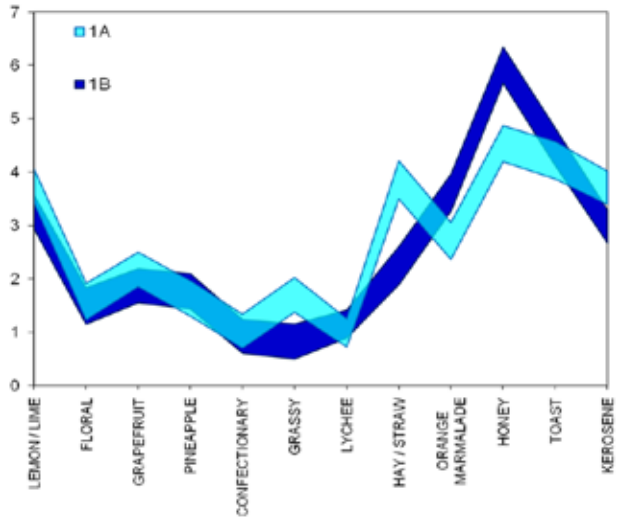


Figure 5.15 Tape graphs of HVS wine (Wine1) tastings in 2007 and 2010. A: light coloured tape indicate 2007 tastings; B: dark coloured tape indicate 2010 tastings. The tape is centred on the sample mean score and the width is +/- one half of Fisher's LSD.

character. This was initially difficult to reconcile as it has been well established that 1,1,6-trimethyl-1,2-dihydronapthalene (TDN) formation with age is the major contributor to kerosene aroma (Marais *et al.* 1992; Winterhalter 1991; Winterhalter *et al.* 1990). It would appear that the other developed characters; orange marmalade, honey and toast, have become more prominent and concealed the kerosene character in the wine.

Whilst all wines have demonstrated a trend of decreased primary fruit character and increased developed character, the ability of all wines to retain some of the fresh fruit character is particularly important to the aging potential of HVS. In particular, the retention lemon/lime provides crispness and freshness. Some wines were able to retain almost their primary fruit character whilst gaining added complexity with the development of aged aromas. Wine 12 shown in *Figure 5.16* clearly demonstrates this evolvement of complexity. These type of aged wines are extremely popular with winemakers and wine show judges whilst some experienced consumers have also been found to prefer this wine (Blackman *et al.* 2011). In this sense, the acquisition of the aged attributes has been illustrated in a positive sense, quite different from previous white wine aging studies that have described aged characters in a negative manner.

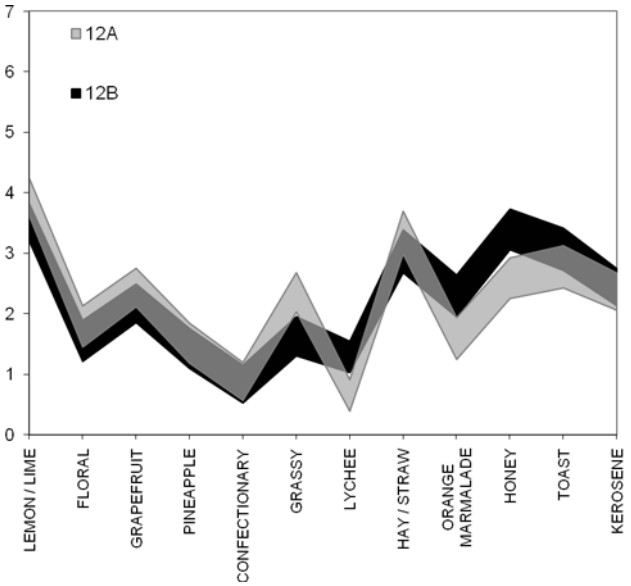


Figure 5.16 Tape graphs of HVS wine (Wine12) tastings in 2007 and 2010. A: light coloured tape indicate 2007 tastings; B: dark coloured tape indicate 2010 tastings. The tape is centred on the sample mean score and the width is +/- one half of Fisher's LSD.

Experiment 5.5
What do consumers think about Semillon?

Materials and methods

Winemakers and writers are wondering how a wine variety that previously was the 'world's most planted white grape capable of premium wine production' has fallen out of fashion (Robinson 1999; Shea 2010). The negative impact on price since the 1990s is considered surprising for a 'long-term classic amongst Australian varietals' (Steiner 2004, *p* 300). In recent years the wine has even been described as a 'little geeky' and 'a workhorse' (Cleary 2007). This view is also held internationally, as summarised by Marsh (2007):

Should you ask me what is the most unfashionable or under-rated white grape variety in terms of quality, in Australia or the world for that matter, my answer would be Semillon.

Sales of the wine and market analyses support the idea that as a varietal, Semillon has lost consumer appeal both internationally and locally. Despite claims such as 'some of the most recent enjoyable drinking has involved [Semillon]' (Port 2004) and the style being 'one of Australia's great gifts to the wine world of wine' (Robinson 2004), consumer resistance to the varietal version has resulted in strategies to downplay the product—usually by blending with a more popular variety. For example, Lehman reports "The consumer preference for Sauvignon Blanc continues to impact on sales of Semillon, the company's white wine mainstay" (Peter Lehman Ltd 2009). The result is that the company, like many, introduced a Semillon Sauvignon Blanc blend. Indeed, Semillon blends are easier to find in most bottle shops than the varietal version. While blending is a recognised method of producing quality wines, the decision to blend Semillon may be often related to consumer resistance to it as a varietal. The weighting in a blend may even change, for example Plantagenet Wines (2010) states that they changed the weighting in one of their blends 'towards the more aromatic Sauvignon which has a bit more pizzazz than plain old Semillon' (Plantagenet Wines 2010).

In Australia, the Hunter Valley is so associated with its Semillon it holds a Festival of Semillon and Seafood and has done so for the past 8 years. Research on Semillon has demonstrated the complexity of the wine across age and style (Blackman and Saliba 2009). In wine shows it receives acclaim; yet one Californian writer has recently claimed, "It occurs to me that we have betrayed Semillon, allowing it to slip through the cracks of our collective wine thoughts," (Bonné 2009). Anecdotally, it certainly does not have the same consumer recognised name as varieties such as Chardonnay or Sauvignon Blanc. If wine producers and distributors are feeling the need to blend Semillon with other varieties, this suggests at least some problem with consumers' perception of the wine—regardless of its status within other circles.

Consumer behaviour is never simple to evaluate or predict. Occasionally, studies reveal basic predictors of sales such as a wine label (Mueller *et al.* 2010); functional additives (Barriero-Hurle *et al.* 2008), or even music played during purchase (North *et al.* 1999). Based on point of sale research from the UK, Adrian North and colleagues found that sales of French wine increased when French music was played in store, and sales of German wine increased when German music was played (North *et al.* 1999). Unfortunately for Semillon producers, there is no "Semillon music" that can help boost sales of this variety! Even if there were, such consumer effects are usually short term. When wine varieties are affected by 'fashion', the impact of reference groups become a salient factor determining choice, but such factors are also time-limited. Over time, a once popular variety is replaced by another, so that there is selective forgetting about some worthwhile wines. In such cases it can be useful to ask "what do consumers think about a 'past' favourite now".

In this study, therefore, we sought to find out what Australian consumers think about both Semillon and the Australian region best known for its Semillon, the Hunter Valley. In this way, we sought to determine factors that may explain either indifference to or preference for the variety in terms the consumers themselves are offering us.

Both men and women attending wine and food festivals in Wagga Wagga and Sydney were given a questionnaire on attitudes to wines. They completed this questionnaire after they blind tasted and rated several wines. Among other questions (to be reported on elsewhere) they were asked, "What are a few words to describe what Hunter Valley Semillon means to you?" Four blank lines were available for writing.

We used a thematic analysis, commonly used to summarise qualitative data. The themes arose from the participants' words, rather than from the authors' knowledge or expectations around Semillon. The verbatim comments were copied into a database spreadsheet. These replies were then scanned for key words or items, especially those recurring across participants, and those items were listed in adjacent columns, taking as many columns as needed to begin categorizing the replies. Some respondents covered a few points in their answer for example 'light, fragrant, steely, lemon, ages well', others only one point, for example 'nice'.

The responses were then sorted into clusters or themes based on similarity of content, and examined again for further similarity or differences of content and re-categorised as necessary. For example, if response content was about taste, those items were clustered into a theme of 'taste'. Themes are thus about the focus of content rather than about the specific judgments or valence of comments.

Results and discussion

Three hundred and eighty one people returned their questionnaires but of these, 111 did not complete the open-ended question. We looked at some additional data to see if these non-completers rated Semillon particularly low or high when they were asked to rate their liking of the variety in a separate aspect of the study (not reported here). There were no differences in that rating, so we feel confident that the final sample of 270 is not biased by having those with a particular attitude to Semillon removed from this analysis.

The thematic analysis revealed that respondents' comments fell into one or more of eight themes. A schematic of the themes is presented below in *Figure 5.17*. Examples of comments categorised into each of the themes are presented in *Table 5.7*. The order of themes does not reflect the frequency of the related comments.

Theme 1: Knowledge of region

Theme 2: Knowledge of grape variety

Theme 3: Acceptance of grape variety

Theme 4: Consistency of style

Theme 5: Age of wine

Theme 6: Taste characteristics

Theme 7: Easy drinkability

Theme 8: Use of wine

The themes in *Figure 5.17* show how the respondents' comments fell into clusters or themes based on similarities of words used. Not all respondents mentioned all these categories but in all cases there were reasonable subgroups.

Table 5.7 expands on the above themes by giving specific examples of the comments people wrote in reply to our open-ended question. These examples are not exhaustive of the number of comments, but are typical of the focus within the themes.

The themes in *Table 5.7* are discreet response clusters, but they allow some further clusters discussed next. Specific examples of responses are in italics.

A. ***Knowledge*** (from Theme 1: Knowledge of region and Theme 2: Knowledge of grape variety): results indicate that Semillon has indeed fallen out of many people's awareness. Over seventy participants answered that they knew very little, if anything, about HVS. These were respondents who did not know Semillon as a variety (*not a thing; never heard of it; very little*). There were also more who did not know that the HV is good for producing Semillon (*know little about the HV*; *[I] don't pair [Semillon] with the HV*). Others were knowledgeable describing the HV-Semillon pairing was iconic (*iconic for Hunter; tradition, history*) or stated the HV had a reputation sufficient to carry over to Semillon (*high quality*). Others did not like HV wines (*not fond of HV wine*).
B. ***Acceptance*** (from Theme 3: Acceptance of grape variety): some respondents knew the variety but didn't like it (*don't like it much; generally don't drink this style*) or liked it a lot (*yummy; favourite; Semillon enthusiast*).



Figure 5.17 Themes emerging from descriptions of Hunter Valley Semillon.

C. ***Variability*** (from Theme 4: Consistency of style; Theme 5: Age of wine; and Theme 6: Taste characteristics): Several people commented around the variable nature of the wine, that is its consistency or otherwise (*sometimes excellent; depends on brand; quality safe always reliable*). This variability could be due to particular styles associated with winemakers, or the age of the wine (*great when aged; reliable quality*), or in the way the taste was described (*toasty; lemony; savoury; light*). The former use of corks as the bottle closure, rather than screws caps as occurs almost exclusively today, has also been considered as a source of variation, although this was not referred to explicitly by our participants. While certain taste descriptions suggest a younger wine (*light, refreshing*) the respondents did not necessarily mention they were describing a young wine. The flavours most often seemed to be typically described as 'opposites' of lemony/citrus/fresh versus toasty/nutty/honey. The comments about age did not always suggest that aged Semillon is better, but there was a trend that way. Some specifically referred to young wines negatively (*young/sharp, old/good*), others commented favourably (*can be consumed young and ages well*) and others saw age as the link to quality (*very special when aged*). While these factors can represent different characteristics, the common thread is that people vary in their descriptors making it difficult to say what most consumers expect of a Semillon on these dimensions.

D. ***A comfortable, easy wine***. Theme 7: Easy drinkability and Theme 8: Use of wine: There were some comments that the variety was harsh or too strong, but these last themes were those where there was greater agreement and positive comments. Responses suggest that people who liked Semillon found it easy to drink (*all occasion wine; yummy; very drinkable wine*) and found it went well with food, especially but not exclusively seafood (*fabulous wine alone and with right food; great with seafood; pairs well with meals*). Their responses often contained words such as

Table 5.7 Themes emerging from subjective meaning of Hunter Valley Semillon, with verbatim examples from which the themes were derived*

Theme 1:	Knowledge of region	Not distinguishable; drank SA for last 10 years; know little about HV; well known region; don't pair with HV; improved over the years; buy it when I like a particular wine; good wine from a good region; the wine of the HV; iconic for the Hunter buy it over others; some of best in Australia; signature wine of the Hunter
Theme 2:	Knowledge of grape variety	Never heard of it; never tried it; know nothing of it; never drank it before; heard of it; my parents drank it
Theme 3:	Acceptance of grape variety	Least favourite wine; don't enjoy drinking semillon; not my style; not a semillon fan; prefer Semillon–Sauv Blanc blend; more of a red wine drinker; good for a white; pretty good drop; favourite; semillon enthusiast
Theme 4:	Consistency of style	Depends on brand; wines vary from producer to producer; changes with age; sometimes excellent; dependable; expect it to be good; always reliable; consistently good
Theme 5:	Age of wine	Like it young; made to drink young or old; young sharp; old good; ages well; very special when aged
Theme 6:	Taste characteristics	Tasteless; sweet; fresh; light, great; light, refreshing; not too dry; dry white; fresh, acidic, citrus; thought sweeter–crisp, fruity; dry, floral, citrus; acidic; good flavour and after taste; toasty; savoury toast; strong gutsy very drinkable
Theme 7:	Easy drinkability	Harsh; flavour too strong; nice; nice drinkable wine; nice easy drinking; easy to drink; pleasant easy drinking wine; very drinkable wine; yum; yummy
Theme 8:	Use of wine	All rounder; enjoy with dinner; good with meals; good with seafood; crisp, good with seafood; great with seafood

* Comments general to more than one theme could relate to region or wine e.g. 'overrated', 'quality', 'good value' 'wonderful'. Quality mentioned several times as both 'good quality' and 'poor quality'.

'nice', 'fresh', 'favourite'. Some simply wrote 'good wine'. These 'nice' terms were not pejoratively used - that is, they were not damning with faint praise. Rather, the comments indicated a level of comfortable expectation and context associated with Semillon.

This analysis shows that respondents considered a variety of aspects of Hunter Valley Semillon when asked what it means for them. The results show that as a group they are not just focusing on image or taste. Our respondents gave a range of responses including variability, drinkability, and the context for drinking Semillon. Across this range of themes, responses were both positive and negative. This research shows on their own, people will consider some aspects more salient than others (at least in terms of answering our question). The further question is then raised, to what extent do these descriptors relate to improving uptake of Semillon by consumers?

For the purposes of re-presenting Semillon to the market, it would seem that Themes 7 and 8 hold special promise. In the comments for Themes 7 and 8 there was a focussed expectation around Semillon, much more so than in the other themes. Focusing on Semillon as an 'easy' wine would mean a change from the image of Semillon as a wine that is best blended with other wines, to a wine with the ability to work with the drinker, forming a comfortable liaison, often in the comfortable context of a meal and relaxation. A recent Australian study found that together with taste, 'pairing with food was the most important motivating factor given for consuming wine' (Charters and Pettigrew 2008, p 23). In an earlier study Charters (2004) reported that a small panel of experts criticised Sauvignon Blanc because, among other factors, it is not a 'food friendly wine'. Based on our results Semillon seems to be well placed to fit this category of 'food friendly wine'. While several people used the term 'easy' positively in our study, the word may have overtones of cheapness; in which case synonyms may be better used such as accommodating, easygoing, flexible, leisurely, and pleasant. Extrapolating from our data, these terms mean that Semillon does not require the drinker to work at enjoyment. This presentation moves the emphasis away from taste. It does not rule out taste, but as our results suggest, consumers are not consistent in what they expect Semillon to taste like.

Experiment 5.6
Winemaking consistency

Materials and methods

The consistency of experimental winemaking is a controversial issue within the wine industry, with some thinking experimental wines are a waste of time and funds due to inconsistency of replicates. Partially due to this notion, a study was conducted on experimental winemaking within the Wine Growing Futures (WGF) program. Nine projects within the program were examined, with remarkable results.

Results and discussion

The consistency of experimental winemaking is a controversial issue within the wine industry, with some thinking experimental wines are a waste of time and funds due to inconsistency of replicates. Partially due to this notion, a study was conducted on experimental winemaking within the WGF program. Nine projects within the program were examined, with remarkable results.

Sensory tests were carried out on 61 comparisons, with a total of 918 participants. Only five comparisons from the entire set of results showed a significant difference in replicates. Six projects showed an ideal result, that 100% of replicates had no significant difference. One project did have only 50% of replicates with no significant difference, but also had a very small number (two) of comparisons.

The results showed the overall consistency of replicates from the nine projects was 92%, which was an excellent result for the WGF program and the experimental winemaking team.

Two projects had one comparison each that was approaching significance, but this was not included in the overall percentage. If these were included, the overall consistency of replicates would have been 88.5%.

The NWGIC Small Lot White Winemaking Protocol shows the experimental winemaking protocols for white wine. The results of this study validate the replicate consistency of these protocols and so, the procedure of small lot white winemaking.

As a similar procedure is in place for red winemaking, we can assume the experimental winemaking procedure for red wine is also validated. Only white wines (Chardonnay and Semillon) were included in this study, as sensory work done on the red wines in the WGF program did not include tests on just replicates.

Experiment 5.7
Wine chemistry analysis of Semillon flavour compounds

Materials and methods

A variety of gas chromatography (GC) techniques were employed for the analysis of Semillon flavour compounds. This included GCMS (gas chromatography mass spectrometry) and GCOMS (gas chromatography olfactometry mass spectrometry) utilising a variety of extraction and injection modes (i.e. solvent extraction, solid phase extraction (SPE), SPE with derivatisation, and solid phase micro-extraction (SPME)). The data arrays generated were treated in a variety of manners. Preliminary studies involved identification and quantification of a range of chemical compounds, via the use of internal standards, whilst later a chemometric approach was taken in the analysis of the bulk data sets. Such analysis, allowed highlighting of markers within GC data for flavour components within the wine. A survey of the potent aroma compounds within Semillon was then conducted by GCO analysis. Although such a diverse range of Semillon wine volatile compounds have been analysed, it is by no means a universal census of the volatile compounds in Semillon, and perhaps one limitation in the data sets obtained is the likely lack of sensitivity to many sulfur-containing aroma compounds. This is mainly due to the fact that many sulfur aroma compounds are inherently difficult to extract and/or detect by conventional GC (i.e. without utilising sulfur-specific GC detectors and/or alternate extraction procedures) than the vast majority of the volatile compounds in wine. The analysis/quantification of the sulfur aroma compounds in Semillon is certainly an area for further work.

Given the emphasis of the project on Semillon flavour, GC analysis was adopted over liquid chromatography techniques (i.e. LC-MS and LC-nmr), as GC is more readily able to analyse the flavour compounds responsible for the nuances of Semillon wine aroma, and these same flavour compounds can also contribute to retro-nasal flavours. Although it was initially planned to perform some LC analysis on the wine, it became apparent that the GC analysis would allow greater comparisons to the sensory data that was dominated by evaluation of the Semillon aroma. Furthermore, the approach of analysing the wines via the 'high powered' technique of liquid chromatography -

nuclear magnetic resonance (LC-nmr) was avoided given the experience of several researchers with this technique (see Final Report GWRDC CSU03_02). The LC-nmr technique is better suited to simpler sample matrices that contain components of high concentrations, that is, conditions far removed from that of Semillon. GCxGC-MS was initially planned as an analytical technique for the Semillon wines. However, it was decided to attempt to link the variety of 'two dimensional' GC-MS analysis data sets (i.e. with variable extraction/injection modes) to the Semillon sensory data before attempting to increase the complexity of the GC data to 'three dimensions'. As the project progressed and the chemometric-linking of GCMS to sensory data was initiated, the use of GCxGC-MS analysis was considered beyond the scope of the project and flagged as an area of further work.

The project was to be tackled in the same fashion as the design of the Wine Growing Futures program. That is, the analysis of volatile aroma compounds was to be conducted initially on wine samples, this data related to sensory data of the wines and then a variety of potential marker compounds highlighted. Where possible, these marker compounds would then be extrapolated back to the effect of wine production processes and/or viticultural management factors on their production. Although in the original scope of the project, measurement of volatile compounds in various juice samples and wine samples throughout the production process were envisaged, they were not achieved for this final report. This was a consequence of a variety of factors including the initial extended time required for optimisation of a variety of GC methods, general GC wine sample analysis and data analysis, and complications due to staff changes.

The following provides a description of the different GC methods utilised to analyse the wine in various parts of this report. Methods not described can be found in the relevant scientific publications attached to this report.

GC Method#1–Solid Phase Micro-Extraction Gas Chromatography–Mass Spectrometry (SPME GC-MS)

Sample preparation

The chosen sample to headspace volume ratio of 1:1 was based on that used in previous studies (Marti *et al.* 2003). Analysis samples (10 mL) were introduced into a 20 mL vial. 50 µL of the standard mixture was added as internal standards to each analysed sample. The standard mixture consisted of methyl isobutyl ketone (MIBK, 1 g L^{-1}), n-dodecane (0.0004 g L^{-1}), 4-methyl-2-pentanol (0.8 g L^{-1}) and ethyl nonanoate (0.001 g L^{-1}) in ethanol. All solutions were stored in darkness at 4°C. Each sample was prepared in triplicate prior to analysis.

For qualitative and quantitative analyses, calibration solutions were prepared by diluting each pure compound with ethanol to five concentrations in the range described further below (*Table 5.10*) using instrument grade water. Appropriate volumes of the calibration solution and the buffer solution (tartaric acid (0.008 M) and potassium hydrogen tartrate (0.011 M)) were mixed to obtain a 10 mL sample with 10% (v/v) ethanol as final alcohol percentage of the synthetic wine.

SPME GCMS analysis conditions

Analyses were carried out on a Varian CP-3800 gas chromatography system equipped with a CombiPAL autosampler and a split/splitless injection port type 1079, interfaced to a Varian Saturn 2000 ion trap mass spectrometer detector. The 20 mL vials were placed in a thermostated oven at 40°C under agitation (500 rpm). A polar capillary column (Varian, CP-Wax 52 CB, 60 m ×0.25 mm i.d. 0.25 mm film thickness) was used for qualitative and quantitative analyses and a nonpolar column (Varian, CP-Sil 8 CB, 60 m ×0.25 mm i.d. 0.25 mm film thickness) was used to compare the generated retention indices with the polar column. The oven temperature program was 5 minutes at 50°C and then increased to 230°C at a rate of 3°C min^{-1}, and held for 5 minutes. The flow rate of the helium carrier gas was constant at 1 mL min^{-1}. The ion trap, manifold and transfer line temperatures were 180°C, 40°C and 230°C respectively. Compounds were detected by electron ionisation. Mass spectra were collected at a rate of 0.6 s $scan^{-1}$ over a range of m/z 35–350.

The volatile compounds were extracted using a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre exposed to the sample headspace for 20 minutes at 40°C without agitation and introduced into the injector inlet at 260°C for 2 minutes under splitless mode and held for a further 3 minutes under split mode (1:100) to clean the fibre. The injector inlet was under splitless mode at the end of the analysis run. The monitoring of DVB/CAR/PDMS fibre quality was performed by following signal intensities of the four internal standards. A response ratio was calculated for each I.S. as the peak area of a specific

internal standard divided by the sum of all I.S. peak areas. For each I.S. ratios were used as a population (n>100 measurements) to construct the Shewhart chart (*Figure 5.18*). The upper and lower warning level (UWL and LWL) was calculated at the 95% confidence levels (Miller and Miller, 2005).

The retention indices (RI) were generated for each reference compound analysed using a commercial mixture of n-alkanes (C8-C25, 10µg L^{-1} in hexane, Sigma) using two different columns (CP-Wax 52 CB and CP-Sil 8 CB). Identification of volatile compounds was achieved by matching the RI and the mass spectrum obtained from pure standards run under similar GC-MS conditions with the two GC columns and by comparing RI and mass spectra from the National Institute Standards and Technology (NIST) mass spectral library, Version 2.0 or from published RI values (Miller and Miller 2005; Howard *et al.* 2005). Quantification was achieved using extracted ion areas and a specific internal standard: 4-methyl-2-pentanol (I.S.3) for alcohol compounds and ethyl nonanoate (I.S.4) for ester compounds. Linear regression equations for each analyte were calculated from the calibration curve of [volatile compound/internal standard] peak area ratio versus analyte concentrations in mg L^{-1}.

GC Method #2—Solid Phase Extraction Gas chromatography Mass Spectrometry (SPE GC-MS)

Extraction procedure: 4 mL dichloromethane, 4 mL methanol and 4 mL of 12% (v/v) ethanol aqueous solution were eluted in sequence through a 200 mg LiChrolutEN solid phase-extraction cartridge in order to achieve conditioning of the cartridge. A 50 mL of Semillon wine sample was spiked with 50 µL of an internal standard solution consisting of 4000 mg L^{-1} BHA, 1000 mg L^{-1} MIBK, 0.4 mg L^{-1} n-dodecane, 800 mg L^{-1} 4-methyl-2-oentanol and 1 mg L^{-1} ethyl nonanoate in ethanol. The sample was subsequently loaded onto the cartridge. Dichloromethane (1.5 mL) was applied to elute the analytes. Another internal standard solution, consisting of 300 µg g^{-1} 4-hydroxy-4-methyl-2-pentanone and 300 µg g^{-1} 2-octanol in dichloromethane, (25 µL) was added to dichloromethane extract for GC-MS analysis in 2 mL injection vials.

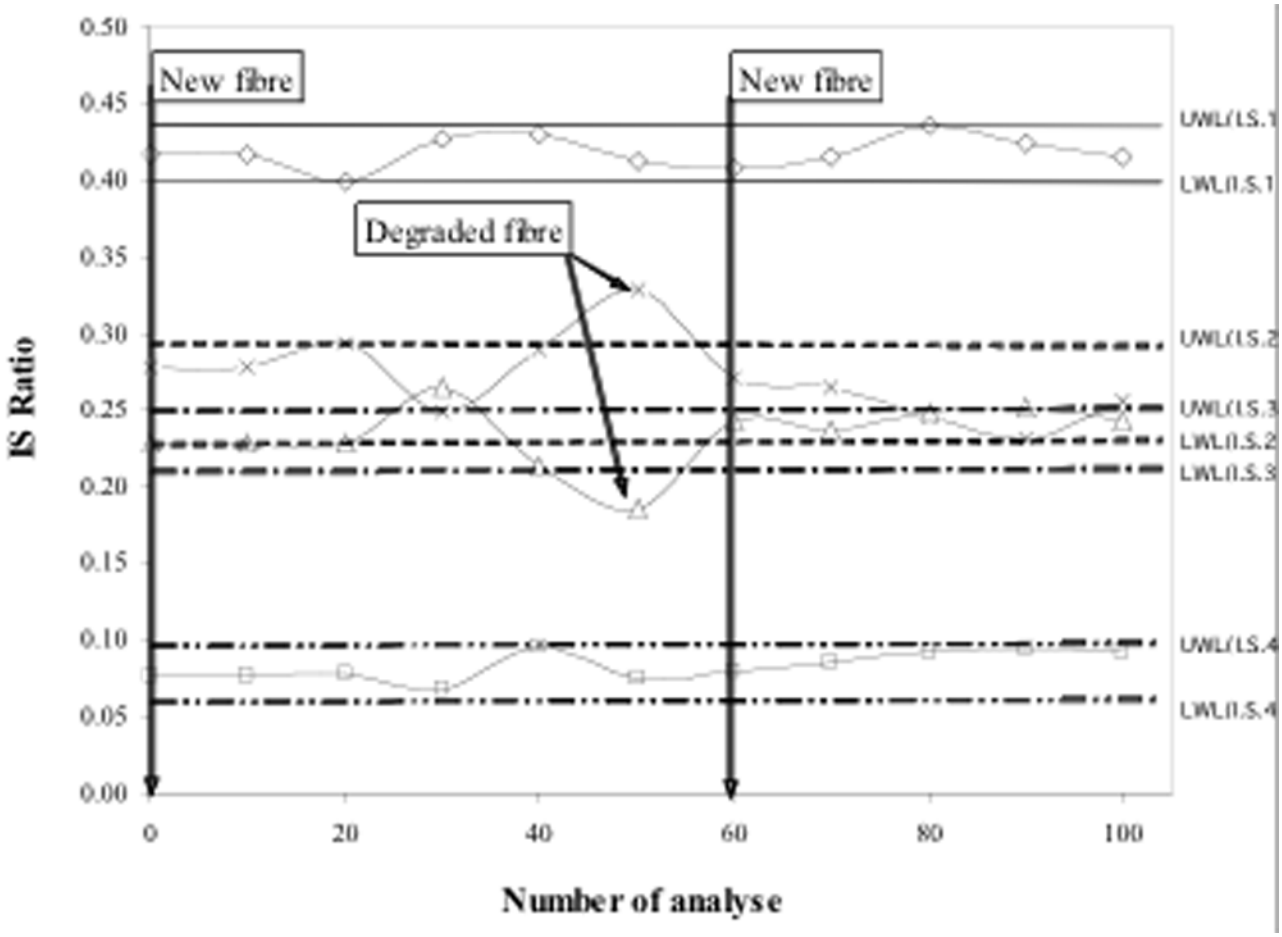


Figure 5.18 Fibre response (I.S. Ratio) during analyses with –◊– I.S.1: methyl isobutyl ketone, –×– I.S.2: n-dodecane, –Δ– I.S.3: 4-methyl-2-pentanol, –□– I.S.4: ethyl nonanoate; I.S. Ratio = I.S.n / (I.S.1+I.S.2+I.S.3+I.S.4) where n=1, 2, 3 or 4

GC-MS conditions: Analyses were carried out on a Varian CP-3800 gas chromatography system equipped with a CombiPAL autosampler and a split/splitless injection port type 1079, interfaced to a Varian Saturn 2000 ion trap mass spectrometer detector. A polar capillary column (Varian, CP-Wax 52 CB, 60 m×0.25 mm i.d. 0.25 mm film thickness) was used for qualitative and quantitative analyses. 2 µL of sample was injected into the column at a temperature of 40°C. The injector temperature was held at 40°C for 0.25 minutes, then heated at 200°C min^{-1} to 250°C. The injector split ratio was set to 100:1 for 0.25 minutes. The split valve closed for 2.75 minutes and opened to 30:1. The oven temperature program was 5 minutes at 40°C and then increased to 210°C at a rate of 2°C min^{-1}, and held for 20 minutes. The flow rate of the helium carrier gas was constant at 1.5 mL min^{-1}. The ion trap, manifold and transfer line temperatures were 180°C, 40°C and 230°C respectively. Compounds were detected by electron ionisation. Mass spectra were collected at a rate of 0.9 s $scan^{-1}$ 4 µscans over a range of m/z 35–350.

GC Method #3—Derivatisation Solid Phase Extraction Gas chromatography Mass Spectrometry (Derivatisation SPE GC-MS)

Extraction/derivatisation of model wine systems: A LiChrolutEN solid phase-extraction cartridge (200mg) was conditioned with 4 mL dichloromethane, 4 mL methanol and 4 mL of 12% (v/v) ethanol aqueous solution. 50 mL of model wine was spiked with 50 µL of an internal standard consisting of 120.5 mg L^{-1} 3-tert-butyl-4-hydroxyanwasole (BHA) and 21.32 mg L^{-1} methyl wasobutylketone (MIBK). After which the sample was loaded onto the extraction cartridge. Acetaldehyde, a interfering agent for the technique, was removed by cleanup of the cartridge with 10 mL of an aqueous solution containing 1% $NaHCO_3$. A 2 mL solution of 5 g L^{-1} o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) aqueous solution was applied to derivatize the retained carbonyls in the carriage. The PFBHA solution level was lowed to approximately 5 mm above the sorbent and allowed the cartridge to imbibe with regent for 15 minutes at room temperature. Excess regent was removed by a 10 mL aliquote of 0.05 M sulphuric acid. The derivatized analytes were eluted by 2 mL dichloromethane. 40 µL of the internal standard containing 46.22 mg L^{-1} 2-octanol and 50.54 mg L^{-1} pentafluorobenzophenone (PFBP) were added to derivatized analytes for GC-MS analysis.

GCMS conditions for the model wine systems: These were identical to that described in GC Method#2, however rather than scanning of the MS, the detection was conducted according to *Table 5.8*.

Extraction/derivatisation of Semillon wine systems: The procedure was the same as for the method immediately above with the following additions: 10 mL of Semillon wine was used in the SPED process, 10 mL of Semillon wine was spiked with the internal standards 800 mg L^{-1} BHA, 100 mg L^{-1} MIBK, 0.04 mg L^{-1} n-dodecane, 80 mg L^{-1} 4-methyl-2-oentanol and 1mg L^{-1} ethyl nonanoate in ethanol. Finally a 40 µL of an internal standard solution containing 46.22 mg L^{-1} 2- octanol and 50.54 mg L^{-1} PFBP in dichloromethane was added to the derivatised analyte.

GC Method #4—Gas Chromatography Olfactometry–Mass Spectrometry (GCOMS) with Solvent Extraction (time-intensity–Food Science Australia).

A Varian 4000 GC-MS/MS fitted with an olfactory port (ODO II, SGE Australia) was used. The GC oven was programmed from 40°C (held 5 minutes) to 240°C at 5°C min^{-1} (held 3 minutes). The injector was held at 250°C. The MS was set to electron ionisation (EI) mode to scan between 50–250 m/z at 0.46 sec $scan^{-1}$ after a solvent delay time of 5 minutes. The transfer line was held at 280°C. The same wines were also subjected to methanol, (MeOH) chemical ionisation (CI) in separate GC/MS runs. MeOH vapour is reacted with volatiles in the ion trap often producing stable protonated M+1+ positively charged ions, which undergo little fragmentation. Cross referencing CI data with EI mass spectra at the same retention time can assist in the identification of compounds, especially for co-eluting or trace concentration peaks.

A large volume injector liner was fitted into the injector port. A pressure pulse of 18 psi was applied during the injection of 1 mL in splitless mode; after 2.5 minutes the split vent was opened (1:25). Chromatographic separation was achieved with a BP-20 WAX-Forte capillary column (30 m, 0.32 mm i.d., 0.5 µm film thickness, SGE, Australia).

Tentative identification of compounds was achieved using the National Institute of Standards Technology mass spectral database program (NIST, Version 2.0, 2002, United States of America). Identification was based on a at least one or a combination of the following criteria; a high match of the electron impact mass spectrum with compounds in the NIST- 2002

database, linear retention index, odour quality and the presence of a consistent [M+1]+ mass spectral ion by methanol chemical ionisation. A database of identified mass spectral peaks was constructed using the Varian MS Workstation Software (Ver 6.41) and used for semi-quantification of (tentatively) identified compounds. The linear retention index (LRI) of compounds was determined using a standard mix of straight chain C7–C22 alkanes.

Simultaneous to the acquisition of GC-MS data, some of the gaseous effluent was split (4:1) between the MS detector and an olfactory port. The odour intensity at the olfactory port was monitored by a panel of five trained assessors, who had previous GC-O and sensory panel experience. A direct intensity approach was used as described in Frank *et al*. 2007. The odour-intensity of eluting compounds was monitored on a scale of 0 to 100 %, not detectable to overpoweringly strong over a 45 minute sniffing period. The output of olfactometry is a 'direct-intensity aromagram' which shows the changing odour intensity associated with volatiles over the duration of the chromatographic separation (~45 minutes).

GC Method #5—Gas Chromatography Olfactometry (GCO) and GCMS with Solvent Extraction (dilution analysis).

Semillon wine samples (200 mL) were placed in a 500 mL extraction flask together with 100 mL of Freon-113 and then slowly agitated at 20°C for 20 minutes. The liquid phases were separated in a 250 mL separation funnel. The lower phase (Freon) was collected and dried with anhydrous magnesium sulfate. The extract was concentrated to 1 mL under reduced pressure (0.5 atm) using a rotary evaporator and water bath (30°C).

1 µL of each concentration was injected into the GCO in splitless mode, a modified Hewlett-Packard 5890 gas chromatograph equipped with a fused silica capillary column (JandW Scientific DB-wax 30 m ×0.25 mm i.d. 0.50 µm film thickness) and a high-resolution olfactometer system that mixed the GC effluent with a stream of humidified air (20 L min^{-1}). This air stream passed through a 10 mm diameter stainless steel tube and was sniffed by a person (the sniffer) after the solvents were eluted. The injector was held at 260°C, and the GC was held at 40°C for 3 minutes following sample injection and programmed at 6°C min^{-1} to 250°C; He at 2 mL min^{-1} was used as a carrier gas. All extractions were sniffed until no odor was detected (successive 3-fold dilutions until 1:729=36) with a repeated measure for the last dilution. The retention time of each odorant was converted to retention indices by adjusting to a series of 7-18 carbon n-paraffins run under identical conditions but detected with a flame ionization detector.

Mass spectrometric characterizations of the aroma extracts were performed using a Hewlett-Packard 5890 GC attached to a 5970 mass selective detector (MSD). One microliter of each 243-fold concentrated extract (i.e. 35) was injected into the gas chromatograph equipped with an HP5-MS capillary column (0.20 i.d. 30 m) and He at 2 mL min^{-1} as a carrier gas. The GC was held at 35°C for 3 min following sample injection and programmed at 4°C min^{-1} to 225°C. The injector was held at 200°C. The compounds eluted from the column were ionized with electrons at 70 eV. An identification was reported only when the spectra, retention time, and odor character matched those of an authentic standard.

Results and discussion

Optimisation of a SPME GCMS method for the analysis of volatile compounds in Semillon wines.

This section highlights the optimisation, validation and application of a SPME-GCMS technique to the analysis of volatile compounds in Semillon wine. Although white wines including Sauvignon blanc, Chardonnay or Riesling have been extensively analysed in terms of chemical composition (Sefton

Table 5.8 Segments identification in GC-MS

Segments	Retention Time (start to end) (minutes)	Mass Scan Range	SWAS Parameters Ion range
2-Octanol	30-40	35-250	44-46, 54-56, 68-80, 96-98, 128-130
MIBK–PFBHA	40-55	45-320	178-299
PFBP	55-71	45-320	102-275
Methional–PFBHA	71-78	45-320	178-302
Phenylacet–PFBHA	78-86	45-320	178-319
BHA	86-95	45-320	134-183

et al. 1994; Sefton *et al*. 1993; Tominaga *et al*. 2000; Petka *et al*. 2001) and the impact of wine production and/or practices on chemical (Francis *et al*. 1992; Ugliano *et al*. 2006) and sensory characteristics (Francis *et al*. 1992; Francis *et al*. 1994; Guitart *et al*. 1999). Relatively few studies have been reported on Semillon wines (Sefton *et al*. 1996; Godden *et al*. 2001; Reynolds *et al*. 2001; Cox *et al*. 2005), although the iconic botrytized Semillon wines (Sauternes) have been examined in more detail with a particular focus on specific aroma compounds (Tominaga *et al*. 2006; Sarrazin *et al*. 2007). To our knowledge, there was only one report describing the analysis of a range of volatile compounds in dry Semillon wines by a headspace purge and trap extraction GC-MS method (Leino *et al*. 1993). Forty compounds were identified in this study and semi-quantified by their relative MS signal response. However, the reported extraction method was not automated and the quantification was based on the relative peak height and hence was not proportional to absolute concentrations present in the wine. This section aimed to generate a fully automated SPME-GCMS method for the analysis of Semillon wine. This method, with a variety of extraction/injection methods, and GCOMS, would then be applied to multiple wine samples as is described in preceding sections of this report.

GCMS background information.

To provide further information on GC methods, volatile compounds in wine are commonly analysed by gas chromatography coupled to at least one detector, such as a flame ionization detector (FID) or mass spectrometer (MS). The latter detection technique is particularly advantageous as it provides fragmentation data that is useful for the identification of molecules. Most studies on wine aroma utilising gas chromatography involve extraction methods including liquid-liquid extraction, headspace extraction, purge and trap extraction, solid phase extraction (SPE) and solid phase micro extraction (SPME) in order to capture volatile compounds from the wine sample (Versini *et al*. 2008; Cabredo-Pinillos *et al*. 2004).

Given the range of concentrations of volatile compounds found in wines a highly sensitive technique for analysis of volatiles is required. The availability of the SPME technique, allowing high sensitivity for detection of wine volatiles, greatly improved the feasibility of automating the analysis of wine volatiles (Pawliszyn 1997; Boutou and Chatonnet 2007). The SPME method is sufficiently sensitive to adsorb small amounts of volatile compounds and the wide range of fibre types allows a large diversity of compounds to be analysed. Extraction and desorption parameters, such as time and temperature, are commonly investigated to optimise SPME methods. It has been reported that agitation time and speed during the extraction times and temperatures can change the headspace composition and thereby result in variations on the extraction capacity and efficiency of the SPME fibre for certain compounds (Rocha *et al*. 2001; Pena *et al*. 2005; Setkova *et al*. 2007). Similarly, addition of salt to samples can increase the concentration of some compounds in the headspace and hence their extraction (Rocha *et al*. 2001; Pena *et al*. 2005).

Despite the advantages of SPME, comparison between GC-MS analyses using this extraction technique can be complicated by the degradation of the fibre with time. The degradation of the fibre can often impart lower response as well as poor repeatability and reproducibility. Changing fibres during an extended study introduces further complications if the fibres do not have identical quantitative responses to analytes under examination. To compensate for these phenomena, internal standards (I.S.) may be added to the samples (Kalua *et al*. 2006) in such a way as to detect fibre degradation and to provide a reliable comparison of extraction efficiency from one fibre to the next. Another function of these internal standards is to mimic the extraction behaviour of compounds by the fibre for quantification study. The ideal internal standard is a deuterated derivative of the target compound. Unfortunately, few deuterated compounds are commercially available and ultimately their addition for each volatile compound in wine is impractical. An alternative mode of quantification is to select compounds as internal standards which have molecular properties that are similar to the target compounds, such as molecular mass, polarity and functional group, thereby showing similar extraction and desorption behaviour to the target compound (Howard *et al*. 2005).

SPME-GCMS Analysis of Wine.

Most aroma studies on wine provide a snapshot of the wine's status at the time of analysis. A longitudinal study of changes in the aroma profile affords the possibility of developing production and marketing strategies to maximise consumer acceptance of the wine. For this to be successful, it is necessary to develop a robust method to analyse volatile compounds that can be fully automated, requires little sample pre-treatment and importantly allows

a comparison of aroma profiles within and between vintages. Furthermore, the technique should be suited to the general composition of Semillon wine, as described in the Sensory section of this report, whereby it has lower alcohol (10%v/v) and higher acidity (pH 3.0 or less) than most white wines and it shows significant changes as it ages. To this end, a new SPME-GC-MS method was developed in this study using a combination of various published approaches to quantify a diverse range of volatile compounds in the headspace composition of Semillon wines.

Optimisation of the SPME-GCMS analysis technique

The development of a HS-SPME-GC-MS procedure for the accurate quantification of this diverse range of volatile compounds was the initial challenge in method development. The procedure was required to be suitably robust to offer accurate quantification over different vintages and hence over different SPME fibre lifetimes. Deuterated standards are available for only a limited number of the volatile compounds under study, requiring an alternate approach to quality control of the measurement. In order to refine the method development, it was decided to focus on twenty-one volatile compounds previously reported in white wine and identified in *Table 5.9*.

Three different types of SPME fibres were investigated and covered a large range of extraction properties (100 µm PDMS, 65 µm PDMS/DVB, 50/30 µm DVB/CAR/PDM). The 100 µm PDMS fibre is well known for its extraction capacity of volatile non-polar compounds, while the 65 µm PDMS/DVB fibre is used for more volatile polar compounds, such as amines and alcohols, which are both adsorbed and desorbed more efficiently on this fibre. To ensure sufficient extraction over an expanded range of compounds (molecular mass 40–275), a three phase DVB/CAR/PDM (50/30 µm) is often more appropriate (Pena *et al*. 2005; Setkova *et al*. 2007; Vaz Freire *et al*. 2001; Zander *et al*. 2005).

The different SPME fibre types were compared using absolute peak areas of the volatile compounds (*Figure 5.19*). For all but two compounds, extractions obtained with PDMS/DVB and DVB/CAR/PDMS fibres showed better recoveries of the volatiles than the PDMS fibre, consistent with previous reports (Howard *et al*. 2005; Marti *et al*. 2003). The PDMS/DVB fibre exhibited greater extraction capacity for higher molecular mass esters, while the DVB/CAR/PDMS fibre had more affinity for low molecular mass compounds, *Figure 5.19*). Furthermore the relative standard deviations for the internal standards were higher with the PDMS and PDMS/DVB fibres than the DVB/CAR/PDMS fibre. Consequently, a three-phase fibre (DVB/CAR/PDMS) was chosen based on

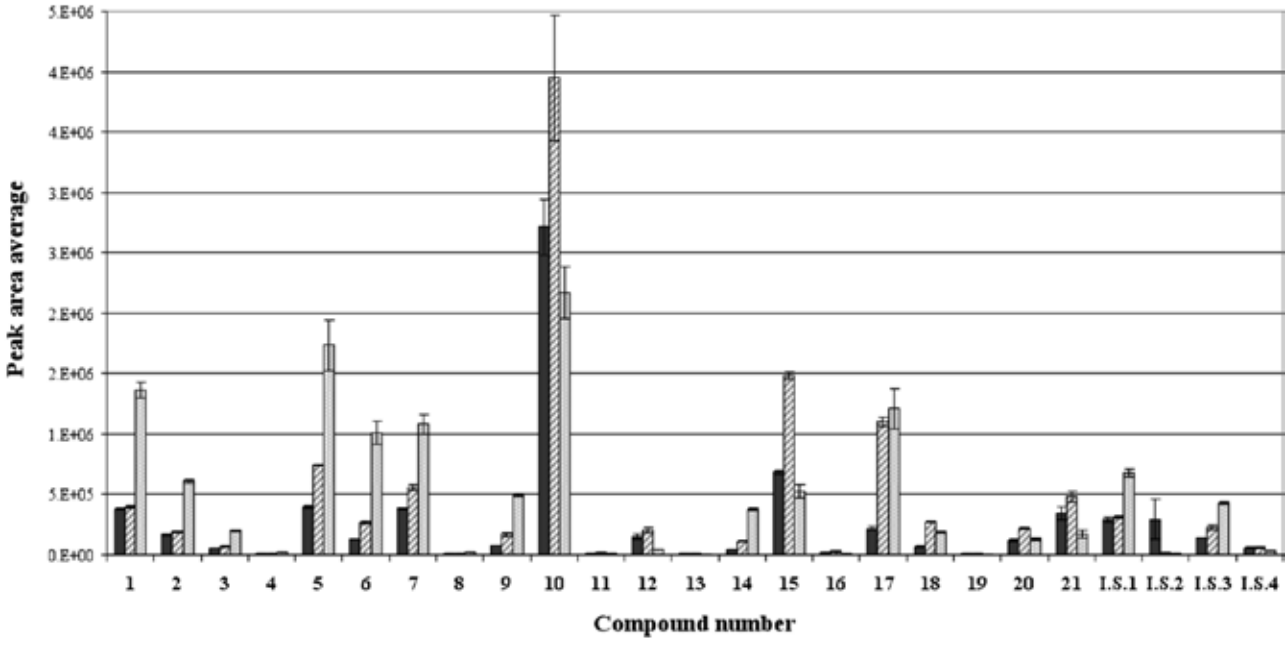


Figure 5.19 Comparison of volatile compound peak areas under identical GC–MS conditions using 100 µm PDMS (solid bar), 65 µm PDMS/DVB (bar with diagonal lines) and 50/30 µm DVB/CAR/PDMS fibres (bar shaded grey with white dots) with corresponding compound numbers (Table 5.1) on x-axis and peak area average on y-axis. Peak identification: 1, ethyl butanoate; 2, ethyl 2-methylpropanoate; 3, isoamyl acetate; 4, ethyl 3-methylbutanoate; 5, 3-methyl-1-butanol; 6, ethyl hexanoate; 7, hexyl acetate; 8, ethyl-S-lactate; 9, 1-hexanol; 10, ethyl octanoate; 11, methyl octanoate; 12, ethyl decanoate; 13, isoamyl octanoate; 14, cis-3-hexen-1-ol; 15, 2-phenylethylacetate; 16, ethyl dodecanoate; 17, hexanoic acid; 18, 2-phenylethanol; 19, methyl decanoate; 20, octanoic acid; 21, decanoic acid.

Table 5.9 Quantification ion, retention indices (RI) and regression parameters of volatile compounds analysed using HS-SPME-GC-MS

	Compounds	Quantification ion (m/z)	RI CP-WAX Calculated (literature)[a]	RI CP-Si 8CB Calculated (literature)[a]
1	ethyl butanoate	71	1048 (1032)	800 (805)
2	ethyl 2-methylpropanoate	117	1067 (975)	985 (761)
3	isoamyl acetate	43+55+70	1115 (1118)	1107 (876)
4	ethyl 3-methylbutanoate	57	1164 (1069)	907 (849)
5	3-methyl-1-butanol	39+55+71	1217 (1206)	892 (736)
6	ethyl hexanoate	144	1221 (1235)	1000 (1002)
7	hexyl acetate	43	1331 (1275)	1014 (1014)
8	ethyl-S-lactate	45	1379 (1345)	1235 (1010)
9	1-Hexanol	56	1396 (1355)	1014 (851)
10	ethyl octanoate	172	1433 (1436)	1201 (1198)
11	methyl octanoate	74	1619 (1389)	1131 (1041)
12	ethyl decanoate	200	1641 (1639)	1396 (1379)
13	isoamyl octanoate	70	1689	1487 (1487)
14	cis-3-hexen-1-ol	67	1709 (1405)	1171 (858)
15	2-phenylethylacetate	104	1809 (1830)	1351 (1260)
16	ethyl dodecanoate	101	1849 (1842)	1607 (1494)
17	hexanoic acid	60+73+99	1864 (1870)	1102 (1020)
18	2-phenylethanol	91	1904 (1909)	1126 (1118)
19	methyl decanoate	74	2022	1333 (1324)
20	octanoic acid	60	2096 (2089)	1318 (1279)
21	decanoic acid	129	2370 (2361)	1512 (1373)
I.S.1	methyl isobutyl ketone	43	1007 (1012)	896 (733)
I.S.2	n-dodecane	57	1250 (1200)	1203 (1200)
I.S.3	4-methyl-2-pentanol	45	1282 (1121)	1067
I.S.4	ethyl nonanoate	88	1531 (1537)	1441 (1319)

I.S. internal standard; RI retention indices

[a] literature sources: Howard *et al.* (2005); Escudera *et al.* (2004); http://www.flavornet.org; http://www.odour.org.uk

its extraction ability and repeatability. The three phase DVB/CAR/PDMS fibre, with its higher flexibility in terms of recoveries of different class compounds, was considered to allow the possibility of extending the analysis of volatile compounds in Semillon wines beyond the twenty-one analytes selected studied here.

Multiple roles were fulfilled by the addition of internal standards to the samples: to assess the quality of the SPME fibre (i.e. detect fibre degradation); to allow direct quantitative comparison of analytes when a new fibre is required; and to provide standards that are representative of the two main classes of volatile compounds in white wines vis. alcohols and esters. Based on past studies (Kalua *et al*. 2006, Howard *et al*. 2005), and as mentioned earlier our internal standards were selected: methyl isobutyl ketone (MIBK) (I.S.1, fibre check), n-dodecane (I.S.2, fibre check), 4-methyl-2-pentanol (I.S.3, fibre check, alcohol quantification) and ethyl nonanoate (I.S.4, fibre check, ester quantification).

Concentrations for the internal standards were chosen such that their respective peak intensities were of a similar magnitude (i.e. within 5-fold peak area units of each other) to ensure that variations in individual responses were not masked when calculating the peak area ratio. This permitted a more consistent and accurate approach to the detection of multiphase fibre performance.

A process of monitoring fibre death and headspace SPME–GC method performance was developed in this work as a modification of that described by Kalua *et al*. (2006). *F*ibre performance was monitored by plotting the ratio of the peak area of one internal standard over the total peak area for the four internal standards (I.S. ratio) against analysis number. Provided the ratios fall within the range for acceptable performance, defined here as the 95% confidence intervals, the fibre can be judged to be working satisfactorily. A degraded fibre is indicated when the I.S. ratio is outside these limits. This occurred for I.S.2, n-dodecane, and I.S.3, 4-methyl-2-pentanol,

at analysis number 50. At this point, I.S.1, MIBK, and I.S.4, ethyl nonanoate, still showed acceptable response on the fibre. If only I.S.1 and I.S.4 had been chosen as internal standards then fibre death would not have been detected, and quantification of alcohols in particular would have been compromised. Visual inspection of a degraded DVB/CAR/PDMS fibre in fact suggested that the different phases degraded at different rates, consistent with the results.

Optimisation of extraction conditions is fundamental for precision and sensitivity. The extraction study was conducted using three fibre exposure times and temperatures (10, 20 and 30 min and 30, 40 and 50°C). Peak area data for the twenty-one compounds of interest were used to compare the results of the different time/temperature combinations. Results for six selected compounds are shown in *Figure 5.20*, illustrating the variable responses of different compounds to different time-temperature combinations. For example, the ester, ethyl 2-methylpropanoate, showed maximum extraction at 30°C and 10 min extraction, whereas for ethyl decanoate the time-temperature combination for maximum extraction was 30 min at 50°C. Similar results for the alcohols were also observed (*Figure 5.20*) and are consistent with previous reports where it was not possible to achieve a globally optimal time-temperature combination where a diverse range of compounds were the target analytes (Kalua *et al*. 2006; Zander *et al*. 2005). The final conditions, 20 min at 40°C, were chosen as a compromise between the need for sufficient sensitivity to detect target analytes at low concentrations, and to limit oversaturation of the fibre with the extraction of compounds at high concentrations.

Identification/quantification/validation of a range of volatile compounds in Semillon.

As a starting point, twenty-one volatile compounds in the GCMS chromatogram were assessed by headspace analysis of the two Semillon wines. These compounds were chosen based mainly on the intensity of their GCMS peak areas. Two capillary columns of different polarity were used (*Table 5.10*) to calculate retention indices of theses volatile compounds on the two phases. Five-point calibration curves were constructed for each reference compound (with I.S.) on the CPWax column taking into account the concentration ranges expected for Semillon wines based on preliminary studies and as reported by Clarke *et al*. (2004). Linearity was tested within the specific range for each compound (*Table 5.10*) and correlation coefficients (R2) were considered precise for R2 ≥0.97 (*Table 5.10*).

Peak area ratios for triplicate runs were averaged and relative standard deviations (RSD) were calculated for each analyte (*Tables 5.11* and *5.12*). The limit of detection (LOD) and quantification (LOQ) were calculated as the minimum concentration that generates a peak signal at least 3 times higher (LOD) or 10 times higher (LOQ) than the signal from adjacent noise. As evident from *Table 5.12*, ethyl dodeconoate was the only volatile compound that was below the LOQ for all the seven wines analysed. The esters, hexyl acetate, methyl octanoate and isoamyl octanoate, all had at least three wines for which the concentrations were below the LOQ.

Recovery experiments in old and young Semillons wines demonstrated that the method was applicable across the two distinct styles (*Table 5.11*), especially given the low concentration of some analytes. Recoveries were not calculated for analytes whose concentration in the wines was less than its LOQ. The high response by MS detection for ethyl decanoate resulted in a more variable recovery in comparison with the other analytes. As noted above, sample dilution could have been used, but with consequent loss of sensitivity for other analytes. At this stage it was not yet clear if ethyl decanoate contributed significantly to the sensory characteristics of the Semillon wines. If shown to be important by, for example GC-O, an improvement in the chromatographic conditions for this analyte may be necessary.

Hunter Valley Semillon is known to have contrasting sensorial characteristics for recent vintage and aged wines and it was of interest to assess the ability of the method to quantify the target analytes in groups of wines with such contrasting aroma profiles. Seven Hunter Valley Semillon wines from a variety of vineyards, producers, and age (2006 and 1996), were selected for analysis (i.e. performed in 2007).

Three esters, namely ethyl hexanoate, ethyl octanoate and ethyl decanoate had the most intense peak heights in the gas chromatograms (*Figure 5.21*), but were not representative of the most concentrated volatile compounds (*Table 5.12*). They actually had rather moderate concentrations, between 0.2 and 2.4 mg L^{-1}. The proportions of ethyl esters of the C4, C6, C8 and C10 acids were as found in other studies (Clarke and Bakker 2004). An alcohol, 3-methyl-1-butanol, was identified as the compound in highest concentration of the twenty-one analysed, 66–159 mg L^{-1}. Several other compounds–ethyl-S-lactate, hexanoic acid,

Table 5.10 Calibration curves and validation parameters of volatiles compounds analysed using HS-SPME-GC-MS.

	Compounds	R^2	Range (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})
1	ethyl butanoate	0.9990	0.10-0.80	0.03	0.08
2	ethyl 2-methylpropanoate	0.9832	0.02-0.20	0.004	0.015
3	isoamyl acetate	0.9988	0.01-0.02	0.003	0.01
4	ethyl 3-methylbutanoate	0.9942	0.02-0.06	0.01	0.02
5	3-methyl-1-butanol	0.9801	20.00-80.00	7.30	7.50
6	ethyl hexanoate	0.9980	0.40-2.00	0.10	0.11
7	hexyl acetate	0.9896	0.02-0.10	nd	0.02
8	ethyl-S-lactate	0.9999	10.00-50.00	3.00	10.00
9	1-hexanol	0.9879	0.20-3.20	nd	0.20
10	ethyl octanoate	0.9929	0.50-2.50	nd	0.50
11	methyl octanoate	0.9878	0.0004-0.002	nd	0.0004
12	ethyl decanoate	0.9717	0.05-0.40	nd	0.05
13	isoamyl octanoate	0.9968	0.00002-0.002	nd	0.0002
14	cis-3-hexen-1-ol	0.9999	0.10-0.50	0.03	0.057
15	2-phenylethylacetate	0.9982	0.002-0.25	0.001	0.002
16	ethyl dodecanoate	0.9970	0.004-0.02	nd	0.004
17	hexanoic acid	0.9895	10.00-30.00	8.30	8.34
18	2-phenylethanol	0.9950	3.00-7.00	nd	3.00
19	methyl decanoate	0.9846	0.001-0.008	0.0009	0.001
20	octanoic acid	0.9918	3.00-10.00	0.37	0.4
21	decanoic acid	0.9902	1.50-5.00	1.34	1.36

R^2 correlation coefficient; LOD Limit of detection; LOQ limit of quantification; nd not determined.

[a] Linear regression equation of the calibration curve obtained by [volatile compound/internal standard] peak area ratio

2-phenylethanol, octanoic acid and decanoic acid–ranged in concentrations of 1.5–24 mg L^{-1}. The majority of compounds had concentrations ≤1.5 mg L^{-1}.

The acetate and ethyl esters are known to undergo acid catalysed hydrolysis or formation during bottle aging (Clarke and Bakker 2004; Roussis *et al.* 2007). In the data presented for the Semillon wines in *Table 5.12*, there did not appear to be any significant trends evident for compound concentration based on the age of the wine. Given that the wines are from different vineyards and producers this is not altogether surprising. However, with a larger data set of wines, one would expect to see a separation of the data based on quantification of acetate and ethyl ester compounds.

A chemometric approach to linking GCMS analysis of volatile compounds to wine sensory data.

The previous section documented the successful validation and application of a sophisticated headspace SPME-GCMS analysis technique to the quantification of a variety of volatile compounds in Semillon wine utilising a range of internal standards. The SPME-nature of the technique meant that it was

Table 5.11 Recoveries of different compounds in 2006 and 1996 Semillon wines.

		Recovery (RSD, %)	
	Compounds	2006	1996
1	ethyl butanoate	92 (1)	65 (4)
2	ethyl 2-methylpropanoate	110 (5)	97 (9)
3	isoamyl acetate	77 (7)	98 (7)
4	ethyl 3-methylbutanoate	82 (3)	81 (2)
5	3-methyl-1-butanol	115 (22)	110 (20)
6	ethyl hexanoate	83 (2)	67 (12)
7	hexyl acetate	ND	ND
8	ethyl-S-lactate	100 (9)	110 (4)
9	1-hexanol	81 (8)	112 (7)
10	ethyl octanoate	106 (7)	88 (2)
11	methyl octanoate	131 (13)	59 (19)
12	ethyl decanoate	67 (7)	127 (3)
13	isoamyl octanoate	93 (10)	ND
14	cis-3-hexen-1-ol	67 (1)	83 (4)
15	2-phenylethylacetate	87 (1)	89 (13)
16	ethyl dodecanoate	101 (2)	ND
17	hexanoic acid	123 (2)	128 (6)
18	2-phenylethanol	80 (3)	85 (14)
19	methyl decanoate	94 (3)	134 (4)
20	octanoic acid	111 (20)	87 (11)
21	decanoic acid	91 (3)	95 (9)

ND, not determined

Table 5.12 Quantification of 21 volatile compounds in seven Semillon wines expressed as mg/L. Orange boxes indicates the RSD for samples is >10%. Odour thresholds are as reported in wine except where flagged by an astrix (i.e., these are the water thresholds). The odours are as described on Flavour Net.

				Average of Concentration							
				Wine 1	Wine 2	Wine 3	Wine 4	Wine 5	Wine 6	Wine 7	
	Compounds	Odour	Odour Threshold (mg L-1)	2006	2006	1996	2005	2006	2006	1998	Overall average
1	Ethyl butanoate	fruity ; apple	0.02	0.52	0.43	0.66	0.58	0.60	0.42	0.41	0.52
2	Ethyl 2-methylpropanoa	strawberry ; sweet, rubber	0.015	0.14	0.15	0.16	0.19	0.12	0.29	0.25	0.19
3	Isoamyl acetate	banana	0.03	0.37	0.06	0.01	0.03	0.03	0.19	0.01	0.10
4	Ethyl isovalerate	fruity	0.003	0.02	0.03	0.04	0.04	0.04	0.03	0.05	0.03
5	3-Methyl-1-butanol	chemical, harsh, stale; whiskey, ma	30	92.86	83.92	66.04	112.78	126.71	158.56	103.19	106.00
6	Ethyl hexanoate	fruity; apple peel, fruit	0.005	1.17	1.02	1.50	1.29	1.04	1.06	1.28	1.19
7	Hexyl acetate	fruity, herb	2-480E-6*	0.2663	<LOQ	<LOQ	<LOQ	<LOQ	0.0429	<LOQ	0.15
8	Ethyl-S-lactate	fruit	0.00005*	19.77	18.09	16.39	16.84	15.45	24.19	20.55	19.00
9	1-Hexanol	grass, green, fresh; resin, flower, gr	8	0.99	0.48	1.14	1.07	0.67	0.95	0.68	0.85
10	Ethyl octanoate	fruity, fresh; fruit, fat	0.002	1.80	1.85	1.48	2.41	1.96	1.79	1.80	1.87
11	Methyl octanoate	orange	0.000004*	0.0006	<LOQ	0.0013	<LOQ	<LOQ	<LOQ	<LOQ	
12	Ethyl decanoate	grape	0.000008*	0.51	0.57	0.08	0.60	0.43	0.45	0.22	0.41
13	Isoamyl octanoate	not reported		<LOQ	<LOQ	<LOQ	0.0002	0.0002	0.0002	<LOQ	
14	Cis-3-hexen-1-ol	grass	0.4	0.20	0.11	0.16	0.16	0.10	0.14	0.13	0.14
15	2-Phenylethylacetate	flowery; fruit, sweet	0.25	0.106	0.028	0.017	0.018	0.017	0.043	0.017	0.03
16	Ethyl dodecanoate	leaf		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	
17	Hexanoic acid	green	0.42	10.63	10.24	10.33	10.91	10.55	10.80	11.22	11.00
18	2-Phenylethanol	flowery, rose; honey, spice, rose, lil	10.00	4.86	5.08	3.70	11.04	10.05	10.24	8.00	7.57
19	Methyl decanoate	wine	200-870E-6	0.009	0.009	0.004	0.007	0.006	0.007	0.003	0.01
20	Octanoic acid	fatty, unpleasant; sweat, cheese	0.5	7.88	6.93	5.21	4.50	8.04	7.02	6.77	6.62
21	Decanoic acid	rancid, fat	1	2.81	2.54	1.53	2.33	2.30	2.62	2.00	2.31

Table 5.12 (continued). The RSD values associated with the quantification of 21 volatile compounds in seven Semillon wines.

		Standard deviation/average (in percentage)						
		Wine 1	Wine 2	Wine 3	Wine 4	Wine 5	Wine 6	Wine 7
	Compounds	2006	2006	1996	2005	2006	2006	1998
1	Ethyl butanoate	7%	5%	5%	7%	3%	8%	4%
2	Ethyl 2-methylpropanoa	14%	4%	6%	19%	6%	5%	12%
3	Isoamyl acetate	15%	3%	10%	4%	2%	2%	7%
4	Ethyl isovalerate	2%	2%	5%	6%	1%	3%	2%
5	3-Methyl-1-butanol	6%	10%	1%	6%	4%	3%	3%
6	Ethyl hexanoate	11%	2%	3%	4%	26%	4%	8%
7	Hexyl acetate	2%					3%	
8	Ethyl-S-lactate	10%	6%	2%	21%	11%	1%	12%
9	1-Hexanol	3%	3%	1%	3%	9%	2%	3%
10	Ethyl octanoate	5%	0%	3%	6%	7%	2%	1%
11	Methyl octanoate	7%		8%				
12	Ethyl decanoate	16%	1%	20%	10%	17%	6%	8%
13	Isoamyl octanoate				14%	3%	1%	
14	Cis-3-hexen-1-ol	6%	2%	7%	6%	11%	2%	6%
15	2-Phenylethylacetate	17%	12%	5%	4%	7%	22%	11%
16	Ethyl dodecanoate							
17	Hexanoic acid	2%	2%	2%	3%	4%	1%	4%
18	2-Phenylethanol	12%	12%	2%	12%	1%	6%	13%
19	Methyl decanoate	4%	1%	3%	7%	4%	14%	2%
20	Octanoic acid	5%	4%	4%	7%	14%	3%	3%
21	Decanoic acid	6%	4%	1%	9%	0%	4%	3%

well automated and could extract a reasonable range of volatile compounds with the tri-phase solid phase material within the SPME fibre. However, one draw back was the fact that the µg quantities of adsoptive material were more prone to interferences from alcohol in the wine matrix, including competitive adsorption processes for certain compounds. Although this wasn't a problem for the volatile compounds quantified in the previous section, it was hoped to maximise the range of compounds detected by GCMS for comparison to sensory data, and therefore to utilise a more generic extraction procedure than SPME. As a result, a well cited and validated SPE methodology was selected that would enable both direct extraction

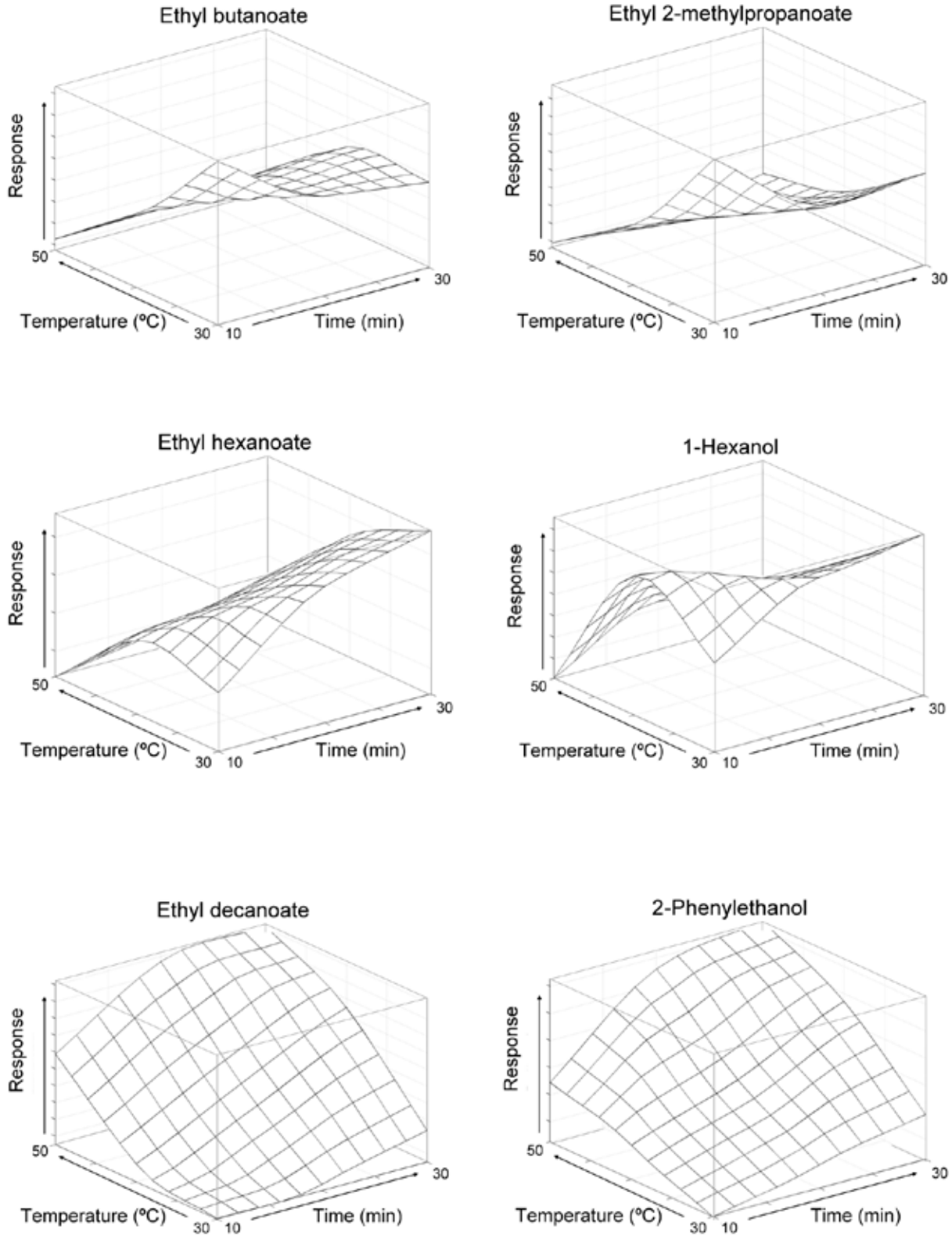


Figure 5.20 Response surface model showing peak response vs extraction time and temperature used for HS-SPME.

of volatile components from wine, and also the derivatisation of certain wine components to enhance their detection. The application of the SPE GCMS techniques, in combination with a chemometric analysis of the GCMS data, would allow a multi-block GMCS analysis of a range of volatile compounds with a diverse range of functional groups to be compared to the sensory data of the wines.

To enable a comprehensive chemometric investigation of the volatile and sensory composition of the wines to be undertaken, the total ion chromatogram (TIC) of the GC-MS data was aligned (time shifted in segments) to correct for retention time shifts. Following data pre-processing the TIC was then subjected to principal component analysis (PCA) to determine potential and interesting areas of the elution profiles that may correlate with experimental factors. Sensory data was investigated using PARAFAC to determine the patterns of sensory data pertinent to the wines and was then used to regress the GC-MS signals to sensory attributes. The use of feature selection and Projection to Latent

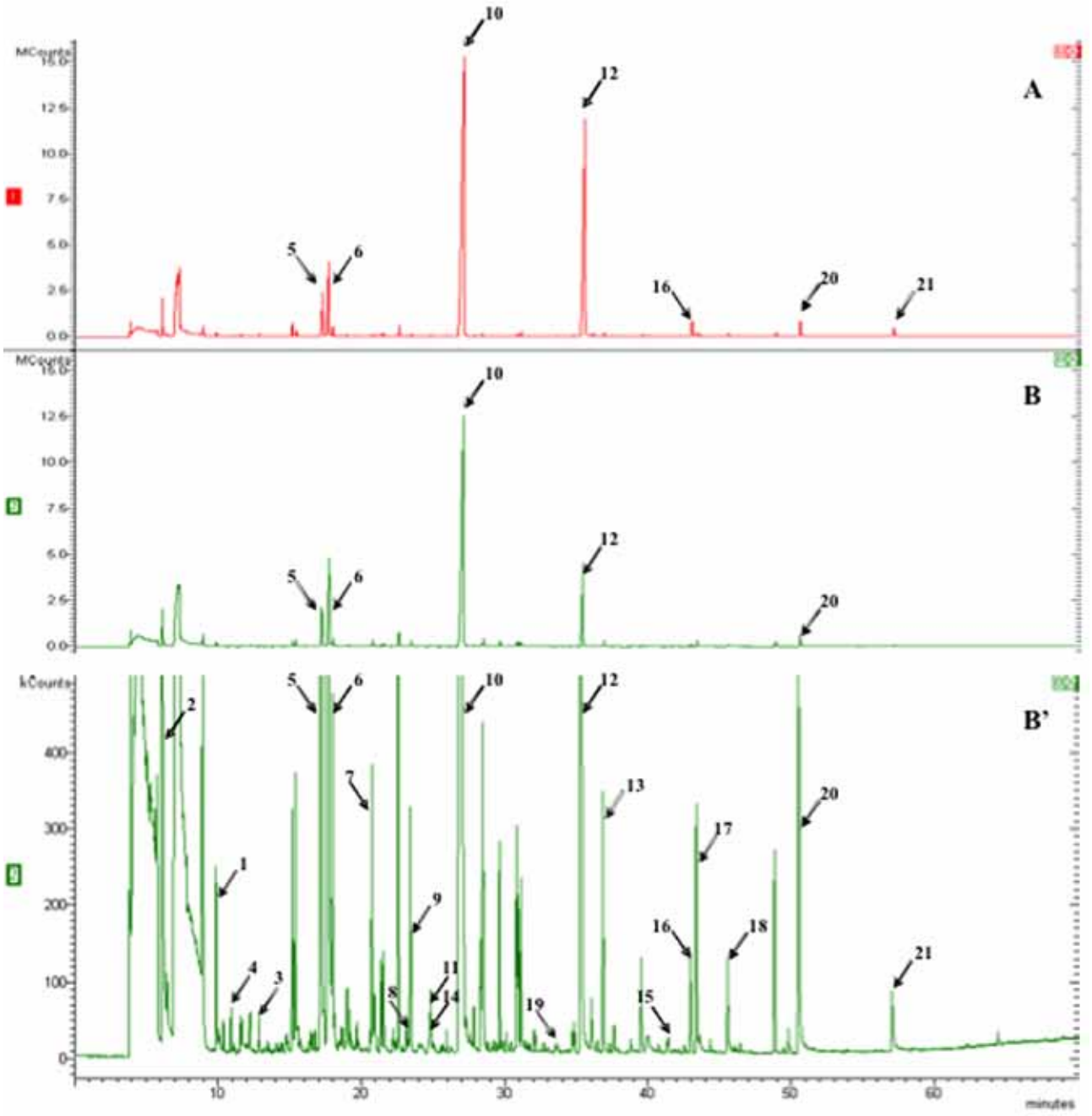


Figure 5.21 Total ion chromatogram of Semillon wines: 2006 (A) and 1996 (B and B' with y axes changed) obtained by HS-SPME at the optimal sampling condition. Peak identification: 1, Ethyl butanoate; 2, Ethyl 2-methylpropanoate; 3, Isoamyl acetate; 4, Ethyl 3-methylbutanoate; 5, 3-Methyl-1-butanol; 6, Ethyl hexanoate; 7, Hexyl acetate; 8, Ethyl-S-lactate; 9, 1-Hexanol; 10, Ethyl octanoate; 11, Methyl octanoate; 12, Ethyl decanoate; 13, Isoamyl octanoate; 14, Cis-3-hexen-1-ol; 15, 2-Phenylethylacetate; 16, Ethyl dodecanoate; 17, Hexanoic acid; 18, 2-Phenylethanol; 19, Methyl decanoate; 20, Octanoic acid; 21, Decanoic acid

Structure regression enabled specific areas of the elution profiles to be identification that correlate with the sensory attributes. Specific compound identification is ongoing that correlates with the sensory attributes.

A third chemometric approach employed Common Components and Specific Weights (CCSW) analysis in which all data sets are decomposed simultaneously. CCSW analysis enables the identification of common data regions with data sets that correspond to specific experimental factors. Thus sensory data and the two GC-MS data sets were be simultaneously mined to identify potential markers associated with vintage and the previously defined wine styles.

The SPE GCMS and derivatised-SPE GCMS techniques adopted throughout this section are outlined in the methods section of this report.

Alignment of GCMS data

A total of 16 Hunter Valley wine samples were extracted in triplicate by the SPE and derivatised-SPE GCMS methods (see *Experiment 5.7, Materials and methods*) at the same time that sensory evaluations were conducted (see *Experiment 5.1, Materials and methods*). The acquired GCMS data was imported into Matlab (i.e. as an ascii file) and the TIC was constructed for all samples (i.e. with a 35–350 m/z scan range). *Figure 5.22* shows an example of the overlaid SPE-GCMS chromatograms for several samples.

In order to successfully apply the chemometric modelling to the chromatographic data, the data first required alignment in order to minimise any apparent retention time fluctuations. *Figures 5.22* to *5.27* show the alignment process (i.e. utilising 'icoshift') for the SPE GCMS data, and an identical procedure was utilised for the derivatised-SPE GMCS data. Once the data was aligned, then 'pre-treatment' was conducted by identifying the lowest signal value and adding this as '+1' to all generated matrix values to enable a logarithmic (base 10) transformation. Log transformations enables relatively small and large variations to be accounted for when conducting PCA and PLS procedures, especially as signals throughout the GCMS chromatograms vary considerably from the baseline to the maximum value.

Initial exploratory analysis revealed that data between 6.8 and 9.5 minutes of the GCMS chromatograms to have an overly large influence on any PCA models thus this region was excluded from all further processing.

PCA analysis of GCMS/wine samples

The variance in the GCMS data, with respect to samples, was then examined by PCA. The number of 'Principal Components' (PC) chosen were based upon the 'Eigenvalue of the Correlation Matrix' and the percent variance was captured for each PC. Seven PCs modelled approximately 67% of data variance. The score plots, coded for wine sample with replications, are illustrated below (*Figure 5.28*). Note that the plots were inspected for replicate number and analysis day to determine if significant influence upon the PCA arose from these factors. No obvious influence could be observed for a seven PC model (data not shown). Good grouping of sample replicates is obvious for the selected PCs and wines appear to be separated by increasing age from bottom right to top left.

Loading plots for each PC plotted against GCMS chromatographic elution time are illustrated below (*Figure 5.29*) with the corresponding 'Durbin Watson Criteria'. These plots give an indication of the elution time for peaks detected by the GC-MS that contribute important variation for each PC. In *Figure 5.29* the loadings have been overlaid with the Total Ion Chromatogram (TIC) from the GCMS data set to assist in identification of peaks of interest. Note that these plots can be expanded when in MATLAB.

Thus the first seven principal components model approximately 67% of data variance (*Table 5.13*). Evident from the plots of principle components is grouping of sample replicates in PC1 and PC2, with wines separated by age in a pattern from bottom left to top right. Plotting the PCs with coded samples for analytical order, replicate order or sample day, did not reveal any common pattern associated with the extracted PC's (data not shown), and thus it can be

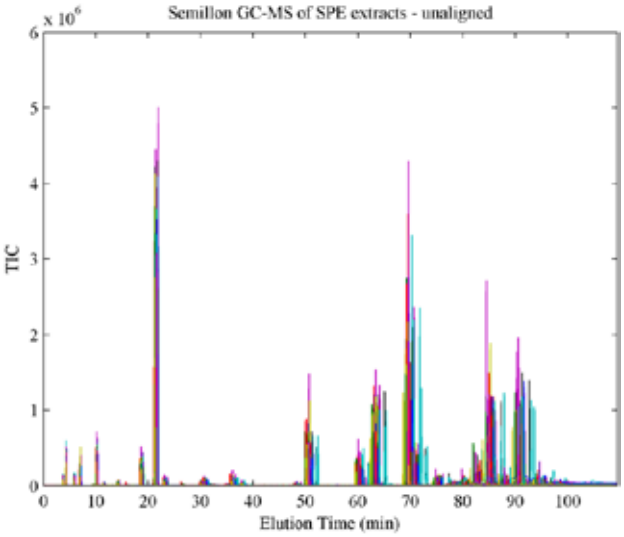


Figure 5.22 SPE-GCMS chromatograms of Semillon wine – unaligned raw data.

concluded that no significant variation is associated with the order of analysis. Variation arising from the actual SPE GCMS analytical process is captured within PCs. This outcome was also observed for a PCA analysis of the derivatised-SPED GCMS data (data not shown).

To facilitate interpretation of the loadings, the scores from the PCA of the SPE GC-MS TIC results were rotated using the Varimax criteria and the

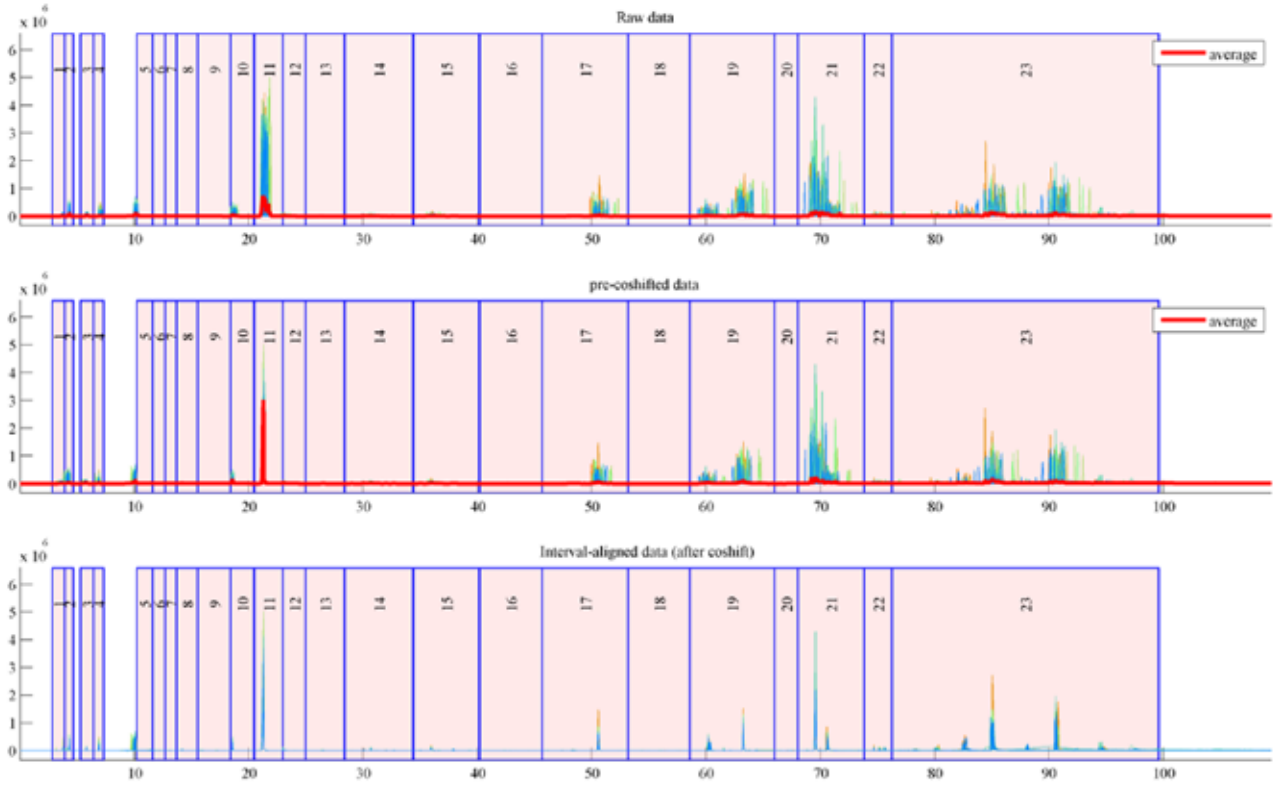


Figure 5.23 GCMS chromatograms subject to first icoshift with two passes to align peaks.

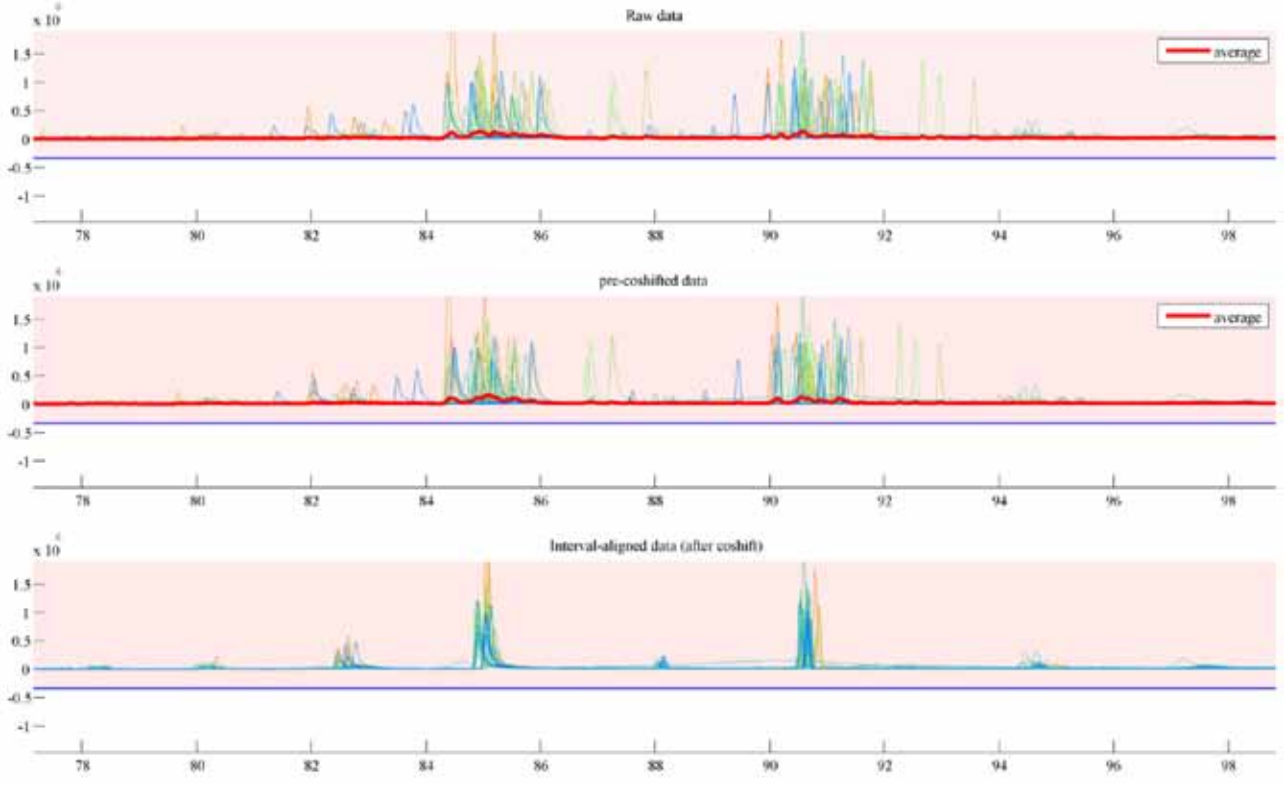


Figure 5.24 Zoomed section (78–98 min) of GCMS chromatograms (from *Figure 5.22*) subject to first icoshift with two passes to align peaks.

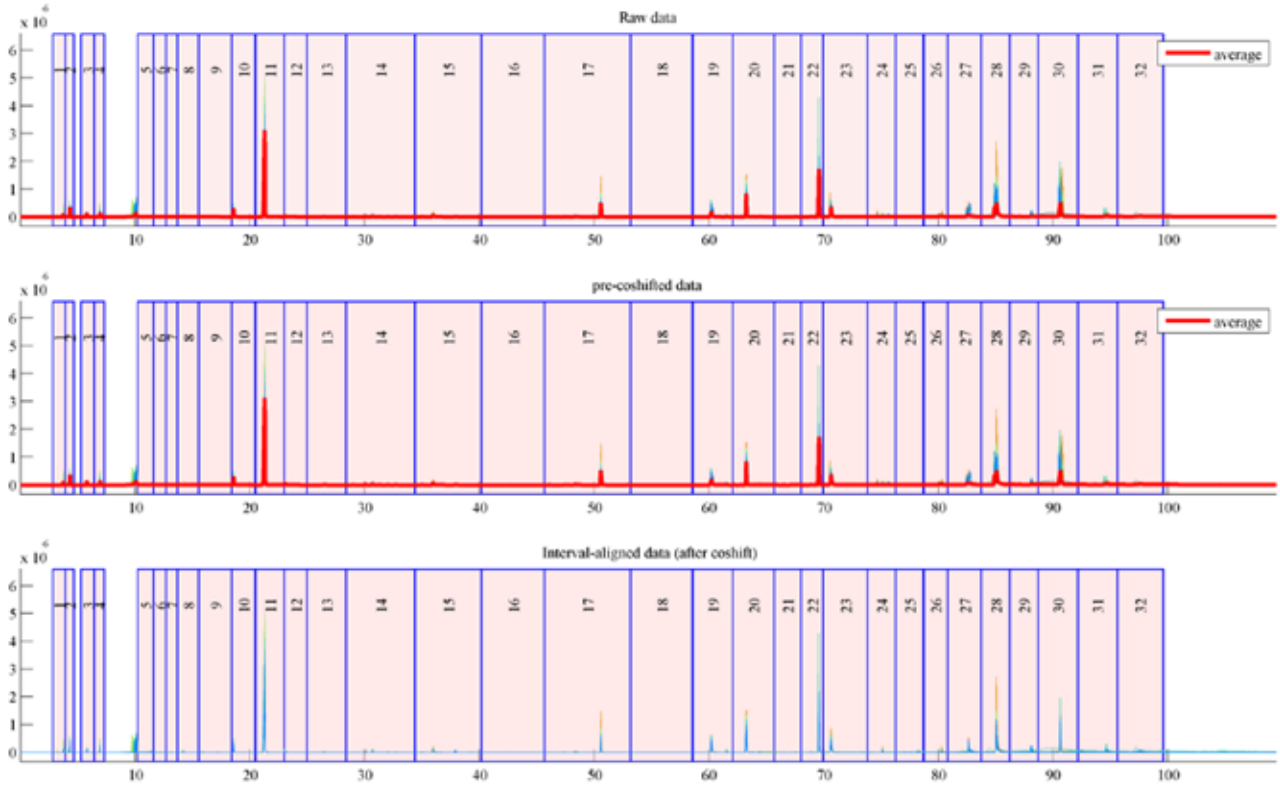


Figure 5.25 GCMS chromatograms subject to second icoshift with two passes to align peaks.

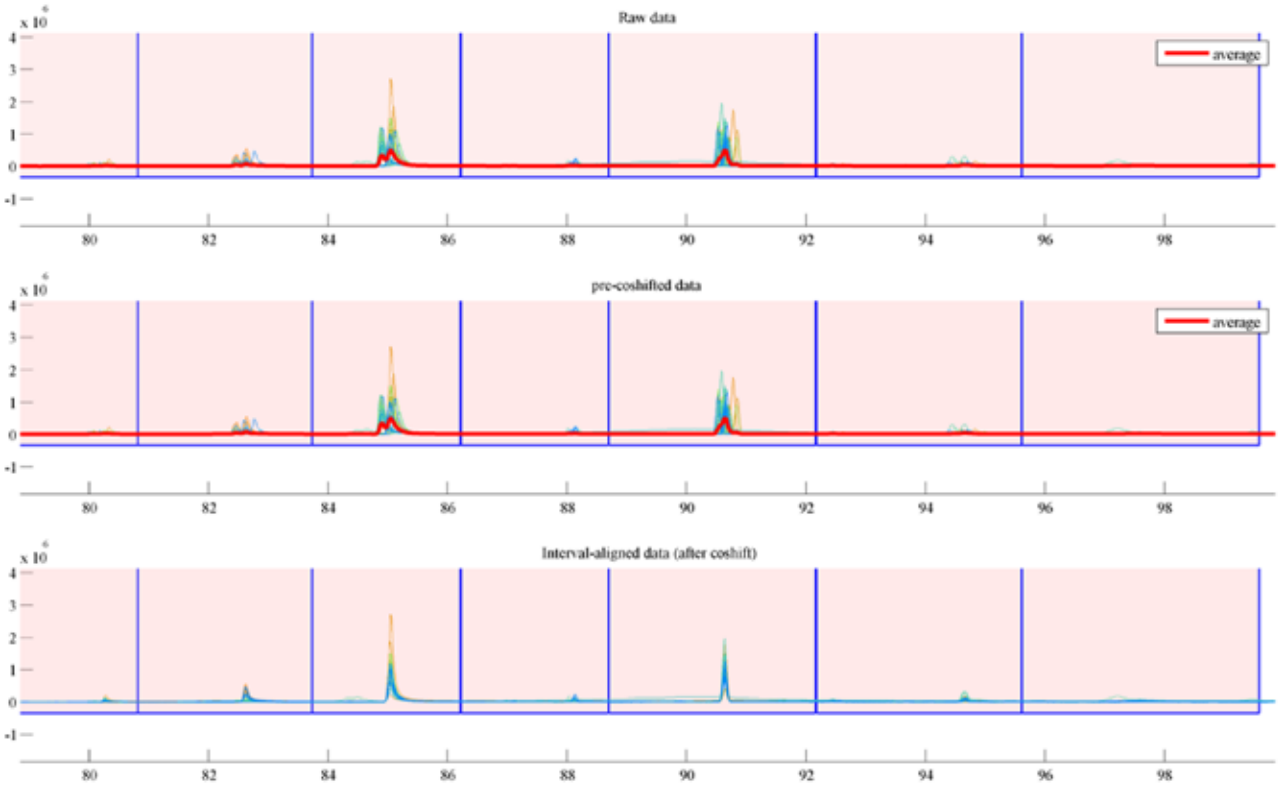


Figure 5.26 Zoomed section (80-98 min) of GCMS chromatograms (from *Figure 5.22*) subject to second icoshift with two passes to align peaks.

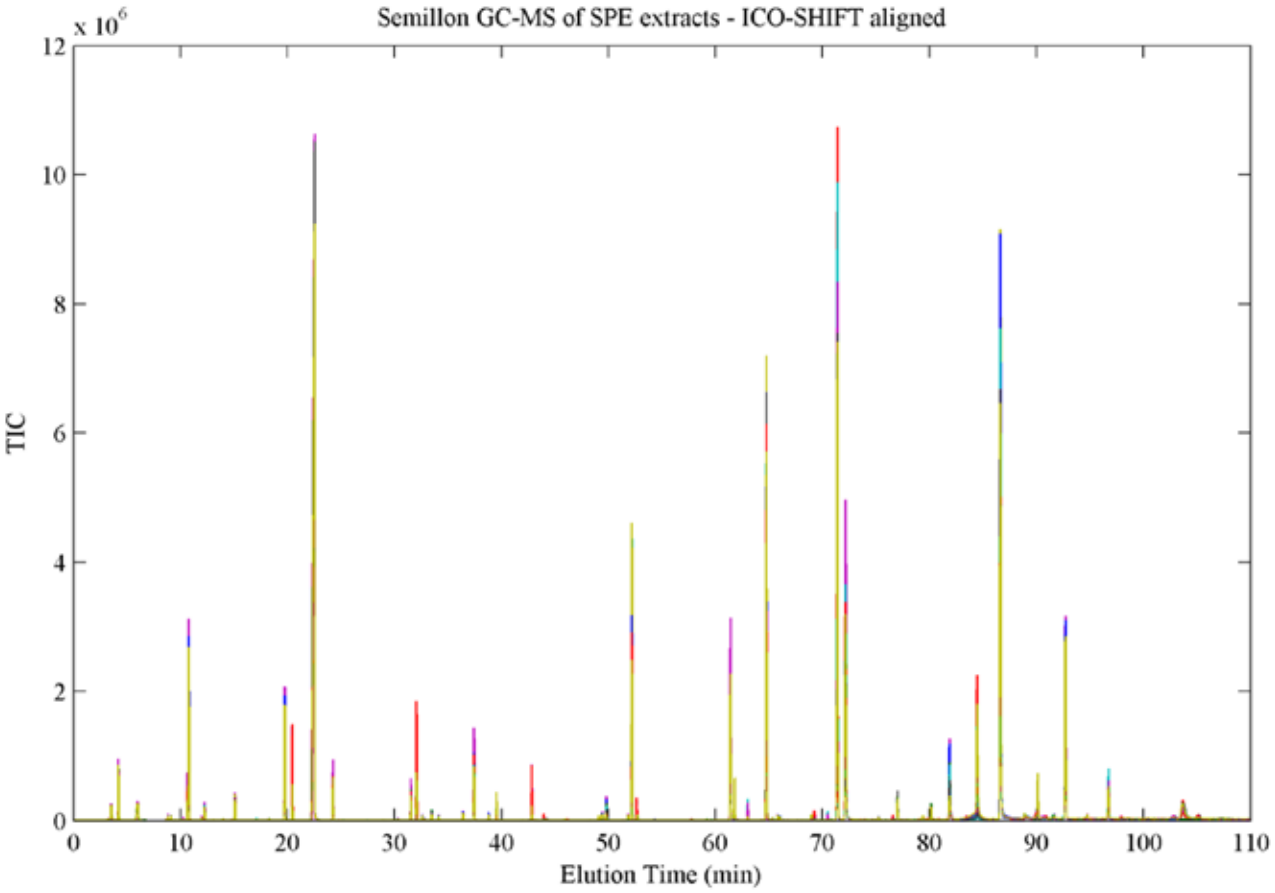


Figure 5.27 27 SPE-GCMS chromatograms of Semillon wine – aligned data.

rotated scores and loadings plotted below with colour coding for wine number, wine style and vintage (*Figure 5.30*, components 1–7). The associated rotated loadings are also included which have been plotted for elution time to assist in the recognition of peaks within the chromatogram associated with the extracted and rotated PC. Ongoing work will identify specific compounds with elution profiles that correspond to the loading plots and these compounds will be identified on the basis of National Institute of Standards and Technology (NIST) mass spectral fragmentation patterns and Kovats retention time index.

From the rotated PCA model excellent groupings of wines in components 2, 4, 5 and 6 are of particular interest and the corresponding loadings plots will assist in the identification of compounds that have significance for modelling the wines. This identification work will form the basis of future investigations for compound identification.

Decomposition of the sensory data utilising PARAFAC modelling.

The sensory data was decomposed using PARAFAC modelling. Raw sensory scores were fitted to a data cube of 16 samples x 15 sensory attributes x 48 (16x3) panellists x replicate. The data was mean centred (across mode 1) and the sensory data decomposed using PARAFAC with decreasing numbers of factors extracted, commencing with 5, until a stable core consistency was achieved. The total number of factors extracted was 3 with a core consistency diagnostic of 98, explaining 18% of data variance (*Table 5.14*).

Table 5.13 The variance (%) captured by the PCA model

Principal component number	% Variance captured this PC	% Variance captured total
1	23.06	23.06
2	14.03	37.09
3	11.00	48.09
4	7.62	55.71
5	5.06	60.77
6	3.71	64.48
7	2.92	67.40

Table 5.14 Percent variance captured by PARAFAC Model (size [16 15 48], decomposed using 3 factors)

	Fit		Unique Fit	
Comp	(%X)	(%Model)	(%X)	(%Model)
1	14.29	69.24	13.96	67.64
2	4.17	20.18	4.07	19.70
3	2.18	10.58	2.18	10.57

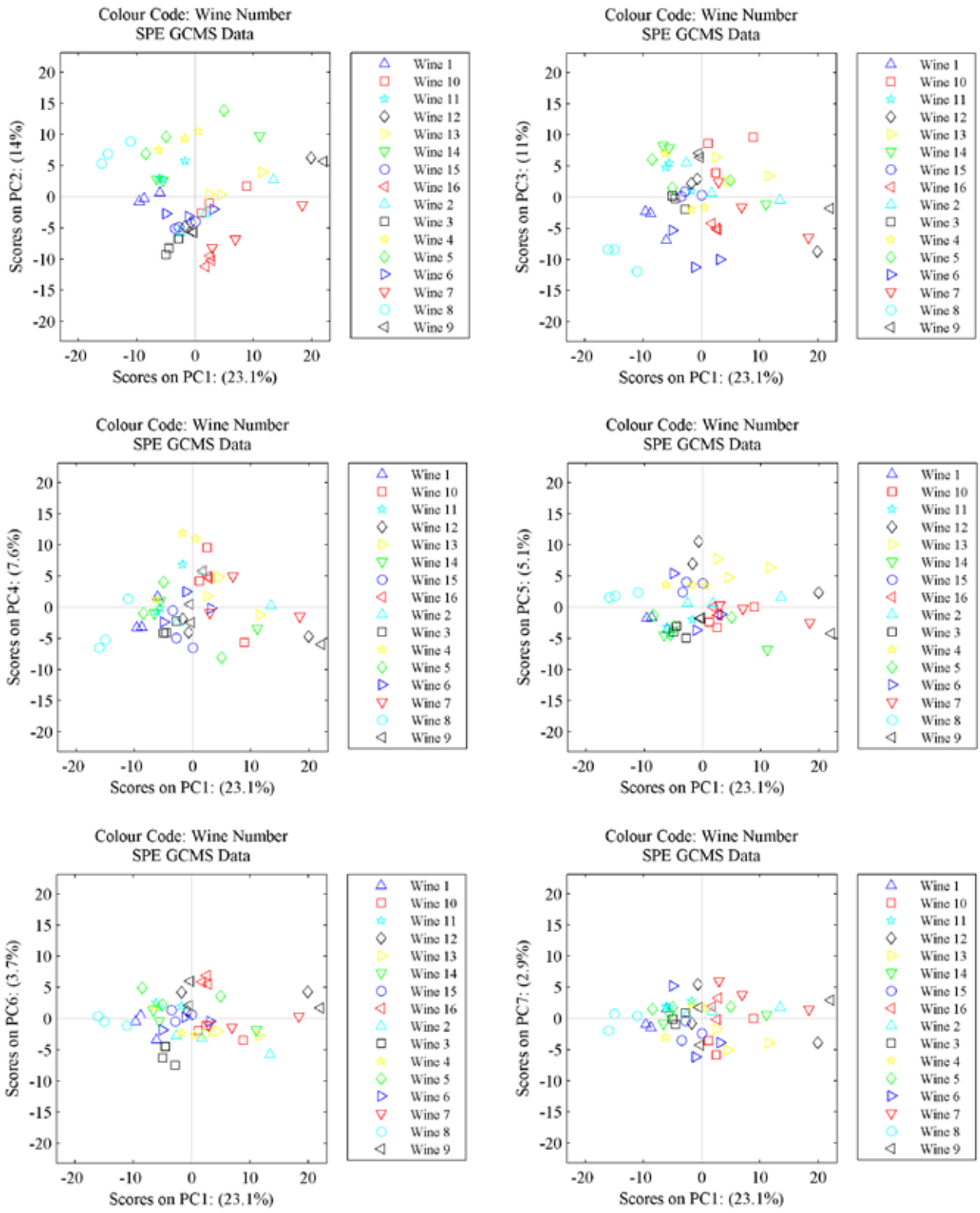


Figure 5.28 PCA score plots for Semillon wine samples based on SPE GCMS data.

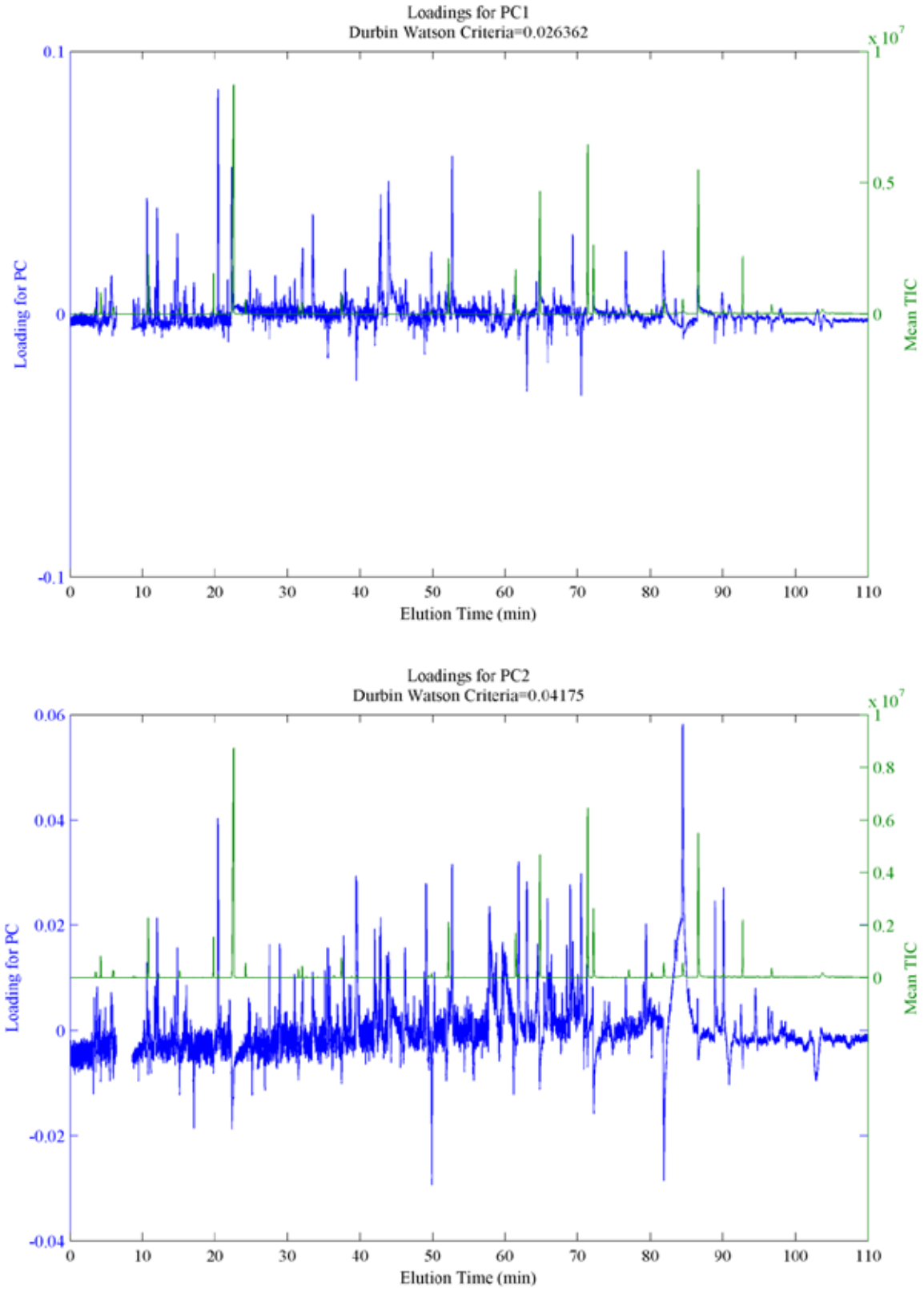


Figure 5.29 Loading plots for the seven PCs of *Figure 5.28* against the SPE GCMS chromatographic elution times, and with the GCMS TIC overlaid.

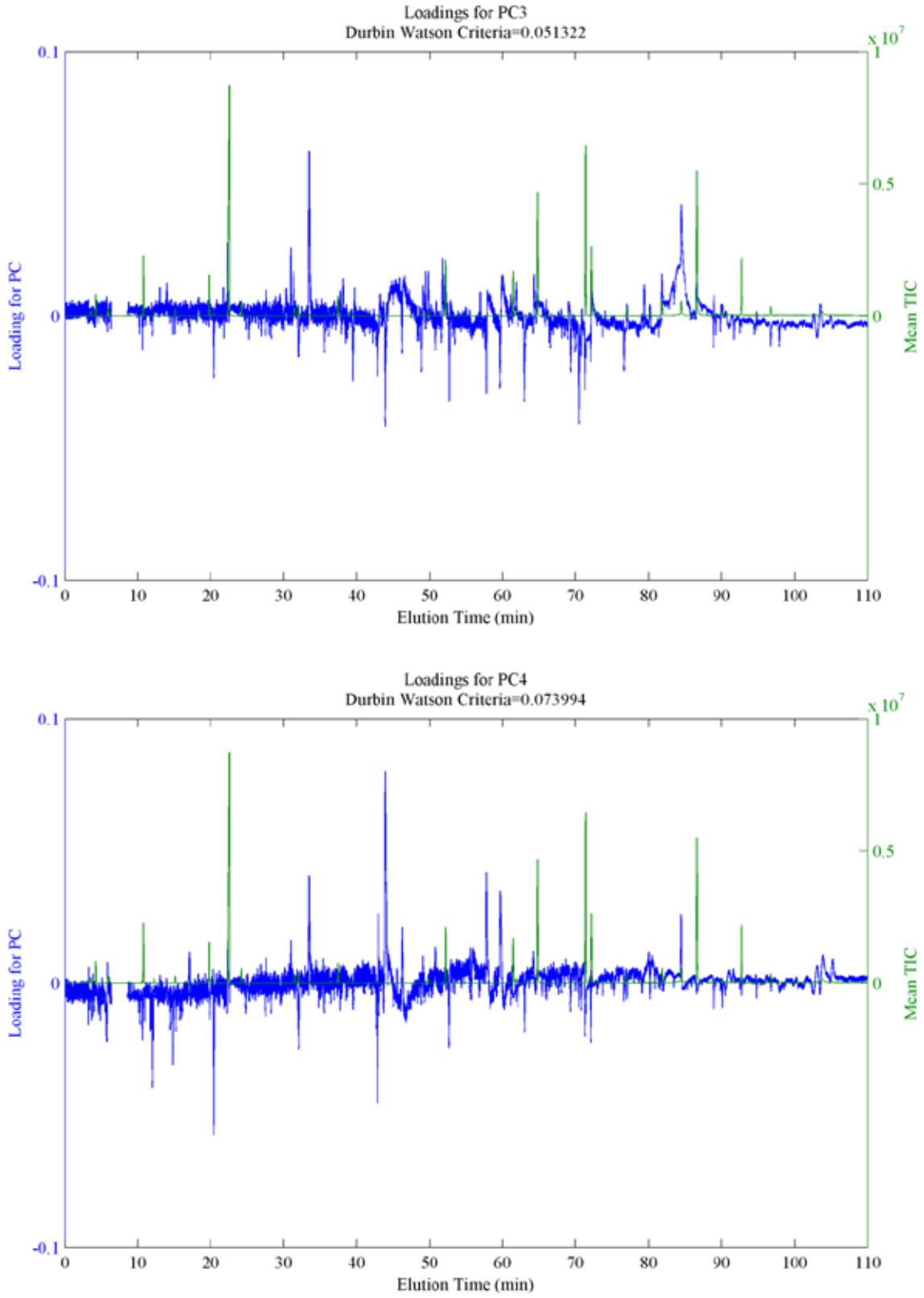


Figure 5.29 Loading plots for the seven PCs of *Figure 5.28* against the SPE GCMS chromatographic elution times, and with the GCMS TIC overlaid.

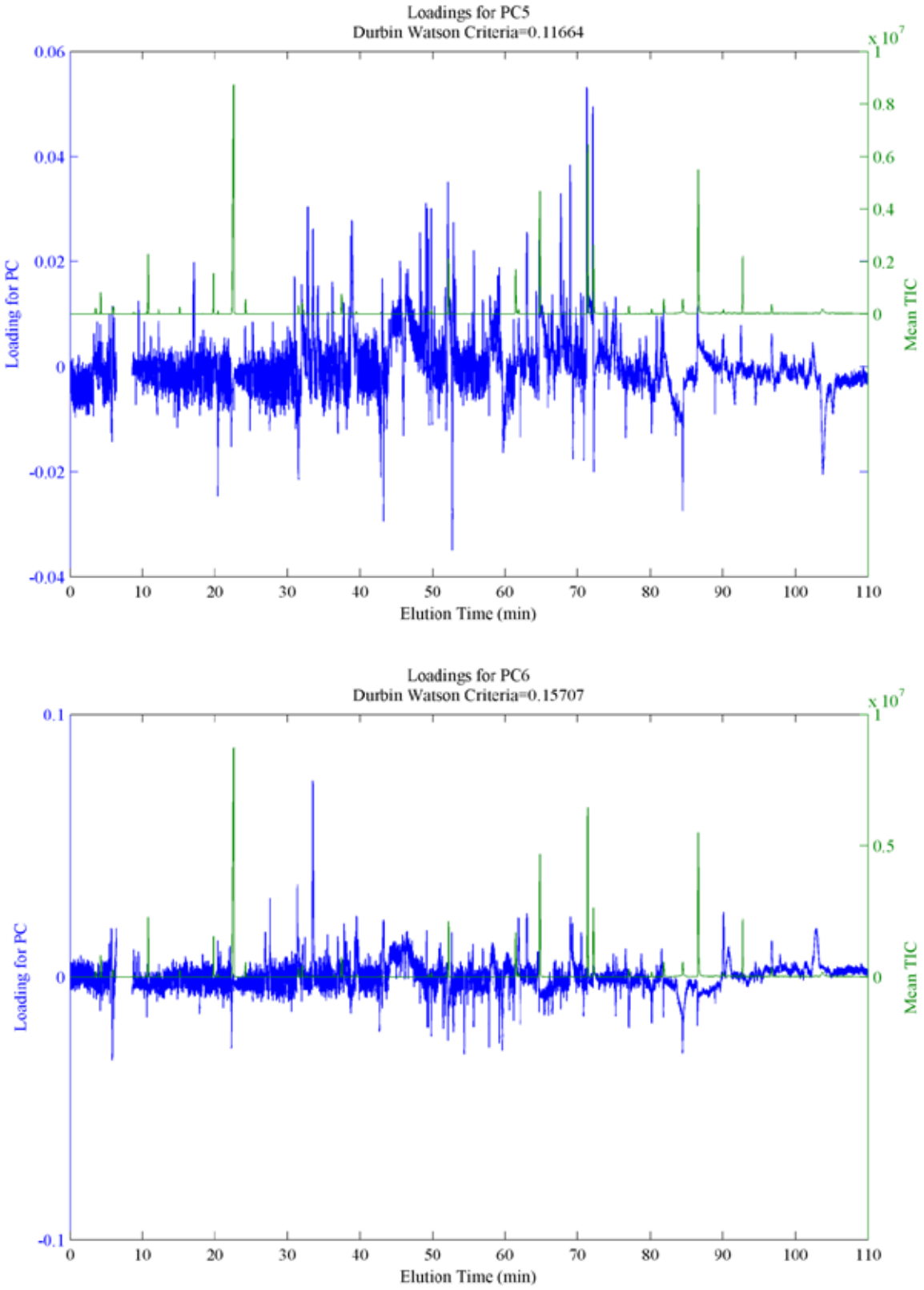


Figure 5.29 Loading plots for the seven PCs of *Figure 5.28* against the SPE GCMS chromatographic elution times, and with the GCMS TIC overlaid.

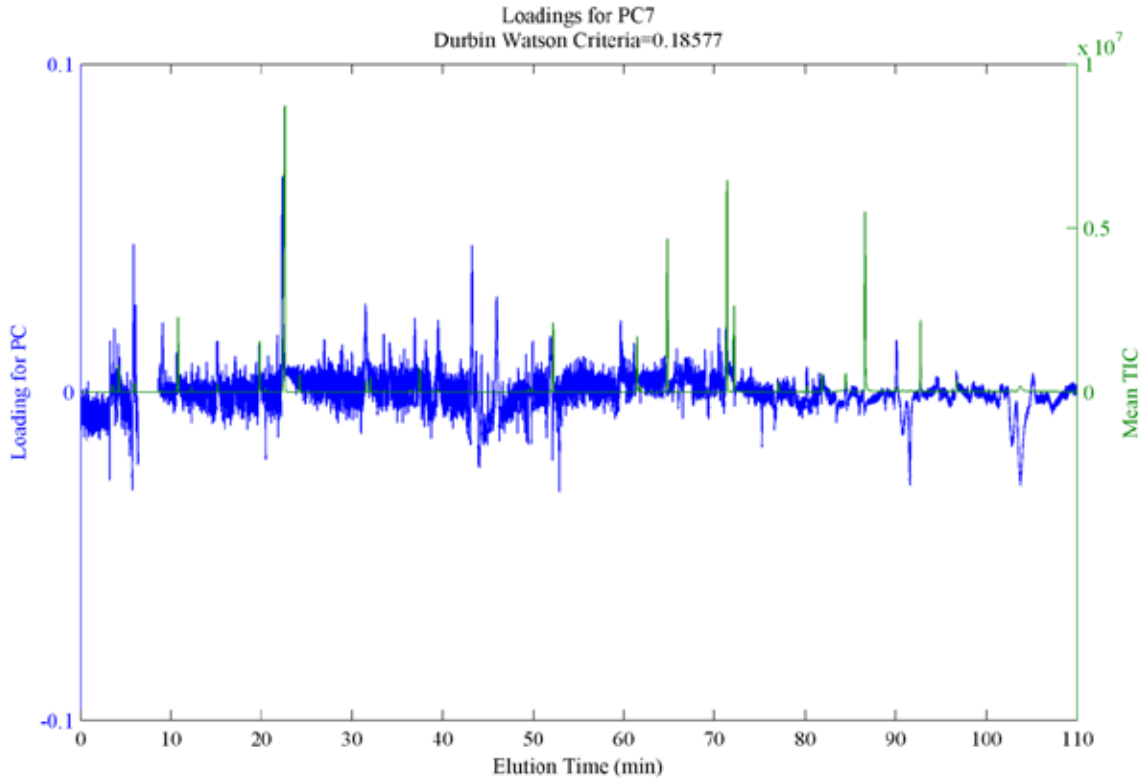


Figure 5.29 Loading plots for the seven PCs of *Figure 5.28* against the SPE GCMS chromatographic elution times, and with the GCMS TIC overlaid.

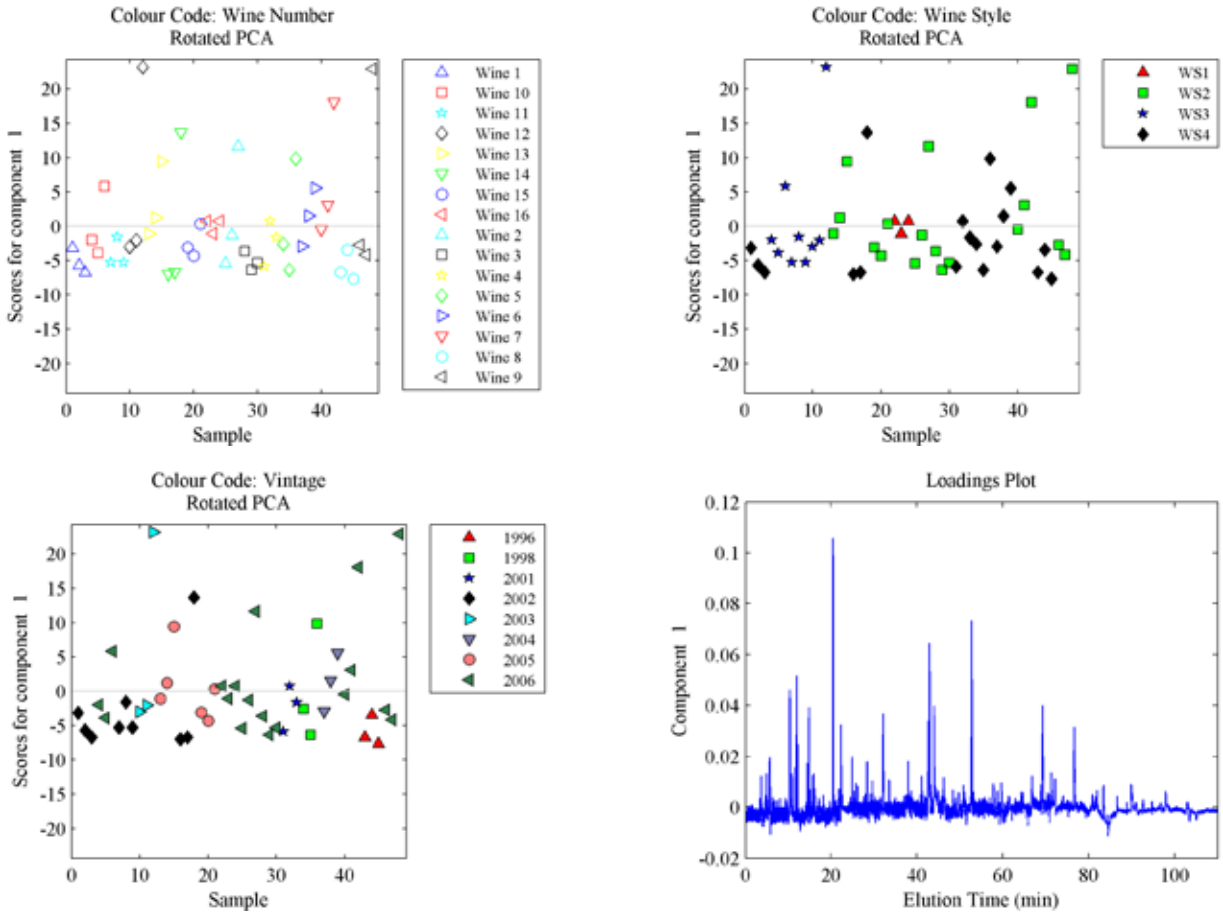


Figure 5.30a Rotated component 1 – SPE GC-MS TIC; WS=Wine Style

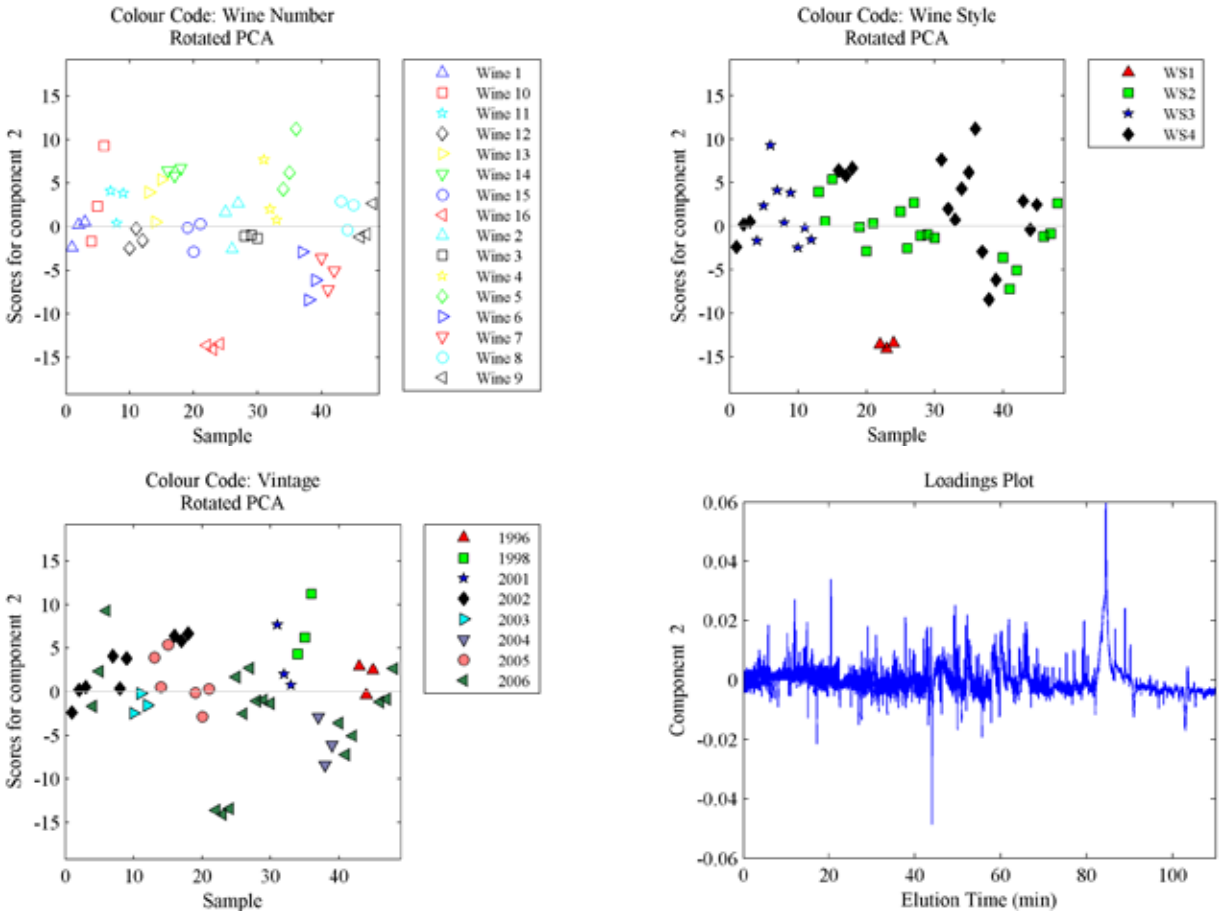


Figure 5.30b Rotated component 2 – SPE GC-MS TIC; WS=Wine Style

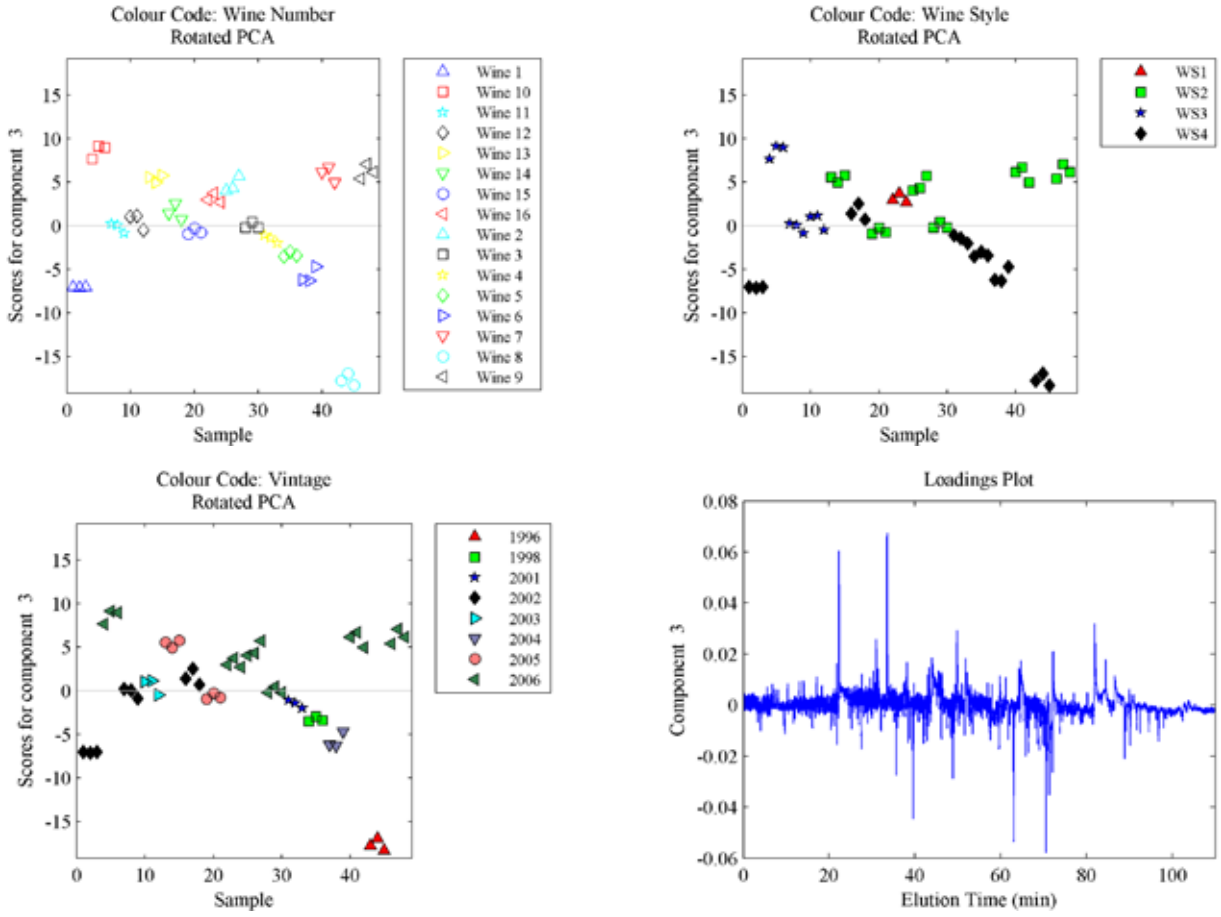


Figure 5.30c Rotated component 3 – SPE GC-MS TIC; WS=Wine Style

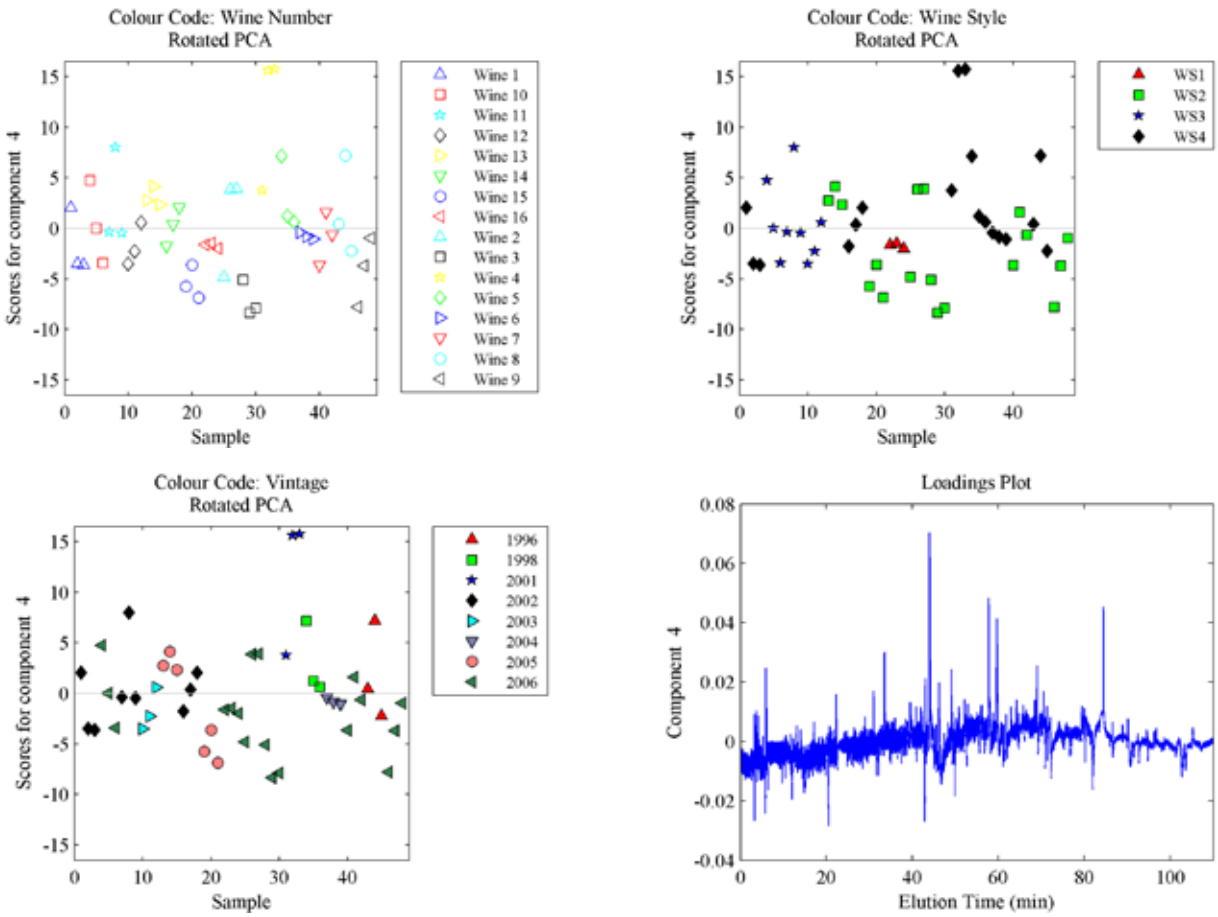


Figure 5.30d Rotated component 4 – SPE GC-MS TIC; WS=Wine Style

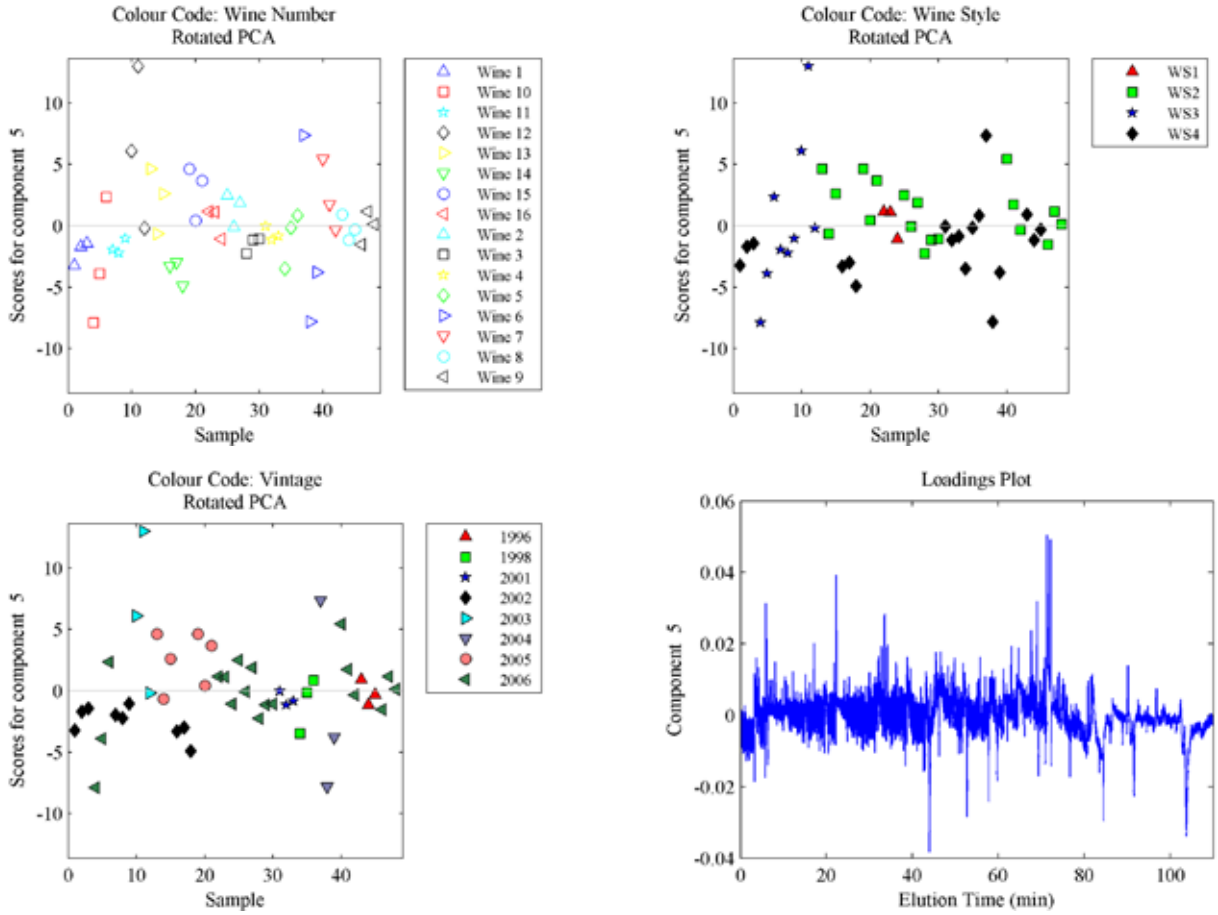


Figure 5.30e Rotated component 5 – SPE GC-MS TIC; WS=Wine Style

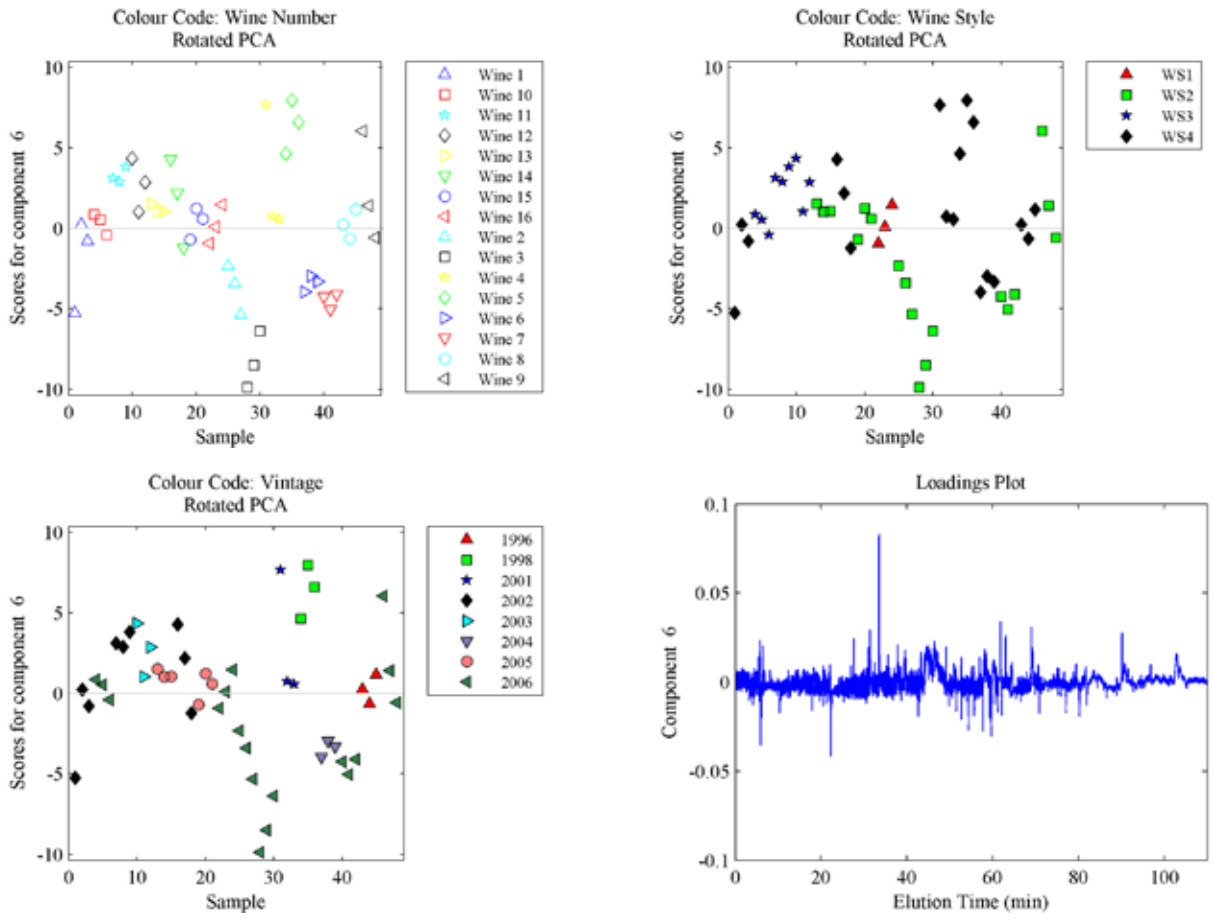


Figure 5.30f Rotated component 66 – SPE GC-MS TIC; WS=Wine Style

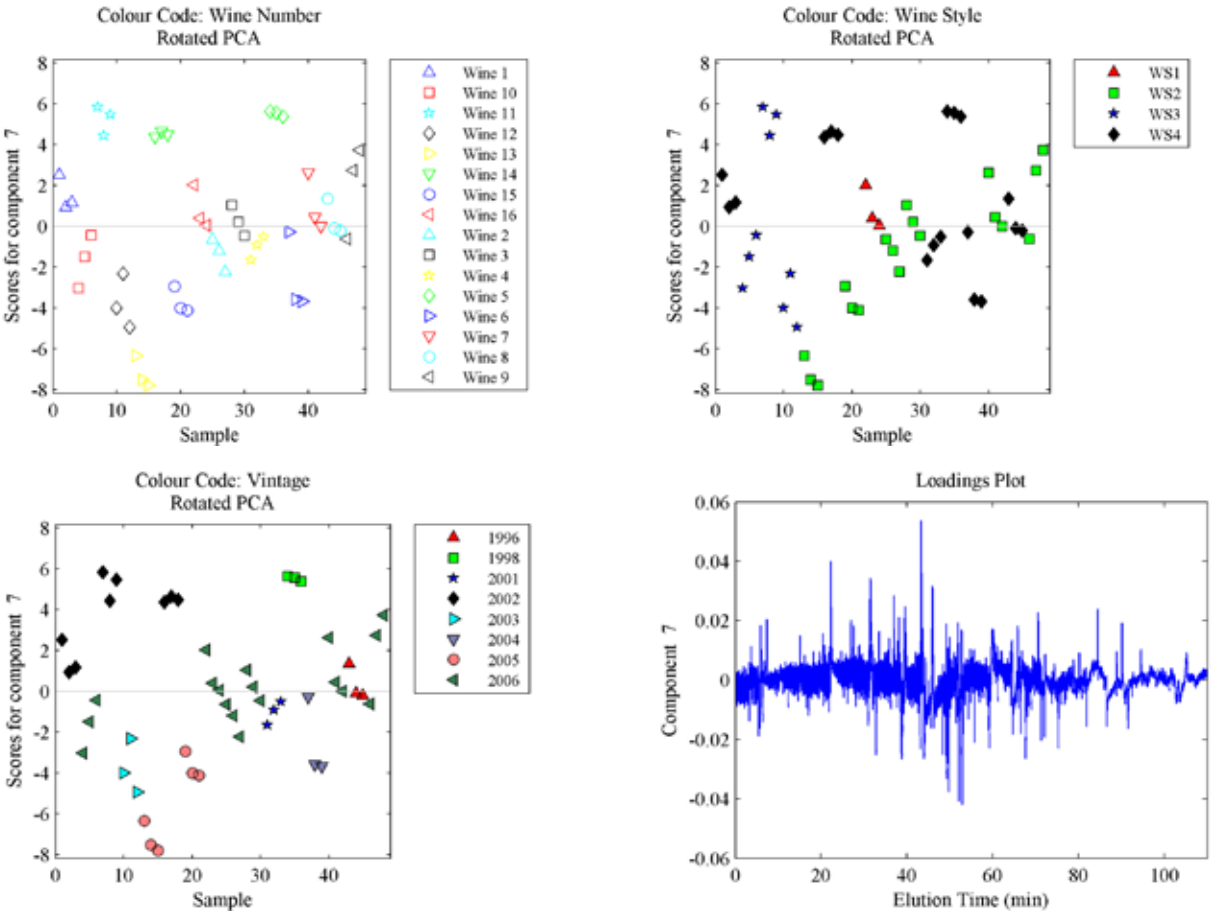


Figure 5.30g Rotated component 7 – SPE GC-MS TIC; WS=Wine Style

To assess the quality of the loadings, the residual sum of squares for each mode (samples, attributes and panellists) were used (*Figure 5.31*) and variables exceeding the 99% confidence interval were flagged as those that were poorly modelled. These variables were removed and the PARAFAC model re-fitted to the data. In summary, one panellist was poorly modelled due to inconsistent attribute identification and ranking, and was thus removed from the data set for the purposes of fitting regression models of GC-MS data to the sensory data. Following removal of this panellist's sensory data, three factors were extracted with a core consistency of 94% explaining 19% of sensory data variation (*Table 5.15*). In *Figure 5.32* loadings are plotted for each mode; wines, sensory attributes and panellists.

Table 5.15 Percent variance captured by PARAFAC Model (size [16 15 45], decomposed using 3 factors)

	Fit		Unique Fit	
Comp	(%X)	(%Model)	(%X)	(%Model)
1	14.33	71.07	14.23	70.59
2	3.69	18.30	3.66	18.17
3	2.14	10.64	2.14	10.64

Loading 1 in the sensory model shows a dispersion of wines with high honey, orange marmalade and toast sensory attributes (*Figure 5.32*). Evident from the plot for wine samples, these wines are typically older wines i.e.: ie vintages 1996 to 2001. Loading 2 is associated with the level of perceived sweetness (positive) and acidity (negative) in the wine samples and one wine is loaded in the positive direction and is identified as wine Style 1 (*Figure 5.32*).

PARAFAC loading three (*Figure 5.33* and *5.34*) clearly models developed Semillon sensory characters and fruitfulness of the wines. Aged Semillon attributes orange marmalade, honey and lemon-lime are positively located in the third loading, whilst less developed characters asparagus and hay/straw are negatively loaded. Of interest is the negative loading for toast in this loading. Generally loading three models wine Style 4 as evident in the plot of wines coded for style.

The PLS regression of SPE GCMS data onto sensory data

This section describes the PLS regression of GCMS data onto sensory scores. Of the 48 samples (16 wines x 3 replicates), each samples was randomly allocated to either a calibration or independent validation data set to give approximately a ²/₃ and ¹/₃ data split.

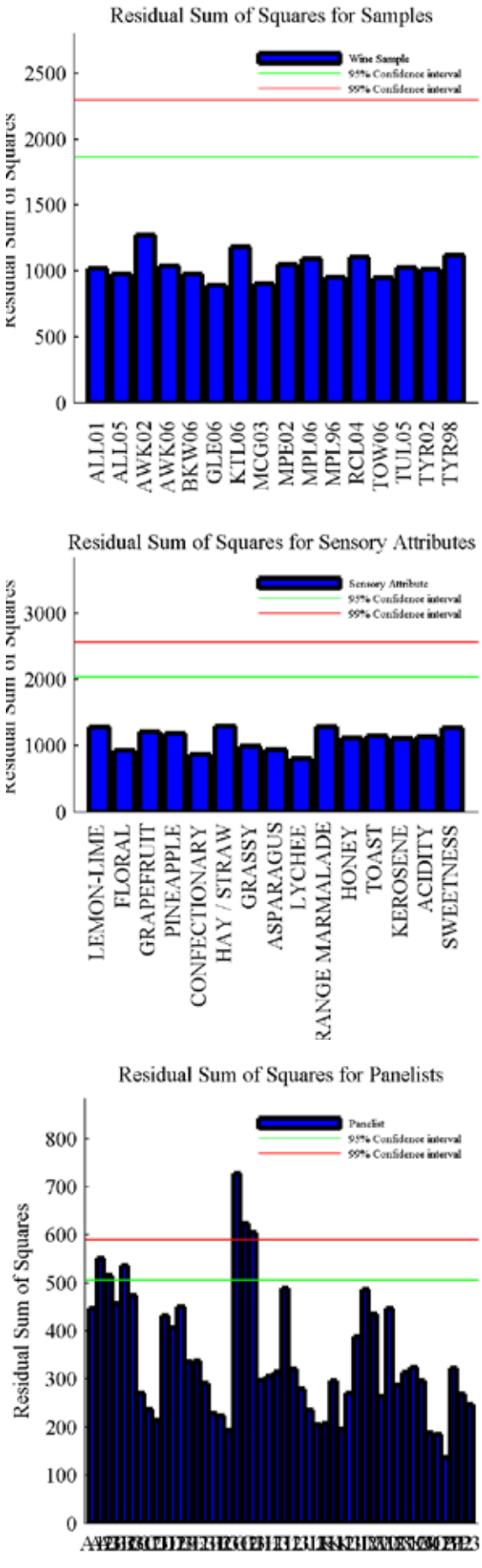


Figure 5.31 Residuals for samples, sensory attributes and panellists.

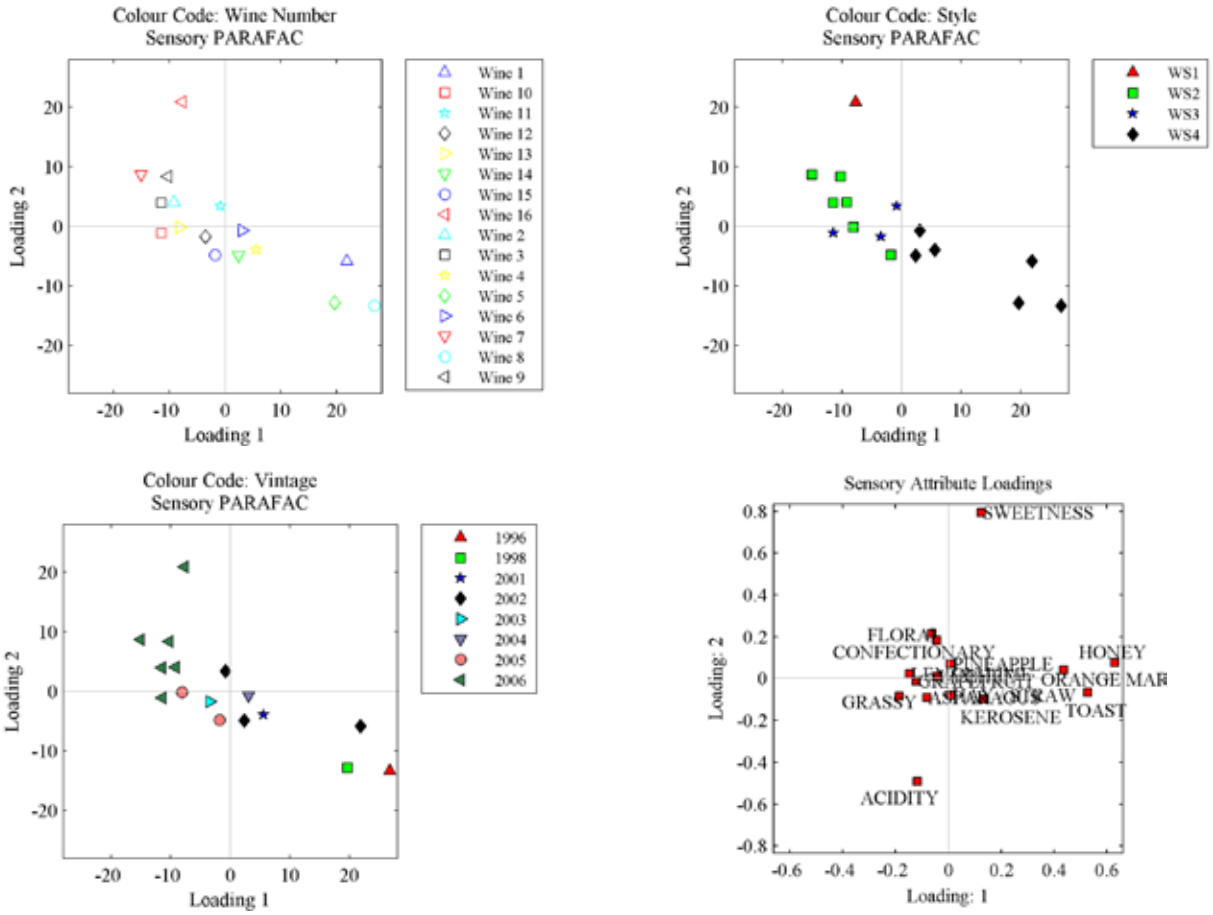


Figure 5.32 Sensory Parafac Model: Loading 1 versus Loading 2 for wines samples and sensory attributes.

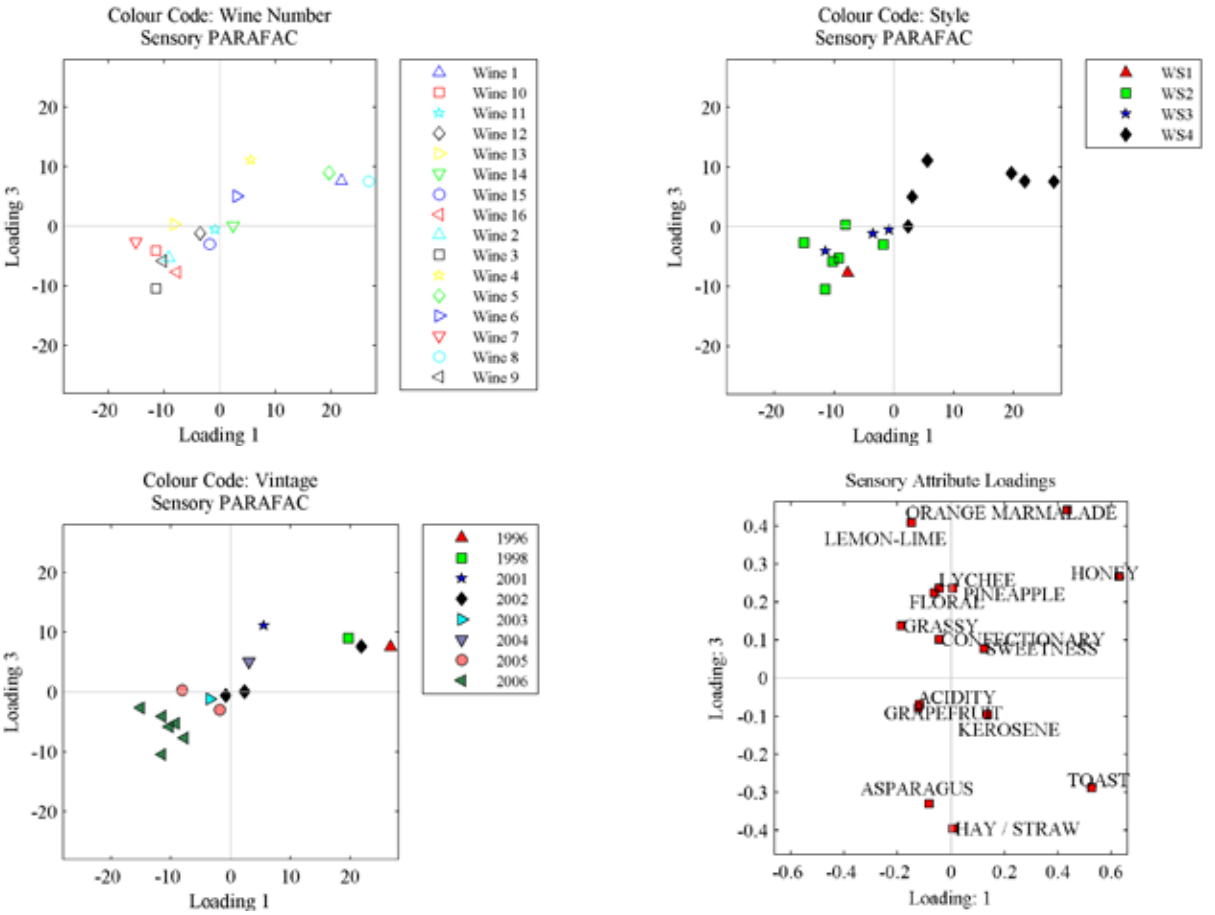


Figure 5.33 Sensory Parafac Model: Loading 1 versus Loading 3 for wines samples and sensory attributes.

An in-house Matlab routine was used for sample allocation and the allocations are shown in *Table 5.16*. Corresponding sensory attribute scores were derived from the replicate means for each wine sample following exclusion of one panellist.

Variable selection for regression analysis of either the GC-MS SPE or GC-MS SPE+Derivatised total ion chromatograms was conducted using the iPLS algorithm. A variable bucket width of 50 or 100 scans representing an elution profile of approximately 30 seconds for each data set was used for feature selection. For the purposes of modelling, multiple y-block variables were chosen chiefly based upon the results of the sensory PARAFAC decomposition. Attributes that were closely positioned in loadings plots were selected to be modelled together rather than as separate attributes. Thus PLS1 or PLS2 regression algorithms were used for modelling single or multiple sensory attributes with the GC-MS data sets. Cross validation of the regression models was performed using random subsets of the calibration data block with six data splits. The validation data set was used as an independent data block for testing the ability of the models to accurately predict the sensory data. *Table 5.17* shows a summary of the regression models from each data set (SPE and SPE + Derivitisation) for each sensory attribute.

Table 5.16 Allocation of samples to PLS calibration and validation data sets.

		Number of samples	
Wine sample	**Coded ID**	**Calibration**	**Validation**
Wine 1	2002	1	2
Wine 2	2006	2	1
Wine 3	2006	2	1
Wine 4	2001	2	1
Wine 5	1998	2	1
Wine 6	2004	1	2
Wine 7	2006	2	1
Wine 8	1996	2	1
Wine 9	2006	2	1
Wine 10	2006	1	2
Wine 11	2002	1	2
Wine 12	2006	2	1
Wine 13	2005	2	1
Wine 14	2002	1	2
Wine 15	2005	2	1
Wine 16	2006	1	2
TOTAL		26	22

Interpretation of the regression model performance must be considered in light of the nature of the data. Sensory data is inherently noisy, and GC-MS data

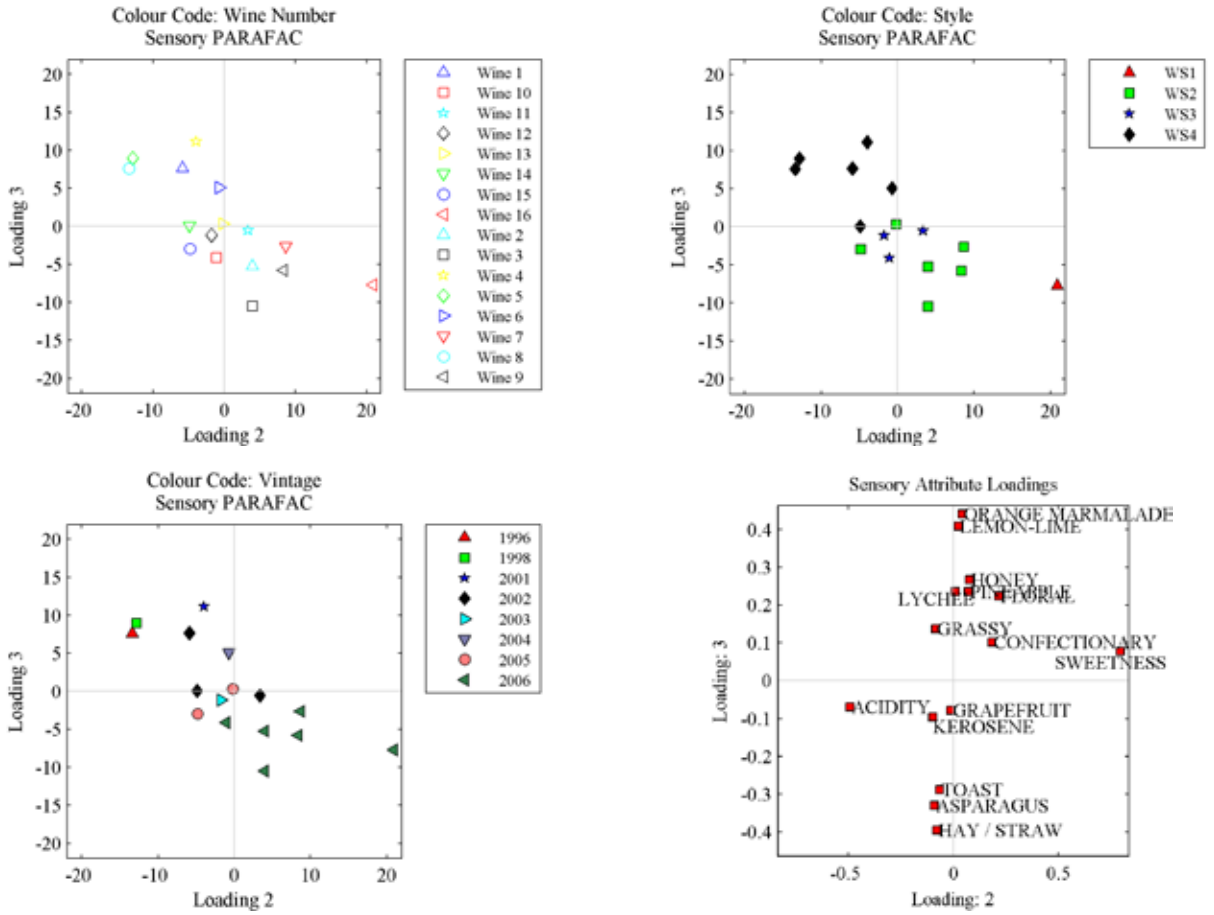


Figure 5.34 SENSORY PARAFAC MODEL: Loading 2 versus Loading 3 for wines samples and sensory attributes.

Table 5.17 Regression models from each data set (SPE and SPE + derivatisation) for each sensory attribute

	Sensory attribute characteristics			Regression model performance					
				SPE			SPE + derivitisation		
Sensory attribute	min	max	mean	R^2	RMSEP	#LV	R^2	RMSEP	#LV
Lemon/lime	2.21	4.67	3.58	0.56	0.53	6	0.40	0.51	5
Floral	0.83	2.97	1.63	0.37	0.58	6	0.34	0.57	7
Grapefruit	1.21	2.83	2.15	0.46	0.44	6	0.42	0.50	8
Pineapple	0.87	2.27	1.61	0.30	0.50	7	0.27	0.40	5
Confectionary	0.52	2.35	1.11	0.43	0.55	7	0.59	0.36	5
Hay/straw	1.49	3.63	2.63	0.40	0.52	8	0.50	0.41	4
Grassy	0.52	2.40	1.37	0.59	0.38	6	0.50	0.49	8
Asparagus	0.50	1.63	0.98	0.54	0.32	8	0.65	0.22	4
Lychee	0.67	1.96	1.22	0.52	0.31	5	0.18	0.48	7
Orange marmalade	1.07	4.77	2.20	0.89	0.47	7	0.75	0.61	6
Honey	2.03	6.87	3.65	0.87	0.69	7	0.83	0.73	6
Toast	1.61	5.83	3.44	0.92	0.47	9	0.77	0.71	4
Kerosene	1.48	3.43	2.49	0.53	0.52	9	0.39	0.45	4
Acidity	4.03	6.10	5.06	0.27	0.67	6	0.03	0.74	5
Sweetness	2.60	5.70	3.57	0.63	0.62	3	0.23	0.91	5

Note: similar coloured sensory attributes were modelled together

even more so. Furthermore, the sensory data has been averaged for replicates thus decreasing the range of values used for regression analysis. Attempting to regress two noisy data sets is unlikely to yield regression models with performance characteristics that can be compared to predictive models for quantifiable analytes such as that use in spectroscopy. Thus a conservative co-efficient of determination (R^2) of 0.7 has been selected to evaluate the PLS models. Unsurprisingly the best predictive models for the sensory characteristics of the wine are associated with the sensory attributes with the largest range of sensory ratings and are associated with the aging characteristics of Semillon wine (i.e. honey, toast and orange marmalade). The worst performing models are associated with the sensory attributes pertaining to sweetness and acidity and again this is somewhat expected as these sensory characters are predominantly related to mouth feel characters rather than volatile components of the wines.

The selected intervals of the SPE and SPE + Derivitisation GC-MS TIC and regression models are illustrated in *Figures 5.35* to *5.38* for the aging characters of Semillon wine (i.e. honey, toast and orange marmalaide). Interval selections are illustrated as green bands in the 'Forward iPLS Results' illustration (i.e. LHS of figure) and predictive model performance is shown as a 'measured versus predicted' graph (i.e. RHS of figure).

The regions selected by the iPLS algorithm and the performance of the regression models can be used to assist in the identification of specific compounds that may be potential markers of these sensory characteristics in Semillon wine. Furthermore, the marker compounds may themselves contribute directly to the sensory character of the wine. These regions are shown in *Figures 5.39* to *5.42*) with interesting regions lightly shaded and enlarged for clarity. To assist in the identification of candidate compounds, an alkane standard series has been overlaid onto the elution times which enables the determination of a retention time index, which can then be matched with published reference data.

Table 5.18 summarises the results of the iPLS variable selection method employed prior to PLS regressions. For each of the regions in the GCMS chromatograms of interest, candidate peaks are numbered in order of elution time and 'Kovats Retention Index' values computed for each. Ongoing work in which candidate peaks are identified using NIST database searches of ion fragmentation patterns, and the use of known standards will form the basis of identity confirmation for these compounds. Several of the highlighted regions can be given tentative identifications such as the following for the 'orange marmalade' and 'honey' sensory attributes from the GCMS SPE data; butanoic acid/ethyl decanoate (compound 3), diethylsuccinate (compound 4) and methionol (compound 5). Similarly for the 'toast' sensory attributes based on the GCMS SPE data; ethyl furoate (compound 1).

Table 5.18 The GCMS elution time and 'Kovats Retention Indices' for PLS identified regions of interest for two sensory attributes.

	Sensory Attributes							
	Orange marmalade and honey				Toast			
	GC-MS SPE		GC-MS SPE+Derivitisation		GC-MS SPE		GC-MS SPE+Derivitisation	
Candidate Compound	Elution time (min)	Kovats Retention Index	Elution time (min)	Kovats Retention Index	Elution time (min)	Kovats Retention Index	Elution time (min)	Kovats Retention Index
1	30.3	1337	34.6	1403	49.0	1642	43.8	1553
2	32.4	1369	39.6	1482	49.6	1653	48.8	1638
3	51.7	1689	45.3	1578	95.9	2623	79.1	2260
4	52.2	1698	45.7	1585				
5	53.4	1719	53.2	1716				
6	74.1	2125	75.1	2148				
7	74.3	2130	81.6	2296				
8	97.9	2659	94.5	2597				
9	98.1	2662	95.0	2607				
Kovat Retention Index determined using a wax column								

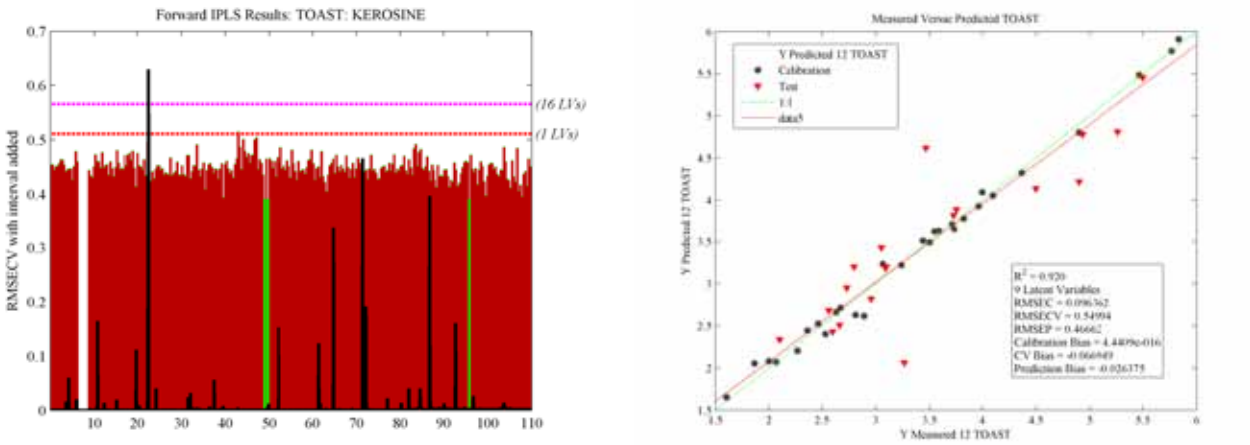


Figure 5.35 PLS analysis: selected SPE GCMS intervals for 'TOAST', and the 'measured versus predicted' graph.

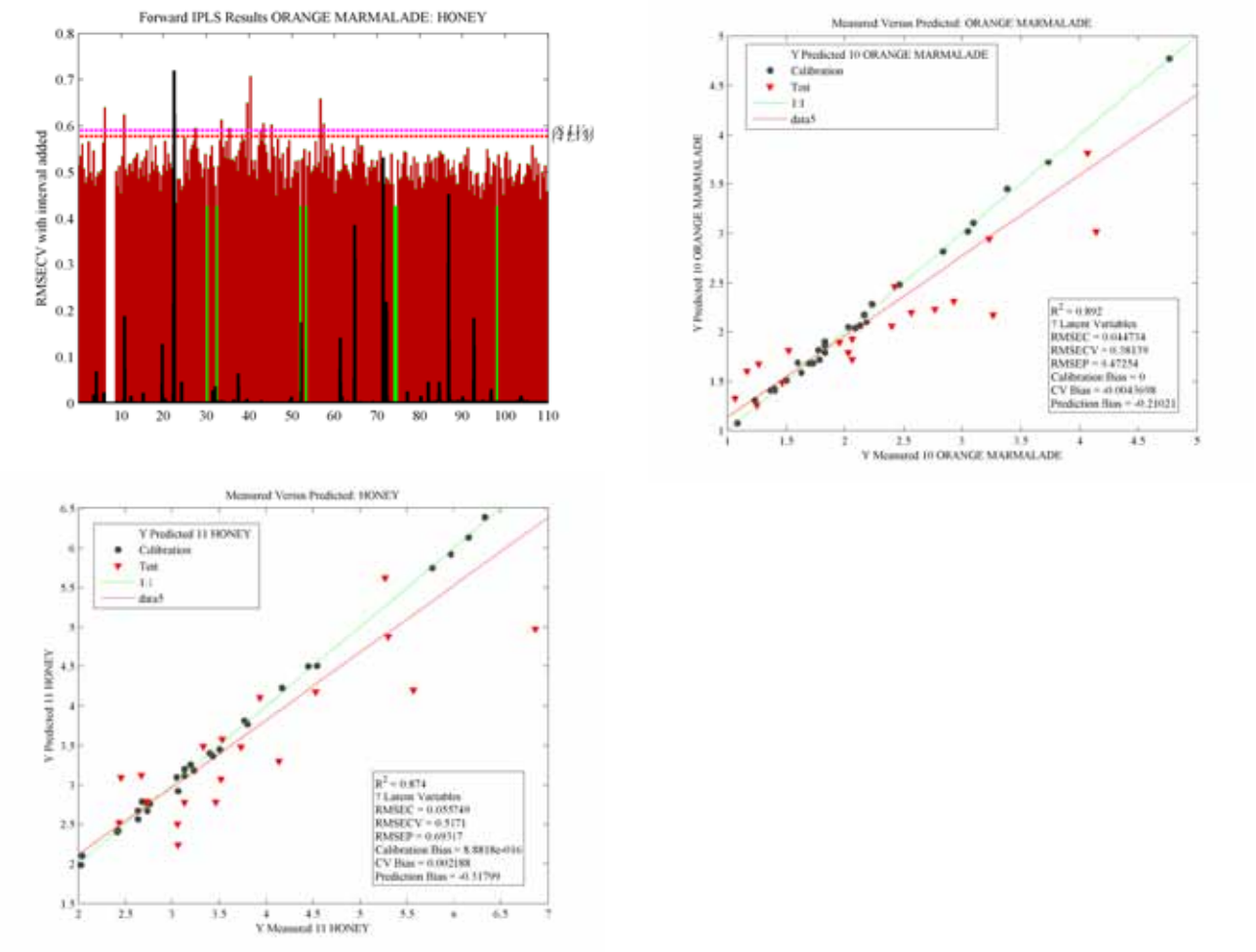


Figure 5.36 PLS analysis: selected SPE GCMS intervals for 'ORANGE MARMALADE + HONEY', and the 'measured versus predicted' graphs.

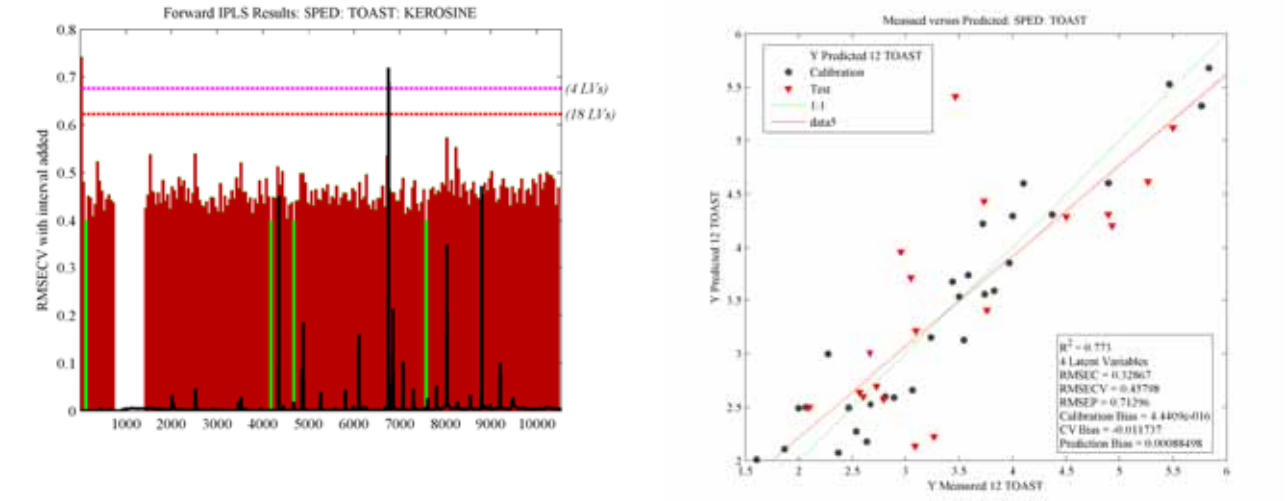


Figure 5.37 PLS analysis: selected derivatised-SPE GCMS intervals for 'TOAST', and the 'measured versus predicted' graph.

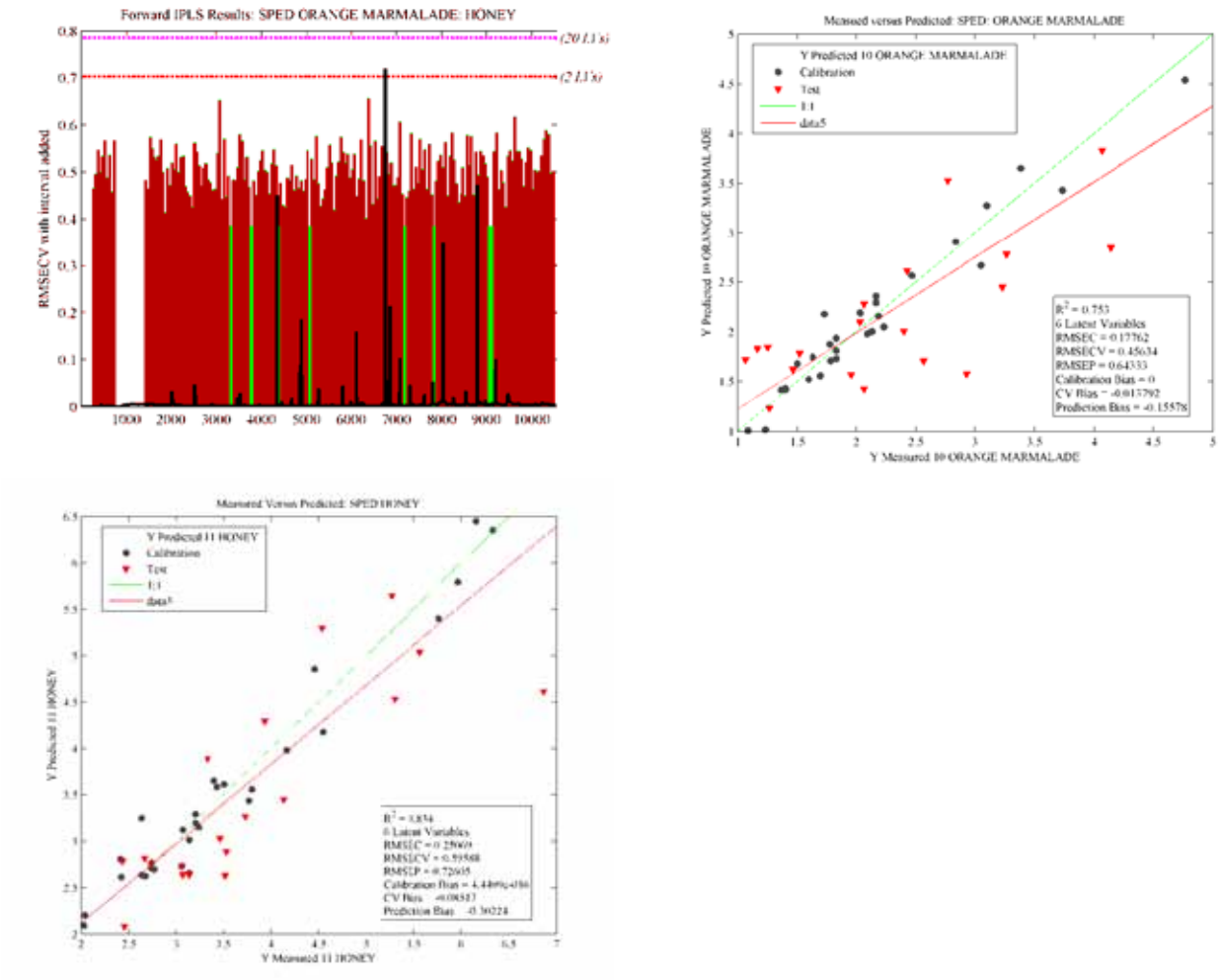


Figure 5.38 PLS analysis: selected derivatised-SPE GCMS intervals for 'ORANGE MARMALADE + HONEY', and the 'measured versus predicted' graph.

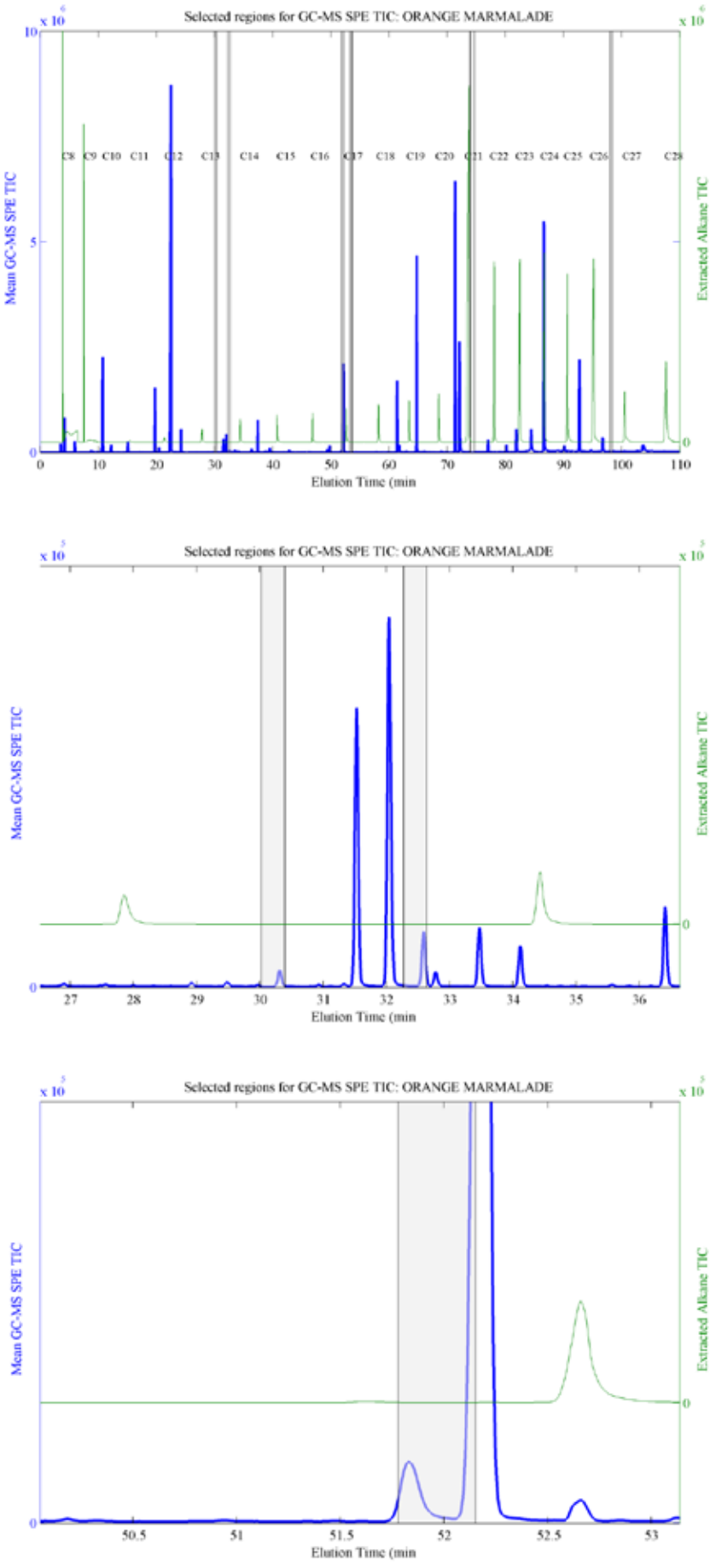


Figure 5.39 (part 1) Selected regions for SPE GCMS: Orange Marmalade

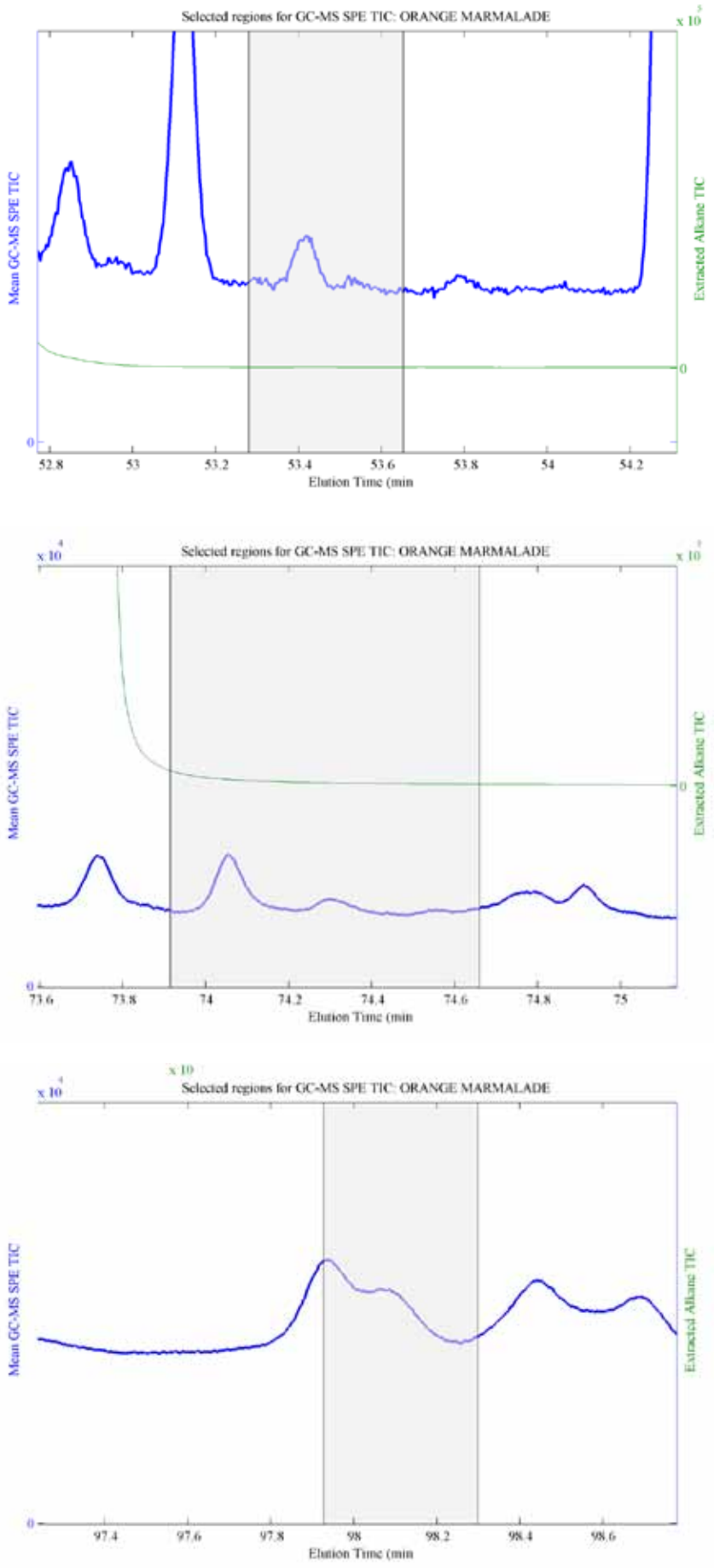


Figure 5.39 (part 2) Selected regions for SPE GCMS: Orange Marmalade

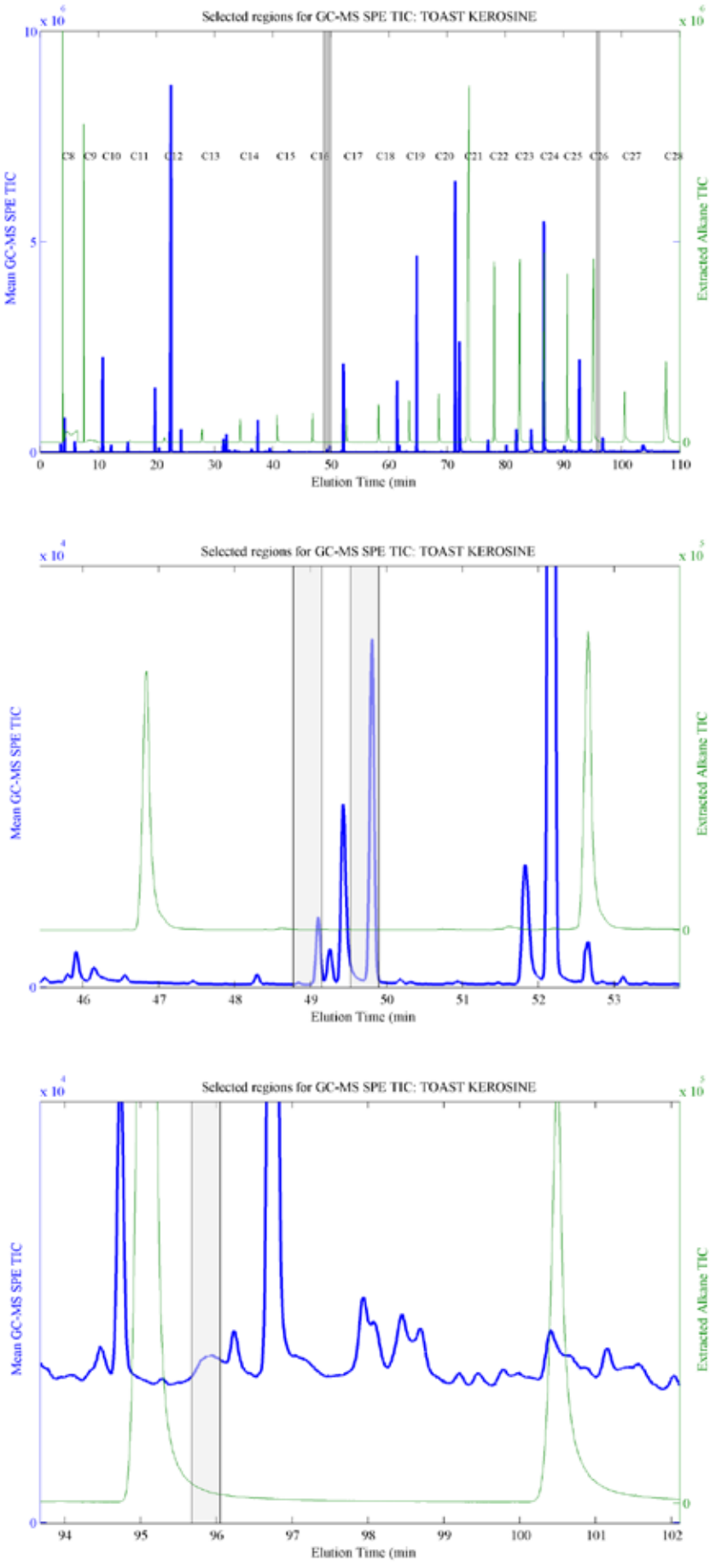


Figure 5.40 Selected regions for SPE GCMS: Toast and Kerosine

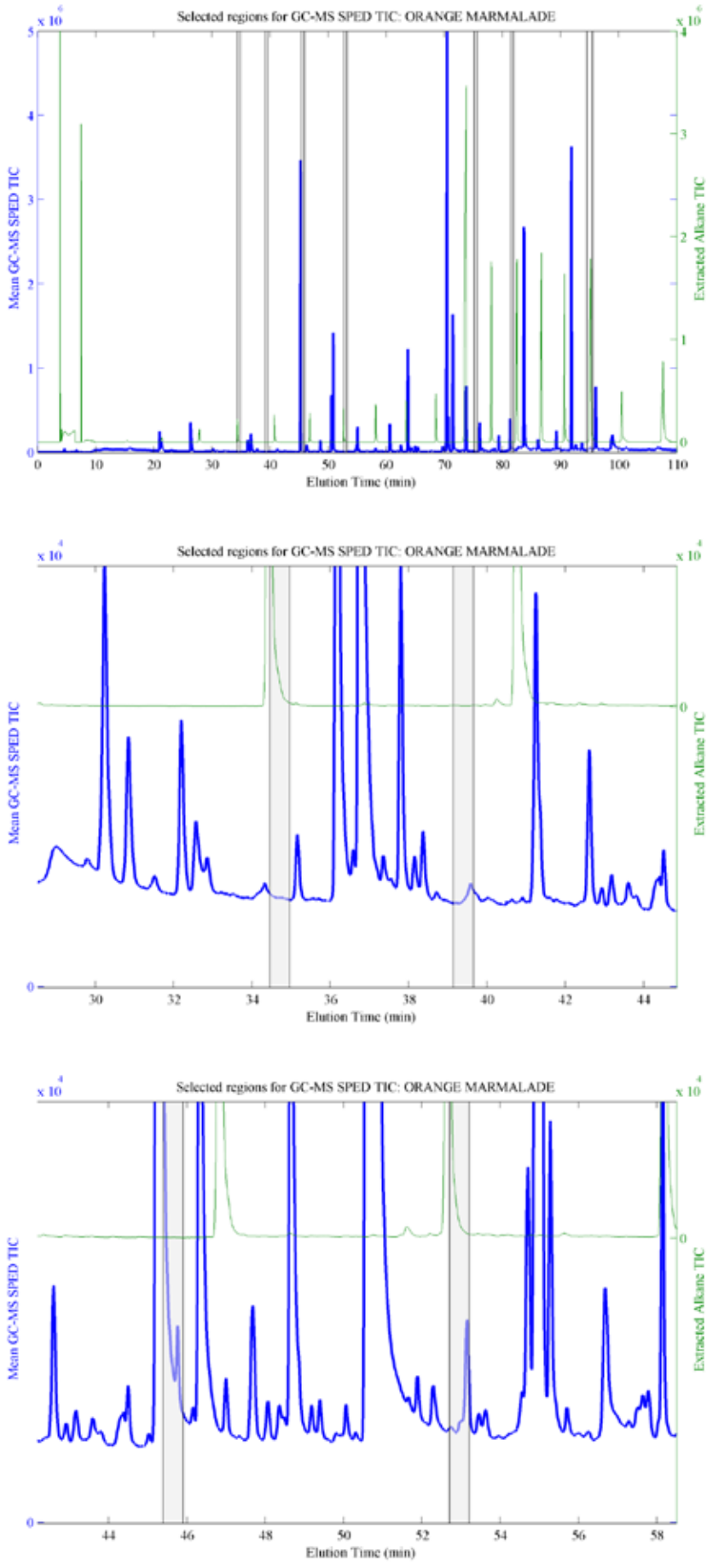


Figure 5.41 (part 1) Selected regions for derivatised-SPE GCMS: Orange Marmalade

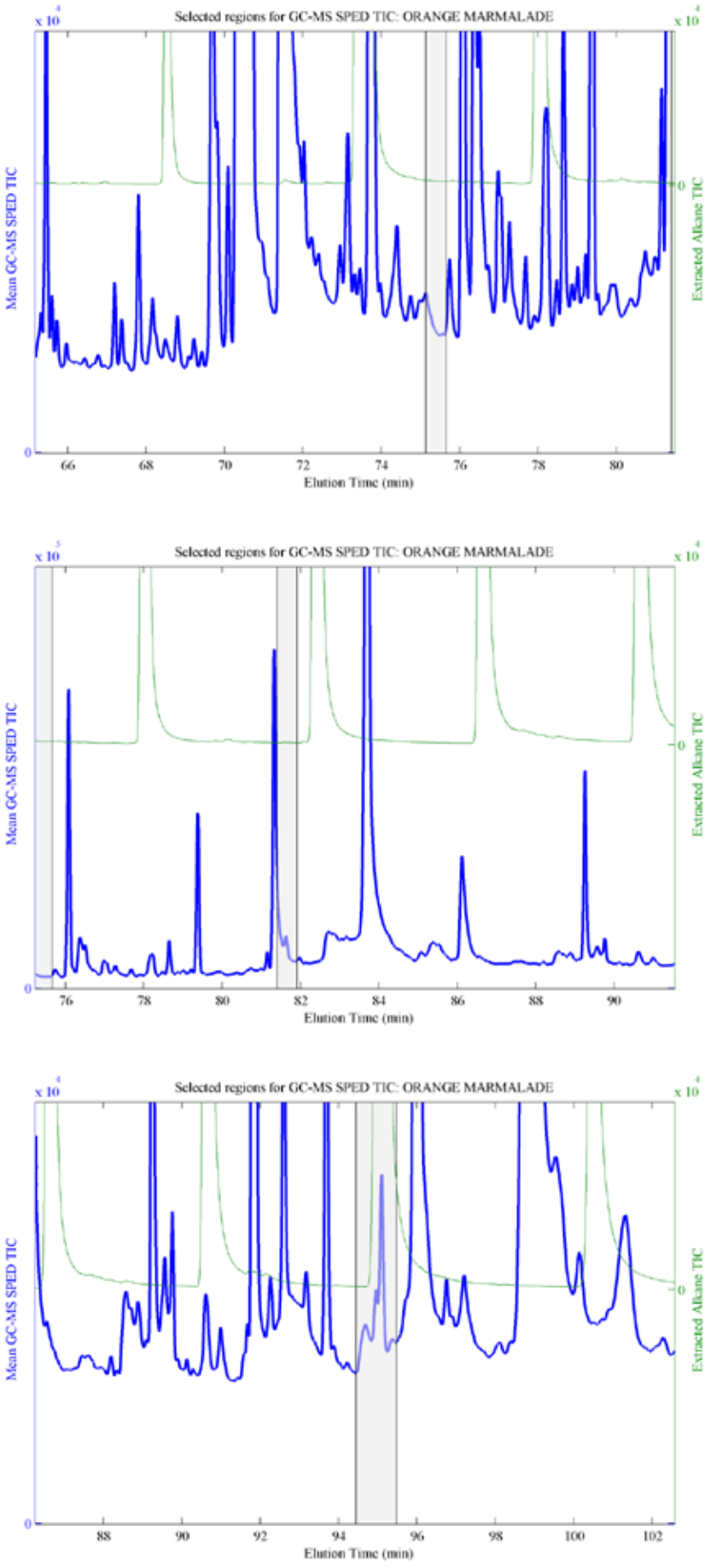


Figure 5.41 (part 2) Selected regions for derivatised-SPE GCMS: Orange Marmalade

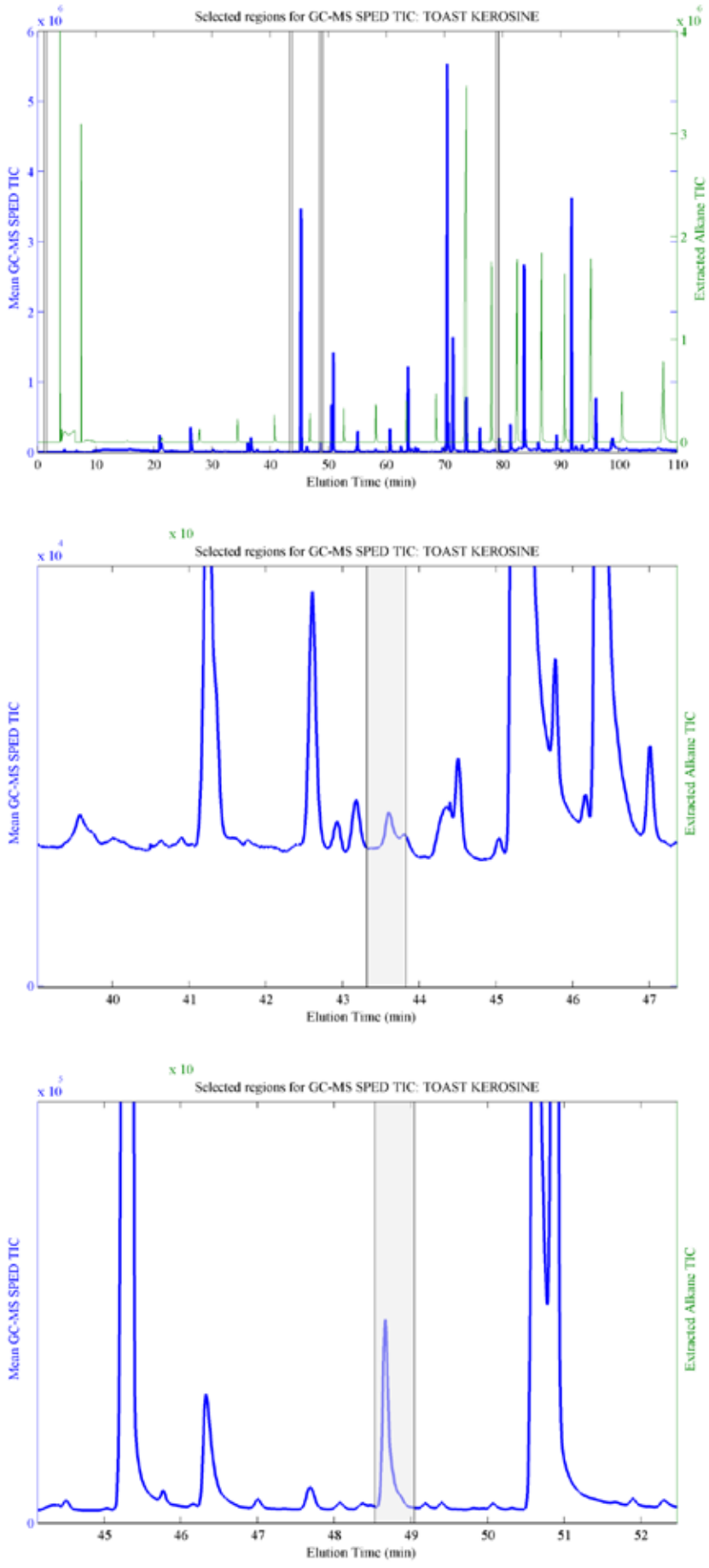


Figure 5.42 (part 1) Selected regions for derivatised-SPE GCMS: Toast and Kerosine

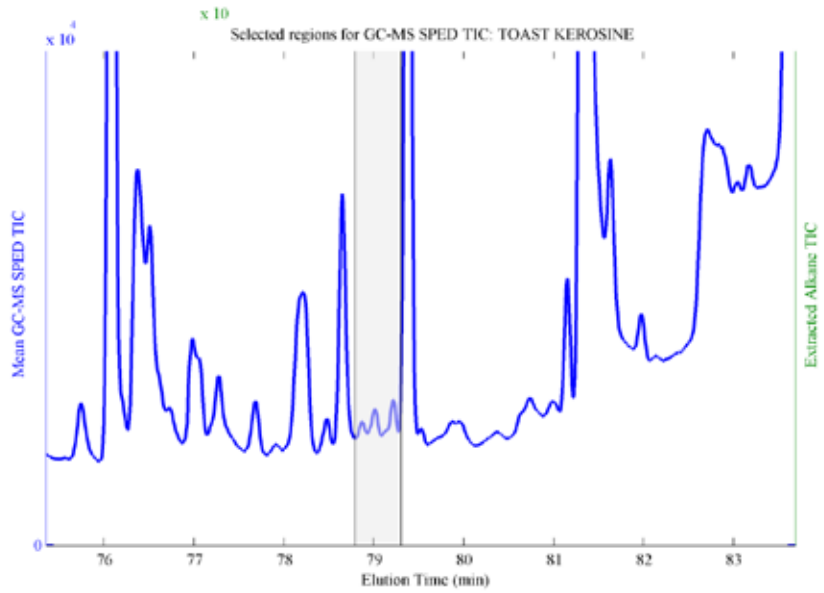


Figure 5.42 (part 2) Selected regions for derivatised-SPE GCMS: Toast and Kerosine

The analysis of GCMS and sensory data by Common Components and Specific Weights (CCSW)

Another chemometric approach to data analysis was undertaken by the use of Common Components and Specific Weights Analysis (CCSW). This method enables the simultaneous decomposition of multiple data blocks, with identical samples, to reveal patterns of data common to the samples. Thus data from samples, which have been analysed simultaneously by multiple techniques, can be decomposed to find the common data structures pertaining to experimental factors of interest. To ensure that each block contributes equally to the data analysis, the variance for each data set is normalised following data centring. Prior to decomposition the GC-MS TIC data blocks were scaled by a log10 transformation to minimise the influence of large values of the variables compared to the sensory data. Autoscaling of the data blocks is a matter of normal data decomposition during the CCSW analysis.

The three data blocks were decomposed simultaneously using CCSW analysis:

Block Number	Data Set	Block Size	Description
1	GC-MS TIC SPE data	48 x 29319	Solid phase extracted wines summed ion counts from 35-350 m/z for 110 minutes elution time
2	GC-MS SPE + derivitisation	48 x 10542	Solid phase and derivitised extracted wines summed ion counts from 35–350 m/z for 110 minutes elution time
3	Mean Sensory data used for PLS1 and PLS 2 regression	48 x 15	Replicate mean sensory data for wine samples

Extraction of common dimensions of the three data sets was under taken and the salience for each block/common dimension is illustrated in *Figure 5.43*. Salience values for data blocks indicate the contribution of the data contained therein, to the overall variance of the combined data sets. Captured variance for each dimension is indicated in parenthesis for each common dimension. If an extracted common dimension gives rise to block saliences of nearly equal weight, the common dimension can be interpreted to contain information that represents commonality across the data sets. Large differences in the block saliences within a common dimension can be interpreted to indicate important differences in the data for each block. Inspection of the block saliences linked with the common dimensions show that most dimensions are strongly associated with one data set for each dimension. Variation within the sensory ratings strongly influences the data decomposition as indicated by the high captured variance for the first common dimension (~86%) and the large

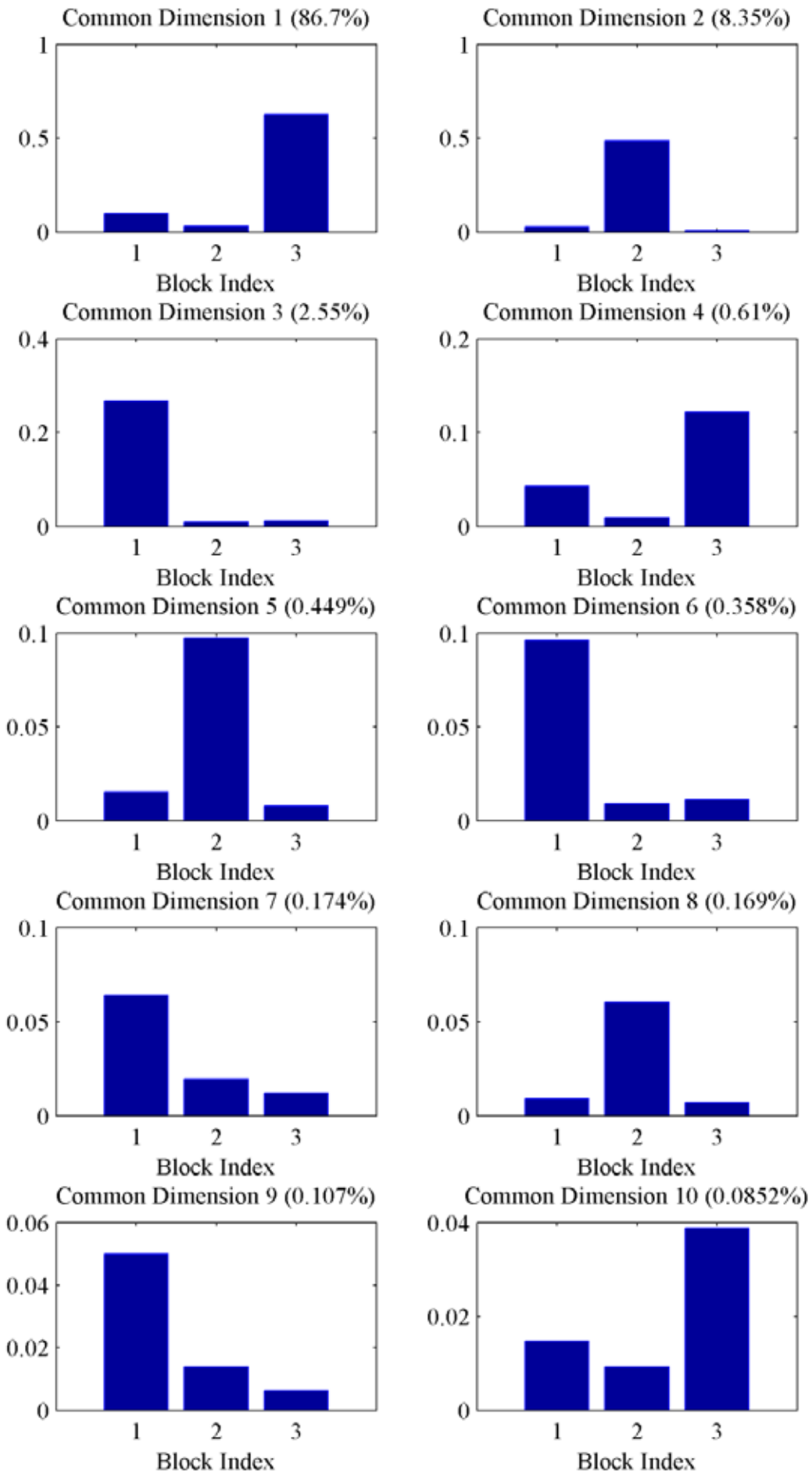


Figure 5.43 Saliences for each block/common dimension of SPE GCMS, derivatised-SPE GCMS and sensory data.

salience for block 3 (sensory data set). As salience values for the GC-MS data sets within dimension 1 are considerably lower, it could be interpreted that the GC-MS data sets do not largely share common information. However, this interpretation should be viewed carefully; as the loadings for the GC-MS data sets can still provide meaningful information that will assist in the location and identification of compounds of interest that may be associated with the corresponding sensory characters.

Ongoing work will be required to align the loadings from all chemometric analysis with the raw data to facilitate the identification of volatile compounds with concentrations that are associated with the sensory dimensions of the wine.

The scores plots for the common dimension show that dimension 1 is associated with Semillon wine age characters. Loadings for the sensory block indicate the strong correlation of attributes orange marmalade, honey and toast within this dimension. Interpretation of the variable loadings must be considered with the position of the aged wines in the scores plots; i.e. aged wines showing high sensory orange marmalade, honey and toast appear in the negative section of dimension 1 thus loadings for variables that are negative in value are in fact positively correlated with these attributes. Scores and loadings for dimensions 1 to 4 are illustrated in *Figure 5.44* to *5.48*.

Grouping of samples by plotting scores within each common dimension can be used to identify interesting patterns of data, and scores for common dimensions 2 to 4 are plotted against CD 1. Score plots for other dimensions were inspected, but no strong grouping of samples was evident for the samples, indicating that most of the captured variance in these dimensions represented sensory or analytical noise. Loading plots, for each data block within the common dimensions of interest, are calculated from the salience and pre-processed data and these are also presented for each common dimension with the score plots. For ease of data presentation, the sensory attributes have been numbered with identification given in *Table 5.19*.

Table 5.19 Identification of sensory attributes–common components and specific weights analysis

Identification number	Sensory attribute
1	Lemon/lime
2	Floral
3	Grapefruit
4	Pineapple
5	Confectionary
6	Hay/straw
7	Grassy
8	Asparagus
9	Lychee
10	Orange marmalade
11	Honey
12	Toast
13	Kerosene
14	Acidity
15	Sweetness

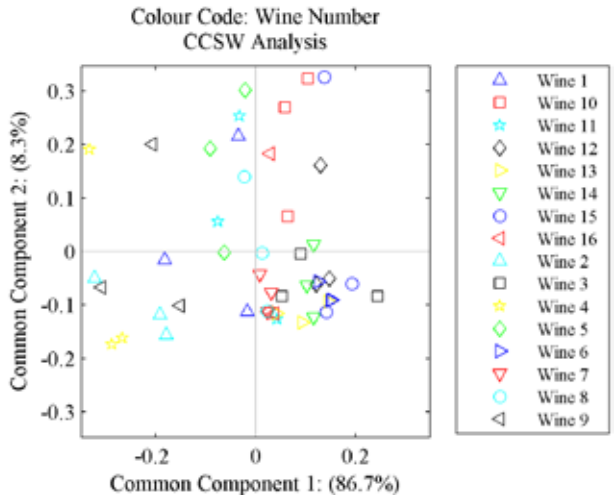
Identification of aroma active compounds in Semillon wines–GCO analysis.

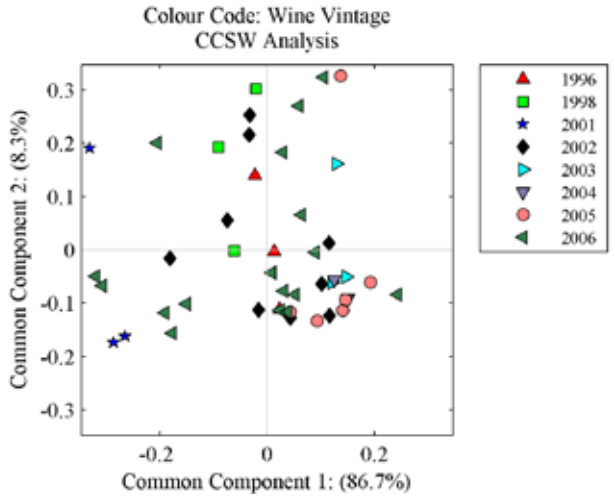
To this point, the data in this report has provided quantification of a range of Semillon wine volatile compounds as well as proposed markers for some sensory parameters of Semillon wines. The following section is focussed on identifying the volatile compounds capable of contributing aroma, and also highlighting the relative, perceived intensity and relative potency of the volatile compounds extracted from Semillon. To achieve this outcome gas chromatography with both olfactory and mass spectrometry detection were utilised.

Gas chromatography olfactometry background and justification of analysis approach.

The 'olfactometry detector' refers to a panellist sniffing an exit stream from the gas chromatography system (i.e. detection of volatiles by the human nose). This detection technique has an importance place in flavour chemistry as many 'chemical' detectors are not as sensitive as the human nose for odour active compounds, and most importantly are not able to describe the specific aroma-note of a compound.

In analysis of the volatile compounds by olfactory detection, the compounds are first extracted from their natural matrix (i.e. from Semillon wine) into a solvent or solid phase adsorbent, separated along the capillary chromatography column in the gas phase, and delivered to a panellist with a humidified and cooled air stream (i.e, exist gas from the GC is normally warm and dry). Consequently, the compounds are detected in isolation and away from their original natural matrix. This means that GCO





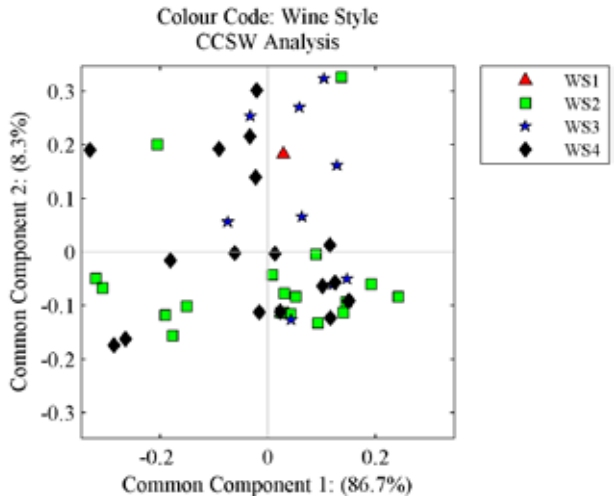
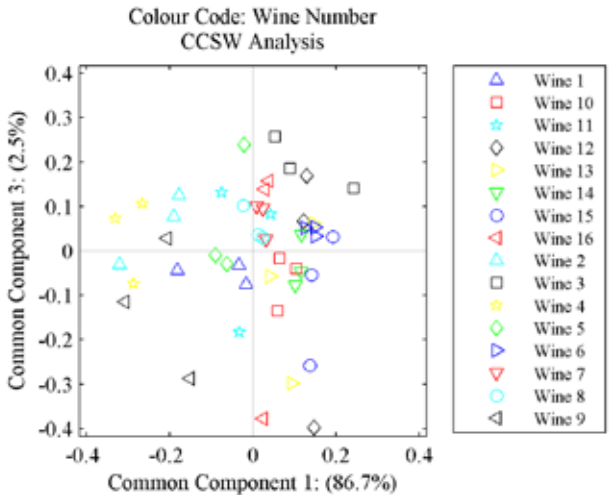
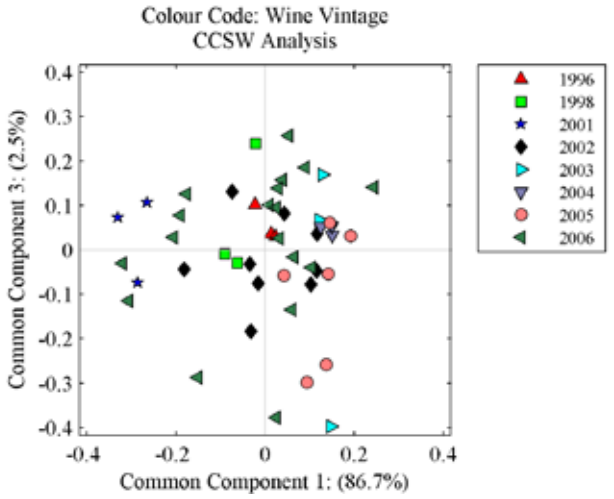


Figure 5.44a CCSW analysis of GCMS and Sensory data: common dimensions 1 vs 2.





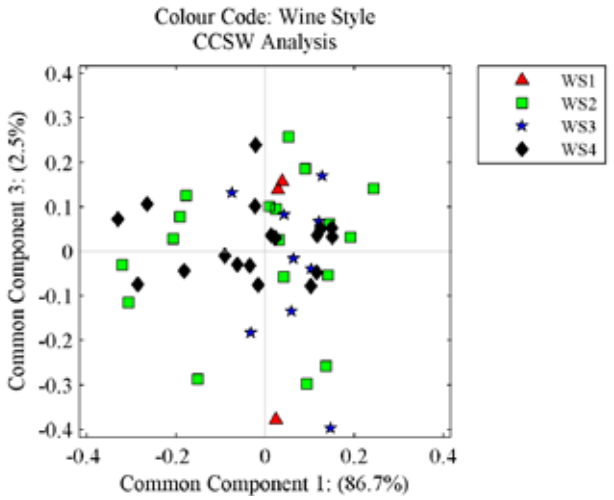


Figure 5.44b CCSW analysis of GCMS and Sensory data: common dimensions 1 vs 3.

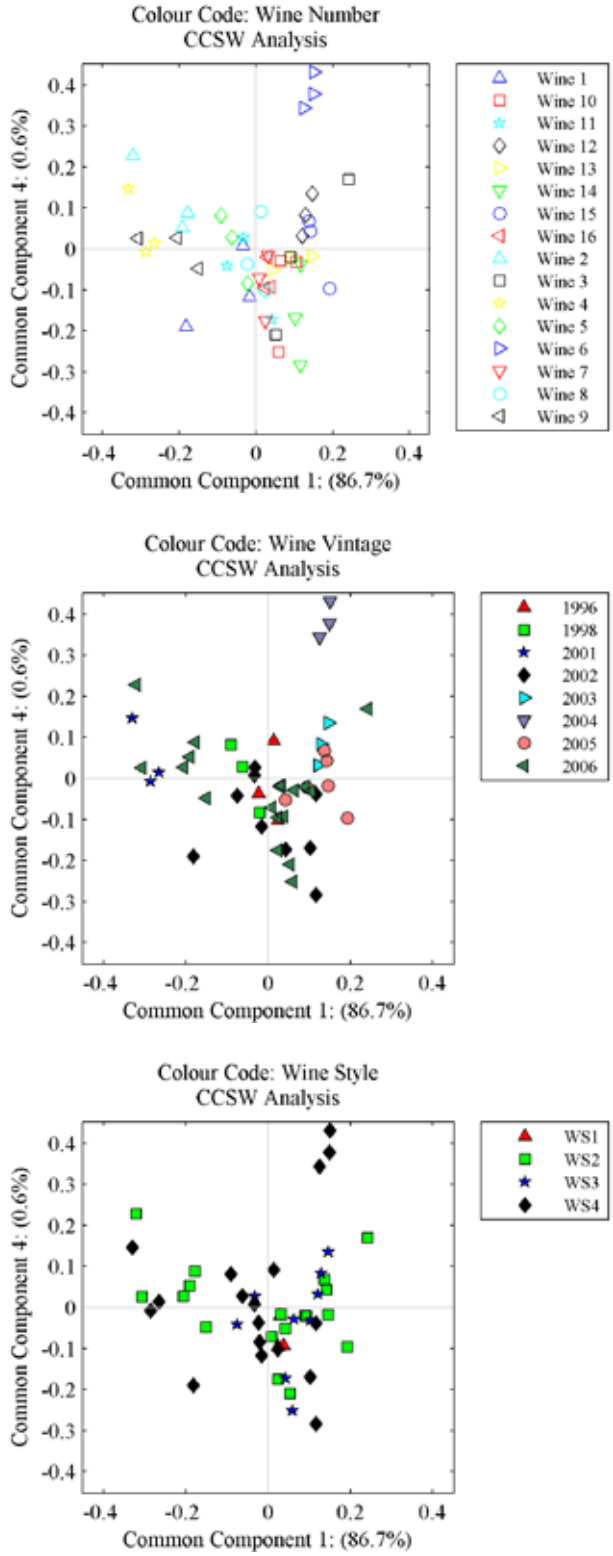


Figure 5.44c CCSW analysis of GCMS and Sensory data: common dimensions 1 vs 4

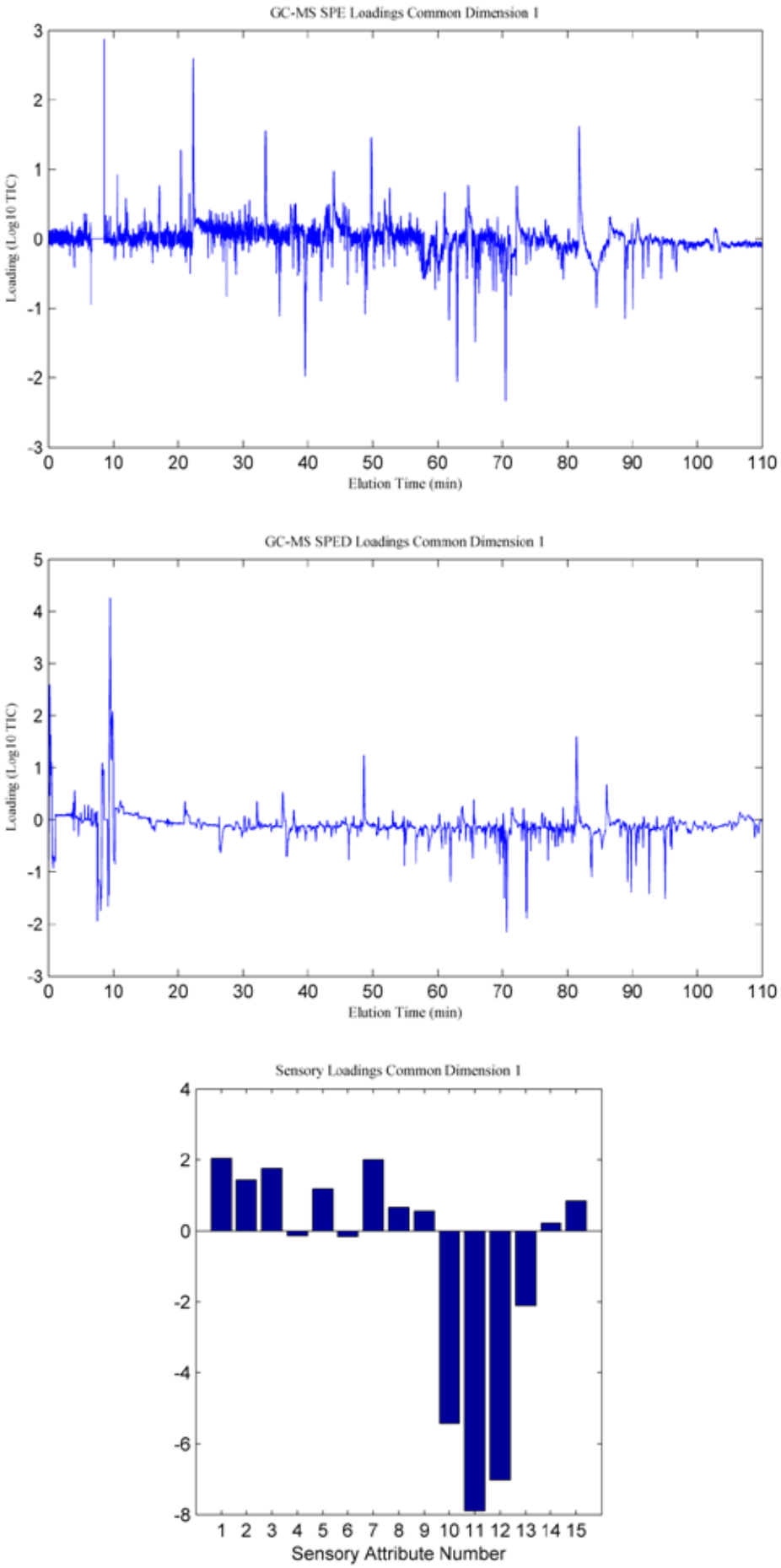


Figure 5.45 CCSW analysis of GCMS and Sensory data: loadings in common dimension 1 for SPE-GCMS, derivatised-GCMS and sensory data

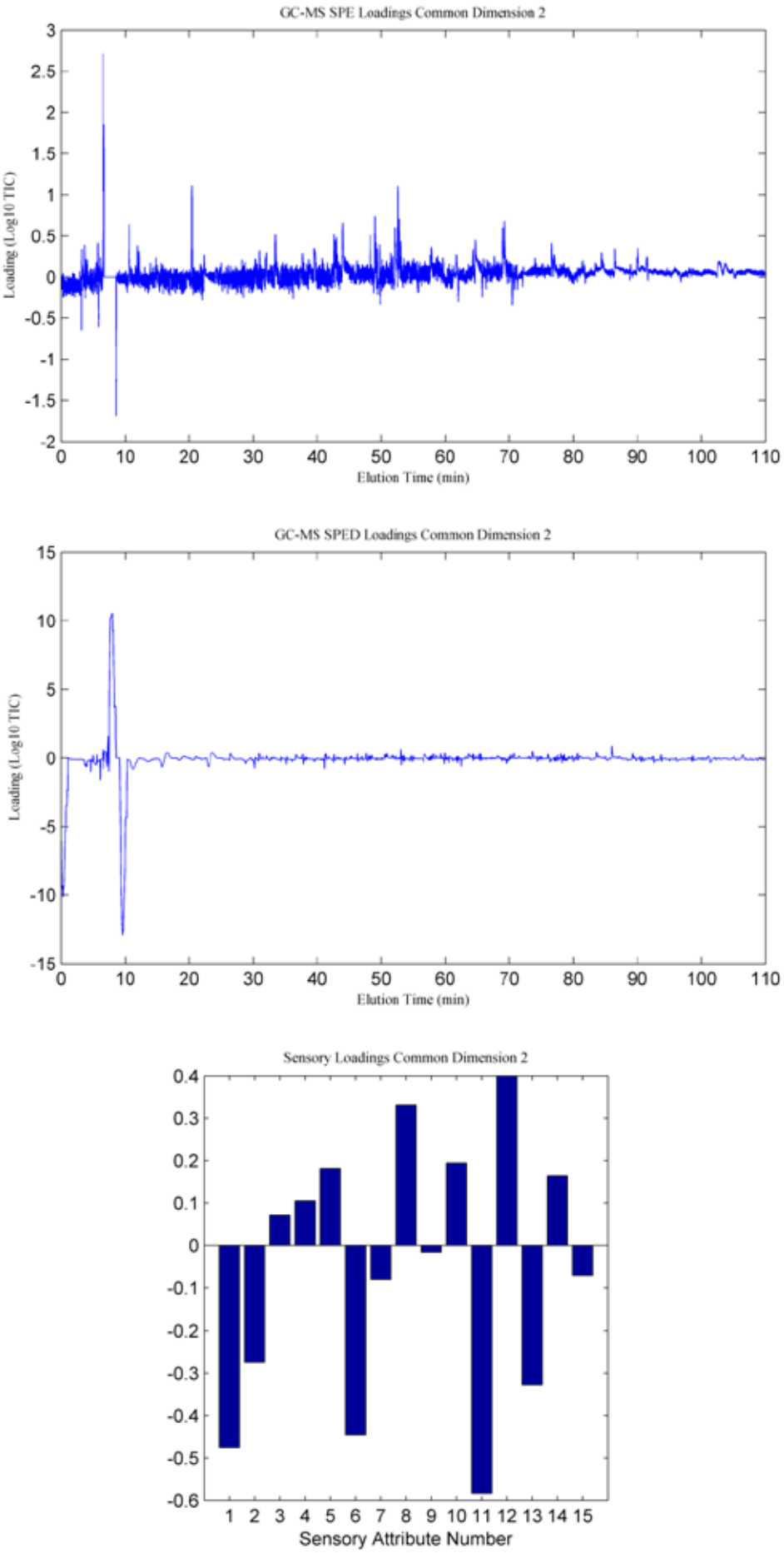


Figure 5.46 CCSW analysis of GCMS and Sensory data: loadings in common dimension 2 for SPE-GCMS, derivatised-GCMS and sensory data

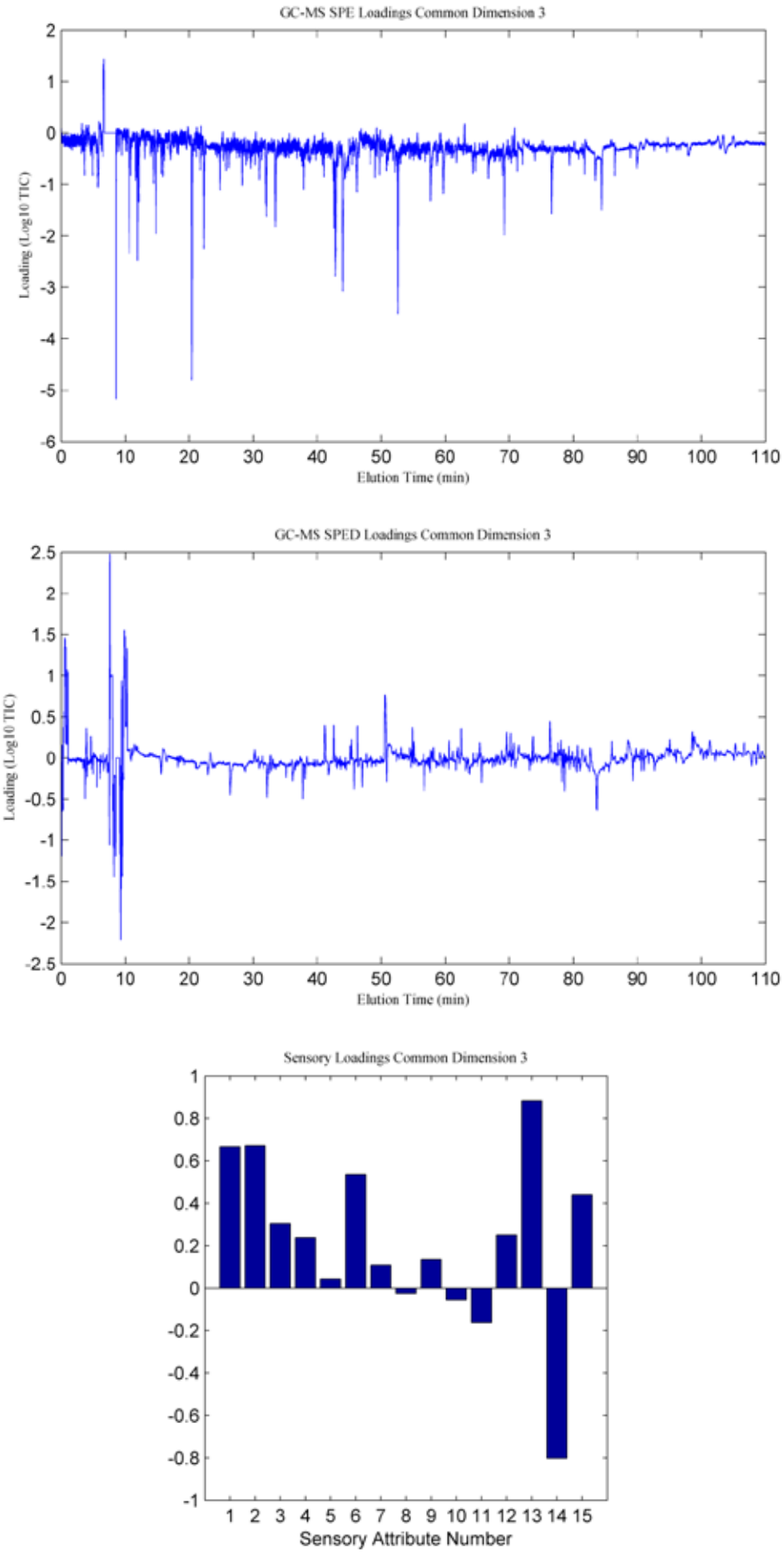


Figure 5.47 CCSW analysis of GCMS and Sensory data: loadings in common dimension 3 for SPE-GCMS, derivatised-GCMS and sensory data

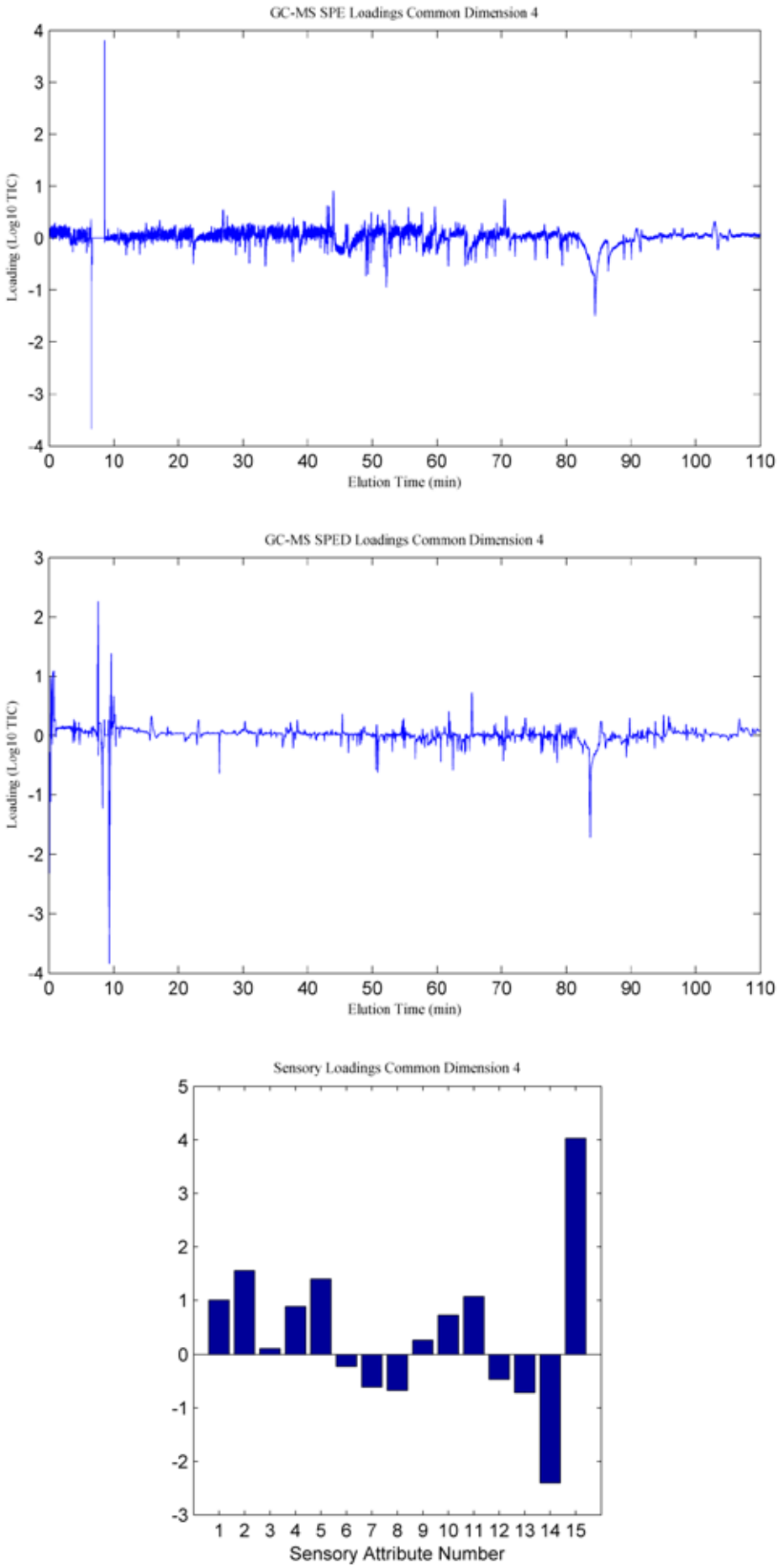


Figure 5.48 CCSW analysis of GCMS and Sensory data: loadings in common dimension 4 for SPE-GCMS, derivatised-GCMS and sensory data.

does not provide exact information on the behaviour of a chemical compound in the original sample, whereby suppression (or enhancement) of 'isolation' aroma may occur due to other components in the original matrix. However, as will be seen below, it does provide identification of likely aroma contributors and their relative aroma potency in the collected sample extract. The collective addition of the most potent aroma compounds to a 12% aqueous ethanol system at pH 3.3 (i.e. 'recombination') can then be investigated in order to assess their relevance to the real sample matrix (i.e. Semillon wine).

Although GCO has been applied to white wines in the past (Guth 1997; Berger 1995), it was of interest to apply it to the Semillon wines under study in this report, particularly to relate it to the sensory and analytical data in the preceding sections. Two different approaches to GCO analysis were taken in order to limit the disadvantages faced by either technique in isolation (van Ruth 2001); time-intensity analysis, and dilution analysis.

The first of these methods (time-intensity) provides information on the relative perceived intensity of the compounds in a sample extract. In this method, the olfactory detection is performed by multiple panellists who not only describe the aroma note of eluting compounds, but also provide an aroma intensity estimate (i.e. as a percentage in the study below), as the analysis is being performed. The aroma intensity data for each aroma compound is then presented as the averaged value as assessed by the collective panellist group. The benefit of the multiple panellists in this method is that it ensures that the diversity in human sensing is taken into account with multiple noses being utilised in the assessment. This usage of 'multiple noses' is of recognised importance considering that certain noses have specific aroma 'gaps', or more aptly, a lowered sensitivity to smell a specific aroma compounds due to physiological predispositions. The disadvantage of this method is the inherent variability in the application of an abstract aroma intensity scale (i.e. 0–100) by multiple panellists.

The second GCO approach (dilution analysis) provides a quantitative measure of the relative odour potency of the compounds within the sample extract. In this method, a single panellist is utilised as the olfactory detector in GCO whereby the sample extract is injected multiple times but at progressively higher and higher dilutions (usually 2-fold or 3-fold dilutions). In this manner, the volatile compounds are effectively rated at the greatest dilution at which they still can be assessed, and are given a dilution factor value (FD=729, which corresponds to the final detection of a compound after a 3-fold dilution of an extract six times, as 36=729). Furthermore, the time for which a panellist senses a compound can also be accounted for in combination with the FD value. Such a measure is provided by a 'charm value' and is equal to the FD multiplied by the time of detection of the compound by a panellist. Both FD and charm value measures are able to provide a relative rank of compounds in order of their contribution to aroma in an extract, with the latter providing greater discrimination power than the former. The disadvantage of this approach is that its time-consuming nature generally precludes more than one panellist (see above), and also it assumes a linear variation of aroma intensity with concentration, which is not always the case for certain aroma compounds.

Gas chromatography olfactometry mass spectrometry analysis: a time-intensity measure (NOTE: Confidential Section)

The work outlined in this section was contracted work performed by Food Science Australia.

Five Hunter Valley Semillon wines (2 old, 3 new) were analysed by GCO with five panellists via the time-intensity method. The eluent GC compounds were annotated with GC-O descriptors and matched with GC-MS data where possible for each of the five assessors. The average response of five assessors for each wine was used to obtain the odour intensity score for a given odour-active compound for a given wine. The average GC-O intensity values for each wine are shown in *Table 5.20*. The odour peaks are displayed in elution order. More than 30 odour-active compounds were detected across the 5 wines; perceived at varying intensity in each. Tentative identification of compounds was made on the basis of the corresponding electron impact mass spectra, linear retention indices, odour quality and with an alternative mode of mass spectrometry ionisation (i.e. chemical ionisation with methanol to provide the existence of an unambiguous M+1+ ion). Some odour compounds were identified only on the basis of odour quality and retention time (diacetyl and 1-octen-3-one) whereas chemical identification of others was not possible; e.g. unidentified–'lime citrus'. In some cases, e.g. mushroom odour (1-octen-3-one) and sweet, floral odour (2-phenylethylacetate) the odour peak was perceived in several of wines and not reported in others.

Table 5.20 List of odour active compounds detected by the trained panel in the Semillon wines. Odour peaks are listed in order of Linear Retention Index (RI), with the most common odour descriptors, tentative chemical identification and average (n=5) perceived intensity (%).

LRI	ODOUR	Tentative identification	Wine 8 1996	Wine 3 2006	Wine 7 2006	Wine 16 2006	Wine 5 1998
<700	caramel, butterscotch, toffee	diacetyl	0	0	33.4	59.2	64.4
<700	sharp, acrylic, glue, lime, chemical, nail polish, remover	ethyl acetate	51.0	68.2	51.8	51.8	68.2
721	fruity, melon, bubblegum	ethyl butanoate	41.8	30.2	57.4	62.4	57
747	fruity, sweet, bubblegum	ethyl2-methylbutanoate	50.8	63.6	61.6	55.4	67.4
778	fruity, floral, sweet	ethyl-3-methylbutanoate	53.8	70.8	61.6	61.2	51.2
886	bubblegum, yeasty, banana	isoamylacetate	0	50.6	49.6	54.8	0
1062	malty, squashed ants, vomit, bluecheese	(2/3)-methyl-1-butanol	72.8	86.4	84.0	85.0	86.2
1077	bubblegum, pineapple	ethyl hexanoate	62.6	82.2	72.6	75.6	78.4
1179	mushroom, fungus	1-octen-3-one	0	0	0	44.8	0
1218	Vegemite, yeasty, cooked, savoury, Vitamin B	hexanol	46.4	38.4	47.4	68.4	36.2
1296	green, crushed leaves, fresh	3-hexen-1-ol-(Z)	21.2	29.0	34.4	33.0	24.4
1400	yeasty, vinegar, boiled potato, salt n' vinegar	acetic acid and methional	52.0	85.2	70.8	69.8	70.8
1413	herbal, peanut, earthy	furfural	36.8	33.6	13.2	31.2	41.2
1623	cheese rotten, parmesan, sweaty	butanoic acid	56.4	31.4	62.2	45	21
1682	cheesy, sharp, broadbeans, vomit, old socks	3-methylbutanoic acid	54.0	54.6	70.8	69.6	48.0
1717	cheesy, old socks, smelly cheese, damp potato	methionol	16.2	18.2	60.8	30.4	37.8
1749	honey, chemical, glue, sneakers, floral, plastic	1, 1, 6-trimethyl-1, 2-dihydro naphthalene (TDN)	65.5	59.5	69.5	61.75	75.75
1828	sweet floral	2-phenylethylacetate	21.0	0	35.6	69.0	0
1845	apple, tobacco,	β-damascenone	54.0	64.0	69.6	71.0	30.4
1939	intense rose, floral	2-phenylethyl alcohol (PEA)	69.4	80.6	87.6	84.2	61.6
1946	honey, sweet, floral	ionone-like#3	34.6	38.0	0	0	27.2
1969	rose sweet, floral honey	2-ethoxybenzyl alcohol	22.2	0	31.6	29.4	21.2
2023	fireplace, wine, fermented, beer	methyl fumaric acid	26.4	39.4	12.6	43.2	36.0
2049	spice, beer, winey, grapey	1-H-pyrrole-2-carboxaldehyde	37.0	27.4	0	36.6	28.2
2060	fermented, yeasty, winey	ethyl malate	18.0	0	34.2	28.8	29.6
2106	fermented, grape, winey, cinnamon	isobutyl hexanoic acid	32.8	33.4	49.4	41.0	35.8
2180	raisins, winey, fermented	2-hydroxy-3-methylsuccinic acid diethyl ester	31.6	0	0	31.6	44.2
2212	sweet, woody, phenol, fermented, muscat, port	p-vinylguaiacol	45.8	18.6	0	18.4	10.6
2244	raisins, fireplace, phenol, applepie	acetosyringone	40.2	46.4	46.8	32.4	31.8
2320	cooked christmas cake, winey,	isobutyl decanoate	49.0	0	0	42.0	35.4
2331	apple, fermented, beer, winey	diethyl tartaric acid	43.0	36.8	0	33.8	46.8

Table 5.21 List of odour active compounds across all the Semillon wines ranked in order of decreasing average perceived odour intensity. Data is from 5 assessors of 5 wines (i.e. 5x5).

LRI	Odour	Tentative identification	Average perceived intensity	Semillon ranking
1062	malty, squashed ants, vomit, blue cheese	(2/3)-methyl-1-butanol	82.9	1
1939	intense rose, floral, sweet	2-phenylethylalcohol (PEA)	76.7	2
1077	bubblegum, pineapple,	ethyl hexanoate	74.3	3
1400	yeasty, vinegar, boiled potato, salt n'vinegar	acetic acid and methional	69.7	4
1749	honey, chemical, glue, sneakers, floral, plastic	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	66.4	5
747	fruity, sweet, bubblegum, melon	ethyl 2-methylbutanoate	59.8	6
778	fruity, floral, sweet, pineapple	ethyl-3-methylbutanoate	59.7	7
1682	cheesy, sharp, broad beans, vomit, old socks, parmesan	3-methylbutanoic acid	59.4	8
<700	sharp, acrylic, glue, lime, chemical nail polish remover	ethyl acetate	58.2	9
1845	apple, tobacco, floral, tea, berry	β-damascenone	57.8	10
721	fruity, melon, bubblegum,	ethyl butanoate	49.8	11
1218	Vegemite, yeasty, cooked, savoury, Vitamin B	hexanol	47.4	12
1623	cheese rotten, parmesan, sweaty	butanoic acid	43.2	13
2244	raisins, fireplace, phenol, apple pie	acetosyringone	39.5	14
2106	fermented, grape, winey, cinnamon	iosbutylhexanoic acid	38.5	15
1717	cheesy, old socks, smelly cheese, damp potato	methionol	32.7	16
2331	apple, fermented, beer, winey	diethyltartaric acid	32.1	17
2023	fireplace, wine, fermented, beer	methylfumaric acid	31.5	18
<700	caramel, butterscotch, toffee	diacetyl	31.4	19
1413	herbal, peanut, earthy	furfural	31.2	20
886	bubblegum, yeasty, banana	isoamyl acetate	31.0	21
1296	green, crushed leaves, fresh	3-hexen-1-ol-(Z)	28.4	22
2049	spice, beer, winey, grapey	1-H-pyrrole-2-carboxaldehyde	25.8	23
2320	cooked christmas cake, winey, muscat, sultana	isobutyl decanoate	25.3	24
1828	sweet floral	2-phenylethylacetate	25.1	25
2060	fermented, yeasty, winey	diethyl malate	22.1	26
2180	raisins, winey, fermented	2-hydroxy-3-methylsuccinic acid diethyl ester	21.5	27
1969	rose sweet, floral honey	2-ethoxybenzyl alcohol	20.9	28
1946	honey, sweet, floral	ionone-like#3	20.0	29
2212	sweet, woody, phenol, fermented, muscat, port	p -vinylguaiacol	18.7	30
1179	mushroom, fungus	1-octen-3-one	9.0	31

Of note is the fact that the aroma compounds identified in *Table 5.20* are relatively common to white wines in general and are not unique to Semillon. The majority are also by-products of yeast fermentation, with the exception of TDN, β-damascenone and furfural. Given the small sample size it is difficult to give definitive comparisons between the old and new styles of wine with too much confidence. However, the data in *Table 5.20* does show no isoamyl acetate (banana aroma) detected by the GCO panellists in the older wines, as one would expect, given that it is well known to be prevalent during fermentation and decrease in concentration thereafter.

The odour intensity for the odour active peaks across all 25 Semillon-extract GC-O runs were averaged, and then ranked in order of decreasing odour impact. *Table 5.21* can be taken as a provisional list of the relative perceived intensity of compounds extracted from Hunter Semillon wine, based on the five wines investigated in this study (i.e. two young and three old). Once again, the yeast fermentation-derived aroma compounds (i.e. higher alcohols, esters, and aliphatic acids) dominate the top 10 and top 20 compounds on this list, with the exception of TDN, β-damascenone and furfural. It must be mentioned that such a list does not indicate the potency of the aroma compound per unit concentration but merely indicates the strength of compounds as extracted from the wine. It is interesting that the a related time-intensity GCO study on Pinot Noir also showed 3-methylbutanol, 2-phenylethanol, methionol and 2-phenylethylacetate with high perceived intensity values (Miranda-Lopez *et al*. 1992), in addition to hexanoic acid and γ-nonalactone for the Pinot Noir. Indeed it is well established that different wines with a similar content of higher alcohols and esters can smell differently, indicating a specific role of minor and trace impact components. As such, this lends weight to chemometric approach of linking GCMS chromatographic data to sensory features, in combination to GCOMS studies.

To build upon the bank of volatile chemicals identified in *Experiment 5.7, Optimisation of a SPME GCMS method for the analysis of volatile compounds in Semillon wines*, a detailed investigation of the volatile compounds eluting in the Semillon wine extract was conducted in conjuction with the GCO analysis. More than 60 volatile compounds were detected in the five Semillon wines (*Table 5.22*). In most cases identification could be made based on the criteria outlined above, however further confirmation (i.e., with authentic reference samples) would be required for those compounds with incomplete data in *Table 5.22*. The early eluting compounds diacetyl and ethyl acetate co-eluted with the tailing of the ethanol and solvent peaks, and were therefore not integrated. Hence they were identified only on the basis of LRI, M+1+ ion and odour quality. Some compounds could not be identified from their electron impact mass spectra, for example unidentified m/z 112, unidentified m/z 111 and ionone-like#1 through to ionone-like# 4. The ionone-like compounds have electron impact mass spectra consistent with that of β-ionone. At least one of these was found to be odour active (ionone-like#3, honey, sweet, floral).

Of the compounds tentatively identified as markers in the previous section, methionol and butanoic acid were the only ones that showed aroma notes in this GCO study. Both were detected as markers for orange/marmalade notes in the previous section, but their isolated aroma-notes provided contrasting descriptions (i.e. cheese, damp potato, sweaty). This demonstrates the complexity in relating isolated-aroma notes from extracts to the contribution to the global aroma of wine. However, it also suggests that these compounds may undergo changes in concentration similar to the likely suite of compounds that may contribute to the orange/marmalade characteristics of wine. Further work is required to relate the compounds of *Tables 5.20* to *5.22* to the highlighted chemmometric chromatographic regions of the previous section of this report (see *Experiment 5.7, Identification of aroma active compounds in Semillon wines – GCO analysis*).

Gas chromatography olfactometry mass spectrometry analysis: dilution analysis

Dilution analysis was conducted, via GCO/GCMS, in order to get further information on the potency of the aroma compounds, with respect to their concentration in the Semillon wine extract. The previous section showed that the majority of the perceived aroma compounds from an extract of Semillon wine were yeast-fermentation products that are fairly common to most wines (of course, the unknown (ionone-like) compound requires further work investigation). It was of interest to assess the aroma potency compounds in a range of Semillon wines and also to assess if any significant differences could be observed for the aroma potency ranking between a selection of young Semillon wines versus older Semillon wines.

Table 5.22 List of volatiles tentatively identified in the Semillon wines.

Time (min)	LRI	Most likely identification	Odour descriptors	Quan Ion a	M+1+b	Wine 8 1996	Wine 3 2006	Wine 7 2006	Wine 16 2006	Wine 5 1998
4.90	<700	diacetyl *	caramel, toffee, butterscotch	nd	87	nd	nd	nd	nd	nd
6.42	<700	ethyl acetate*	sharp, acrylic, glue, lime, chemical, nail polish remover	nd	89	nd	nd	nd	nd	nd
7.10	730	ethyl butanoate	fruity, melon, bubblegum, lolly, candy	88	117	3.5	108.0	183.5	219.9	245.4
7.42	747	ethyl-2-methylbutanoate	fruity, sweet, bubblegum, melon	102	131	0.7	7.8	7.3	4.6	23.0
8.03	778	ethyl 3-methylbutanoate	fruity, floral, sweet, pineapple	88	131	1.2	13.5	20.1	7.7	25.1
8.22	788	ethanethiol		62		29.4	0.0	0.0	0.0	0.0
10.12	886	isoamyl acetate	bubblegum, yeasty, banana, nail polish remov.	70	131	0.0	56.6	114.0	339.9	17.5
11.47	955	ethyl-2-butenoate		69	115	0.0	6.5	2.9	5.9	3.9
13.56	1062	(2/3)-methyl-1-butanol	malty, squashed ants, vomit, blue cheese	70		2361.5	9445.8	23614.6	8265.1	10626.6
13.83	1077	ethyl hexanoate	bubblegum, pineapple, artificial fruit, lolly	88	145	989.6	2548.6	1975.4	2247.6	2503.7
14.30	1101	4-penten-1-ol		67		0.1	1.1	1.5	1.9	0.3
14.95	1134	hexyl acetate		67	145	0.0	0.7	1.7	10.1	0.1
15.83	1179	1-octen-3-one	mushroom, fungus	nd		nd	nd	nd	nd	nd
16.27	1202	hexenol acetate		67		0.7	1.2	1.0	4.6	1.6
16.57	1218	hexanol	vegemite, yeasty, cooked, savoury, Vitamin B	69		3.8	11.0	9.5	9.0	13.8
17.57	1269	3-hexen-1-ol-(E)		67		7.0	4.7	6.7	14.3	13.1
17.87	1284	3-ethoxypropanol		71	105	1.0	0.3	30.1	38.8	32.5
18.10	1296	3-hexen-1-ol-(Z)	green, crushed leaves, fresh, clean	67		36.9	41.7	45.5	67.5	53.6
19.36	1361	ethyl octanoate		88	173	380.7	517.0	386.5	496.7	456.6
19.68	1378	*cis* -linalool oxide		94		3.5	0.7	0.8	0.6	4.5
20.12	1400	acetic acid/methional	yeasty, vinegar, boiled potato, salt n' vinegar	60		33.7	170.8	119.4	109.3	82.8
20.8	1413	furfural	herbal, peanut, earthy, plastic, rubber tires, diesel	96	97	192.9	53.9	31.7	51.9	220.2
21.35	1463	acetyl furan		95	111	7.5	3.5	2.0	2.5	17.2
21.81	1487	vitispirane #1		192		4.2	1.8	1.1	0.8	2.2
21.91	1492	dihydro-2-methyl-3(2H)-thiophenone		60	117	2.7	13.7	14.7	15.2	23.0
23.88	1593	ethyl levulinate		99	145	3.2	0.4	0.3	0.3	1.8

Time (min)	LRI	Most likely identification	Odour descriptors	Quan Ion a	M+1+b	Wine 8 1996	Wine 3 2006	Wine 7 2006	Wine 16 2006	Wine 5 1998
24.18	1609	ethyl-2-furoate		95	141	47.6	13.3	10.4	10.7	66.7
24.38	1619	ethyl decanoate		88	201	54.3	147.7	78.1	59.8	49.1
24.46	1623	butanoic acid	cheese rotten, parmesan, sweaty	60		59.3	55.7	68.6	85.4	54.6
25.42	1673	diethyl succinate#1		RIC	175	8265.1	4722.9	4722.9	2361.5	11807.3
25.60	1682	3-methylbutanoic acid	cheesy, sharp, broad beans, vomit, old socks, parmesan	60		0.0	17.5	26.5	23.0	1.7
25.82	1693	α-terpineol		93		2.1	1.5	1.3	1.8	0.3
25.85	1695	ionone-like#1		163		6.7	2.4	1.9	1.1	4.5
26.24	1715	unidentified m/z		192	192	0.0	0.6	0.6	0.8	0.0
26.28	1717	methionol	cheesy, old socks, smelly cheese, damp potato, floral	106	107	8.5	14.8	15.1	7.8	8.1
26.54	1730	ionone-like#2		163		3.7	1.5	1.1	0.7	2.9
26.90	1749	1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	honey, chemical, glue, sneakers, floral, plastic	157	173	2.9	2.0	1.8	1.1	2.4
27.62	1785	diethyl glutarate		143	189	7.6	6.1	9.1	5.2	11.0
28.22	1817	tetrahydro-2-methylthiophene		87		12.7	9.4	9.2	7.4	5.9
28.44	1828	2-phenylethylacetate	sweet floral	104		20.9	10.0	22.8	46.5	23.7
28.77	1845	β -damascenone	apple, tobacco, floral, tea, berry	121	191	0.1	0.4	0.1	0.2	0.1
28.98	1856	ethyl dodecanoate		88	229	2.1	3.0	2.8	1.7	11.4
33.52	2089	hexanoic acid		RIC		8265.1	11807.3	8265.1	11807.3	10626.6
29.72	1894	benzyl alcohol		79		136.7	3.8	3.2	9.3	22.0
30.60	1939	2-phenylethylalcohol	intense rose, floral, sweet	91		1788.1	1885.3	3108.9	2256.6	2365.3
30.74	1946	ionone-like#3	honey, sweet, floral, rose	163		49.5	15.9	11.7	9.1	32.6
31.04	1961	ionone-like#4		163		74.0	25.0	19.4	15.7	50.0
31.18	1969	o- ethoxybenzylalcohol	rose sweet, floral honey	78		23.5	0.7	0.3	0.5	5.2
32.04	2013	unidentified m/z 111		111		0.1	3.1	2.2	4.7	0.7
32.24	2023	methylfumaric acid	fireplace, wine, fermented, beer	112		45.0	15.2	19.0	22.0	57.4
32.74	2049	1-H-pyrrole-2-carboxaldehyde	spice, beer, winey, grapey, spice	95	96	84.0	0.1	0.5	0.6	8.7
32.96	2060	diethyl malate	fermented, yeasty, winey, grapey, honey	RIC	191	94458.4	35421.9	23614.6	11807.3	59036.5
33.85	2106	isobutyl octanoate	fermented, grape, winey, cinnamon	127	187	3975.8	5925.8	3745.5	4418.1	6084.6
35.29	2180	succinoic acid, 2-hydroxy-3-methyl-,diethyl ester	raisins, winey, fermented	85	205	318.4	233.1	328.9	164.4	255.6

Time (min)	LRI	Most likely identification	Odour descriptors	Quan Ion a	M+1+b	Wine 8 1996	Wine 3 2006	Wine 7 2006	Wine 16 2006	Wine 5 1998
35.90	2212	4-vinyl guaiacol	sweet, woody, phenol, fermented,muscat, port	135	151	20.1	9.0	7.2	8.8	17.0
36.30	2232	unidentified m/z 112		112		63.4	6.8	8.3	5.7	84.7
36.53	2244	acetosyringone	raisins, fireplace, phenol, apple pie, cooked raisins	181		8.7	1.7	2.6	7.6	4.8
36.57	2246	unidentified m/z 85		85	159	302.2	295.5	405.6	210.1	284.4
38.00	2320	isobutyl decanoate	cooked christmas cake, winey, muscat sultana	155		36.5	634.2	169.1	450.5	73.3
38.21	2331	diethyl tartrate	apple, fermented, beer, winey	RIC	207	1958.1	875.1	33.7	1.4	7772.2
39.30	2386	diethyl succinate#2		147		1703.3	1057.6	2102.6	346.3	2503.7
39.98	2421	2,6-dimethoxytoluene		151		5.7	0.3	0.0	0.0	0.0
40.49	2447	ethyl citrate		157	277	389.7	14.1	15.7	5.5	600.8
42.13	2532	vanillin		151	153	11.9	0.4	0.0	1.8	13.0
42.70	2561	methylvanillate		151		5.7	5.3	0.0	0.0	0.0
43.12	2583	3-oxo- -ionol		108	207	51.6	157.7	18.5	23.9	24.7
43.25	2590	acetovanillin		151	167	7.4	18.8	6.7	11.4	9.3

a Quan ion = m/z ion used for integration of peak areas (i.e., semi-quantitative data).
b M+1+ = m/z of prominent ion (parent + 1+) at the same LRI by methanol chemical ionisation

The data in *Tables 5.23* to *5.25* provide measures of the potency of the extracted aroma compounds in terms of dilution factor (FD), charm analysis (Charm) and odour spectrum values (OSV). The FD represents the highest dilution of the wine extract for which a GCO panellist could still detect compounds. The Charm value is often generated by the FD multiplied by the time of detection of the compound by a panellist, but in *Table 5.24* the Charm value was instead given by the FD multiplied by the area of the peak in the Charm chromatogram (i.e., generated by the GCO panellist during sniffing). The Charm value is proportional to the concentration of the component in the extract divided by the gas-phase detection threshold. The OSV is the normalized Charm value modified with an approximate Steven's law exponent (Stevens 1958). OSV values are independent of total concentration and approximate the relative potency of each component.

Of the compounds identified/quantified in *Experiment 5.1 Results and discussion*, fourteen were found to have aroma associated with them from the wine extracts. Another 16 compounds, unidentified at this stage, also had aroma associated with them and most were still detected after at least some dilution. These 16 compounds require further identification based upon analysis of the collected MS data, co-elution of standards (where possible), and also cross referencing with the data from *Experiment 5.7, Identification of aroma active compounds in Semillon wines – GCO analysis* and *Table 5.28*. Large variations were observed for the aroma potency data for particular compounds across the wines as is best exemplified in the spider plots of *Figure 5.49*. For example, unknown 12 (fruity, floral) ranges from OSV values of 14–100. As a consequence no significant differences (P=0.05) were observed with respect to the aroma potency for old wines versus young wines. However, the data does indeed provide potential compounds to target for identification, that is, those that would appear to be of high importance to wine styles. For example, wine D (1996) contains the highly potent unknown compounds 16, 13 and 10, which provide 'smoke, chocolate', 'fruity, floral' and 'smoke, ham', respectively. Another would be unknown 16 (i.e. 'cosmetic'-aroma) that would appear more potent in the older wines. Once identified, this data would also allow recombination experiments to be conducted to assess if the different Semillon wine style nuances could be generated with the compounds listed in *Tables 5.23* to *5.25*.

Chemistry of aged and/or oxidised Semillon flavours: phenylacetaldehyde and methional.

In parallel to the analytical GC work described above, attempt was made to examine volatile compounds relevant to the aged/oxidised character of Semillon and the impact of various wine components (i.e. sulfur dioxide, ascorbic acid, oxygen consumption rate) on the concentration of these volatile compounds. The compound assessed in detail was phenylacetaldehyde ('honey' aroma), along with a related aged/oxidative aroma compound methional ('boiled potato'). Both compounds are suggested to be produced by similar mechanisms as described below.

Background to phenylacetaldehyde and methional as aroma contributors in wine.

Phenylacetaldehyde is known to be responsible for the 'honey-like' aroma of wines subjected to elevated oxygen concentrations and higher temperatures (Ferreira *et al*. 2002; Culleré *et al*. 2007). Although not specifically researched/reported as a function of white wine aging, its concentrations certainly increases during the aging of red wines (Culleré *et al*. 2007). Its production was suggested to occur from the amino acid phenylalanine via a Strecker reaction (*Figure 5.50*). This mechanism requires the amino acid to interact with a carbonyl compound that ultimately, via intermediate compounds, generates the aldehyde derivative of the amino acid. The role of oxygen is proposed to provide carbonyl compounds, either through oxidation reactions directly (i.e., acetaldehyde production, ortho-quinone production etc) or through consumption of sulfur dioxide and subsequent release of bound carbonyl compounds.

The equivalent strecker reaction product from the amino acid methionine was also examined, as it is known to contribute significantly to the oxidation aroma of oxidised wines (Cullere *et al*. 2007; Escudero *et al*. 2000). Although an added carbonyl compound (methyl glyoxal) induced conversion of methionine to the aldehyde in a model wine system (Ferreira *et al*. 2002), it was suggested that the ortho-quinone compounds, formed from the oxidation of phenolic compounds, was a more likely was more likely to afford this step in wine (Escudero *et al*. 2000). In any case, the addition of sulfur dioxide to the solution of methionine and methyl glyoxal led to no methional being detected. The addition of methionol, the alcohol equivalent of methional, to a wine that was then oxygenated lead to increased levels of methional, and consequently methionol (i.e. meaty/damp cheese aroma) was suggested to

Table 5.23 The FD values for components of the Semillon wine GCO aroma extraction dilution analysis.

Compound	Found GCO	odour description	FD WINE A 1999	FD WINE B 2006	FD WINE C 2006	FD WINE D 1996	FD WINE E 2006	FD WINE F 1998
Ethyl butanoate	1017	fruity; apple	81	81	81	81	81	81
Ethyl isobutyrate	974	strawberry; sweet, rubber	81	243	243	243	243	243
Isoamyl acetate	1078	banana	81	81	243	27	81	243
Ethyl isovalerate	1045	fruity	81	243	243	81	27	81
3-Methyl-1-butanol	1201	chemical, stale, whiskey, malt, burnt	ns	ns	ns	ns	ns	3
Ethyl hexanoate	1227	fruity,apple peel, fruit	9	81	27	1	27	27
Hexyl acetate	1270	fruity, herb	ns	ns	ns	ns	ns	1
1-Hexanol	1360	grass, green, fresh,resin, flower, green	ns	ns	ns	ns	ns	1
Ethyl octanoate	1452	fruity, fresh, fruit, fat	1	ns	1	ns	3	3
Ethyl decanoate	1690	grape	9	9	1	1	9	243
2-Phenylethylacetate		flowery, fruit, sweet	27	27	81	1	243	81
Hexanoic acid	1867	green	9	ns	9	27	9	27
Octanoic acid	2130	fatty, unpleasant, sweat, cheese	81	27	1	81	ns	27
Decanoic acid	2399	rancid, fat	1	ns	ns	1	1	1
unknown1	1061	fruity, apple	81	81	243	243	27	243
unknown2	1249	fruity, apple, estery	81	81	9	243	81	243
unknown3	1305	chemical, chocolat, green, plastic	9	3	1	1	1	3
unknown4	1322	mushroom	ns	243	ns	ns	27	9
unknown5	1406	floral, green	9	1	1	1	9	1
unknown6	1423	green	ns	ns	ns	1	ns	ns
unknown7	1472	bread, almond, sweer	ns	1	1	ns	ns	1
unknown8	1495	fruity, mushroom, chemical	ns	1	1	ns	3	1
unknown9	1692	chocolat, chemical,	1	9	1	1	9	243
unknown10	1785	smoke ham	81	9	81	243	81	243
unknown11	1909	smoke	9	ns	9	81	9	27
unknown12	1955	fruity, floral	81	3	243	243	243	7
unknown13	2184	fruity, floral	81	27	9	81	3	27
unknown14	2247	fruity, wood, smoke	1	9	27	9	9	243
unknown15	2275	smoke, chocolat	ns	27	27	729	9	3
unknown16	2325	cosmetic	81	3	1	81	9	243

Table 5.24 The Charm values for components of the Semillon wine GCO aroma extraction dilution analysis.

Compound	Found GCO	odour description	CHARM WINE A 1999	CHARM WINE B 2006	CHARM WINE C 2006	CHARM WINE D 1996	CHARM WINE E 2006	CHARM WINE F 1998
Ethyl butanoate	1017	fruity; apple	210	238	130	322	168	227
Ethyl isobutyrate	974	strawberry; sweet, rubber	358	1075	548	891	1023	546
Isoamyl acetate	1078	banana	219	213	734	72	209	830
Ethyl isovalerate	1045	fruity	211	544	420	279	85	196
3-Methyl-1-butanol	1201	chemical, stale, whiskey, malt, burnt	ns	ns	ns	ns	ns	15
Ethyl hexanoate	1227	fruity,apple peel, fruit	35	240	74	4	78	104
Hexyl acetate	1270	fruity, herb	ns	ns	ns	ns	ns	1
1-Hexanol	1360	grass, green, fresh,resin, flower, green	ns	ns	ns	ns	ns	2
Ethyl octanoate	1452	fruity, fresh, fruit, fat	2	ns	4	ns	8	6
Ethyl decanoate	1690	grape	38	45	3	3	26	510
2-Phenylethylacetate		flowery, fruit, sweet	116	167	275	5	243	984
Hexanoic acid	1867	green	32	ns	27	88	25	96
Octanoic acid	2130	fatty, unpleasant, sweat, cheese	261	96	4	284	ns	146
Decanoic acid	2399	rancid, fat	4	ns	ns	6	3	3
unknown1	1061	fruity, apple	169	249	506	531	77	547
unknown2	1249	fruity, apple, estery	230	181	23	616	237	688
unknown3	1305	chemical, chocolat, green, plastic	18	4	1	3	2	12
unknown4	1322	mushroom	ns	535	ns	ns	43	26
unknown5	1406	floral, green	23	3	2	2	22	4
unknown6	1423	green	ns	ns	ns	3	ns	ns
unknown7	1472	bread, almond, sweer	ns	1	3	ns	ns	3
unknown8	1495	fruity, mushroom, chemical	ns	1	3	ns	7	2
unknown9	1692	chocolat, chemical,	3	45	3	3	26	510
unknown10	1785	smoke ham	368	34	81	1883	397	127
unknown11	1909	smoke	32	ns	27	308	25	96
unknown12	1955	fruity, floral	450	21	916	1628	1227	7
unknown13	2184	fruity, floral	261	96	15	284	11	146
unknown14	2247	fruity, wood, smoke	3	39	49	34	37	686
unknown15	2275	smoke, chocolat	ns	114	192	7061	30	10
unknown16	2325	cosmetic	500	20	4	745	42	2296

Table 5.25 The OSV values for components of the Semillon wine GCO aroma extraction dilution analysis.

Compound	Found GCO	odour description	OSV WINE A 1999	OSV WINE B 2006	OSV WINE C 2006	OSV WINE D 1996	OSV WINE E 2006	OSV WINE F 1998
Ethyl butanoate	1017	fruity; apple	65	47	38	21	37	31
Ethyl isobutyrate	974	strawberry; sweet, rubber	85	100	77	36	91	49
Isoamyl acetate	1078	banana	66	44	90	10	41	60
Ethyl isovalerate	1045	fruity	65	71	68	20	26	29
3-Methyl-1-butanol	1201	chemical, stale, whiskey, malt, burnt	ns	ns	ns	ns	ns	8
Ethyl hexanoate	1227	fruity,apple peel, fruit	26	47	28	2	25	21
Hexyl acetate	1270	fruity, herb	ns	ns	ns	ns	ns	2
1-Hexanol	1360	grass, green, fresh,resin, flower, green	ns	ns	ns	ns	ns	3
Ethyl octanoate	1452	fruity, fresh, fruit, fat	6	ns	7	ns	8	5
Ethyl decanoate	1690	grape	28	20	6	2	15	47
2-Phenylethylacetate		flowery, fruit, sweet	48	39	55	3	45	65
Hexanoic acid	1867	green	25	ns	17	11	14	20
Octanoic acid	2130	fatty, unpleasant, sweat, cheese	72	30	7	20	ns	25
Decanoic acid	2399	rancid, fat	9	ns	ns	3	5	4
unknown1	1061	fruity, apple	58	48	74	27	25	49
unknown2	1249	fruity, apple, estery	68	41	16	30	44	55
unknown3	1305	chemical, chocolat, green, plastic	19	6	4	2	4	7
unknown4	1322	mushroom	ns	71	ns	ns	19	11
unknown5	1406	floral, green	22	5	5	2	13	4
unknown6	1423	green	ns	ns	ns	2	ns	ns
unknown7	1472	bread, almond, sweer	ns	3	6	ns	ns	4
unknown8	1495	fruity, mushroom, chemical	ns	3	5	ns	7	3
unknown9	1692	chocolat, chemical,	8	20	6	2	15	47
unknown10	1785	smoke ham	86	18	30	52	57	24
unknown11	1909	smoke	25	ns	17	21	14	20
unknown12	1955	fruity, floral	95	14	100	48	100	6
unknown13	2184	fruity, floral	72	30	13	20	9	25
unknown14	2247	fruity, wood, smoke	8	19	23	7	17	55
unknown15	2275	smoke, chocolat	ns	33	46	100	16	7
unknown16	2325	cosmetic	100	14	7	32	19	100

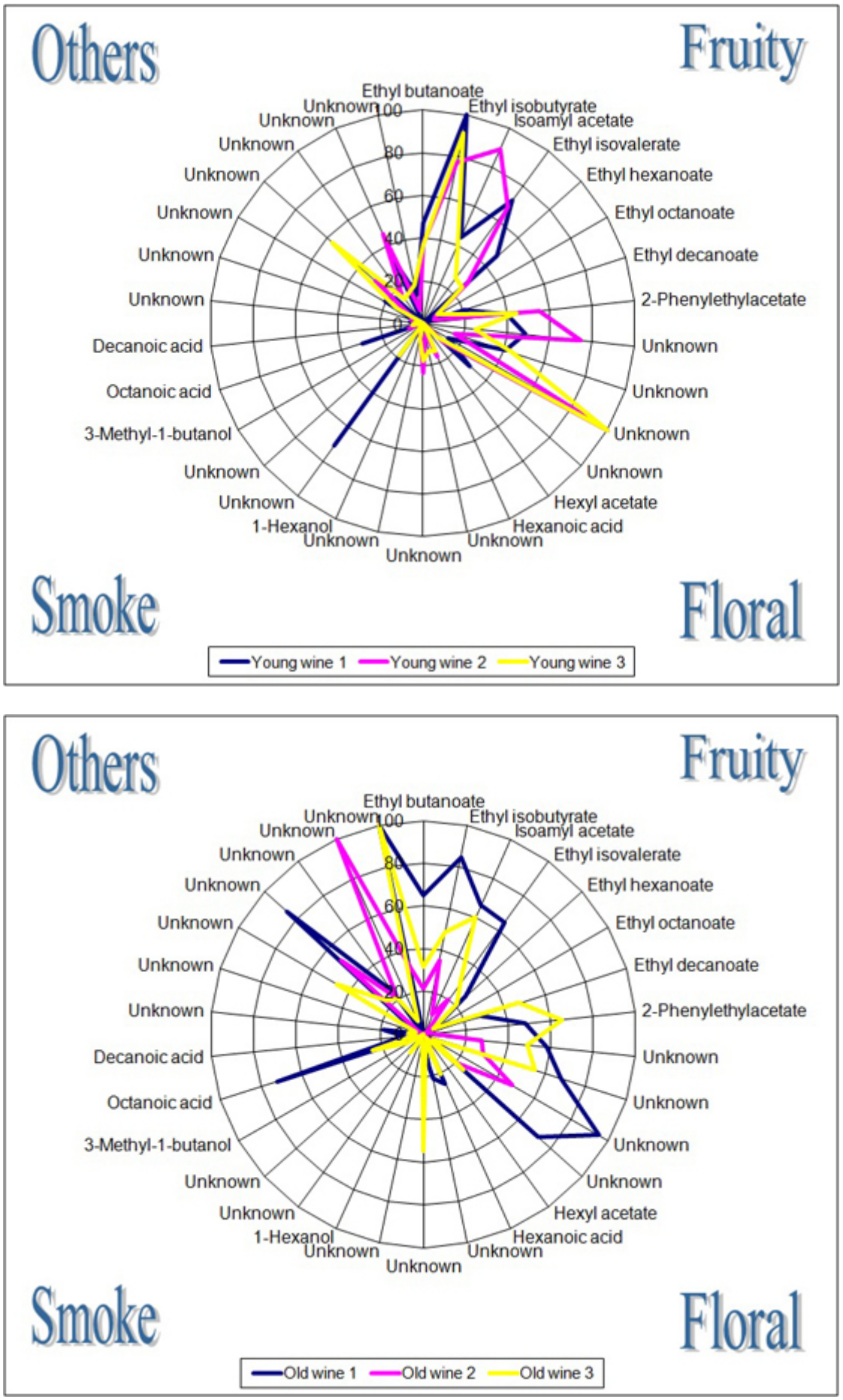


Figure 5.49 Spider plot for the aroma potency (i.e., OSV values) for aroma extract components in young (upper) and old (lower) Semillon wines.

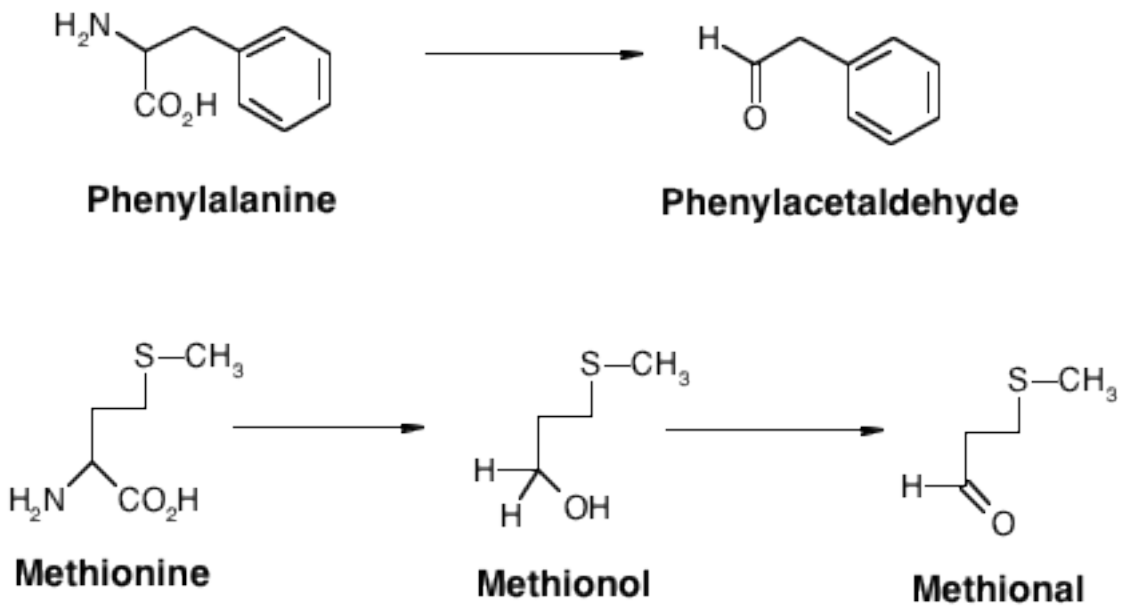


Figure 5.50 The production of phenylacetaldehyde and methional from their corresponding amino acids. Carbonyl compounds are known to induce the conversions shown below.

be an intermediate in the conversion of methionine to methional (*Figure 5.50*) (Escudero *et al.* 2000). Although not shown, it is likely that phenylethanol (i.e. a compound known to contribute floral aroma) would similarly be an intermediate in the conversion of phenylalanine to phenylacetaldehyde. Both methionol and phenylethanol (i.e. also referred to as 2-phenylethylacohol (PEA)) were identified/quantified in Semillon wines in the previous sections of this report. Similarly, the amino acids phenylalanine and methionine are known to be present in Semillon wine as described in a separate section of this final report.

It has been suggested that the precursors of methional and phenylacetaldehyde (*Figure 5.50*) are more easily oxidised than phenolic compounds and, consequently, these aroma compounds are generated before oxidative discolouration of the wine (which is a consequence of phenolic compound polymerisation). Therefore, given the propensity of the precursors to oxidise, the rate at which oxygen is consumed in wine may be important to their production.

Aims for investigations into phenylacetaldehyde and methional

The purposes of the study was to:

1. Confirm the ability of the aldehyde compounds (i.e. phenylacetaldehyde and methional) to be produced in a simplified oxygenated model wine system (i.e. without added carbonyl compounds such as methyl glyoxal) and with no other precursors to the aldehydes other than the amino acids;
2. Assess the impact of 'antioxidants' in such high oxygenated conditions;
3. Establish binding of the aldehydes at typical wine sulfur dioxide concentrations; and
4. Assess any links between the rate of oxygen consumption in wine and the production of these aldehyde compounds.

Measurement of aldehydes and the model wine system utilised.

The aldehydes were measured by the derivatisation-SPE-GCMS method, outlined in the methods section that was slightly adapted from Ferreira *et al.* (2006).

A model wine system was utilised that contained 12%(v/v) aqueous ethanol and buffered to pH 3.2 with tartaric acid. To this was added 200 mg L^{-1} caffeic acid to model the non-flavonoid phenolic compounds in white wine, and 50 mg L^{-1} catechin was added to model the smaller concentration of skin-derived phenolic compounds. Also added were the amino acids, phenylalanine and methionine, at

concentrations of 100 mg L^{-1} and 50 mg L^{-1}, which are at the upper ranges for these amino acids in white wines (Ali *et al.* 2010). The metal ions copper(II) and iron(III) were added to the model system at concentrations of 0.2 mg L^{-1} and 5 mg L^{-1}, respectively, as these are typical concentrations of these metal ions in white wine (Danilewicz 2007). Finally, sulfur dioxide was added at a concentration of 45 mg L^{-1}. A 300 mL volume of samples were prepared in triplicate and stored in 1000 mL bottles, whereby the sample was saturated with oxygen gas (i.e., to achieve

~30 mg L^{-1} dissolved oxygen) and the headspace was also purged with oxygen.

Production of phenylacetaldehyde and methional in high oxygenated systems: impact of temperature and antioxidants.

The initial experiment examined the ability of the aldehyde compound to be generated in the model wine under conditions of high oxygen (i.e., saturation of solutions with pure oxygen and headspace over the samples with pure oxygen gas), and at temperatures of 25°C and 45°C, over a 7 day period. The temperature of 25°C was more relevant to general wine storage conditions whilst the higher temperatures had previously been shown in wine to induce more of the aldehyde compounds in wine. At the same time the addition of the antioxidants, ascorbic acid (100 mg L^{-1}), erythorbic acid (100 mg L^{-1}) or glutathione (174 mg L^{-1}), was made to different versions of the model wine systems in order to assess their impact on the production of the aldehyde compounds. Although the tripeptide glutathione (consisting of glutamate, cysteine and glycine) is not yet a permitted additive to wine, recent reports have shown that it has some antioxidant capabilities in wine conditions (Roussis *et al.* 2008), whilst others have shown that during wine aging it has been linked to raised hydrogen sulfide concentrations (Ugliano *et al.* 2011). The concentration of 174 mg L^{-1} glutathione was utilised to enable it to be used at a 1:1 ratio to ascorbic acid and erythorbic acid and thereby to enable a direct comparison between the three.

After 7 days, the free sulfur dioxide concentration was 0 mg L^{-1} in all samples, as was the concentrations of ascorbic acid, erythorbic acid and glutathione in the respective samples. The results in *Figure 5.51* show that after 7 days, minimal aldehyde production occurred in the control and glutathione samples at 25°C whilst appreciable levels were observed in the ascorbic acid and erythorbic acid samples. In fact, the concentrations generated are far above the aroma threshold levels quoted within Cullere *et al.* (2007) (i.e., 1.0 µg L^{-1} for phenylacetaldehyde, and 0.5 µg L^{-1} for methional). Remembering, that as such oxygenated conditions are far removed from normal wine aging, it is likely that ascorbic acid and erythorbic acid accelerated production of the aldehydes due to their ability to accelerate consumption of oxygen, thereby leading to rapid depletion of sulfur dioxide and increased rates of carbonyl compound production. In typical bottled wine conditions, where access to oxygen is limited (and will be investigated below), the absolute depletion of sulfur dioxide as induced

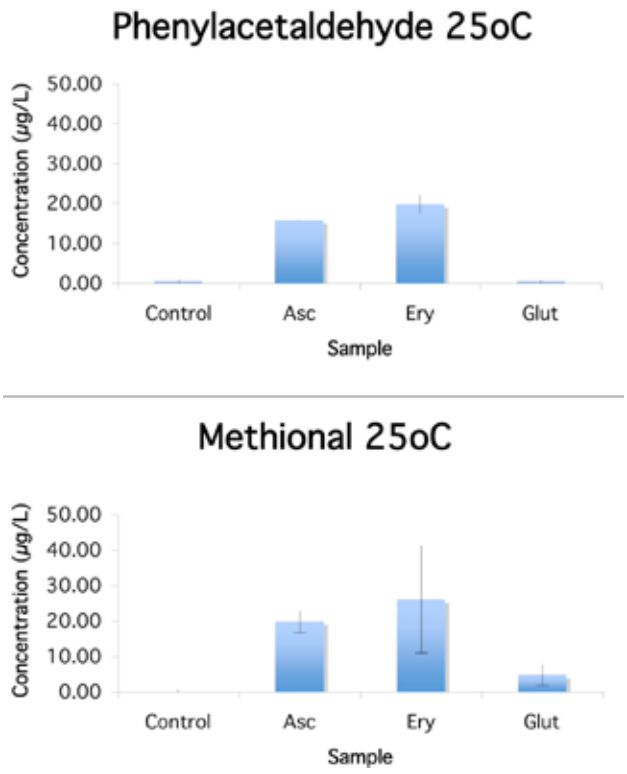


Figure 5.51 Concentrations of phenylacetaldehyde and methional in the model wine systems stored at 25°C.

by ascorbic acid/erythorbic acid would be less likely, but in any case it is interesting to note that in high oxygen conditions they can induce formation of these aldehyde compounds.

In the equivalent samples stored at 45°C (*Figure 5.52*), all samples had the expected higher concentrations of the aldehydes compared to the samples stored at lower temperatures (*Figure 5.51*), and all were above the aroma thresholds mentioned above. This increase included the control sample that had concentrations of phenylacetaldehyde and methional of 160 ±13 and 65 ±12 µg L^{-1} (note: quoted uncertainty is the standard deviation), respectively. Similar to the results at 25°C, ascorbic acid and erythorbic acid had higher concentrations than the control, but in contrast to the results at the lower temperature, the sample with glutathione had the largest levels of the aldehyde compounds at 45°C. Glutathione is known to undergo hydrolysis at higher temperatures and it is possible that release of the more reactive cysteine may have contributed to the higher aldehyde production. This may have been due to accelerated oxygen consumption, or again direct degradation to carbonyl compounds via a Strecker reaction.

These results showed that in this simplified oxygenated model wine system, with no other precursors present besides the parent amino acids,

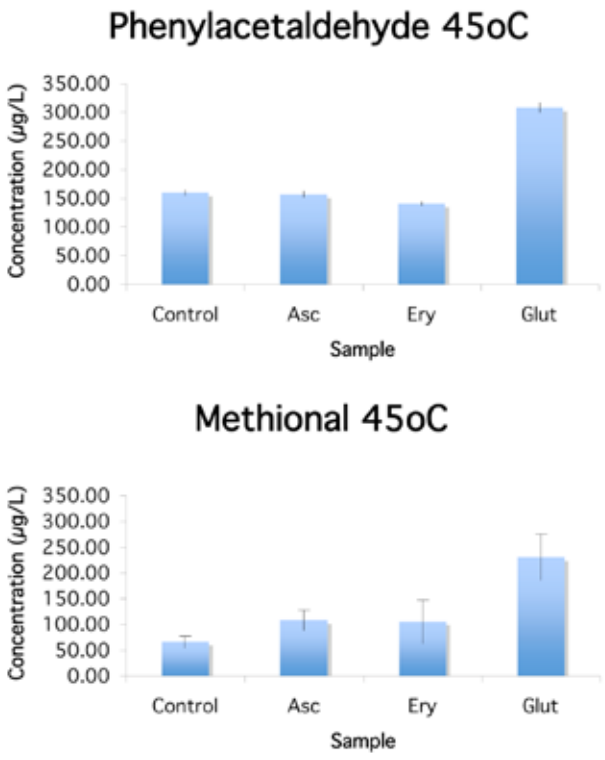


Figure 5.52 Concentrations of phenylacetaldehyde and methional in the model wine systems stored at 45°C. Note the different scale used to that in Figure 5.51

both phenylacetaldehyde and methional were generated. This was despite the fact that the measured concentration of amino acids did not significantly ($P<0.05$) change during the experiment (data not shown). Given that the concentrations of aldehydes generated were less than 300 µg L^{-1}, in any sample, and the concentration of amino acids were 50–90 mg L^{-1}, it is not surprising that the change in amino acid concentration could not be detected by the analysis method utilised (i.e. amino acid derivatisation and LC analysis with fluorescence detection). That is, the maximum molar yield of equivalent aldehyde from the amino acids was less than 0.5% for phenylacetaldehyde and less than 0.7% for methional.

In summary, the higher temperatures favoured the production of the 'honey-like' aroma compound (i.e., phenylacetaldehyde) and the 'boiled potato-like' compound (i.e., methional). As did the presence of ascorbic acid and erythorbic acid, but most likely due to the increase oxygen consumption induced by these agents in the high oxygen environment, but also possibly due to the array of carbonyl products that they yield. Glutathione induced the highest aldehyde concentrations but only at the higher temperature adopted. Given the viability for the production of the aldehydes in the model wine system, before looking at more wine relevant conditions, with reduced oxygen concentrations and maintaining sulfur dioxide concentrations, the interaction of sulfur dioxide with the aldehyde compounds was examined.

The binding of sulfur dioxide to phenylacetaldehyde and methional

Standards of phenylacetaldehyde (250 µg/L) and methional (100 µg L^{-1}) were prepared in a simplified model wine system, consisting of 12 %(v/v) aqueous ethanol buffered to pH 3.20 with tartaric acid. The concentrations were chosen as they were within the range encountered for the aldehyde compounds in *Figure 5.52*. The solution was divided up into 30 samples, and 40 mg L^{-1} sulfur dioxide added to 24 of the samples and all were left to equilibrate overnight (16 h). After 16 hours, acetaldehyde, hydrochloric acid and hydrogen peroxide were each added to six different samples containing sulfur dioxide. The acetaldehyde and hydrogen peroxide were added at a 1:1 molar ratio to sulfur dioxide whilst the hydrochloric acid was added to reach a pH of 1.0. Half of the samples were analysed for phenylacetaldehyde and methional after 17 hours stored at 25°C (1 hour of equilibration after acetaldehyde, acid or hydrogen peroxide additions), whilst the remaining solutions were analysed 40 hours later stored at 25°C (i.e., 25 hours after the addition of the above).

The results (*Figure 5.53*) show that 40 mg L^{-1} causes binding of both phenylacetaldehyde and methional. However, based on the data in *Figure 5.53* and similar data collected at different sulfur dioxide concentrations (data not shown), dissociation constants can be calculated for these aldehyde compounds and provide values of $(4 \pm 2) \times 10^{-4}$ M (SD, n=6) and $(6 \pm 2) \times 10^{-4}$ M (SD, n=4) for phenylacetaldehyde and methional, respectively. This places these volatile aldehyde compounds on par with keto acids and oxidised sugars for their potential to be bound by sulfur dioxide in wine conditions. It also means that once these volatile aldehyde compounds are generated it is particularly difficult to lower their concentration below the reported aroma thresholds in 12%(v/v) aqueous ethanol solutions. For instance, the maximum total concentration of phenylacetaldehyde in wine that would co-exist with 30 mg L^{-1} of free sulfur dioxide and still be below the aroma threshold (i.e. of 1.0 µg L^{-1}) would be 1.7 µg L^{-1}. For methional, whose aroma threshold is 0.5 µg L^{-1}, the maximum concentration would be 0.90 µg L^{-1}.

Similarly, during the aging of wine, the decrease in sulfur dioxide may result in release of the aldehyde from their bisulfite addition form and they may

become perceptible once a threshold concentration is reached. In order to confirm and assess the rate of release of the aldehyde compounds from the bisulfite addition form, various means of removing sulfur dioxide from the model wine systems were utilised. The addition of either acetaldehyde, concentrated acid (pH<1.1), or hydrogen peroxide is well known to release sulfur dioxide from carbonyl compounds or even anthocyanins (in the case of red wine). *Figure 5.53* shows that on addition of these agents to the model wine solution that contained 40 mg L^{-1}, little change occurred in the samples when analysed for the aldehydes after one hour. However, 25 hours after the addition the treatments of acetaldehyde and hydrogen peroxide both had appreciable release of the aldehyde compounds, albeit more so for phenylacetaldehyde. This result demonstrated that the rate of establishing the equilibrium between sulfur dioxide and the aldehydes occurred in the order of days rather than hours when conducted at 25°C.

The production of phenylacetaldehyde and methional in conditions of excess sulfur dioxide.

Given that most Semillon is bottled under screw cap, with access to minimal oxygen other than that present at bottling, further experiments were conducted to examine the effect of lower oxygen conditions, and residual sulfur dioxide concentrations, on the production of the methional and phenylacetaldehyde. It was also the aim to note how the different antioxidants impacted on the rate of oxygen decay, particularly glutathione, and consequently if this related to aldehyde production. That is, to relate molar oxygen consumption to aldehyde production, and whether this changed at substantial rates of oxygen consumption.

Samples were prepared in gas tight containers such that the dissolved oxygen concentration was 20 mg L^{-1} (using pure oxygen gas), and the decay in oxygen concentration was monitored whilst storing the sample at 45°C until its concentration reached 5 mg L^{-1} (i.e. consumption of 15 mg L^{-1} oxygen). The same model wine system was utilised as in *Figure 5.49*, although higher concentrations of ascorbic acid, glutathione, erythorbic acid and sulfur dioxide was utilised, with 200, 400, 200 and 120 mg L^{-1}, respectively. These higher concentrations were utilised to assure that some portion of each of these components would

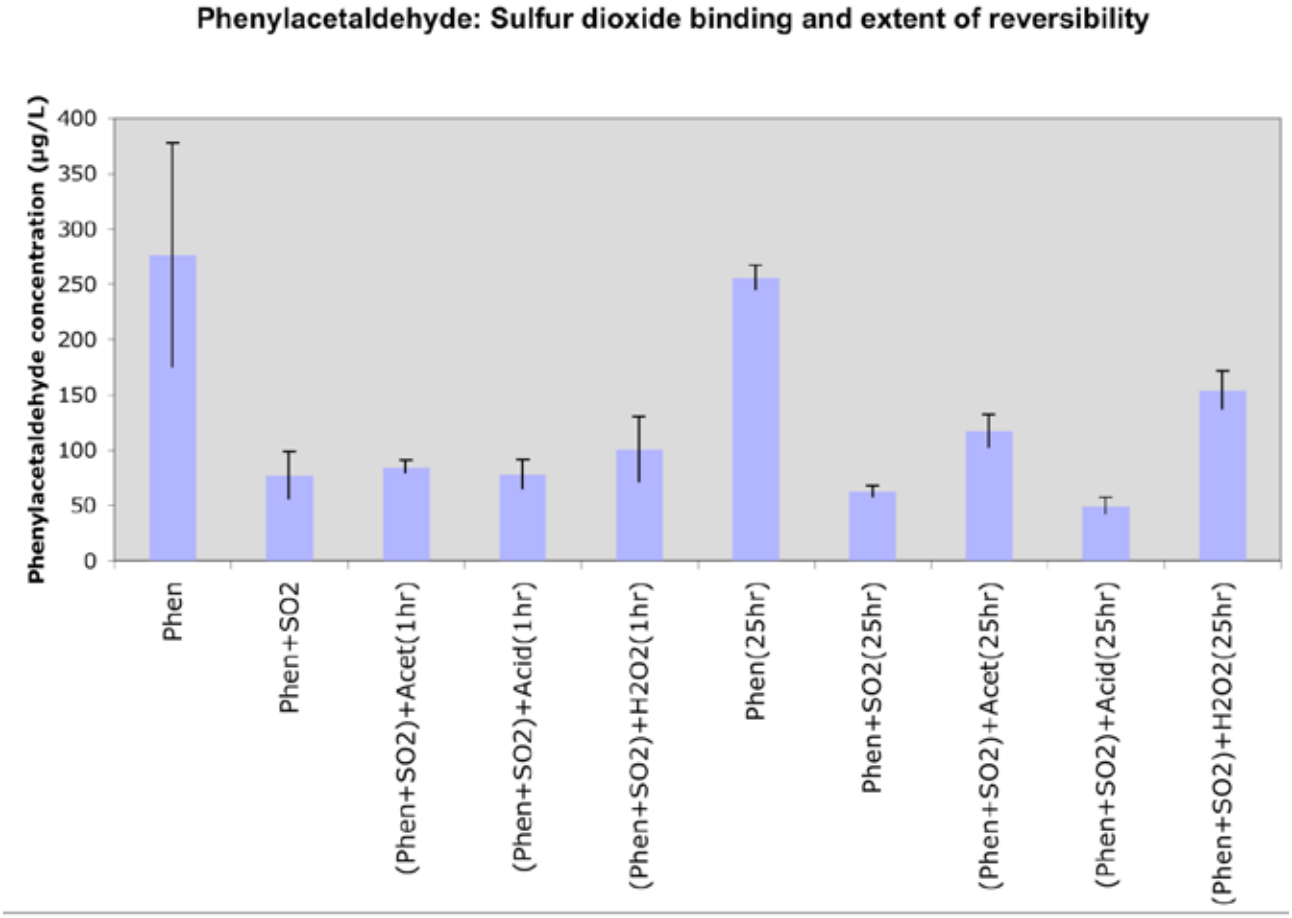


Figure 5.53 The impact of sulfur dioxide on phenylacetaldehyde concentration and investigations into the reversibility of the interaction.

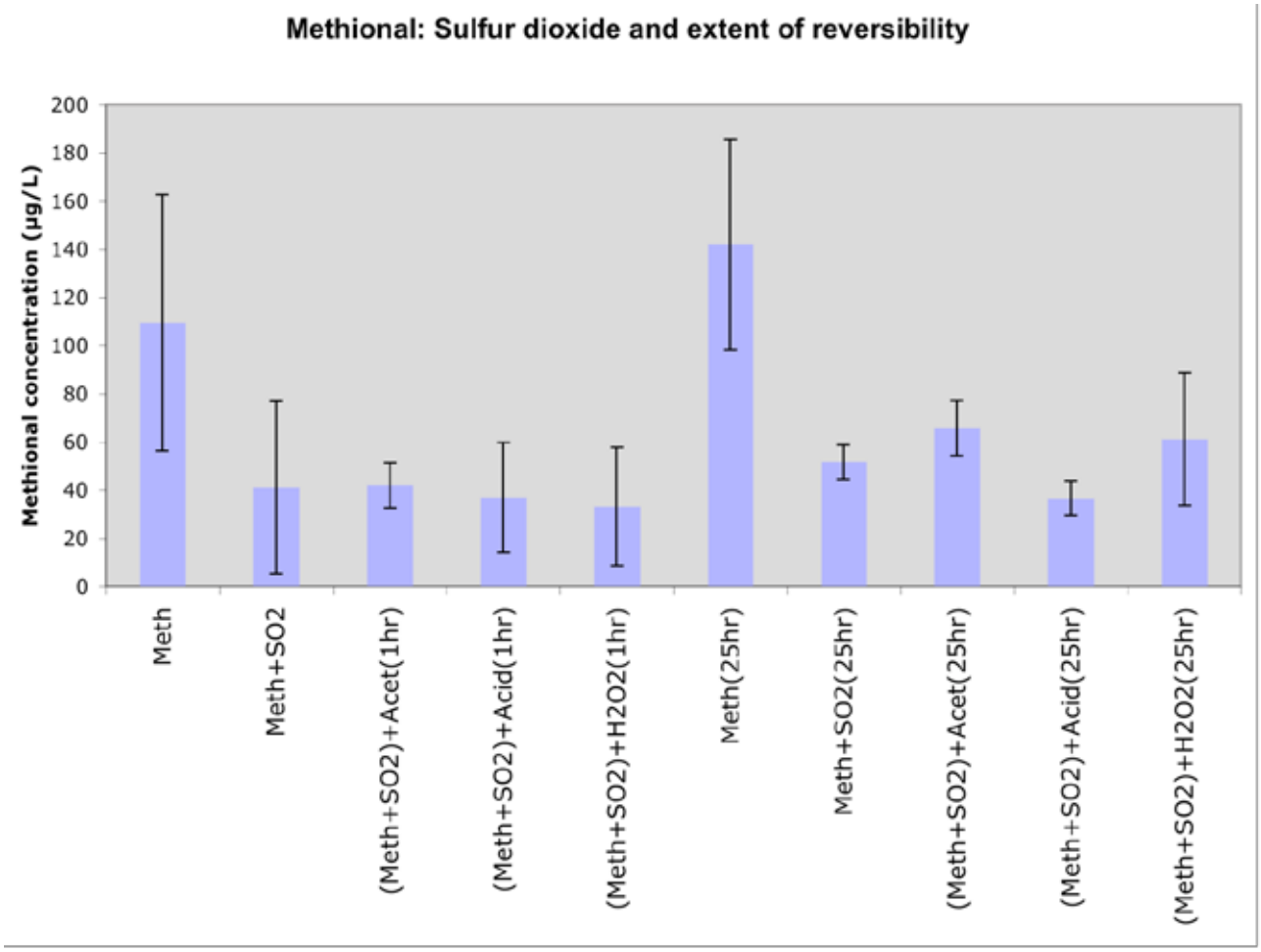


Figure 5.54 The impact of sulfur dioxide on methional concentration and investigations into the reversibility of the interaction.

remain by the end of the experiment (and this was confirmed), and also enabled 1:1:1 molar ratios of ascorbic acid:glutathione:erythorbic acid (for direct comparison of results). Another erythorbic acid sample was prepared with a ten-fold lower sulfur dioxide concentration (12 mg L^{-1}) that was depleted by the end of the experiment.

As expected, the samples containing ascorbic acid and erthorbic acid consumed oxygen much faster than the control sample. In the samples with erythorbic acid and ascorbic acid, the 15 mg L^{-1} oxygen was consumed in around 1.5 h, whilst the control samples and that with glutathione required ~15 h. This also meant that accelerated production of aldehydes in the earlier experiment conducted with excess oxygen was not due to accelerated oxygen consumption by glutathione.

The concentrations of the generated aldehyde compounds in this experiment were far below that obtained for the experiments conducted with excess oxygen as shown in *Figure 5.52*. *Figure 5.55* shows the results for methional whereby little methional was generated in any of the samples other than that containing erythorbic acid with the low initial concentration of sulfur dioxide. The concentration of phenylacetaldehyde was minimal in all the samples (including that with low sulfur dioxide) (data not shown) with no significant differences ($P<0.05$) between the samples. The results show that sulfur dioxide is efficient in protecting against the production of these volatile aldehyde compounds from their parent amino acids regardless of the supporting antioxidant present.

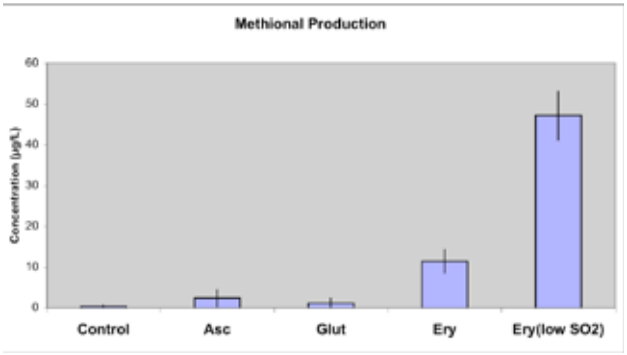


Figure 5.55 The production of methional in model wine samples after the consumption of 15mg/L oxygen. The error bars represent the standard deviation (n=3).

Examination of the potential for using 'simple' chemical analysis (i.e., non-volatile measures) as a surrogate for sensory analysis.

Given the data sets obtained, and the work outlined in *Experiment 5.7, A chemometric approach to linking GCMS analysis of volatile compounds to wine sensory data*, it was decided to assess the potential of using a multi-block chemometric technique known as Common Components and Specific Weight Analysis (Qannari *et al*. 2000) to the comparison of sensory descriptors and the 'simple' chemical analysis of Semillon white wine (i.e., sourced from the Hunter Valley). By 'simple' it is meant the measures of wine chemistry that are routinely measured in commercial wineries of all sizes, that is, measurements that do not require the more research oriented techniques of gas chromatography and liquid chromatography, but instead only require wet chemistry techniques, pH meters and/or enzymatic kits (i.e., with access to single wavelength spectrophotometers). *Table 5.26* lists the 'simple' chemical measures utilised in this work, along with the measurements in sixteen Semillon wines, whilst *Table 5.27* shows the sensory descriptors for the same wines. The advantages of such research are to potentially provide a surrogate to expensive and time-consuming sensory panels as described below. For specific details on chemical measurements, sensory analysis and chemometric treatment of data see the relevant publication attached to this report (Blackman *et al*. 2010).

Background for links between sensory and chemical analysis

Sensory analysis involves the application of human senses to the description and/or evaluation of a product for consumer use. Rigorous sensory analysis involves a panel of assessors that have been trained for a specific evaluation. For example, the determination of descriptors to characterise a wine style or to assess the impact of a processing step on the wine style is now routine practice. Each separate sensory exercise, however, requires an intensive training program for the assessors.

A full sensory analysis, particularly descriptive, texture and time-intensity analyses, are complex processes demanding considerable time with an associated high cost. A proper sensory analysis of a food or beverage, including wine, can take at least three months and possibility six months of training and application. Extensive training is necessary to ensure consistency in and between assessors. The time period demands commitment from panel members and this in turn implies a high cost of the operation (Meiselman 1993). The time and cost factors restrict the extent to which full sensory analysis can be routinely applied.

The generation of analytical measurements for a range of quality parameters related to aroma, flavour and texture is faster, generally less expensive and more objective than sensory analysis. That is analytical measurements, when properly collected, do not suffer from bias due to personal preference (Jack and Steele 2002). The application of chemometrics to the interpretation of analytical data has opened up many interesting possibilities in food and beverage studies, particularly with respect to process monitoring, determination of geographical origin, authentication, adulteration and substitution (Masoum *et al*. 2006a, Masoum *et al*. 2006b; Cunny *et al*. 2008; Cunny *et al*. 2007).

The possibility of using analytical data as a surrogate for sensory data is less well examined, although Lesschaeve (*personal communication*) argues that this has been a sought after goal in many studies over the last 20 or more years. This position is supported by Piggott (1990), who argues that flavour cannot be measured directly by instruments. That is, only individual chemical compounds can be measured quantitatively by instrumental analysis and not the interactions between them that give rise to flavour.

While there have been several studies examining the link between sensory properties and aroma compounds, the main focus has tended to be on validation of the product type or characterising its origin. For example, there have been studies examining the link between sensory properties and non-volatile and volatile parameters on dry-cured ham (Careri *et al*. 1993), drinking water (Meng and Suffet 1997), balsamic vinegar (Durante *et al*. 2006), broiler chicken cuts (Vainionpää *et al* 2004) and netted muskmelon (Senesi *et al*. 2002). Baker and Arnold (Bakker and Arnold 1993) have described the positive relationship between sensory perception and chemical data for colour in port wines, while Kennedy *et al*. (2006) have examined the relationship between several methods of tannin analysis in red wines and perceived astringency. Neural networks have been applied to modelling the sensory characteristics in Scotch Whiskey (Jack and Steele 2002) and beer (Wilson 2003). The potential of instrumental texture measurements as a substitute for the sensory assessment of grape berry ripening properties has been evaluated (Le Moigne *et al*. 2008).

Table 5.26 Analytical parameters for the 16 Semillon wines. Values are in g L-1, except alcohol (%v/v).

Analysis code	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
	Glucose	Fructose	Total (G+F)*	Sugar sweetness response**	Titratable acidity	pH	Alcohol	Malic acid	Volatile acidity	Glycerol
WINE CODE										
A	0.61	5.93	6.54	14.74	6.7	3.25	10.48	2.3	0.2	4.96
B	0.32	0.31	0.63	1.06	6.5	3.15	10.32	1.7	0.3	3.97
C	ND	0.08	0.08	0.19	7.7	2.84	10.64	1.4	0.2	3.94
D	0.13	ND	0.13	0.13	6.3	3.09	10.43	3.2	0.3	4.93
E	0.16	0.27	0.43	0.80	8.2	2.92	11.33	1.7	0.3	5.11
F	0.24	2.04	2.28	5.10	7.5	2.89	10.37	1.7	0.2	4.08
G	0.93	0.97	1.9	3.24	7.3	3.05	10.71	2.7	0.2	5.49
H	0.43	0.78	1.21	2.29	6.2	3.09	10.50	2.6	0.3	4.45
I	0.23	3.39	3.62	8.30	6.9	2.93	10.68	1.9	0.2	5.88
J	0.49	3.48	3.97	8.78	7.6	2.89	10.09	1.6	0.1	4.04
K	1.49	1.73	3.22	5.61	7.1	3.16	11.37	2.9	0.2	4.86
L	0.13	1.25	1.38	3.11	6.8	3.08	11.87	1.5	0.2	5.91
M	0.17	0.03	0.2	0.24	7.5	2.89	10.12	1.3	0.1	3.99
N	1.41	1.56	2.97	5.13	7.7	3.09	10.13	2.5	0.3	3.77
O	0.05	0.16	0.21	0.43	6.5	3.24	11.57	2.3	0.2	4.01
P	1.55	2.11	3.66	6.58	6.9	2.97	10.64	2.4	0.3	4.50

* Total G+F = sum of the glucose (G) and fructose (F) concentration

** Sugar sweetness response: calculated as (glucose + 2.382 fructose) concentration to allow for the enhanced sweetness of fructose with respect to glucose and to allow for its earlier perception (Shallenberger 1993).

Table 5.27 Sensory descriptors for the 16 Semillon wines. See reference 'ISO 11035 1994' for details.

SENSORY CODE	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
	LEMON / LIME	FLORAL	GRAPE FRUIT	PINE APPLE	HAY / STRAW	ORANGE MARMALADE	HONEY	TOAST	GRASSY	ASPARAGUS	LYCHEE	KEROSENE	CONFECTIONARY	ACIDITY	SWEETNESS
WINE CODE															
A	5.24	4.10	2.63	3.13	2.27	0.98	2.28	1.08	2.55	1.30	1.67	1.46	3.48	4.49	5.05
B	3.73	1.51	2.19	1.67	3.76	2.74	4.62	4.23	1.76	1.51	1.12	3.74	0.98	5.06	2.52
C	4.29	2.40	2.83	2.09	3.06	0.79	1.59	1.51	2.49	1.06	0.94	1.77	1.67	5.89	3.05
D	4.59	2.59	2.43	2.17	2.83	1.43	2.57	2.21	2.26	1.57	1.40	1.68	1.83	4.91	3.33
E	4.09	2.17	2.17	2.38	3.01	2.14	3.66	2.72	1.78	0.87	1.01	2.61	1.24	5.30	2.89
F	3.20	1.62	1.63	1.26	3.78	2.18	4.15	5.33	1.27	1.04	0.96	2.83	1.01	5.14	2.88
G	5.37	4.13	2.85	3.22	2.21	1.47	1.80	1.17	2.55	1.33	2.04	1.24	2.47	5.42	3.86
H	3.75	1.77	1.72	1.95	3.79	3.64	5.55	5.46	1.33	0.82	1.27	3.13	0.78	4.87	2.79
I	4.50	3.91	2.91	3.13	2.59	1.04	1.78	1.03	2.97	1.13	1.49	1.18	2.72	5.77	3.83
J	4.18	1.99	2.26	1.85	3.26	1.09	2.76	2.15	2.16	1.04	0.89	1.79	1.28	5.70	3.07
K	3.98	1.77	2.47	1.52	3.34	1.55	2.55	2.72	2.33	1.45	0.70	2.32	0.90	5.00	3.37
L	4.55	2.27	2.66	1.98	3.19	1.21	2.21	1.96	2.18	1.07	1.16	2.09	1.45	5.01	3.67
M	3.74	1.91	2.19	1.94	3.13	1.34	2.81	3.54	1.79	0.95	1.00	2.24	1.28	5.53	3.09
N	4.50	2.72	2.54	1.98	2.85	1.93	2.66	2.13	2.17	1.22	1.29	2.11	1.67	4.90	3.67
O	3.77	1.77	2.23	2.48	3.01	2.39	3.28	3.06	1.72	1.31	1.14	2.43	1.51	4.21	2.93
P	4.51	3.60	2.45	2.23	3.05	1.30	2.46	1.26	2.45	1.34	1.81	1.61	2.54	4.81	3.99

A predictive model for the characterisation of the aromas in coffee based on the correlation between descriptive sensory profiling and analytical measurement obtained by proton transfer reaction –mass spectrometry (Lindinger *et al*. 2006) opens up new possibilities for using chemical analysis as a surrogate for sensory analysis. Principal Component Analysis was used in this coffee study. The recent advances in chemometric methods for the interpretation of multi-block data have provided new methodologies for data treatment (Hanafi *et al*. 2006). Multi-block methods facilitate the comparison of different blocks of variables describing the same samples, highlighting similarities and differences among the blocks and also among the variables within each block. The present study investigates the potential of using a multi-block technique known as Common Components and Specific Weight Analysis (Qannari *et al*. 2000) to the comparison of sensory descriptors and chemical analysis of Semillon wine.

Initial linear regression analysis of sensory vs. chemical analysis data

The correlations among all the analytical variables (columns and rows 1 to 10) and sensory variables (11 to 25) are presented graphically in *Figure 5.56* where it is clear that the positive and negative correlations are much stronger within the sensory variables block than between the analytical and the sensory variables blocks. Supplementary *Figures 5.57* show the correlations between all pairs of analytical parameters, all pairs of sensory parameters and sensory and analytical parameters respectively.

The initial statistical analysis used simple linear regressions to determine if there were any potentially significant correlations between the sensory and analytical data that would suggest that there may be value in a more detailed analysis. *Table 5.28* presents a summary of the correlations found by this approach.

The sensory ***sweetness*** (S15) score is clearly significantly correlated with the analytical measures of residual sugar; that is with the total glucose plus fructose concentration (Total (G+F) – *Table 5.1*) and the sugar sweetness response, the total sugar concentration adjusted for the enhanced sweetness of fructose (A2, A3, A4). Similarly, ***confectionary*** (S13), a sensory descriptor also related to sweetness shows a positive correlation with the total residual sugar concentration, although not as strong as with the ***sweetness*** score (*Table 5.28*).

The sensory score for ***acidity*** (S14) is significantly correlated with titratable acidity (A5, *Table 26*) and shows significant, but negative, correlations with malic acid (A8) and pH (A6). Although a strong negative link between pH and sensory assessment of acidity is logical, it is somewhat unexpected here, given the narrow range of pH values of these wines, from pH 2.84 to pH 3.16 (*Table 26*). The malic acid correlation is discussed in more detail below.

Intriguingly, positive and significant correlations were also found between some analytical measurements and aroma sensory scores (Table 5.28). For example, ***floral*** sensory score (S2) is positively correlated with fructose (A2), the total residual

Table 5.28 Correlation parameters for linear regression analysis between sensory scores and analytical data

Sensory descriptor	Analytical parameter*	Correlation (r)	Probability	Standard error of estimate
Sweetness	Total (G+F)	0.756	0.0007	0.423
Sweetness	Sugar sweetness response	0.746	0.0009	0.430
Confectionary	Total (G+F)	0.594	0.0152	0.632
Confectionary	Sugar sweetness response	0.611	0.0120	0.623
Acidity	pH	-0.829	0.00007	0.265
Acidity	TA	0.563	0.0231	0.392
Acidity	Malic acid	-0.511	0.0430	0.395
Floral	Total (G+F)	0.561	0.0239	0.297
Floral	Sugar sweetness response	0.551	0.0271	0.792
Floral	Glycerol	0.536	0.0322	0.800
Lemon/lime	Glycerol	0.553	0.0264	0.491
Pineapple	Glycerol	0.553	0.0263	0.491
Grassy	Glycerol	0.506	0.0453	0.417
Asparagus	pH	0.572	0.0206	0.191
Asparagus	Malic acid	0.577	0.0194	0.190
Orange marmalade	Volatile acidity	0.508	0.0448	0.677

* Total G+F = sum of the glucose (G) and fructose (F) concentration; Sugar sweetness response: see text for details of calculation

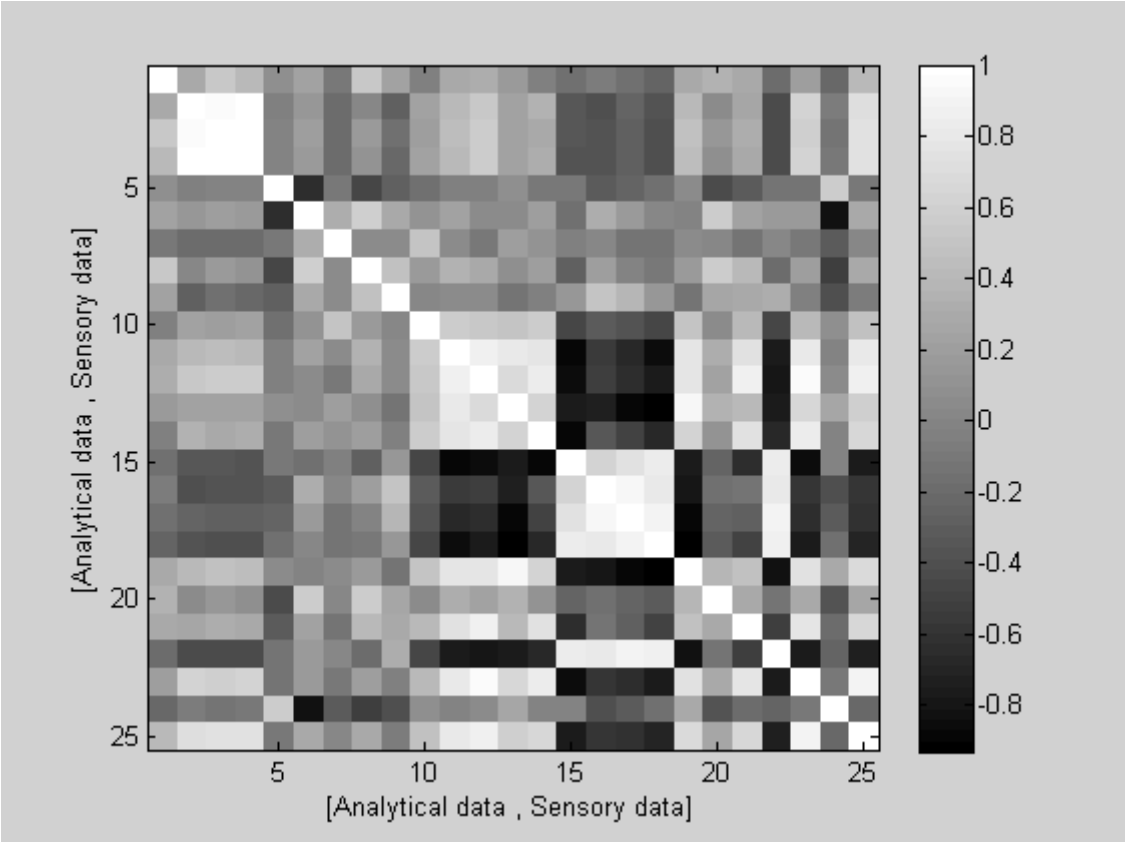


Figure 5.56 Correlations between all pairs of sensory and analytical parameters

sugar score (A3) and sugar sweetness response (A4) as well as with the glycerol concentration (A10). Glycerol also showed a relationship with ***lemon/lime, floral, pineapple*** (S1-S4) and ***grassy*** (S9). There was a positive correlation between ***asparagus*** (S10) with pH (A6) and malic acid (A8) as well as between ***orange marmalade*** (S6) and volatile acidity (A9), a reflection of acetic acid in the main.

This initial linear regression analysis implied that the potential for developing a model that used analytical data as a surrogate for sensory descriptors might be achievable. Several of the sweetness and acidity correlations are very significant when the probability values and standard errors (*Table 5.28*) are considered. Several of the other correlations in *Table 5.28* are also moderately strong, implying that there may be a real relationship. This suggested that an in-depth analysis of the relation among sensory and analytical parameters using the more sophisticated multi-block ComDim analysis would be of value.

Multi-block ComDim analysis of sensory vs. chemical analysis data

Several relations of proximity and opposition can be seen in the projection of the Sensory and Analytical parameters onto the CD1-CD2 plot (*Figure 5.57*). It is clear that there are several regions of related descriptors in the CD1-CD2 plot. The region labelled Sweetness represents the commonality between the mouthfeel sensory parameter of ***sweetness*** (S15), the aroma parameters of ***lemon/lime*** (S1), ***floral*** (S2), ***grapefruit*** (S3), ***pineapple*** (S4), ***grassy*** (S9), ***lychee*** (S11) and ***confectionary*** (S15) and the analytical parameters of fructose (A2) and glycerol (A10) and the calculated parameters of total glucose + fructose (A3) and sugar sweetness (A4). These groupings of descriptors are in general agreement with the linear regression analyses (*Figure 5.56* and *Table 5.28*), but of course the multi-block ComDim data are presented on a single map, rather than calculated individually as in linear regression.

Glucose (A1) is not part of the Sweetness region, but perhaps this is not surprising, given its zero to low concentrations in the wines examined here (*Table 5.26*). Ethanol (A7), which is sometimes considered to show sweetness (Zamora *et al.* 2006), is not part of the Sweetness region. The ethanol concentration in the 16 wines is essentially invariant (*Table 5.26*), so it is reasonable to expect that it would not have any commonality with the common dimensions identified here.

The inclusion of glycerol (A10) in the Sweetness region is intriguing. Glycerol is known to affect various sensory attributes including sweetness, acidity, mouthfeel and viscosity. However, the actual concentration at which these attributes are expressed

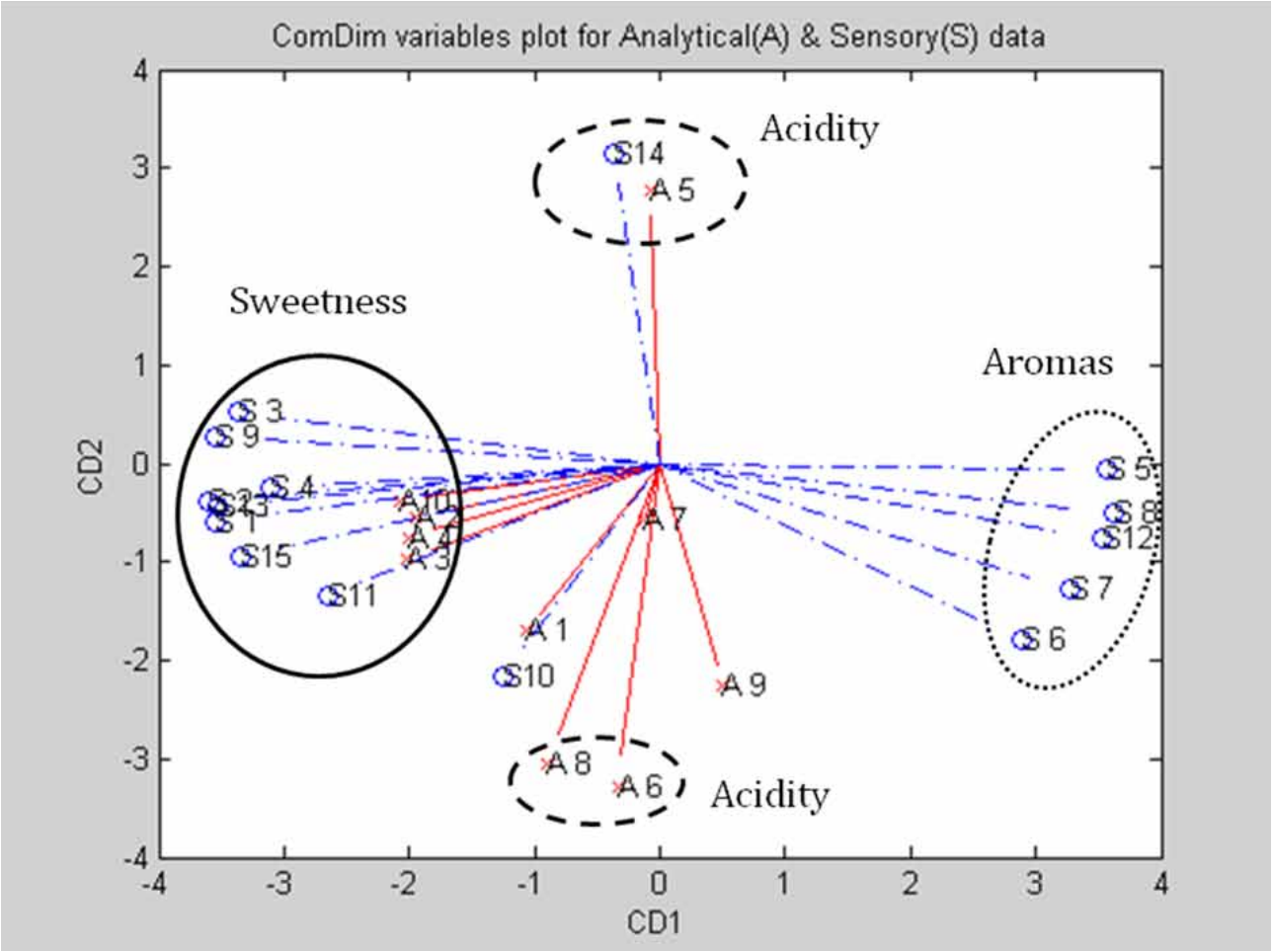


Figure 5.57 Scatter plot of scores for Analytical variables (stars) and Sensory variables (circles) onto Common Dimension 2 vs. Common Dimension 1, calculated by ComDim.

is the subject of considerable debate. Gawel *et al.* (2007) found varying taster responses to different concentrations of glycerol, Noble and Bursick (1984) suggested that additions of 26 g L^{-1} of glycerol were necessary before an increase in viscosity could be perceived, while Nurgel and Pickering (2005) claimed a perceived increase in viscosity could be detected as the glycerol concentration increased from 10 to 25 g L^{-1} in a model wine. Intriguingly, the glycerol concentration in the wine studied here is much less than that for the levels examined by others. Clarification of these conflicting reports is outside the scope of this present work, but the ComDim analysis used here may well have the capacity to provide a better interpretation of published data and lead the way to a better understanding of the sensory aspects of glycerol in wine.

Two opposing relations of Acidity are identified in the ComDim variables plot for CD1 and CD2 (*Figure 5.57*). One zone in the positive CD2 direction contains the sensory attribute ***acidity*** (S14) and the analytical parameter titratable acidity (A5). Titratable acidity is a measure of the amount of acid present in the wine, so the commonality with the sensory acidity attribute is to be expected. The second Acidity zone in the negative CD2 direction contains only the analytical parameters of pH (A6) and malic acid (A8). The placement of pH opposed to sensory acidity is also as expected, given its negative relationship with acid concentration, as noted above with the linear regression analysis. The placement of malic acid is unexpected, but also in accord with the linear regression analysis (*Table 5.28*).

Malic acid is sometimes regarded as 'green', while tartaric acid is considered to be 'hard' (Peynaud 1987). The relative acid taste of these two acids in white wine is still the subject of debate. Early work by Amerine *et al.* (1965) on white wine showed that at the same titratable acidity, malic acid is perceived as more acidic, reflected as an increase in sourness. Relative sourness was also found to be higher for malic acid when the wines were adjusted to the same pH (Amerine *et al.* 1965). Noble *et al.* (1986) focused on a comparison of sourness of organic acid anions at equal pH and equal titratable acidity in binary acid solutions. Only succinic acid (a minor component of wine) was found to be more sour than malic acid. Lugaz *et al.* (2005) in a study of the time-intensity

effects of organic acids on saliva suggested that it is the hydrogen ion that is the stimulus and not the neutral acid molecule or its monoanion.

In the Hunter Valley Semillon wines used here, the pH is generally around 3.2 or less and the malic acid concentration is only a small component of the titratable acidity (*Table 5.26*). The main acid is tartaric acid, a stronger acid than malic acid (pKa1 (tartaric): 2.93; pKa1 (malic): 3.46), suggesting that the hydrogen ion may well be the dominating factor in determining sensory acidity. Clearly more work is required, and the multi-block analysis approach used here may well provide greater insight into the competing effects than has been possible in earlier studies.

The sensory aromas fall into two categories: one group (labelled Aromas in *Figure 5.57*) consists of ***hay/straw*** (S5), ***orange marmalade*** (S6), ***honey*** (S7), ***toast*** (S8) and ***kerosene*** (S12). There is no commonality between these sensory attributes and the analytical measurements used here. These same attributes are in opposition to the sweetness aroma attributes of ***lemon/lime*** (S1), ***floral*** (S2), ***grapefruit*** (S3), ***pineapple*** (S4), ***lychee*** (S11) and ***confectionary*** (S13). ***Lemon/lime*** (S1), ***grapefruit*** (S3) and ***pineapple*** (S4) are orthogonal to the acidity parameters (*Figure 5.57*), despite the strong association of acidity with the taste of these fruits.

Interestingly, the sensory attribute ***asparagus*** (S10) is midway between the sweetness region and the acidity region that is in opposition to titratable acidity (*Figure 5.57*). There is no proximity between ***orange marmalade*** (S6) and volatile acidity (A9) in the CD1-CD2 plot, despite the correlation observed using linear regression (*Table 5.28*). This confirms the weakness of that correlation.

The CD2-CD3 plot (*Figure 5.58*) confirms the strong connection among the analytical sweetness parameters A2, A3, A4. The unexpected proximity of pH (A6), malic acid (A8) and volatile acidity (A9) with Titratable acidity (A5) and ***acidity*** (S14) in opposition, is also confirmed in this plot. ***Honey*** (S7) and ***sweetness*** (S15) are this time in the same quadrant as glucose (A1).

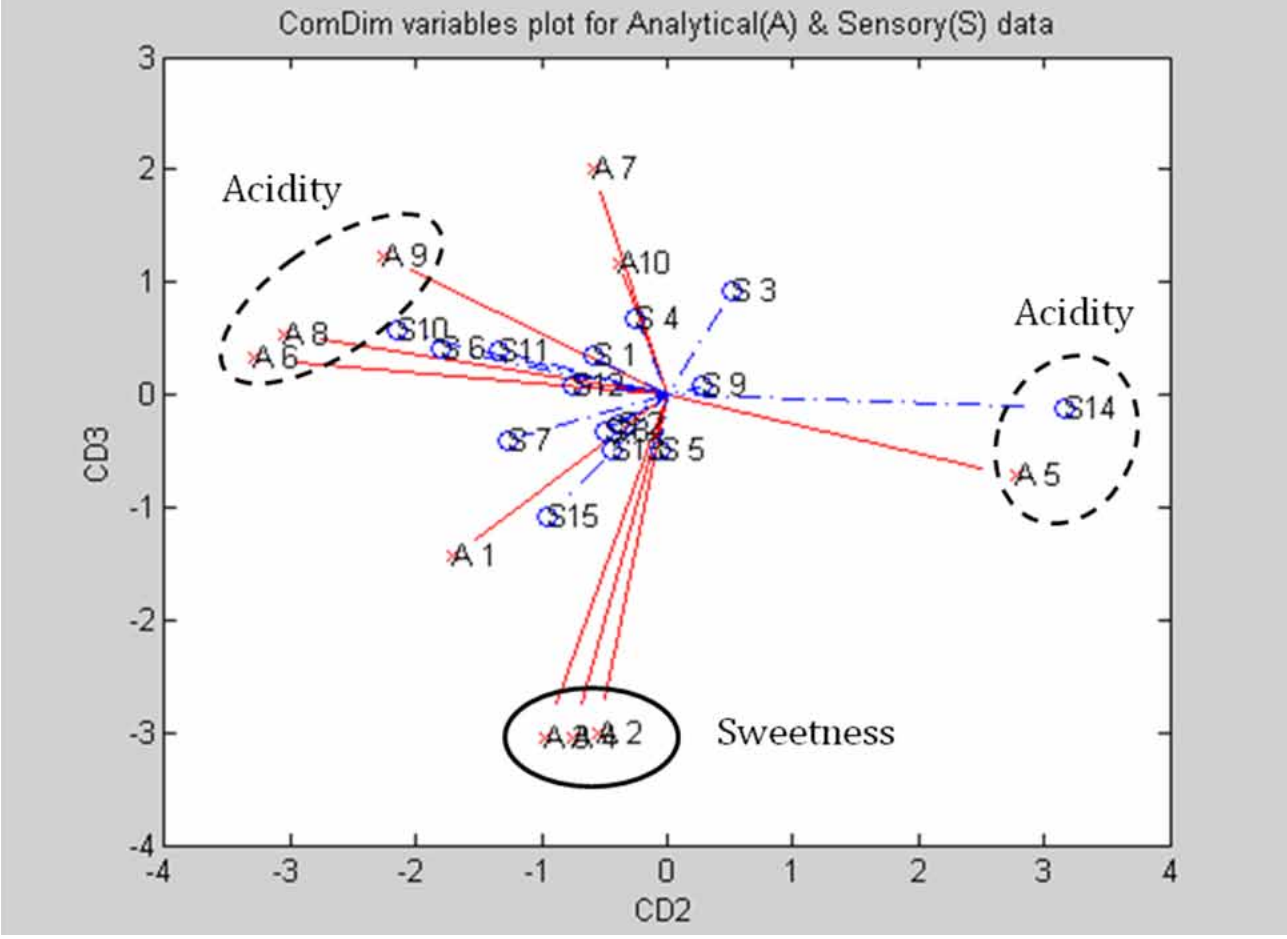


Figure 5.58 Scatter plot of scores for Analytical variables (stars) and Sensory variables (circles) onto Common Dimension 3 vs. Common Dimension 2, calculated by ComDim.

Experiment 5.8
Grape and wine composition in Semillon influenced by vine resources under different environmental conditions

Materials and methods

Grape production and vineyard management aims are commonly to produce a certain quality of grapes or yield level, for this purpose vine balance (vegetative/reproductive) is altered and consequentially the carbon allocation to the parts of the vine.

Carbohydrate source-sink relationships are complex and dynamic, and the allocation of carbohydrates to the different vine parts during the season changes with developmental stage and activity of the various sinks. The stored nutrient and carbohydrate reserves from one season are required to support shoot growth in the following spring (Conradie 1992; Zapata *et al.* 2004). Nitrogen reserves are important because early demand in spring cannot be ensured by root uptake (Cheng *et al.* 2004). Carbohydrate reserves are essential because the canopy is not able to produce sufficient carbohydrate from photosynthesis until around flowering, although it has been suggested that reserve status may still influence fruit-set under adverse weather conditions (Zapata *et al.* 2004).

Vine reserves accumulated during maturation are influenced by crop load (Koblet *et al.* 1996). Similarly, because of the influence on berry ripening, harvest dates are also altered by yield levels. Yield levels may therefore determine the time remaining after harvest for carbon production and nutrient uptake. Although photosynthesis declines after harvest (Scholefield *et al.* 1978) a considerable amount of carbohydrates can be accumulated during the post-harvest period (Williams, 1996). Significant root growth and nitrogen uptake also occurs after harvest in hot irrigated climates (Bates *et al.* 2002; Conradie 2005); meaning that nutrient and carbohydrate reserves in the permanent structure are highest in winter (Conradie 1980; Sommer and Clingeleffer 1996). Reserve levels vary between parts of the vine; roots have been reported to have much higher levels than elsewhere (Uys and Orffer 1983).

This information together with assessment of vine or vineyard carbohydrate reserve status at key times could be an essential tool to assist the vineyard management to achieve the targeted vine balance.

Every management factor influences the performance and size of the sources (canopy, perennial structure) or sinks (vegetative and reproductive growth), ultimately impacts on the amount of reserves stored in a vine. The variety planted and/or the rootstock used will affect the partitioning of the vines in a vineyard. The condition of canopy in relation to crop load is also important and can be altered by vineyard management, since leaf damage, nutrient deficiency and leaf age lowers assimilate production.

Irrigation and nutrition are interactive inputs reflected in the vines ability to produce assimilates for current growth and storage in the perennial structure. The supply of nutrients such as nitrogen (Williams and Smith 1985) and phosphorus are important for the rate of assimilates produced (Skinner and Matthews 1990). N deficiency lowers the carbohydrate demand and indirectly effects photosynthesis (Wermelinger *et al.* 1991). The application of nitrogen affects the source assimilation capacity and the sink activity to utilize the carbon (Xia and Cheng 2004), vigorous roots can divert the assimilated carbon away from the storage sinks to the vegetative structures. In addition, the applied N can lower carbohydrate reserves, by allocating it to nitrogenous compounds (Xia and Cheng 2004). N deficiency might lead to an increase in root growth (Keller and Koblet 1995), nutrient deficiency leads to growth limitation, roots become a stronger sink in relation to the shoots to improve nutrient uptake (Keller 2005). Under limited water and nutrient supply yield is reduced, particularly if this occurs early in the season (Williams and Mathews 1990). Stress occurring after fruit set generally does not affect fruit growth and maturation at the cost of shoot and root growth (Williams and Mathews 1990) and the replenishment of the storage reserves (Smith 2004). Restricted water supply shows more of an impact on nutrient status if less N applied (Wade *et al.* 2004; Holzapfel *et al.* 2008), suggesting that a higher N supply is required to achieve similar photosynthetic rates of fully irrigated vines (Alleweldt *et al.* 1982). The optimum supply of nitrogen and water can also extend the effectiveness of the leaves and therefore extend the growing season (Lawlor 2002). Water and nutrient supply reflect on canopy size and leaf capacity to produce assimilates, the effectiveness of the canopy and its development in a season are also important factors to determine the vine reserves and the dynamics.

The nitrogen status of vines influences the form and concentration of must nitrogen compounds, which ultimately affects fermentation rate via yeast biomass and activity, wine microbial stability and final wine character. Moreover nitrogen level in must also affect the residual nitrogen content in the wine influencing secondary fermentations, like malolactic fermentation and tirage by sparkling wine production.

Nitrogen is the most important nutrient in the must, necessary for correct yeast growth, proper fermentation of the must and the quality of the wine obtained. A value of 500 mg L^{-1} N was reported by Agenbach (1977) as necessary to achieve maximal yeast biomass production. An assimilable amino acid N concentration of 140 mg L^{-1} (Agenbach 1977) and of 150 mg L^{-1} (Spayd *et al.* 1995) assimilable N respectively, are interpreted to be the minimum for complete fermentation.

When vineyard N is low, arginine and proline are the major amino acids in grapes (Sponholz 1991). Arginine and proline are generally present in the highest concentrations, followed by glutamine, alanine, histidine, threonine, not necessarily in this order (Kliewer 1968; Bisson 1991; Hernandez-Orte *et al.* 1999; Spayd and Andersen-Bagge 1996; Treeby *et al.* 1998; Miele *et al.* 2000). Proline usually increased very sharply during the period of rapid sugar accumulation (Kliewer 1968; Coombe and Iland 1986). Arginine also usually increased rapidly during fruit ripening, but after fruit were mature the concentration of arginine decreased (Kliewer 1968). Also Hernandez-Orte *et al.* (1999) reported a decline of the amino acid content before harvest in one season. Stines *et al* (2000) reported that the proline accumulation occurred late in ripening, about four weeks after veraison. In contrast, arginine accumulation began before veraison. Moreover most of the amino acids were found in the pulp compared to skin or seeds by Riesling as well as by Cabernet Sauvignon.

The metabolic preferences of yeasts with respect to N sources have been described and classified in four categories by Jones and Pierce (1964). Class A amino acids (aspartarte, asparagine, arginine, glutamate, glutamine, lysine, serine, threonine) were consumed first and class B amino acids (histidine, isoleucine, leucine, methionine, valine, tyrosine) started to be consumed prior to depletion of the class A compounds. Class C (alanine, ammonium, glycine, phenylalanine, tryptophan) amino acids were not taken up until the medium was depleted of the class A compounds. Class D (proline) amino acid did not appear to be consumed under typical anaerobic conditions of wine fermentations.

Inadequate concentration of utilizable N sources in grape juices and musts resulting in sluggish or stuck (incomplete) fermentation (Monteiro and Bisson 1991; Ingledew and Kunkee 1985).

One of the very important objectives during most winemaking processes is the achievement of complete or "dry" alcoholic fermentation, so that the residual sugar level in the wine after fermentation is less than 4 g L^{-1}. Slow and incomplete alcoholic fermentations are frequent problems for the wine industry. Fermentations leaving higher than desired residual sugar levels are defined as incomplete or stuck fermentations. Slow or sluggish fermentations are characterized by very low yeast viability at the end of fermentation and they require a longer than average time to reach a low residual sugar content (Bell and Henschke 2005).

The main macronutrient most frequently implicated as cause of stuck and sluggish fermentations when present in insufficient quantities is nitrogen in form of amino acids and ammonia (Agenbach 1977; Fleet and Heard 1994; Henschke and Jiranek 1994; Alexandre and Charpentier 1998; Blateyron and Sablayrolles 2001).

The decrease in rate of sugar consumption is correlated with a decrease in sugar uptake capacity (Salmon *et al.* 1993; Bisson 1999). This limitation of fermentation rate is due to a targeted loss of hexose, amino acid and ammonium transport capacity of yeast cells as well as glycolytic enzymes and ATPases, which are all highly nitrogen depended. The sugar transporters maintain high rates of turnover in stationary phase cells (Salmon 1989; Salmon *et al.* 1993; Butzke 1998; Dittrich and Grossmann 2005). Thus a supply of nitrogen must be available to allow continued resynthesis of these proteins. It is well established that increasing N content in the must or addition of ammonium salts improve fermentation kinetics by two aspects. Increasing initial N level increases biomass production and stimulates the rate of sugar utilization and, thus, reduces fermentation lag, increases maximum fermentation rate and decrease fermentation time (Agenbach 1977; Tromp 1984; Monteiro and Bisson 1991; Jiranek *et al.* 1993).

With regard to the nitrogen demand of yeast strains substantial differences were found. Some strains appear to be able to use nitrogen more effectively for

protein syntheses and need, therefore, less nitrogen to complete alcoholic fermentation (Tromp, 1984; Monteiro and Bisson 1992; Manginot *et al*. 1998). It should, therefore, be possible to select strains for optimal fermentation activity under nitrogen limited conditions.

It must be noted that sluggish and stuck fermentations are not exclusively caused by nitrogen deficiency. Factors such as high initial sugar content (Lafon-Lafourcade *et al*. 1979), vitamin deficiency (Ough *et al*. 1989), high ethanol content (Henschke and Jiranek 1994), anaerobic conditions (Alexandre and Charpentier 1998), low pH level of the juice (Kunkee 1991), excessive temperature (Ough 1966), excessive clarification of the must (Groat and Ough 1978), lack of lipids (Belviso *et al*. 2004), presence of toxic fatty acids (Viegas *et al*. 1989), high concentrations of volatile acidity (Alexandre and Charpentier 1998), excessive sulphite contents (Kunkee 1991) and presence of fungicides residues (Feet and Heard 1994) have all been considered to be the cause of fermentation problems.

The work will lead to an improved understanding of the role of carbohydrate and nutrient reserves during grape maturation under different environmental conditions, particularly with regard to fruit and wine composition.

The aim of this project is to clarify the link between vine nutrient reserve, fruit and wine composition in Semillon by investigating the vine resource distribution during grape maturation under different environmental conditions.

Site details and treatments

The field trial was established in a Semillon (TO 90817) vineyard over the growing seasons 2007–08 and 2008–09, located in Griffith (35°19'55"S, 145°59'44"E), NSW (*Figure 5.59*). The vines were planted in 1995 on own roots in heavy clay loam, with a row spacing of 3.6 m and an inter-vine spacing of 1.8 m. The trellis system was a single wire cordon and the drip irrigated vines were spur pruned by hand. Griffith is hot and semi-arid area with a mean daily January temperature of 24.2°C and average annual rainfall of 406 mm.

Nitrogen has been applied in different levels (0 kg N ha^{-1} and 50 kg N ha^{-1}) and at different stages during the growing season (post harvest or at bloom) in form of ammonium nitrate. These N treatments were combined with two different water supply levels, one simulating water stress during the early ripening period, approximately two weeks before and after veraison. Therefore six treatments replicated four times were applied in a fully randomized block design. Each plot consisted of 12 vines, with the first



Figure 5.59 Aerial image of the experimental site in Griffith, NSW

and last vine acting as a buffer. Water status during the water stress period was monitored weekly by predawn water potential measurements.

Table 5.29 Predawn leaf water potential values at the end of the water withholding period. Standard errors of the means are shown in small type.

	Plant water potential (MPa)			
	15 January 2008		14 January 2009	
N 50, bloom, no water stress	-0,23	-0,01	-0,33	-0,01
N 50, bloom, water stress	-0,42	-0,02	-0,51	-0,02
N 50, post harvest, no water stress	-0,20	-0,01	-0,35	-0,01
N 50, post harvest, water stress	-0,53	-0,01	-0,56	-0,03
N 0, no water stress	-0,22	-0,01	-0,31	-0,01
N 0, water stress	-0,44	-0,01	-0,50	-0,01
N 50, bloom	-0,32	-0,02	-0,42	-0,02
N 50, post harvest	-0,37	-0,03	-0,46	-0,02
N 0	-0,33	-0,02	-0,41	-0,02
No water stress	-0,22	0,00	-0,33	-0,01
Water stress	-0,46	-0,01	-0,52	-0,01

Plant measurements and sampling

Root and wood samples were collected throughout the two seasons at five phonological key stages (dormancy, budburst, flowering, veraison, harvest) commencing at dormancy 2007 and ending at dormancy 2009. At each stage five vines out of all 24 plots were sampled. Root samples were manually dug from near the base of each vine ranging between three and seven mm in diameter. Wood samples were collected with a 4.8 mm drill bit to a depth visually estimated as the centre of the cordon and trunk (*Figure 5.60*).

A 100 berry samples out of each plot were collected randomly in weekly intervals during berry ripening starting from veraison, yields as well as yield components were determined at harvest.

Fruit analysis

The berry samples were stored after collection at -20°C for subsequent analysis. The berry samples were weighed and juiced. Total soluble solids (°Brix) were measured using a digital refractometer (ATAGO PR-101, Tokyo, Japan). A Titrando System autotitrator (Metrohm, LMWI 40-15, Herisau, Switzerland) was used to determine pH and titratable acidity.

Nutrient analysis

Twenty petiole samples of each plot were collected to determine the nutrient status at flowering. The samples were oven dried at 70°C and then ground through a 0.12 mm sieve (Retsch ZM200, Retsch GmbH, Haan, Germany). Total N content of the tissues was determined with a total combustion gas chromatograph using a 50 mg sub-sample (Vario Macro, Elementar, Hanau, Germany). The other macro nutrient (P, K, Ca, Mg and S) analysis was undertaken with an inductively coupled plasma optical emission spectrometer (ICP-OES) (ARL 3580B, Applied Research Laboratories, Ecublens, Switzerland) on a 300–400 mg sub-sample.

Carbohydrate analysis

Roots were washed in phosphate free detergent and rinsed with de-ionized water, both wood and root tissues freeze dried). Following the drying processes the samples were ground to 0.12 mm with an ultra-centrifugal mill (Retsch ZM200, Retsch GmbH, Haan, Germany). Non-structural carbohydrate concentrations were determined on a 20 mg sub-sample of each tissue as outlined below. For analysis of non-structural carbohydrates a 20 mg sub-sample



Figure 5.60 Collection of wood and root tissue for carbohydrate analysis

of each tissue above was weighed, and soluble sugars extracted with 80% ethanol at 80°C. The concentration of sugars were determined with enzyme assays (MEGAZYME International Ireland Ltd, Bray, Ireland) and a Konelab Arena 20 XT at 340 nm. After extracting soluble sugars with 80% ethanol the remaining starch in the wood and root samples were solubilised with dimethyl sulphoxide. Starch concentrations were determined by colorimetric assay for released glucose after enzymatic digestion with α-amylase and amylglucosidase (MEGAZYME International Ireland Ltd, Bray, Ireland). The released glucose concentrations were determined at 510 nm using the instrument as above. The concentrations of sugars and starch were used to calculate the total concentration of carbohydrate reserves in the root and wood tissues.

Experimental winemaking

The small lot winemaking facility at the National Wine and Grape Industry Centre in Wagga Wagga was used to prepare wines from the field trial on a 30L scale. Therefore the NWGIC's standard winemaking procedure was used, with the exception of not adding any additional N source for yeasts in form of diammoniumphosphate or GoFerm. The fruit was hand harvested and an equal proportion of fruit from the four replicates in the vineyard was bulked. After pressing and sedimentation the clear racked juice was transferred in stainless steel variable lid tanks in equal 30 liter batches. All ferments were replicated three times and inoculated with DV 10. The juice was fermented at 16°C in a temperature controlled room. Samples will be taken before, during and after fermentation of the must or wine for observations of fermentation and further analysis. Baume and temperature was controlled once a day. Must and wine was analysed using a FOSS WineScan (FOSS, Hillerød, Denmark). Sensory analysis of the experimental wine was performed 12 month after bottling by Griffith winemakers.

Results and discussion

Carbohydrate reserves

The carbohydrate concentrations varied between tissues, seasons and during the season as observed for N. Generally the concentrations were about double in the roots compared in the wood, showing the importance of the roots as a carbohydrate reserve pool. Root carbohydrate concentrations were highest at dormancy during the growing seasons (around 20%). Generally carbohydrate reserves depleted till harvest to about 6% followed by a strong replenishment in the post harvest period (*Figure 5.61*). This pattern is observed for both seasons since resources are mobilized to support berry maturation. Wood carbohydrate concentrations are also highest at dormancy ranging between 12% and 10% dry weight. In the 2007-08 season wood reserves followed the same changes than root reserves being at its lowest at harvest (6.5%). In the following season wood reserves decreased to its lowest value of 7% at flowering indicating carbohydrate requirement for early canopy

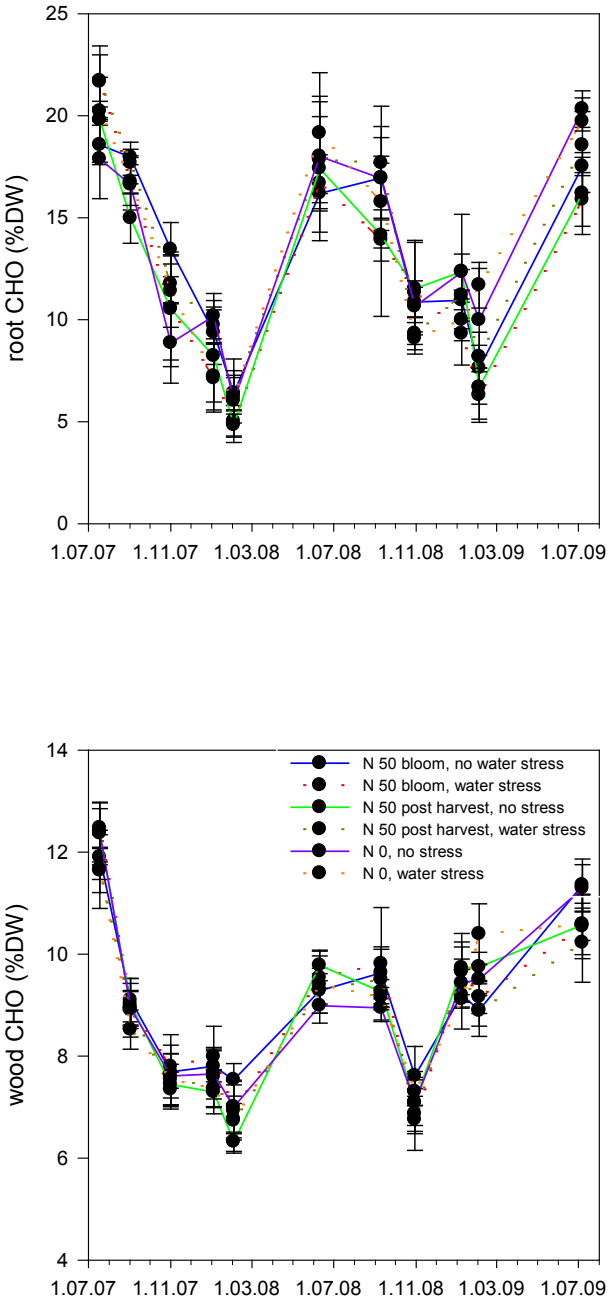


Figure 5.61 Total CHO concentrations in root (top) and wood tissue (bottom) of Semillon grapevines in the seasons 2007/08 and 2008/09 influenced by different vineyard management practices. Standard errors are indicated as bars below and above the mean.

development. Similar seasonal changes and higher root carbohydrate levels in vines compared to wood have been observed in previous studies. Recent work has shown, that altered assimilate production or demand of carbohydrates impacts mostly on the root reserves (Smith and Holzapfel 2009), indicating that carbohydrates in the root tissue is more influenced by assimilate supply than wood tissue. No significant differences between treatments were monitored during the study.

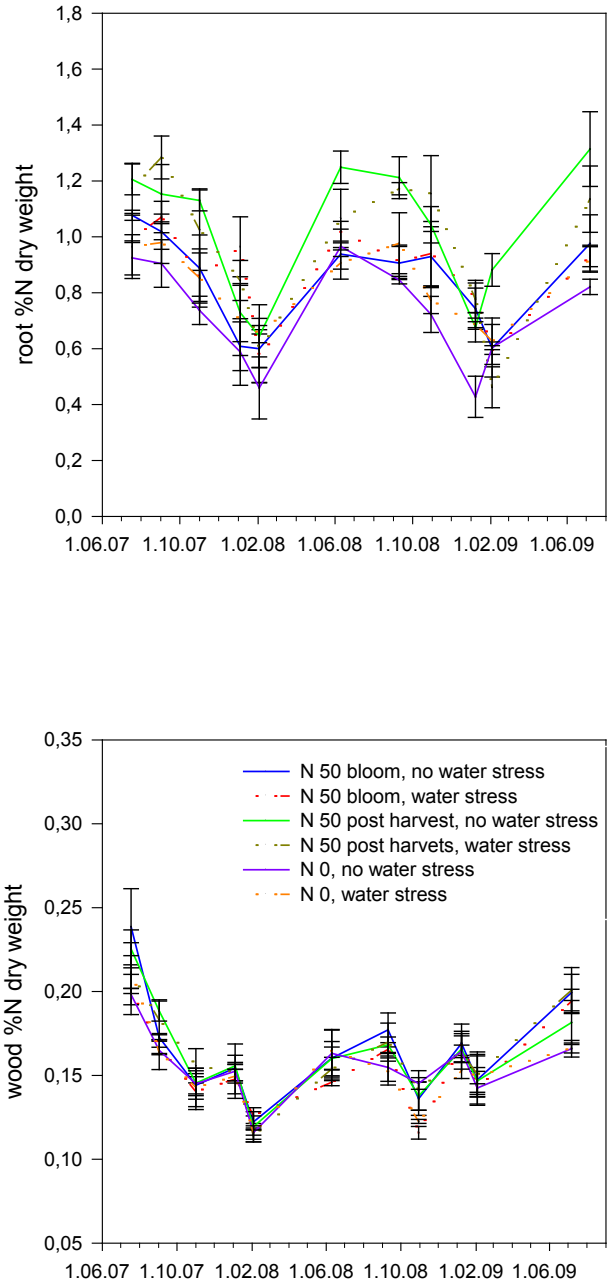


Figure 5.62 Total N concentrations in root (top) and wood tissue (bottom) of Semillon grapevines in the seasons 2007/08 and 2008/09 influenced by different vineyard management practices. Standard errors are indicated as bars below and above the mean.

Total N concentration in roots followed the seasonal changes as descript above with highest values at dormancy and lowest N levels at harvest. The concentration of total N ranges between 1.3% at dormancy and 0.5% at harvest. All treatments receiving nitrogen after harvest had higher N levels in roots at dormancy, showing a greater N recovery in root tissue due to the availability of N and the non existence of a competing sink. Wood tissue N content ranges from 0.23% at dormancy to 0.12% at harvest 2007. The concentration of N in roots was fivefold compared to wood samples emphasizing the importance of the root system as an N storage organ. Like in the wood carbohydrate pattern the lowest level of total N in the 2008-09 season was reported at flowering. No significant treatment results were monitored in wood samples.

Nutrient status

Petiole nutrient levels at flowering are often used as indication of whole plant nutrient status. The post harvest fertilization increased N and Mg levels at flowering of the following season 2007-08. P, K, and Ca were lower compared to N application at bloom or N deficiency (*Table 5.30*).

In the 2008-09 season a similar pattern was observed. N and Mg in petiole tissue was highest for post harvest fertilized vines with lower levels for P, K and Ca. Water stressed vines showed N levels and lower P and K values. Ca and Mg being not affected by any water treatments. N levels in both seasons across all treatments are below the limit of 0.8–1.1% dry weight, which indicates the recommended nutrient status of grapevines (Robinson *et al.* 1997). S was not altered in any of the two seasons by any of the treatments.

Berry and harvest parameters

Berry weight increases during the ripening period 2008 with the exception at the 15 January (*Figure 5.63*). At this date the berry weights across all treatments decline. All treatments sharply increase in berry weight and at the end of berry maturation the weight increase slows down to reach its peak at harvest. Water stress two weeks before and after veraison reduced significantly berry weights. During the ripening period in 2009 berry weight of all treatments increases rapidly after veraison to a maximum value shortly before harvest. Although the berry weight development of all treatments follows the same pattern the mean weight differs upon the water availability around the start of berry ripening. Like

Table 5.30 Nutrient concentration of petiole tissue at flowering of the seasons 2007-08 and 2008-09. Standard errors of the means are shown in small type.

	Tissue concentration (%DW)											
	N		P		K		Ca		Mg		S	
2007-08												
N 50 bloom, no water stress	0,691	0,017	0,405	0,031	2,725	0,085	1,248	0,056	0,485	0,019	0,240	0,011
N 50 bloom, water stress	0,627	0,013	0,395	0,022	2,800	0,178	1,303	0,047	0,520	0,021	0,238	0,005
N 50 post harvest, no water stress	0,723	0,007	0,383	0,036	2,550	0,096	1,253	0,058	0,540	0,027	0,233	0,005
N 50 post harvest, water stress	0,695	0,022	0,363	0,011	2,350	0,119	1,133	0,068	0,515	0,018	0,235	0,012
N 0, no water stress	0,680	0,023	0,418	0,036	2,600	0,135	1,335	0,055	0,483	0,005	0,258	0,012
N 0, water stress	0,661	0,019	0,400	0,023	2,950	0,166	1,348	0,034	0,483	0,017	0,238	0,014
N 50 bloom	0,659	0,016	0,400	0,018	2,763	0,092	1,275	0,035	0,503	0,015	0,239	0,005
N 50 post harvest	0,709	0,012	0,373	0,018	2,450	0,080	1,193	0,047	0,528	0,016	0,234	0,006
N 0	0,671	0,014	0,409	0,020	2,775	0,119	1,341	0,030	0,483	0,008	0,248	0,009
No water stress	0,689	0,010	0,401	0,018	2,706	0,060	1,296	0,032	0,498	0,013	0,242	0,006
Water stress	0,661	0,013	0,386	0,011	2,700	0,112	1,261	0,039	0,506	0,011	0,237	0,006
2008-09												
N 50 bloom, no water stress	0,587	0,017	0,560	0,018	3,925	0,189	1,038	0,054	0,413	0,023	0,165	0,010
N 50 bloom, water stress	0,618	0,014	0,545	0,003	3,500	0,242	1,085	0,006	0,443	0,028	0,147	0,008
N 50 post harvest, no water stress	0,652	0,020	0,538	0,017	3,525	0,111	1,020	0,033	0,438	0,015	0,160	0,003
N 50 post harvest, water stress	0,680	0,035	0,518	0,013	3,750	0,247	1,065	0,032	0,460	0,015	0,154	0,006
N 0, no water stress	0,587	0,013	0,578	0,009	4,125	0,111	1,163	0,034	0,410	0,016	0,171	0,007
N 0, water stress	0,569	0,019	0,568	0,015	4,000	0,187	1,083	0,025	0,403	0,021	0,157	0,005
N 50 bloom	0,603	0,012	0,553	0,009	3,713	0,163	1,061	0,027	0,428	0,018	0,156	0,007
N 50 post harvest	0,666	0,019	0,528	0,010	3,638	0,132	1,043	0,023	0,449	0,011	0,157	0,003
N 0	0,578	0,011	0,573	0,008	4,063	0,103	1,123	0,025	0,406	0,012	0,164	0,005
No water stress	0,599	0,013	0,561	0,009	3,894	0,105	1,076	0,029	0,416	0,010	0,163	0,004
Water stress	0,622	0,019	0,543	0,009	3,750	0,133	1,078	0,013	0,435	0,014	0,153	0,004

in 2008 berries from non water-stressed treatments show higher berry weights.

The development of sugar accumulation followed similar patterns in both growing seasons (*Figure 5.64*). A steady increase of total soluble solids was observed from version to harvest. Water stress generally increases sugar accumulation during ripening. However interactive effects of N fertilization at bloom combined with water stress around veraison resulted in decreased sugar levels in berries at harvest. This could be observed to a lesser extent in 2009. Also in the second season an increased sugar accumulation was noticed for the N deficient vines without water stress.

Titratable acidity decreased in both season from the start of berry ripening till harvest for all treatments (*Figure 5.65*). In 2008 water stressed grapes started at a lower acid concentration, but at harvest no significant differences were observed. Simultaneously pH values increased during berry maturation. At harvest no significances were detected between all treatments in both seasons (data not shown).

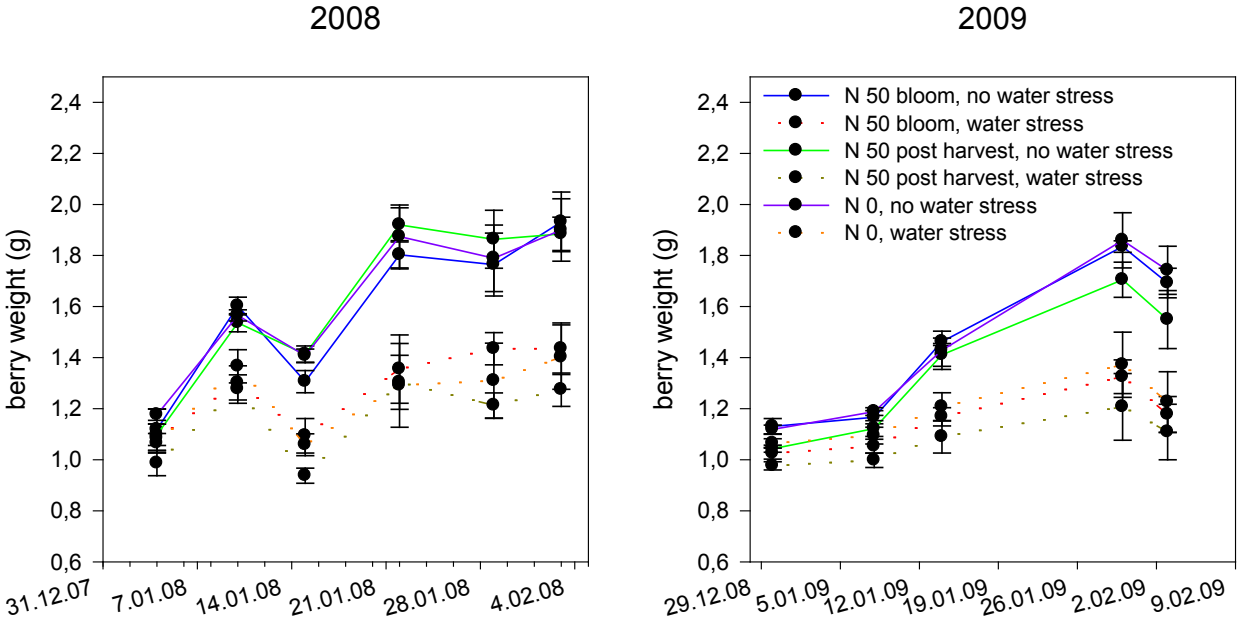


Figure 5.63 The Impact of different nitrogen and irrigation strategies on berry weight during berry maturation. Standard errors are indicated as bars below and above the mean.

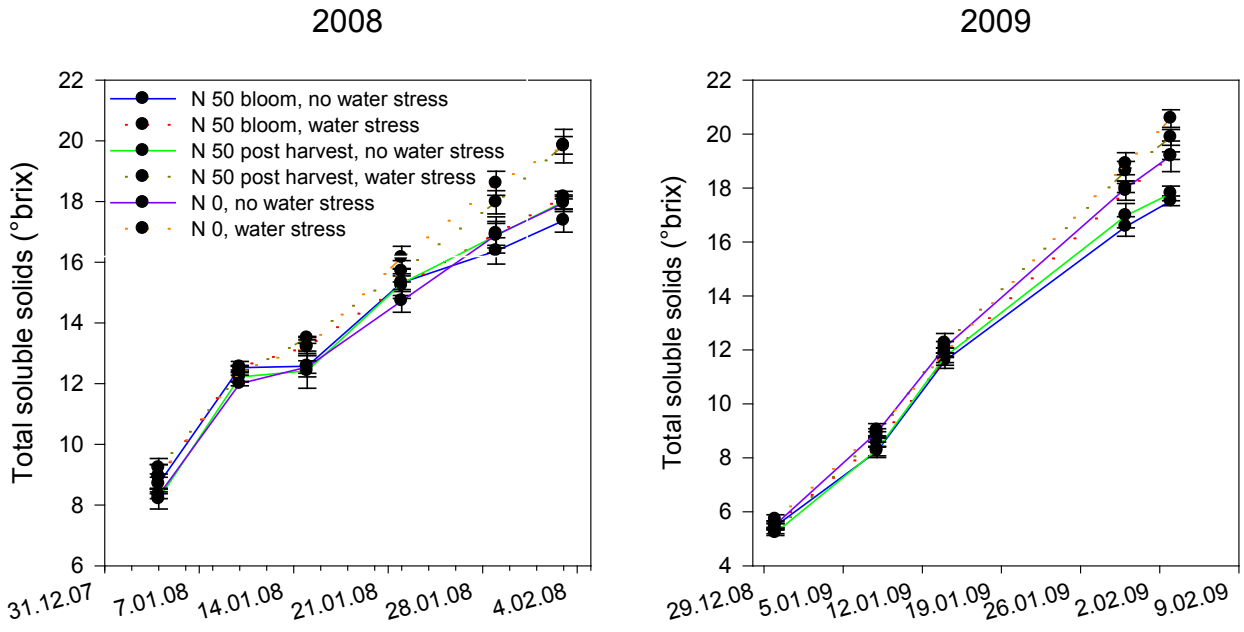


Figure 5.64 Effects of different nitrogen and irrigation strategies on sugar accumulation during berry maturation. Standard errors are indicated as bars below and above the mean.

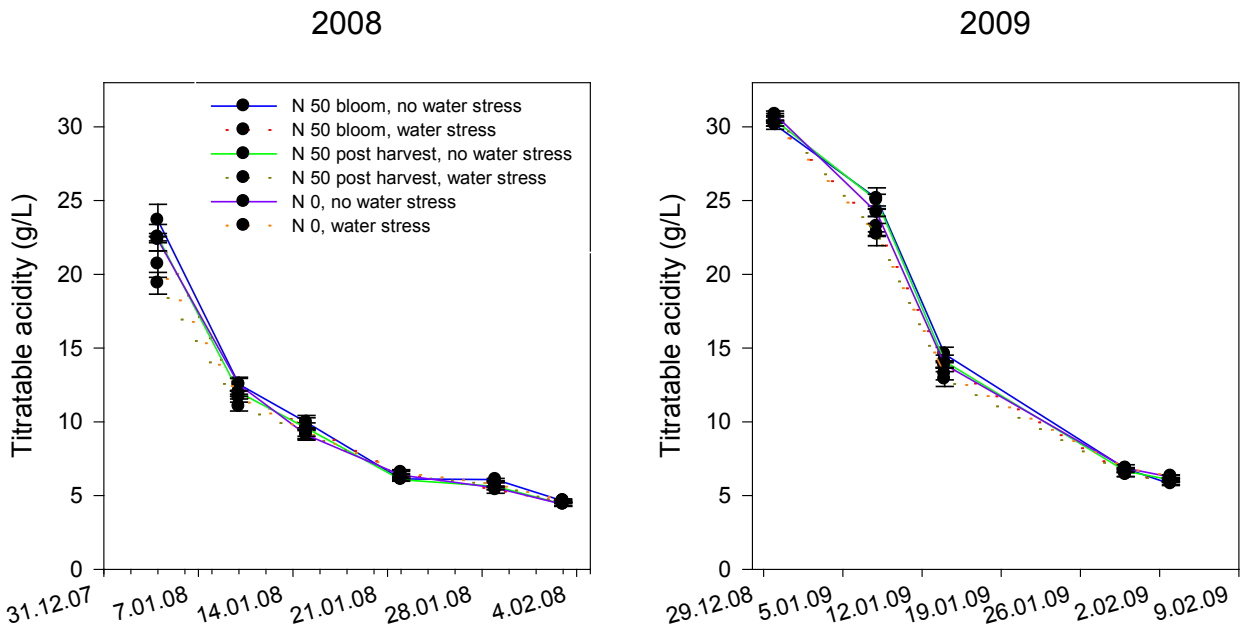


Figure 5.65 The Impact of different nitrogen and irrigation strategies on titratable acidity during berry maturation. Standard errors are indicated as bars below and above the mean.

Yield was considerately influenced by water stress at the beginning of berry ripening (*Table 5.31*). Due to higher bunch weights yield was increased in both season for fully irrigated treatments. Nitrogen applied at bloom also increased bunch weights and therefore yield. The combined treatment of nitrogen applied at bloom without water stress resulted highest yields in both seasons. In 2008 N deficient vines yielded slightly higher than post harvest fertilised vines. As the N deficiency progressed in the second season yield further declined and were lower than the post harvest fertilsed vines. Bunch numbers were not influenced by any of the treatments in either the 2007-08 season nor in the 2008-09 season.

Vinification process

Nitrogen application increased both α amino N and ammonia content and therefore yeast available nitrogen (YAN) in both seasons (*Table 5.32*). Both season confirmed highest YAN levels of must fertilized in bloom, followed from grapes receiving nitrogen after harvest. YAN levels of the nitrogen deficient musts were low in both years compared to Agenbach (1977) and Spayd *et al* (1995) minimum values of 140 and 150 mg L^{-1} yeast assimilable N, therefore increasing the risk of complications during fermentation.

In 2008 α amino N values were slightly higher than ammonia N levels. Water deficit around version had no influence on N levels at harvest. In contrast water stress increased YAN levels, particular α amino N in 2009. Also the relation from ammonia to α amino N shifted towards a higher α amino N level in this season.

The N deficient must lead to a lag phase and slower fermentation rates in 2008 and to a lesser extent in 2009 (*Figure 5.66*). Both N stressed treatments started fermenting 4 days later in 2008. The following fermentation phase is similar across all treatments and characterized by rapid sugar metabolisation. However, the N deficient batches slowed down at the end of fermentation and finished seven to eight days later than N fertilized treatments.

In 2009 the lag phase for N deficient musts lasted only for two days. These batches fermented slower than all the other treatments resulting in a four day longer fermentation time. Musts received N at bloom fermented quickest in 2009.

Wine evaluation showed preferences for wines of the 2008 vintage made from water stressed grapes. Sensory analysis indicates no difference of nitrogen treatments for this vintage. In contrast, the wines made from grapes coming from vines, which did not receive an N application for two years, were preferred for the 2009 vintage. Water stress before and after veraison did not influence the preferences of the 2009 wines (data not shown).

Table 5.31 Comparison of yield components at harvest for the seasons 2007-08 and 2008-09 influenced by different vineyard practices. Standard errors of the means are shown in small type

	Number of bunches		Bunches per vine		Yield per vine (kg)		Yield (t ha^{-1})		Ave bunch weight (g)	
2007-08										
N 50 bloom, no water stress	553	58,57	138,3	14,64	27,60	1,81	42,59	2,80	203,68	15,64
N 50 bloom, water stress	602	34,18	150,4	8,55	20,30	2,31	31,32	3,56	136,64	17,26
N 50 post harvest, no water stress	569	47,52	142,1	11,88	27,27	2,70	42,09	4,17	191,42	6,19
N 50 post harvest, water stress	636	30,30	159,0	7,57	18,83	1,01	29,05	1,56	119,08	7,92
N 0, no water stress	555	32,51	138,6	1,91	26,32	0,36	31,32	0,55	189,89	1,08
N 0, water stress	585	42,70	146,3	8,13	19,32	1,94	33,04	2,99	131,90	9,72
N 50 bloom	577	32,70	144,3	8,18	23,95	1,94	36,96	2,99	170,16	16,64
N 50 post harvest	602	29,04	150,6	7,26	23,05	2,08	35,57	3,21	155,25	14,44
N 0	570	16,50	142,4	4,12	22,82	1,61	35,22	2,48	160,90	11,86
No water stress	559	22,95	139,7	5,74	27,06	1,00	41,77	1,54	195,00	5,41
Water stress	608	18,08	151,9	4,52	19,48	0,98	30,06	1,51	129,21	6,81
2008-09										
N 50 bloom, no water stress	648	29,33	161,9	7,33	24,08	1,10	37,16	1,70	149,50	8,47
N 50 bloom, water stress	620	47,14	155,0	11,78	15,58	2,11	24,04	3,25	99,37	6,71
N 50 post harvest, no water stress	650	49,94	162,6	12,48	22,43	2,06	34,62	3,18	140,14	15,92
N 50 post harvest, water stress	650	41,57	162,4	10,39	13,88	1,53	21,42	2,36	86,10	9,27
N 0, no water stress	663	39,50	165,8	8,24	22,00	1,01	24,04	1,55	132,92	3,46
N 0, water stress	650	31,60	162,6	9,88	13,95	1,03	23,99	1,59	86,67	8,33
N 50 bloom	634	26,22	158,4	6,56	19,83	1,95	30,60	3,01	124,43	10,71
N 50 post harvest	650	30,08	162,5	7,52	18,16	2,01	28,02	3,09	113,12	13,31
N 0	657	23,94	164,2	5,98	17,97	1,66	27,74	2,56	109,80	9,69
No water stress	654	20,19	163,4	5,05	22,84	0,81	35,24	1,26	140,85	5,90
Water stress	640	22,78	160,0	5,70	14,47	0,88	22,33	1,35	90,71	4,65

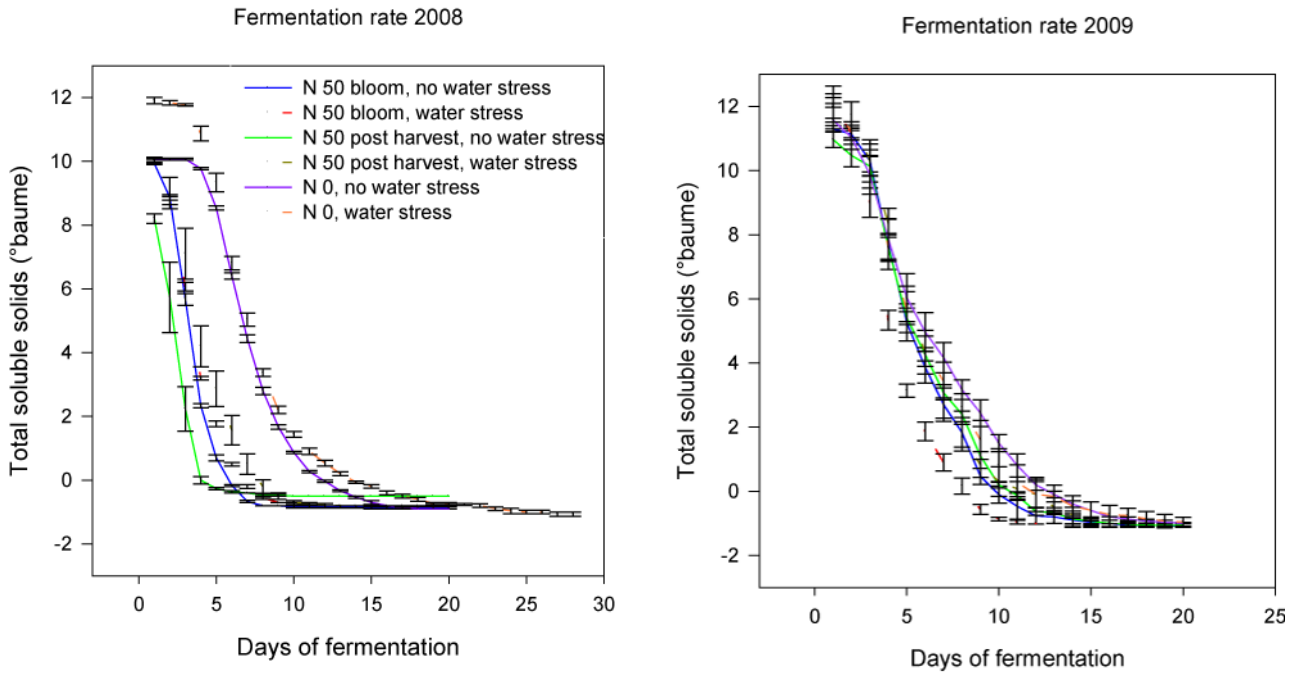


Figure 5.66 Fermentation rates of experimental wines influenced by irrigation and nitrogen management in 2008 and 2009. Standard errors are indicated as bars below and above the mean.

Table 5.32 Effects on must of different nitrogen and water management on yeast available nitrogen. Standard errors of the means are shown in small type

	α-Amino-N (mg L^{-1})	Ammonia (mg L^{-1})	YAN (mg L^{-1})
2007-08			
N 50 bloom, no water stress	110	103	213
N 50 bloom, water stress	112	105	217
N 50 post harvest, no water stress	99	91	190
N 50 post harvest, water stress	98	82	180
N 0, no water stress	97	80	177
N 0, water stress	96	77	173
N 50 bloom	111	104	215
N 50 post harvest	99.5	86.5	185
N 0	96.5	82.5	175
No water stress	102	91.3	193.3
Water stress	102	88	190
2008-09			
N 50 bloom, no water stress	121	77	198
N 50 bloom, water stress	141	75	216
N 50 post harvest, no water stress	109	67	177
N 50 post harvest, water stress	128	65	193
N 0, no water stress	97	48	145
N 0, water stress	120	57	177
N 50 bloom	131	76	207
N 50 post harvest	118.5	66	185
N 0	108.5	52.5	161
No water stress	109	64	173.3
Water stress	129.7	65.7	195.3

Experiment 5.9
Consumer wine show days and what they offer: Do the right consumers attend?

Some Australian wine businesses have known the benefit of using a consumer led product development approach to wine making for some years. Consumer preference mapping is one technique that helps wine makers understand which sensory attributes drive consumer preferences. Although the benefits of such an approach are well known (Costa and Jongen 2006), the cost involved in consumer preference testing (Resurreccion 1998) which is the underlying method of a preference mapping technique means that not all wine businesses can afford to incorporate this method into their production process. In Australia a unique opportunity to make consumer preference testing readily available to wine makers presents itself in the form of the consumer public tasting days associated with wine shows; for brevity we'll call them 'public tastings' and 'wine shows' will refer to the whole show event including the expert judging. The history of Australian wine shows has been documented (Dunphy and Lockshin 1998*c*), and the two important roles of these events is appreciated within the wine industry; first they provide a forum to educate wine makers on technical trends in wine making to improve quality and second, the medals awarded to outstanding wines play a marketing role (Dunphy and Lockshin 1998*b*). Medalled wines are thus believed to have higher saleability in competitive wine markets. The public tasting events however, are not well understood within the wine industry and not much is known about their purpose. Some wine show organisers consider these events a good way to utilise excess wines after the judging event while others consider it a good way to educate wine consumers and to promote wines from particular regions. We suggest that public tasting events could become an important avenue to provide wine exhibitors with useful information about consumers' wine preferences and we have been exploring suitable methods to obtain that information.

What are wine show public tastings?

Public tastings, like wine festivals, are open to the public. The major point of difference between these two events is that the wines available at public tastings have been judged by experts before being exhibited to consumers. No pre-judging occurs with wine exhibited at wine festivals. There is currently no formalised structure on how public tastings should be organised; thus giving rise to variations between different shows. Below is an overview of the Australian wine show system and how the public tasting days fit into it.

Australian Wine Show and Consumer Tasting Days

The Australian wine show system has been in existence since the 1800s although not in its current form (Dunphy and Lockshin 1998*c*). Over time it has undergone various changes and is constantly being modernised to embrace challenges and changes in the industry. The show system has played an important role in raising the quality standards and image of Australian wines in both domestic and international markets (Dunphy and Lockshin 1998*a*). They have also been useful for tracking and developing emerging or evolving styles from the winemakers' perspective (Dunphy and Lockshin 1998*a*). The two main functions of the show system that have been identified are a technical function aimed at assisting the winemaker to reduce technical faults and defects and a commercial function involving consumers and marketing of the wines using the medal system (Dunphy and Lockshin 1998*a*).

There are several types of wine shows in Australia. Although no literature is currently available on the types or classification rules that exist for the different shows, a sort of hierarchal system exists with the National Show in Canberra being recognised as the premier wine show. Most wine makers aspire to be able to exhibit at this show (White 2010, e-mail communication). Exhibitors at this show however are only able to exhibit their wines if they have won medals previously at other recognised shows. It is worth noting that there is no public tasting day organised as part of this show.

Recognition of a wine show is generally based on the location of the show, thus there may be 'Big City' shows (e.g. Sydney, Melbourne and Perth shows); State shows (e.g. Victorian wines show and New South Wales wine shows); or Regional (e.g. Barrosa Valley, Yarra Valley, Rutherglen, Daylesford, Mudgee wine shows). Other smaller shows are also possible. Wine shows may further be recognised based on the type of grape variety used to make the wines (i.e. varietal shows) e.g. Canberra Riesling Challenge, International Riesling Challenge, The Great Australian Shiraz Show and The Alternative Varieties wine show. The volume of wine produced is another way to identify particular wine shows; e.g. NSW Small Wine Makers show. In

addition to these examples of wine show classification, individual event organisers may organise wine shows which are not restricted to locality, variety or volume of production as mentioned before. Such shows are open to all wineries. For instance the Le Concours des Vins du Victoria in Melbourne is organised by French Australian Chambers of Commerce and Industry (FACCI). It is a collaborative show organised between the French Embassy and the Australian Government to promote commerce between the two countries and open to all wineries. It is important to note that not all wine shows include consumer public tasting days. Majority of the shows however have an exhibitors' tasting day to encourage interaction between wine makers and expert judges.

Exhibitor tasting days illustrate one of the two main roles of wine shows in Australia; a technical role in educating the wine maker on product quality from the experts' view, the second is a marketing role through the advertisement of medal winning wines (Dunphy and Lockshin 1998*a*). Both roles are laudable but can be fine-tuned to become even more successful should a system that includes consumer opinion on quality be encouraged.

A significant feature of wine shows in their existing format is the inability to consistently identify and promote wine styles that consumers are certain to find appealing. This deficiency may be attributable to the total reliance on expert judges and little involvement of consumer opinion at these shows.

The judging process at wine shows require experts to identify wines that typify particular styles similar to a quality grading system. There is literature available on the standards that guide judges in this task (ASVO 2001). Deviation from a typical wine style is therefore penalised with low scores and such wines do not win medals. The argument is that amongst the non-medal winning wines there may be new wine flavours acceptable to consumers but not experts.

At some wine shows however, the chief judge may influence the promulgation of particular styles by suggesting that these styles be encouraged with high scores to win medals (Saliba and Blackman 2010, verbal communication). In judging circles this is often regarded as biasing the results hence this practice is not encouraged. Also, the use of expert judges to identify wines preferred by consumers may have unfavourable repercussions; it is known that from a sensory point of view, not all wines that are considered quality wines by experts are acceptable to consumers (Johnson and Bastian 2007; Quester and Smart 1998). Purchase decision and customer loyalty are influenced by taste, such that exposing consumers to wines that they find unpalatable is not a sensible practice (Lockshin *et al*. 2006; Johnson and Bastian 2007; Jaeger *et al*. 2009).

Wine makers will benefit from consumer feedback in addition to the technical feedback already received from expert judges at wine shows. Such information will be valuable in the product development process and will ensure that wines will appeal to consumers, as well as experts.

There is scant information in the literature on the purpose of the consumer or public tasting days included in some wine shows in Australia. The norm at these tasting events is that, award winning wines are displayed after the expert judging event and for a small fee the general public are allowed to come and taste as much of the displayed wines as they wish. In addition to displaying award winning wines, at some shows all wines entered are made available for consumers to taste. The organisation of the event as described above suggests the purpose of the experience is to educate the consumer to appreciate award winning wines. It is debatable though whether this approach is effective as most consumers already have preconceived ideas of likes and dislikes; further, the period of exposure to the wines is short and not likely to change robust opinions.

How can the public tasting benefit the wine industry?

As indicated, given the high cost involved in carrying out consumer product testing, a suggested avenue would be to tap into existing public tasting events as a way to obtain consumer preference information on wines exhibited at the wine shows. In order to do this however, a validated method is required. It is important that the right consumer is used for a preference test so that the results may reflect the general consumer population (McDermott 1990).

For example, it has been suggested that wine festivals could be a good way to tap into future target markets and attract new consumers to be interested in wine (Yuan *et al*. 2005). It has been found that wine festivals do attract a mix of consumers including the demographic group who eventually go on to purchase wine (Houghton 2008). However is not known if the same demographic groups that attend wine festivals also attend public tastings. Perhaps consumers at public tastings could be the right people to provide

meaningful consumer insight into wines exhibited at wine shows, giving wine exhibitors a little more information about their wines. On the other hand, there is the possibility that only highly experienced consumers attend public tastings making them unrepresentative of the general wine drinking population. This in itself may not be a problem. But a key question is who attends wine show public tasting events? We discuss the data we collected to answer this question in the next section.

Hitherto, no studies had been carried out in Australia, to determine if consumers who attend wine show public were representative of the general wine drinking population in Australia and therefore suitable to provide wine preference information at the public tasting days. In the 1950s, some researchers in the United States collected wine preference information from consumers attending a fair in California 1950s quite successfully (Berg *et al.* 1955). The first part of their study was a survey to determine the demographics of consumers within the area to determine if consumers attending the fair would be a representative sample of this demographic. This further supports the need to determine if consumers who attend wine shows constitute the right demographics of Australian wine drinking consumers.

Material and methods

Consumers who attended 10 different wine shows across Australia were identified and characterized using a written questionnaire. A total of 610 consumers took part in this questionnaire. The wine shows that participated in the study were geographically distributed as follows: six shows in Victoria; two shows in New South Wales; one show in Queensland; and one show in Tasmania. At least 30 consumers participated in the survey at each location (*Figure 5.67*). The percentage participation in the survey at each location was between 12% and 82%.

Results and discussion

Gender

Overall there were approximately equal proportions of men and women who took part in the survey across all 10 wine shows. At some shows however slight differences between gender proportions were noted. For example at location L2 in Cairns, Queensland, there were more women than men while the opposite was true at L7 in Hobart, Tasmania (*Figure 5.68*)

Age categories

The age demographic was normal distributed with majority of respondents falling within the 25–64 years category (*Figure 5.69*). Slight differences were noted on a location basis. For example, the highest percentage of 18–24 year olds was found at L6 in

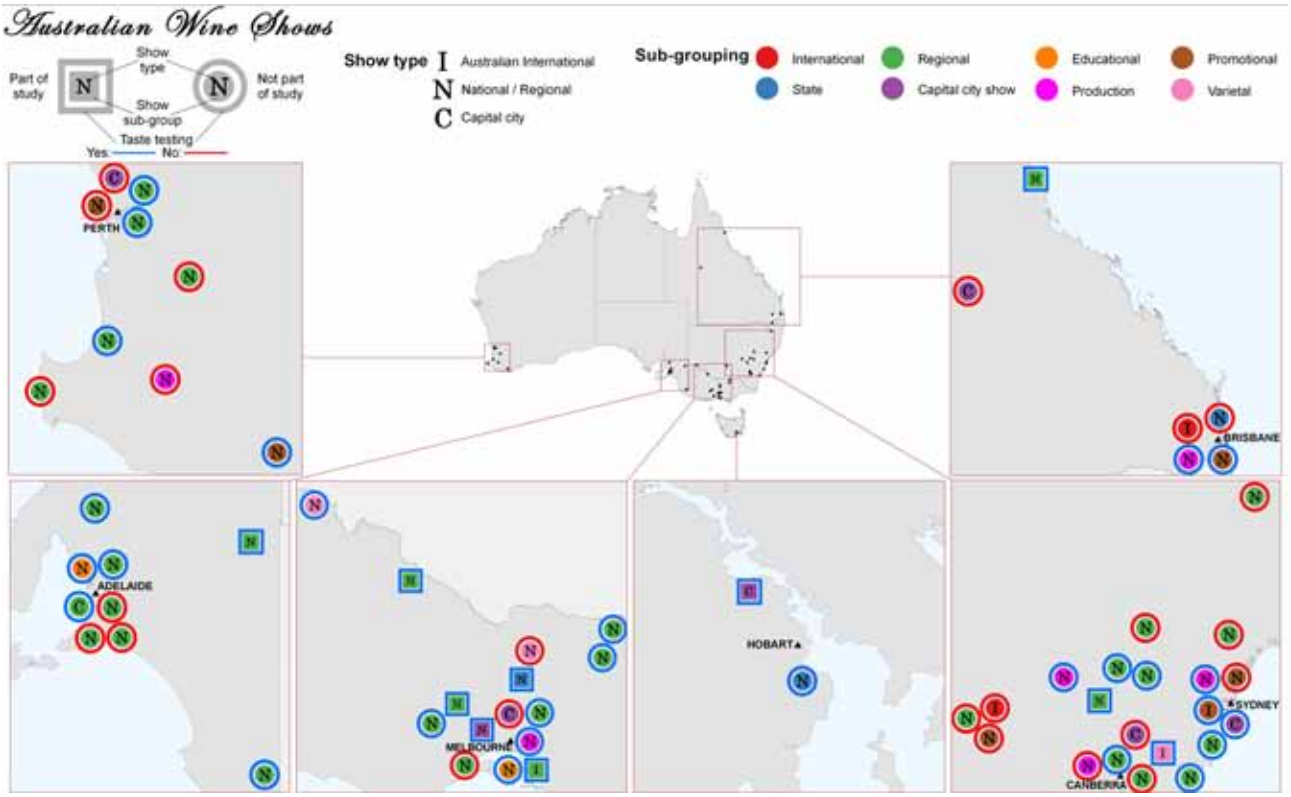


Figure 5.67 Wine show locations across Australia.

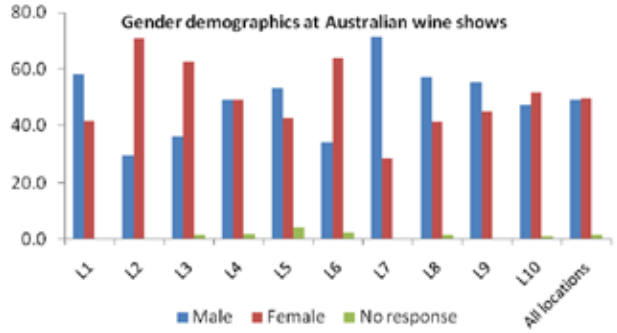


Figure 5.68 Gender demographics at Australian wine show public tastings in 2010.

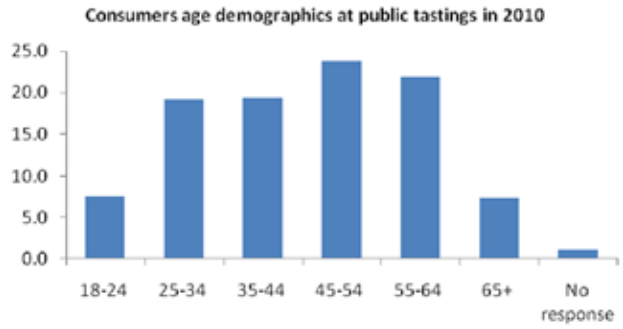


Figure 5.69 Consumers age demographics at Australian wine show public tastings in 2010.

Bathurst, NSW but none of this age group was found at L9 in Daylesford, VIC (figure not shown here).

Household income

About 60% of respondents had an estimated house hold income lower than AU$100,000 while 25% had a household income between AU$100,001 and AU$200,000 (*Figure 5.70*).

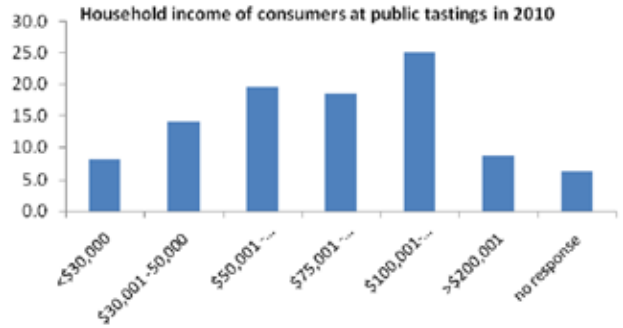


Figure 5.70 Average household income of consumers at Australian wine show public tastings in 2010.

Highest education level and occupation type

The highest education level for most respondents was a Bachelors degree with most respondents employed as professionals (*Figures 5.71* and *5.72*). A small percentage of students and retired people were also present. Most of the students came from L6 in Bathurst, which also had the highest percentage of 18–24 year olds.

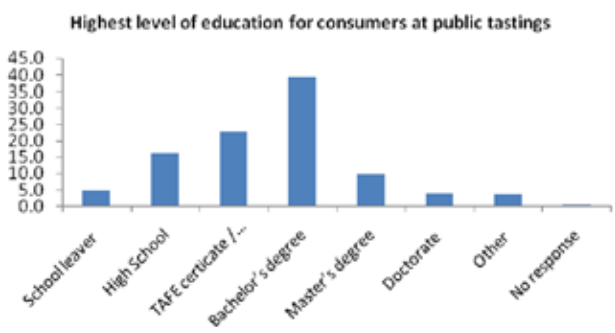


Figure 5.71 Highest Education level for consumers at Australian wine show public tastings in 2010.

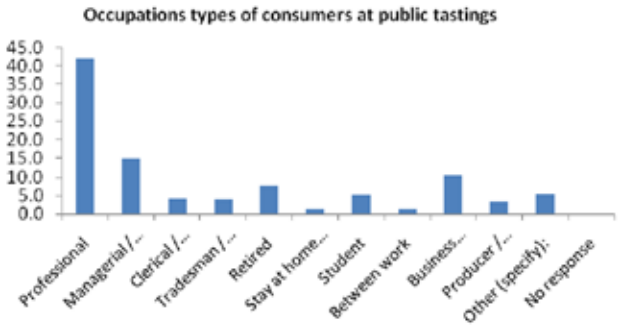


Figure 5.72 Occupation types of consumers at Australian wine show public tastings in 2010

Wine involvement

Consumer involvement with the wine industry may influence their level of expertise with wine. A highly involved consumer may be too expert to qualify as a general wine consumer. This is another way to characterize consumers at wine shows to determine their suitability as representative of the general wine drinking population. Based on their involvement with the wine industry, two main types of consumer attendees were identified; affiliated consumer and unaffiliated consumer. The unaffiliated consumer had no involvement with the industry while the affiliated consumer was involved in the wine industry as: winemaker, grape grower, wine student, wine writer, wine trade person or in other ways such as hospitality, tourism, education or research.

Overall more unaffiliated consumers attended the public tastings days than affiliated consumers i.e. 66:33%. On a location basis, significant differences were noted as shown in *Figure 5.73*. At locations where this happened, the exhibitor and public tasting were held on the same day but at different times. There is a chance that some exhibitors were included in the survey.

Enthusiasm, knowledge and years of drinking [wine]

Some researchers have used knowledge of wine (Dodd *et al.* 2005), interest in wine and the number

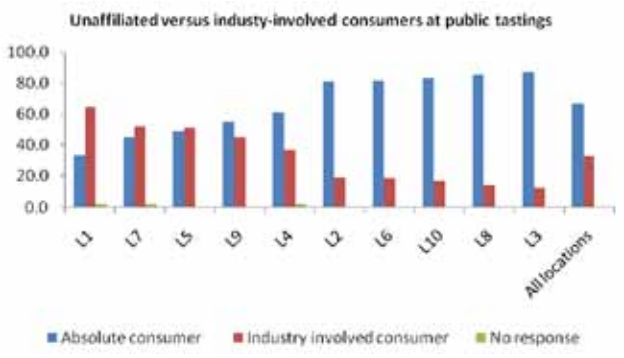


Figure 5.73 Percentage of unaffiliated and affiliated consumers at Australian public tastings.

of years of drinking wine as indices to segment wine drinkers into experienced or novice consumers (Blackman *et al.* 2010). In our research, enthusiasm rather than interest was measured.

Overall, consumers were quite enthusiastic (5.7) about wine and moderately knowledgeable (4.1) about wine. Both enthusiasm and knowledge for wine were self rated by respondents on a 7-point scale where 1 meant 'not at all' and 7 meant 'very much'. Enthusiasm for wine and knowledge of wine were found to be positively correlated, thus consumers who rated highly for one rated highly for the other also.

Years of drinking wine

Overall the average number of years of drinking wine for all consumers across all the public tastings shows was 21.8 years (*Figure 5.74*). Significant difference in the average number of years of drinking wine was noted between consumers at location L1 in Mornington, VIC and L6 in Bathurst, NSW. These differences could be due to the age group of consumers at these shows. Consumers at location L6 had the highest proportion of young wine consumers hence the significantly lower average number of year of drinking wine.



Figure 5.74 Average number of years of drinking wine across different wine show locations.

Wine consumption pattern

Another useful way to segment wine consumers is by considering their wine consumption pattern (Spawton 1991). In this study consumption indices measured were: frequency of wine consumption, volume of wine consumed on weekends and weekdays, the style of wine often consumed and amount spent on the different styles consumed.

Frequency and volume of wine consumption

Overall, most respondents could be considered regular wine consumers with more than 70% consuming wine at least several times a week (*Figure 5.75*). Only 14.5% consumed wine once a week and less than 5% consumed wine less than once a month; these respondents could be considered non-wine drinkers and may be at the shows as designated drivers. Respondents reported consuming more on a weekend (5.6 standard drinks) than on a weekday (2.6 standard drinks). Consumers from location L6 however consumed almost as much on a weekend as on a weekday.



Figure 5.75 Frequency of wine consumption of consumers at Australian wine shows in 2010.

Style of wine consumed and amount spent on style

The wine style most often consumed by respondents was red wine followed by white wine then sparkling wine. Rose wine was the least often consumed wine. A similar trend in the amount spent on these styles was noted. Consumers spent more on red wine than on white wine. The least amount was spent on Rose (*Figure 5.76*).

Public tasting attendance

Generally, consumers hear about which shows through word of mouth from event organizers or friends. Local news papers are also common channels for information on which shows to attend. Surprisingly, tourist information centres were not a popular source of information on shows to attend. This suggests emphasis further the difference between

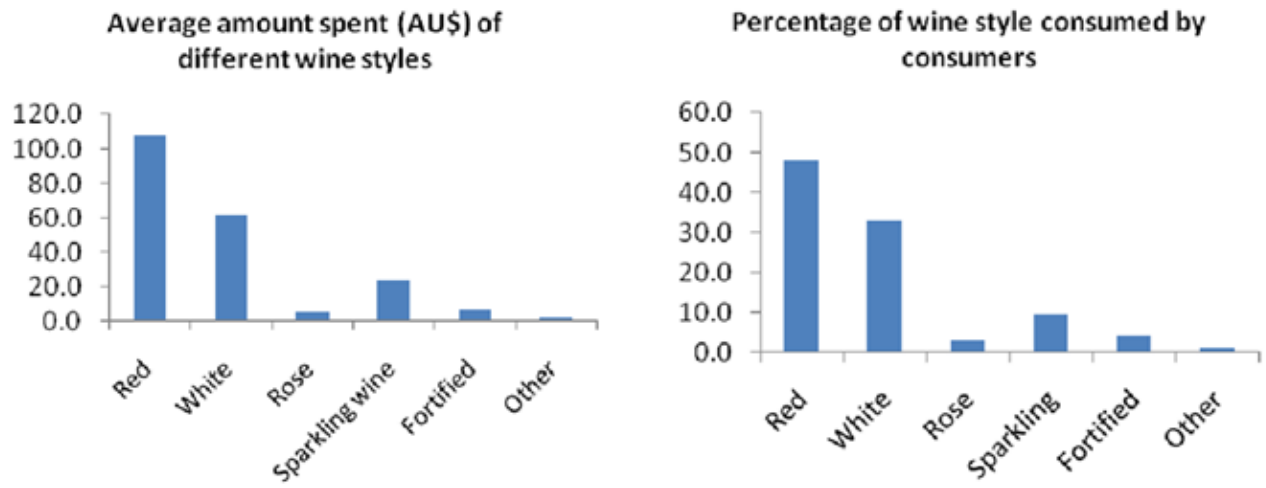


Figure 5.76 Wine styles often consumed and amount spent on different styles.

wine festivals and public tastings. On average across all the shows, consumers travel about 150 km to attend wine shows. The majority of respondents however travel less than 100 km to wine shows suggesting a fairly local demographic. At a few shows however, there was an international presence with consumers travelling from other countries to attend.

Reason for attending public tastings

The most important reason why consumers attend public tastings is because they have an interest in wine (*Figure 5.77*). This was not unexpected. Another frequently mentioned reason was to learn about different wines and to socialise. Interestingly, knowing which wines won medals was not a major popular reason to attend wine shows for most consumers.



Figure 5.77 Frequently mentioned reasons for attending wine show public tastings.

The results suggest that consumers who attend wine show public tastings are enthusiastic product users, with moderate subjective knowledge about wine with most of them having no involvement in the wine industry. This is important since it has been established that experts have different wine preferences to novices (Blackman et al., 2010). This implies that consumers who attend public tastings would be non expert consumers and may represent the general wine drinking population.

The age group demographics of consumers who attend public tastings are similar to the age demographics of Australian wine drinkers as noted by Stanford (1999). It should be noted however, that compared to Stanford's demographics, the 65+ years age group was slightly lower compared to the wine shows demographics possibly because of the travel involved in getting to wine shows. The original data used in Stanford's methodology, involved home visits hence a higher percentage of 65+ would have been interviewed. This similarity further illustrates the suitability of using consumers from wine show public tasting events as a sample to represent the general Australian wine show drinking population. That overall the gender demographics was balanced would imply that consumers from different shows would need to be pooled together to give a better representation of the general wine drinking population if results are to be extrapolated.

For consumers included in this survey, the reasons for attending wine shows were quite similar to reasons why consumers attended wine festivals (Houghton 2008) suggesting that they may also be the target market for the wine industry.

Dodd *et al.* (2005) has suggested that consumers with high self rated or 'subjective' knowledge of wine are able to make informed choices during the wine purchasing process. Although he does not provide an empirical value of subjective knowledge, for comparison, it could be deduced that the consumers included in the 2010 survey had a moderate level of subjective knowledge of wine (i.e. 4.1 on a scale of 1–7 where 7 is very knowledgeable). Thus it is not surprising that to know which wines to buy was an

important reason for these consumers to attend public tastings. They may represent informed wine buyers in the population who base their wine choice on subjective knowledge of wine and not external cues such as medals. This is further supported by the low interest shown by these consumers in knowing which wines won medals. This is an important finding as most wine exhibitors enter shows to win medals with the impression that medalled wines have high saleability. It will appear that this is not always the case, at least not for consumers with moderate subjective knowledge of wine.

The above results suggest that it is possible to use consumers at wine show public tastings for preference tests. A useful addition to the traditional medals awarded at wine shows could be to have a consumer 'judging' session included as part of the public tasting, as some less formal shows already do. Since consumers were not interested in knowing which wines won medals, perhaps, the consumer 'judging' need not be in a format similar to the medal awards system used by the experts to select a single wine as the winner, but rather an interactive method which takes into account the social ambience of these events which is important to consumers.

Experiment 5.10
Consumer wine show days and what they offer: Coming up with a valid measure

From the results of the 2010 survey, it was determined that people who attended the public tasting days were usually wine enthusiasts who consume wine on a regular basis and have a moderate knowledge of wine. These consumers were found not be expert wine drinkers but were representative of the general wine drinking population in Australia. Although some international consumers attended certain shows included in the study, they represent a very small percentage of the total consumer demographics included in the survey that their results would not significantly influence the data. There were some attendees however who do not consume wine often but had a high interest in wine. These people may purchase wine for consumption by others or perhaps collect wine like art (speculation). The atmosphere at a wine show public tasting event is often social; relaxed and informal atmosphere. Most shows provide food which the organizers believe sets the mood for a relaxed social gathering for wine tasting.

Consumers at wine show public tasting days attend these events to learn about new wines, to learn about different wines and wine styles and also to know which wines to buy. At the wine show judging not all wines win medals; some wines which may be appreciated by consumers may not meet required style specification of judges and thus not be awarded. It will be relevant for wine exhibitors at these shows to know how differently consumers perceived their wines to be compared to other wines and how much these wines were preferred at the same time. This information will not replace the medal system at wine shows but could become an additional benefit to wine exhibitors at the show.

In developing a valid method to use within the wine show public tasting event context the following considerations were made:

The people at the show

People who attended public events in 2010 came from a diverse socioeconomic background with varying age categories. Although majority of the people at the public tasting were professionals their level of education ranged from high school leavers to those with a doctorate degree. Most were enthusiastic

about wine but moderately knowledgeable. It was deduced that any test developed for this consumer group should be simple enough that the least educated would understand it, but stimulating enough that those with high intellectual ability will not be excluded from being a part of it. The fact that consumers at such events will be consuming alcohol was also be taken into account as the effect of alcohol on cognition is well known.

The purpose for their being there

Majority of people attended wine show public tasting events because they had an interest in wine. A large percentage also attended because they wanted to learn about new wine styles and about different wines. Others were there to decide on which wines to buy. Importantly, most attendees were there to socialize. A test which detracts from the social ambience regardless of how much the consumer will learn about the wine will not be popular, may reduce patronage at the events or may cause the events to become selective with a skewed demographic patronage. The fact that people wanted to learn about different wines and the different styles should also be considered. A test which encourages this learning process will most likely be stimulating to the consumer and enhance participation in the test. Some people attend the shows because they want to know which wines won medals. This is an important reason as one of the important factors organizers use to judge the success of their show is the increase in wine awards. Incorporating medaled wines in the methodology will not only satisfy the consumers interested in the medals but will facilitate organizers' interest to adopt such a method at their show. Award winning wine makers will also gain satisfaction in knowing that they can get added information about how consumers liked their wines relative to other wines.

The social ambience

The social ambience at public tasting events is what distinguishes this test location from a central location test, home use test or laboratory test often used in sensory consumer preference test. The social ambience of the location makes the public tasting environment an appropriate test is what makes the context a natural one for consumers to taste wine regardless of the time of day. Although the extent of this social ambience may differ for different shows depending on the food available and the kind of entertainment provided, the interaction between people at the show creates this ambience. A method that will fit well in this context will need to be socially-friendly and not detract from the social experience at the show.

The test method

The ultimate use of the data collected from consumers at wine shows will be used in techniques such as preference mapping which has the ability to provide wine exhibitors with information on which sensory attributes in their wines appeal to consumers when consumer data is regressed against sensory attribute data on the wines. For this purpose, the method used should be able to give numerical data; ordinal or categorical. The data would need to be able to be subjected to statistical testing. The results should be able to differentiate between products based on their sensory appeal to the consumer. The method should satisfy simple (psycho-) sensory evaluation principles such as order effect, ceiling effect, and halo effect.

Methodology for a new test protocol: relative acceptance mapping test

The method was developed by taking into consideration all the factors outlined above. The concept was based on Napping® a relatively new method that developed by Pagès (2005). In Napping® assessors place samples on a nape or napkin according to their own selected criteria of similarity and differences to each other. They may then give verbal descriptions of the groups on the nape. The data from a Napping® procedure is analysed using Multiple Factor Analysis which is a multidimensional analysis technique. The difficulty with Napping however is that it may be difficult to interpret the dimensions of the consensus map produced since each assessor uses their own unique descriptor list. When used with trained or expert assessors who all use similar vocabulary for a particular product this may not be a major issue for concern, however, for consumers this method will not be appropriate as the dimensions will have little meaning based on consumers' differing criteria.

For this new method, the dimensions of the map on which consumers would group the products were provided. The challenge was selecting two meaningful dimensions which would be understood by all consumers. After pilot trials using different attributes, 'liking' and 'difference' were selected as appropriate dimensions to use for the mapping technique.

These two dimensions of the map may suggest a basic sensory evaluation error as outlined by Stone

and Sidel (2004 #269), however the concept of relative difference used in this test is not the same as the overall difference test described by these authors. The concept of relative difference used in this method was similar to the difference from control test method described by Meilgaard *et al*. (2006) while the liking dimension of the map stems from the popular 9-point hedonic scale with two variations. First, liking was scored relative to a reference (Bergara-Almeida *et al*. 2002) and secondly a continuous line scale with only the two extreme end descriptors was used rather than the traditional category scale with descriptors.

Test protocol

Two scale types were developed during laboratory test trials of the new method. The two scales used are shown below (*Figure 5.78*).

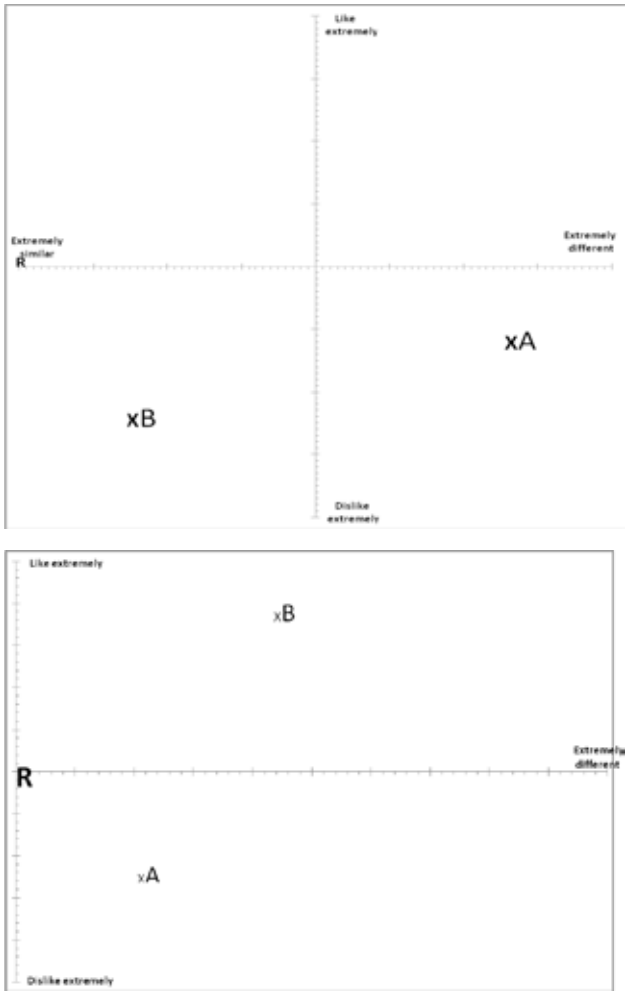


Figure 5.78 The cross scale (top) and the T-scale (bottom)

Participants were asked to taste a reference sample marked R, no other information about this sample was provided. Assessors were asked to keep this sample with them and compare each of seven other test samples to the reference. Assessors indicated by placing a cross on a two dimensional map how different they perceived the test sample was to the reference and simultaneously how much they liked or disliked the sample compared to the reference.

Advantage of a perceptual map approach

1. A two dimensional map is instantly obtained that gives an indication of the direction of product liking relative to a selected reference and direction of product sensory difference relative to a reference. To fully understand which sensory attributes are important to the consumer, sensory profile of selected products in the map will be carried out by a trained panel to interpret the sensory space.
2. Consumers have the option to write on their maps the reason for their differentiation.
3. The process may be seen as a fun thing to do at a public tasting event
4. Consumers may feel a sense of import as they will be giving their opinion about the wines and not only how much they like or dislike them but also how they differ from each other.
5. Consumers may feel they are gaining knowledge about new wine styles by differentiating between wines based on their sensory perception and not only on their preferences.
6. The method has the potential to be turned into a group activity which will encourage the social ambience of the show and enhance the learning process

Disadvantages

1. This method may be tasking for consumers
2. The initial set up of the method may be more time consuming, although once it is set up, it may be very quickly administered
3. It will require consumers to carry out multiple comparisons each time which could become tedious if too many products are evaluated at the same time.

Experiment 5.11
Consumer wine show days and what they offer: Testing the method in wine shows

Comparing two pilot trials of a new method in field

The mapping test was carried out a two test locations. At the first location – International Cool Climate Wine show (ICCWS), the cross map was used. The test was carried out outside the public tasting hall and was set up like in a central location test venue. An outline of the test protocol was as follows:

- 17 consumers were recruited by method of interception at the wine show to take part in the study.
- The cross scale version of the Relative preference mapping technique was used and assessors completed the tasting individually on A4 sheets of paper with the printed map on it.
- The test was set up at the entrance to the public tasting, outside the public tasting area in a layout similar to a central location test environment.
- Assessors evaluated 7 test samples against a reference R for degree of liking/disliking and degree of similarity/difference to R.
- Two glasses were used throughout the test; one for the reference sample, which was kept the same throughout all comparisons and the second glass for the test samples. After each test sample, the glass was rinsed out with a little of the following test sample prior to it being served.
- The Williams design was used to randomize the sample presentation order to reduce order effect of the wines.
- All assessors tasted all 8 samples (reference included).
- Demographic data was collected when all tasting was completed.

In a separate study, assessors were recruited after a seminar at the National Wine and Grape Industry Centre to participate in a group version of the relative preference mapping (RPM) technique, using the inverted T-scale map. The test protocol used is outlined below:

- 15 assessors were recruited to take part in the study via announcements made at the end of the seminar.
- The T-scale of RPM was used.
- The mapping test was carried out as a group task.
- All assessments were made by placing labelled coloured dots onto a large map 90x100cm wide.
- Assessors were randomly assigned labelled coloured dots with unique 3-digit randomised codes. These represented the samples to be tasted.
- The order of the samples was presented using the Williams design to reduce order effect.
- Similar to the ICCWS study, assessors evaluated the same 7 test samples against the same reference R for degree of liking/disliking and degree of similarity/difference to R.
- Two glasses were used throughout the test; one for the reference sample, which was kept the same throughout all comparisons and the second glass for the test samples. After each test sample, the glass was rinsed out with a little of the following test sample prior to it being served.
- Demographic data.

The difference between this study and the ICCWS study was that assessors in the NWGIC study did not use individual maps for their evaluations but rather worked on a large map together. Protocols to ensure that individual assessments of the wines were made included giving each assessor a unique set of blinding codes for all the samples. In the ICCWS study, the same blinding code was used for each sample seen by different people, but in the NWGIC study, different blinding codes were used the same sample seen by different people. This was done to help reduce external influence from other people also tasting the wines. Assessors were made aware that they tasted different samples at the same time and so would not be able to compare or discuss their preferences or perceived differences in the wines.

Analyses

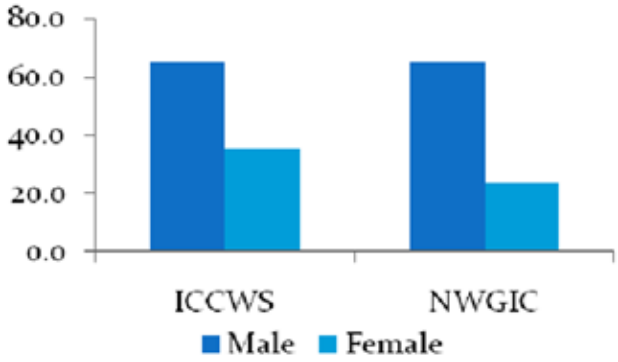
1. Observations on assessor participation and general test environment.
2. Counts, frequencies and percentages of demographic data.
3. Statistical analyses on preference data.

All statistical analyses were carried out in XL-Stats

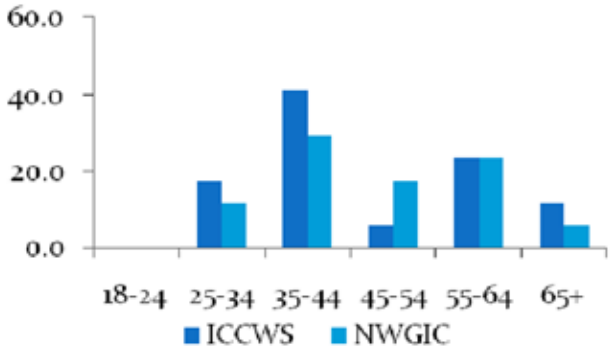
- ANOVA of individual axes (i.e. liking and difference).
- Pearson's correlation between liking and difference axis.
- GPA of individual XY coordinates
- ANOVA of Dimension 1 and 2 from GPA stretched data.

Results and discussion

Gender



Age groups



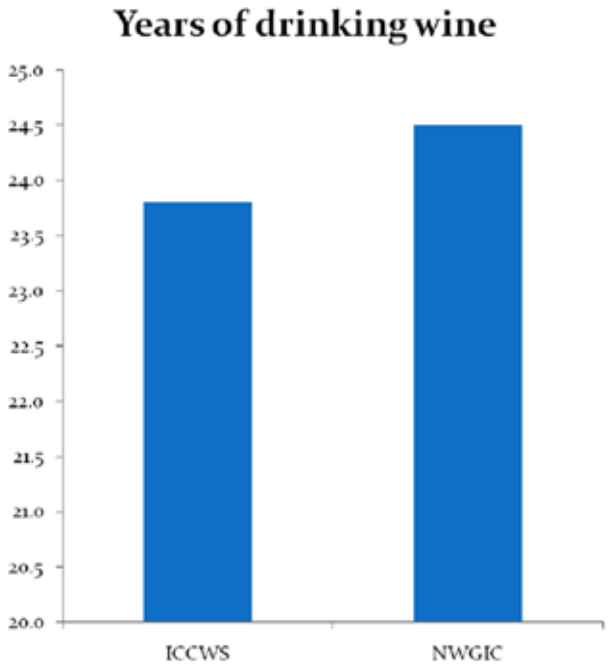
Wine involved consumers

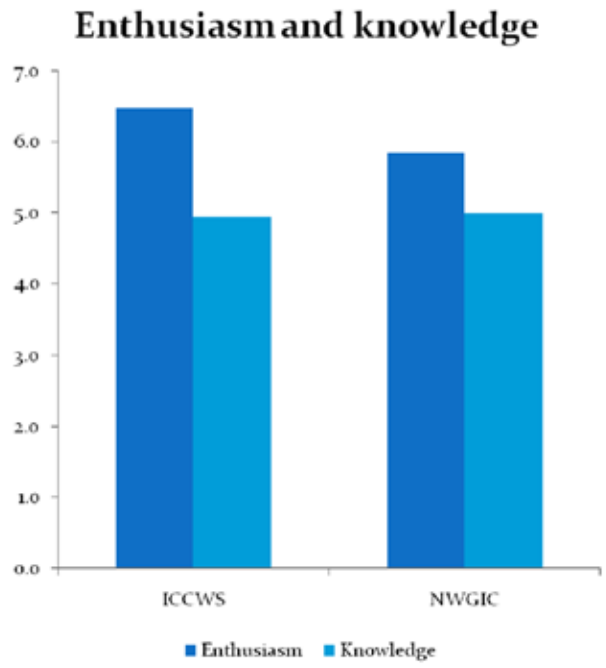


Preferred style consumed



Wine experience indices





Observations

ICCWS

- Consumers arriving earlier than show start time were happy to take part in the test.
- Consumers arriving after show started were not keen to take part in the test and had to be intercepted.
- Took longer time to serve samples as assessors were seated.
- Research staff had to run the samples.
- Once they started they were happy to complete the test.
- Comments about the method included, interesting, easy to differentiate wine based on method, accurate, innovative.
- General level of satisfaction for study was 5 out of 7 (7 means very satisfied and 1 means not at all satisfied.

NWGIC

- Test was carried out between end of the day and reception dinner.
- Announcement was made for people to take part in the study.
- People generally came straight away.
- Others were motivated to take part on seeing the process being carried out.
- Group activity may have aroused interest.
- Participants using dots, were excited and seemed to enjoy the tasting experience.
- People were relaxed, atmosphere was quite social and not 'sterile'.

Map tests

Correlations

- For both ICCWS and NWGIC data, the two variables, liking and difference were not significantly correlated.
- Pearson's r = -0.027 for ICCWS, 0.08 for NWGIC data.

Product differentiation using ANOVA from raw data

- For both tests, there was no significant difference between products on the liking axis, i.e. Y-axis.
- Significant difference between products for difference axis, i.e. X-axis.

Generalised Procrustes Analysis

Overall, the consensus map from ICCWS was slightly better than from NWGIC.

- Rc from PANOVA fell in the 91st Quartile – ICCWS.
- Rc fell in the 70th Quartile – NWGIC.
- Rc – consensus test, the higher the quartile within which Rc is found, the better the consensus map.
- PANOVA showed lower residual values for products position on the map generated by ICCWS consumers than that generated by NWGIC consumers.
- Better assessor agreement on position of products on the maps with ICCWS data than NWGIC data.
- In both maps dimension 1 explained most of the variance in the data.
- For ICCWS map, difference from R and liking to R loaded positively on factor 1.
- For NWGIC map, the two variables loaded in orthogonal directions.

Differences in map results

- The two maps used were different.
- Previous tests suggests that the cross scale (used at ICCWS) gives better consensus configuration than the T-scale (used at NWGIC), although assessors preferred to use the T-scale to the cross scale as it was more logical.
- Higher quartile value.
- Lower product residuals.
- Lower assessor residuals.
- Perhaps the group task reduced assessor agreement?
- This has not been tested and will be confirmed using data from Carins.

Table 5.33 Statistical analysis

		ICCWS		NWGIC	
Effect	**Test statistic**	**X**	**Y**	**X**	**Y**
Overall model	R^2	0.345	0.284	0.346	0.211
	F	2.215	1.664	2.304	1.165
	Pr>F	0.006	0.057	0.003	0.298
Assessor	F	1.906	1.724	1.098	1.308
	Pr?F	0.037	0.066	0.368	0.209
Products	F	2.936	1.525	5.520	0.783
	Pr>F	0.012	0.180	<0.0001	0.585

X - difference; Y - liking

- Perhaps the different cohorts of assessors may have influenced the results.
- More industry involved consumers at NWGIC, similar level of knowledge, lower enthusiasm, more highly educated.

Next steps

- Data collection from wine shows using the T-scale map version will continue. Laboratory tests suggest that consumers preferred to us the T-scale map compared to the cross scale map.
- Indices used to measure the significance of the consensus map using a GPA technique suggests that cross map may give a more statistically significant consensus map compared to the T-scale.
- The cross scale appears to be better able to allow for greater consensus; this is not conclusive however as the results presented here were carried out in two different ways (individual assessment *vs* group task).
- It is hypothesised that the cross scale in a group task environment will provide better consensus results as it allows more scope for differentiating between products while the T-scale might be more suited to highly trained assessors or product experts and may need to be carried out individually.
- This hypothesis will be tested in a different tested.

It should be noted that the results presented here on the new method that has been developed is still being analyzed, particularly data which has been collected from the wine shows. The results so far have been meaningful to progress the research. Final analyses and reporting on the new method will be submitted in completed PhD thesis work of this project.

Experiment 5.12
Vineyard management and Semillon wine styles in the Riverina

The key objective of this study was to clarify the link between vineyard yield management, vine physiology, fruit composition and wine composition in Semillon grown in the Riverina. Relationships between yield and fruit composition and wine quality are complex (Chapman *et al*. 2004; Keller *et al*. 2005). Pruning and fruit or shoot removal can manipulate vine cropping levels. These different crop regulation methods can have varying levels of accuracy in targeting desired yields, different effects on vine physiology and fruit composition, and some are be more expensive than others. Although this area has been a subject of some study (Clingeleffer 2002) there is still a much uncertainty and few scientific publications on this topic relevant to Australia. Vine cropping levels will also tend to vary with site and season.

With the sharp drop in grape prices, there is naturally increasing interest among grape growers to maximise yields while maintaining quality. This is especially relevant for the bulk and popular premium wine production in the Riverina. What consequences will these maximised yields have on fruit composition and wine attributes, and how will they affect vine physiology and short- and long-term carbohydrate partitioning (itself affecting vine health, productivity and longevity, Lakso 2006) warrants an investigation. There are significant financial benefits for growers in knowing the maximum sustainable cropping level of their Semillon vines that will have no appreciable negative effects.

Adding to the complexity of the issue, the behaviour of vines under different yield levels is widely perceived to be affected by vineyard site (and season). This has not been sufficiently scientifically tested, not under Australian conditions.

Different vineyard sites impose different environmental conditions on vines that, even when in a close physical proximity, can sometimes be quite distinct (Tesic *et al*. 2002). The effect of site on Semillon ripening and eventually on wine attributes, at different price point quality categories, needs to be examined. In addition, sites can exhibit a varying degree of interaction with seasonal conditions.

Affecting the ratio between vegetative and reproductive organs is known to influence final grape quality. This experiment aimed to investigate what

percentage of bunches needs to be removed before changing the aroma profile of the wine.

Material and methods

Vineyard selection

Three Semillon blocks across vineyards of the Griffith area were selected for the study based on long-standing relationships with the vineyard managers and a similarity in vineyard practices. Long-term background history was available from these vineyards. On each block, three levels of crop load were applied post veraison when berries were around pea size (EL stage 31). The crop load was set by removing a percentage of bunches for three consecutive seasons:

1. Control – no bunch removal (high)
2. 33% removal (medium)
3. 50% removal (low).

Treatments were replicated 4 times in a randomised complete block design (*Figure 5.79*). Each replicate comprised twenty vines of similar size and vigour (visually assessed) over four rows. The measurements were taken from the middle two rows.

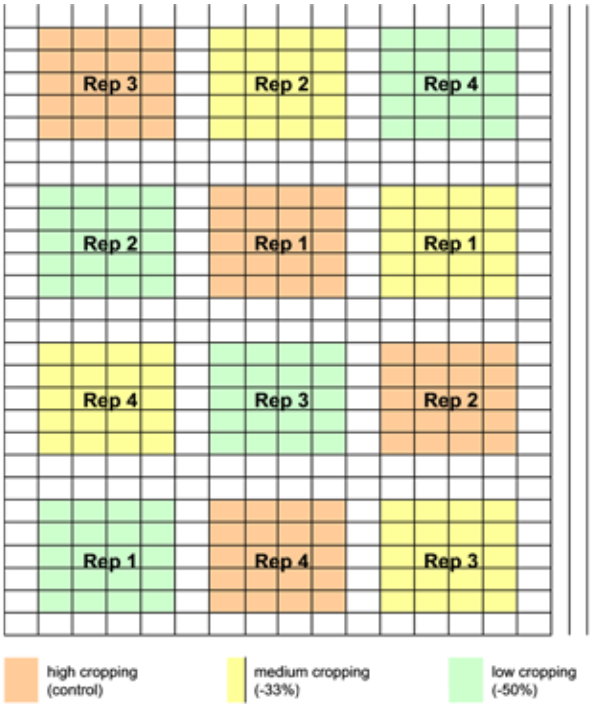


Figure 5.79 Experimental design

Site descriptions

See *Tables 5.34* and *5.35*.

Table 5.34 Site characteristics

	Vineyard C	Vineyard H	Vineyard Y
Year of plantation	**1996**	**1995**	**2001**
Trellis system	Single cordon VSP	Single cordon VSP	Double cordon VSP
Vine/row spacing		1.8 x 3.6 m	
Rootstock	own root	own root	own root
Clone	Macedone	DA16162	not specified

Vine measurements

Measurements were made over three growing seasons, from 2007 to 2010. Vegetative growth and vigour were followed from budburst to leaf fall, whereas berry growth and composition were measured from veraison to harvest.

To measure shoot growth, one shoot from each of the vines from the middle two rows of each replicate was randomly selected and tagged after budburst. Shoot length was measured weekly from budburst to veraison, and then monthly until leaf fall.

Pruning weight was assessed after leaf fall, in June.

To assess berry growth and composition, 100 berries were collected from each replicate at veraison and at harvest. Berries were collected randomly from top, middle and bottom of the bunches and from both sides (Iland). After collection, they were placed in an esky for transport back to the lab. In the laboratory berries were weighed, and then juiced for further composition analysis. The sugar content (TSS) was measured with a bench refractometer (ATAGO PR-101, Tokyo, Japan) and juice pH and titratable acidity (TA) were measured with an automatic titrator (Titrando System LMWI 40-15, Metrohm Ltd, Herisau, Switzerland).

After filtering the juice, the phenolic composition was also assessed by spectrometry (UV-1700

Table 5.35 Yield per vine and per hectare

	2007-08		2008-09		2009-10	
	per vine (kg)	per ha (t ha^{-1})	per vine (kg)	per ha (t ha^{-1})	per vine (kg)	per ha (t ha^{-1})
Vineyard C	10.2	22.7	11.2	24.9	7.3	16.2
Vineyard H	20.6	31.5	10.7	23.8	14.2	22.0
Vineyard Y	16.3	36.2	17.3	38.4	15.2	33.8

PharmaSpec, Shimadzu Scientific Instruments) at 280 nm, 320 nm and 420 nm, after Iland:

- total phenolics (a.u.) = $A_{280} - 4$
- total hydroxycinnamates (a.u.) = $A_{320} - 1.4$
- total flavonoids (a.u.) = $(A_{280} - 4) - 0.66 \times (A_{320} - 1.4)$
- estimate of brown pigments (a.u.) = A_{420}

Extra measurements were taken at particular stages. Active buds were counted at budburst; shoot number was assessed at flowering and bunch number was assessed at harvest. The leaf area of all tagged shoots was measured at flowering, using standardised leaf sizes collected from a typical plant of Semillon. The selected leaves were scanned through a special scanner (Leaf Area Meter, Li 3100C, LI-COR Environmental) that delivered each of their area in cm^2. The method consists of stripping the leaves of a two shoots from buffer vines. The leaves are then ranked by sizes, with a minimum of 8 levels usually required. The leaves are also scanned to get the precise leaf area of each one. Adding the area of all the leaves will give an estimation of the leaf area of a shoot. This area is then multiplied by the shoot number determined previously to get an estimation of the leaf area per vine.

At veraison, the canopy density was assessed using the point quadrat method. Furthermore, petioles were collected at both flowering and veraison, then dried and ground for nutrient analysis.

All three treatments were harvested on the same date. The sugar levels at harvest may differ, but to avoid introducing an extra parameter into the winemaking, sugar and acidity levels were adjusted to similar values for all treatments in the winery.

At harvest, as well as counting the bunches per vine, weight of fruit per vine was measured. Finally, the pruning wood from each vine was weighed. Pruning was performed manually after leaf fall (around June) on the same vines as per vigour measurements.

Experimental winemaking

At harvest, experimental wines were made separately from the three treatments and three separate blocks. Fruit was hand picked and put in 20 kg foam boxes with SO_2 for transport back to the experimental winery in Wagga Wagga. It was then stored in the cool room overnight until crushing the next day. 100 kg of fruit per replicate were used. For logistic reasons, three replicate plots out of the four were picked. After pressing, the wines were adjusted in sugar and acidity to similar level to avoid differential effects during fermentation.

Experimental wines were made following the protocol in this report and summarised in *Figure 5.80*. The neutral yeast DV10 was chosen, as it is appropriate for white winemaking from fruit from this region, including Semillon.

Wine tasting

Duo-trio tests or difference tests: each panellist is presented with a series of three glasses. One glass is labelled as the reference, the other two glasses, labelled with random numbers, contain different wines that are tested. The reference is actually a repeat of one of the two wines tested. The panellist is asked to determine which wine is the same as the reference. A statistical test is then applied to the whole panel to check if the panel can pick the difference between the wines.

Preference test: after the difference test, another simple question is asked as which wine each panellist prefers.

Descriptive analysis (DA): for this test, a series of descriptors typical for Semillon wines was determined by a panel of experts and winemakers. The NWGIC panel was then trained on typical wines with either low or high occurrence of a certain descriptor to rate each aroma on a scale from 0 (absent) to 9 (intense). The wines from the trial were then tested the same way and rated for each of the descriptors. Principal component analysis (PC) was then applied to the results to identify the descriptors that separated the best the different treatments.

Statistical analysis

The data were compiled and manipulated in the data base Excel 2002 SP3 and further analyzed using the graphic program Sigma plot (version 8.0). The statistical analysis was conducted by using Genstat release 11.1 (Rothhamsted Experimental Station, Harpenden, Herts, United Kingdom). Analysis of variance (ANOVA) and restricted maximum likelihood (REML), for unbalanced dataset, were used as statistical tests; Fisher's LSD test was used to interpret differences in means at $P < 0.05$.)

Results and discussion

Vineyard C

In vineyard C, bud and shoot numbers were not affected by bunch thinning; however, pruning weight and inflorescence number were increased substantially by 20% and 7% respectively when the crop load was reduced (*Table 5.36*).

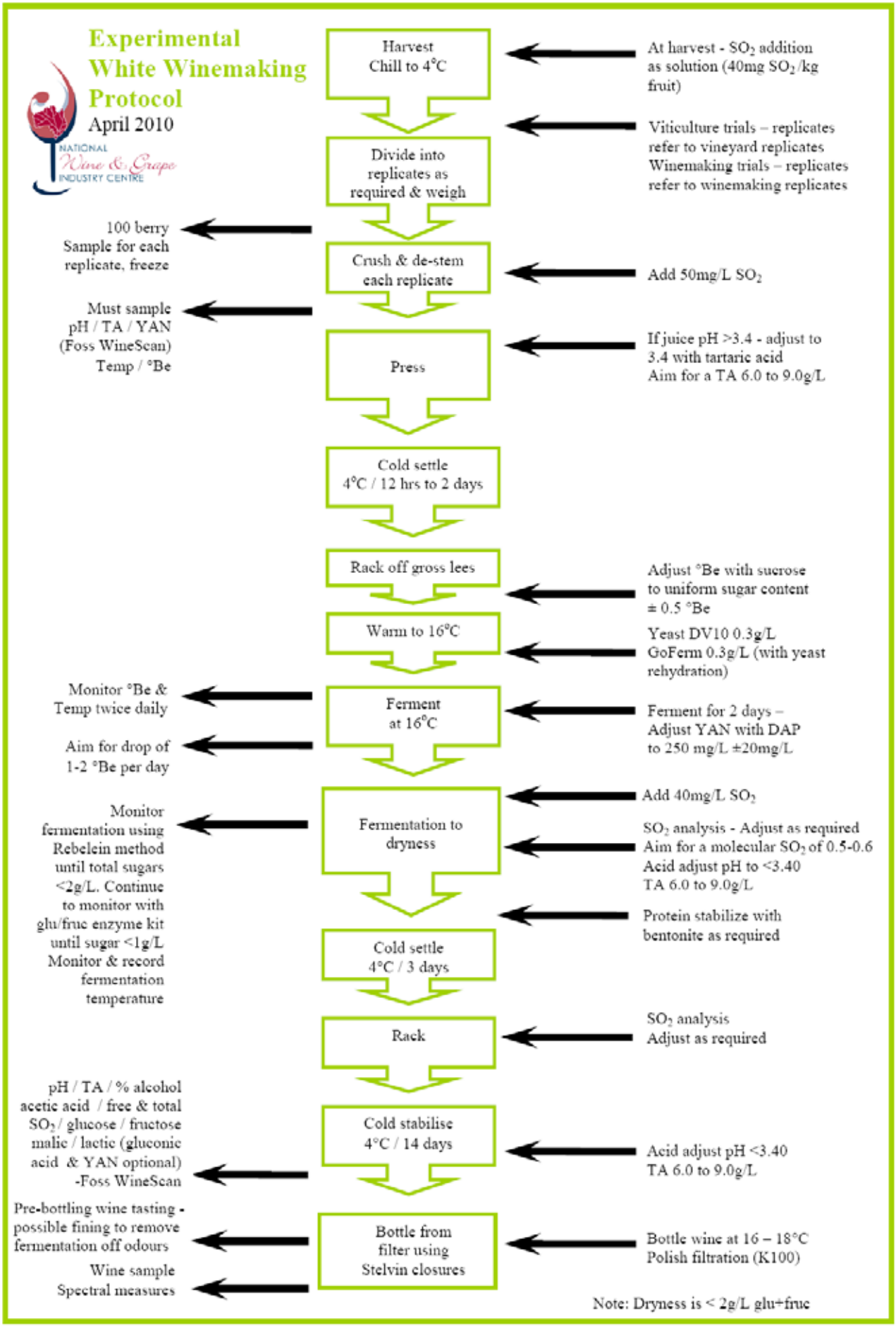


Figure 5.80 Winemaking protocol

Table 5.36 Effect of crop load in vineyard C

	High	Medium	Low	p value
Bud number	36.3	38.7	36.5	0.088
Shoot number	48.7	51.3	49.8	0.126
Inflorescence number	63.5[a]	70.2[b]	67.9[b]	0.003
Pruning weight	0.71[a]	0.85[b]	0.86[b]	<0.001

Mineral composition in petioles was also altered by bunch thinning (*Table 5.37*). Petiolar aluminium, calcium, potassium, manganese, phosphorus and zinc were increased significantly (P<0.05), by about 20%, 5%, 9%, 23%, 17% and 8% respectively; whereas magnesium was decreased by 5%. The other elements were not significantly (P>0.05) affected.

Table 5.37 Effect of crop load on petiole mineral content on vineyard C

	High	Medium	Low	p value
Aluminium	20.9[a]	22.0[a]	25.8[b]	0.002
Boron	47.9	47.6	49.6	0.155
Calcium	10387[a]	10933[b]	10971[b]	<0.001
Copper	13.8	15.4	15.0	0.182
Iron	27.7	25.6	26.0	0.782
Potassium	21514[a]	23938[c]	23012[b]	<0.001
Magnesium	10373[a]	9733[b]	9932[b]	0.017
Manganese	23.6[a]	28.1[b]	30.1[c]	<0.001
Sodium	971	810	808	0.382
Phosphorus	3406[a]	3938[b]	4059[b]	<0.001
Sulphur	1057	1109	1090	0.189
Zinc	41.0[a]	44.9[c]	42.5[b]	0.002

Vineyard H

In vineyard H, shoot number and pruning weight were not affected by bunch thinning; however, bud and inflorescence numbers were steeply increased when the crop load was lighter (*Table 5.38*), by 6% and 9% respectively.

Mineral composition in petioles was only slightly altered by bunch thinning (*Table 5.39*): the content of zinc was increased by 7%; whereas manganese was decreased by 16%. The other elements were not modified.

Table 5.38 Effect of crop load in vineyard H

	High	Medium	Low	p value
Bud number	37.8[a]	40.8[b]	42.7[b]	0.010
Shoot number	59.5	56.7	59.9	0.084
Inflorescence number	80.5[a]	80.8[a]	88.0[b]	0.014
Pruning weight	0.85	0.92	0.91	0.075

Vineyard Y

In vineyard Y, pruning weight, as well as bud and shoot numbers were not affected by bunch thinning; however, inflorescence number was steeply increased by 10% for the lightest crop load (*Table 5.40*).

Mineral composition in petioles was only slightly altered by bunch thinning (*Table 5.41*): the content of manganese was increased by 34%; whereas the other elements were not modified. Zinc showed an

Table 5.39 Effect of crop load on petiole mineral content on vineyard H

	High	Medium	Low	p value
Aluminium	25.9	28.7	25.3	0.072
Boron	49.3	50.1	49.4	0.481
Calcium	17538	16900	16933	0.110
Copper	14.0	14.5	13.8	0.174
Iron	28.8	29.5	29.1	0.601
Potassium	38125	38792	39000	0.360
Magnesium	7029	6971	7008	0.900
Manganese	291.8[a]	299.8[a]	247.1[b]	0.007
Sodium	463	503	360	0.135
Phosphorus	6092	6146	6004	0.626
Sulphur	2082	2157	2095	0.253
Zinc	47.8[a]	50.7[b]	51.8[b]	0.003

Table 5.40 Effect of crop load in vineyard Y

	High	Medium	Low	p value
Bud number	87.0	92.1	93.0	0.072
Shoot number	111.4	107.3	108.9	0.246
Inflorescence number	140.1[a]	137.0[a]	151.9[b]	<0.001
Pruning weight	0.915	0.871	0.869	0.399

Table 5.41 Effect of crop load on petiole mineral content on vineyard Y

	High	Medium	Low	p value
Aluminium	22.3	19.8	23.6	0.208
Boron	47.2	47.3	44.0	0.196
Calcium	15675	15450	15379	0.758
Copper	6.8	6.9	6.6	0.597
Iron	24.0	189.0	25.0	0.380
Potassium	31708	32708	32012	0.616
Magnesium	8829	8650	9150	0.572
Manganese	172[a]	178[a]	234[b]	0.032
Sodium	1636	1704	1465	0.653
Phosphorus	3735	3739	3918	0.501
Sulphur	1296	1338	1292	0.743
Zinc	42.9	47.8	45.0	0.068

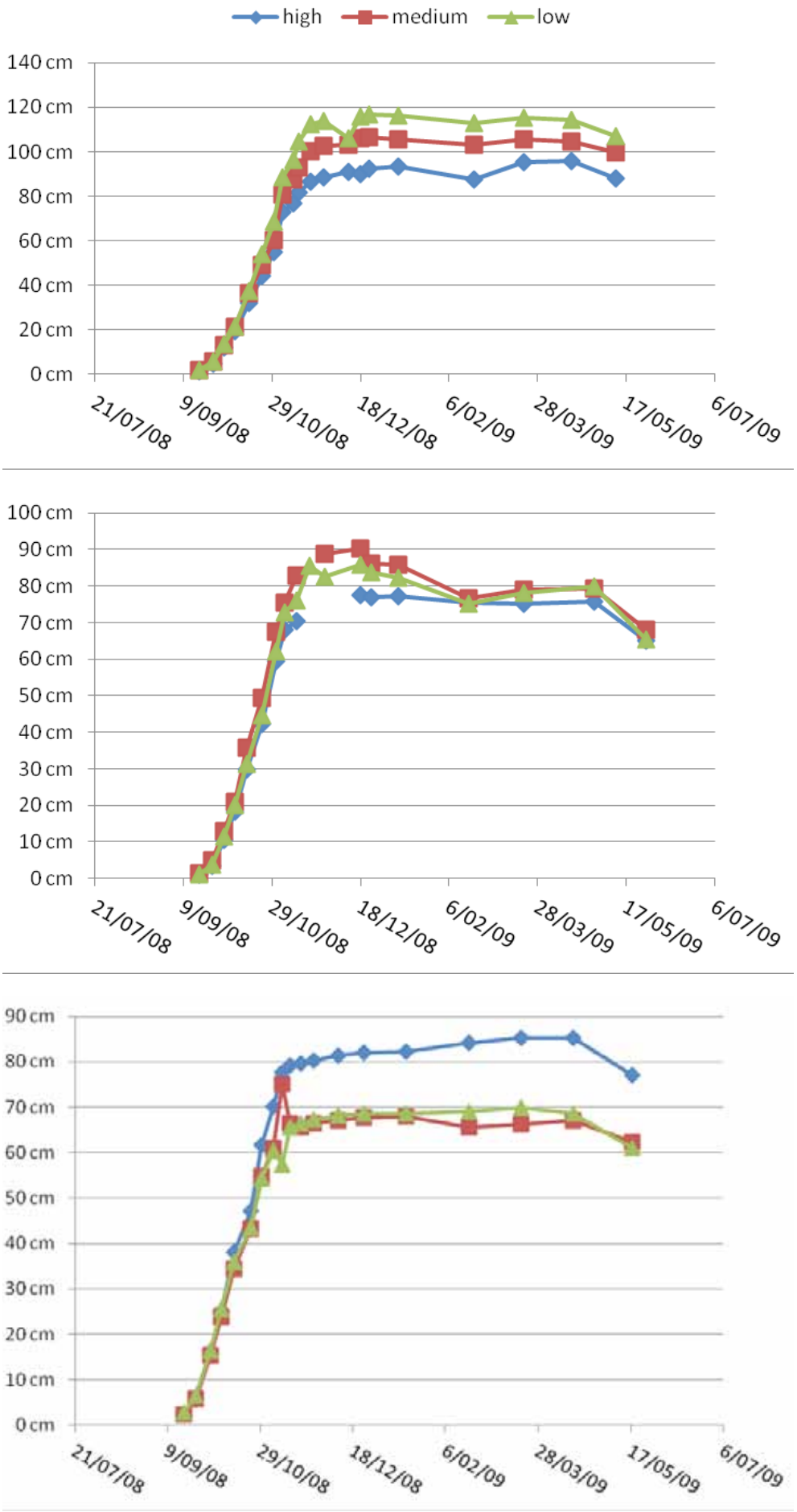


Figure 5.81 Effect of crop load shoot elongation in 2008-09 in vineyard C (top), H (middle) and Y (bottom)

increasing trend with the lower crop load, increased by 4.8%.

In summary, inflorescence number was substantially (on average by 8%) and significantly ($P<0.05$) increased by bunch thinning on all three vineyards,. Similarly bud number was increased significantly in vineyards C and Y but not for vineyard Y. Shoot number, on the other hand, was not affected by the bunch removal. Pruning weight was strongly increased by bunch removal on vineyard C; the trend was only minimal on vineyard H and no difference was observed on vineyard Y. The number of inflorescences per shoot was significantly increased by bunch removal on all three vineyards (data not shown).

The effect of bunch removal on mineral composition of petioles was not consistent between vineyards. Manganese content in petioles was increased in both vineyards C and Y, but decreased in vineyard H. Zinc content, on the other hand, was significantly increased in vineyard H, but peaked for the medium level of cropping for the other two vineyards.

The mineral composition was also modified for several other elements on vineyard C; this included a decrease in magnesium and an increase in aluminium, calcium, potassium and phosphorus.

Finally, regarding the shoot length, the variability inside the treatments was high with no significant differences between the treatments on vineyard C or H suggesting that sampling intensity (i.e. the number of shoots assessed) may not have been adequate to detect treatment differences.

Berry growth and ripening

Vineyard C

After bunch removal on vineyard C, berry weight was increased by 6%. Similarly, the sugar content (TSS) was increased by 8% at veraison and 11% at harvest, as well as the berry pH by 3%. Surprisingly, titratable acidity (TA) was not significantly modified; there was still a decreasing trend from the highest to the lowest crop load, especially at veraison (*Table 5.42*).

Vineyard H

After bunch removal on vineyard H, berry weight was not changed. Similarly, the sugar content (TSS) was not different between the treatments. pH, however, was significantly higher by 4% and titratable acidity (TA) significantly lower by 8% at veraison and 15% at harvest (*Table 5.43*).

Vineyard Y

After bunch removal on vineyard Y, berry weight was increased by 9% with the lowest crop load and already 6% with the intermediate treatment. Similarly,

Table 5.42 Effect of crop load on berry characteristics on vineyard C

	High	Medium	Low	p value
Berry weight	1.40[a]	1.49[b]	1.49 b	0.004
TSS (Brix) veraison	15.65[a]	16.71[b]	17.16[c]	<0.001
TSS (Brix) harvest	20.99[d]	22.77[f]	23.77[g]	
pH	3.55[a]	3.58[b]	3.65[c]	<0.001
TA veraison	9.36	9.13	8.69	0.170
TA harvest	3.59	3.73	3.51	
Brown pigments	0.402	0.410	0.434	0.315
Total phenolics	2.401	2.408	2.695	0.495
Total flavonoids	1.734	1.643	1.858	0.472
Total hydroxycinnamates	1.024	1.142	1.266	0.877

Table 5.43 Effect of crop load on berry characteristics on vineyard H

	High	Medium	Low	p value
Berry weight	1.89	1.91	1.94	0.969
TSS (Brix) veraison	15.63	16.64	16.86	0.988
TSS (Brix) harvest	22.53	23.90	23.53	
pH	3.42[a]	3.52[b]	3.56[c]	<0.001
TA veraison	10.57[a]	9.73[b]	9.63[b]	<0.001
TA harvest	3.82[c]	3.22[d]	3.25[d]	
Brown pigments	0.238	0.250	0.232	0.355
Total phenolics	1.351	1.277	0.892	0.041
Total flavonoids	0.823	0.764	0.559	0.064
Total hydroxycinnamates	0.801	0.766	0.667	0.479

the sugar content (TSS) was increased by 13% at veraison and 10% at harvest, as well as the berry pH by 4%. Titratable acidity (TA) was not consistent between the treatments and stages: at veraison, it increased with bunch thinning between the extreme treatments by 12%, whereas it decreased at harvest by 9% (*Table 5.44*). The medium crop load also had the lowest TA at veraison (16% lower than control) and highest TA at harvest (10% higher than control).

In summary, berry weight was significantly higher after bunch removal on vineyards C and Y. At vineyard H, there was no significant effect (*Table 5.43*).

Looking at the composition, pH was significantly higher on all three vineyards for the lower crop levels. There was a similar pattern with sugar accumulation.

Finally, phenolic composition was mostly not affected by bunch thinning. On vineyard H, the total amount of phenolics was decreased by about 30% with the low cropping. Total flavonoids also tended to decrease with the thinning, but not significantly. On vineyard Y, the amount of brown pigments was augmented by about 20% when thinning was applied.

Wine sensory

The repeatability of the wine assessment was tested by comparing the three replicates. Most of the wine replicates could not be differentiated (*Table 5.45*), except for the medium crop level in vineyard Y where replicates 1 and 3 were significantly different and thus only replicate 2 was used. Furthermore, for the high crop level of the same vineyard, the third replicate was found faulty at a pre-bottling tasting, thus it was not analysed later on. For all the other treatments, either replicate could be used for the rest of the tasting without bias.

Difference test results

For each vineyard, the wines from the different crop loads were tested for differences. In the first season (2008), only the extreme treatments, control and 50% bunch removal, were assessed. The following year, all three levels of cropping were investigated.

In 2008, a difference was observed between the wines from high and low cropping levels only for vineyard C; there was also a strong trend for vineyard H, but not for vineyard Y.

In 2009, the difference between high and low cropping was observed again on vineyard C. the medium treatment, however, did not show any difference with either the high or the low cropping level.

In contrast, no difference was observed on vineyard H between the extreme cropping levels. The medium treatment was thus not analysed.

Finally, even if the difference was not significant, there was a strong trend between high and low cropping levels, but also between medium and low cropping levels on vineyard Y. this means that removing 50% of the crop had only a slight impact on the final aroma profile of the wine in 2008-09.

In 2009, the wines were also assessed in a descriptive analysis test (DA). There was no significant effect of any of the treatments on any of the descriptors measured. More likely, the difference found in the duo-trio test was too subtle to describe in the DA.

Even if sugar and acidity were different on the vineyard, before adding the yeasts, the different treatments were brought the similar levels of sugar and acidity to avoid possible problems during fermentation of one of the treatment. This normalisation of acid and sugar of the must could have masked any aroma differences in the final wine.

Table 5.44 Effect of crop load on berry characteristics on vineyard Y

	High	Medium	Low	p value
Berry weight	1.50^{a}	1.54^{b}	1.64^{c}	<0.001
TSS (Brix) veraison	16.43^{a}	16.53^{a}	18.59^{b}	<0.001
TSS (Brix) harvest	19.39^{c}	19.52^{c}	21.41^{d}	
pH	3.46^{a}	3.52^{b}	3.59^{c}	<0.001
TA veraison	6.64^{b}	5.73^{b}	7.43^{a}	0.003
TA harvest	3.60^{cd}	3.97^{c}	3.29^{d}	
Brown pigments	0.277^{a}	0.314^{b}	0.335^{b}	0.009
Total phenolics	1.125	1.169	1.361	0.154
Total flavonoids	0.836	0.805	0.926	0.276
Total hydroxycinnamates	0.452	0.529	0.650	0.232

The vineyard differences might still have been too subtle to be translated into the wine profile. Other components of the juice that have not been analysed might be more important in the aroma synthesis.

However, the floral character differed significantly between the vineyards C and Y, with means of 1.76 and 2.5 respectively.

Table 5.45 Difference test between wine replicates (example of 2009 vintage) NS: non significant; * p<0.05.

Block / Crop load	Comparison (reps)	Same (correct)	Total no. of people	p-value
C low	1 vs. 2	8	15	0.500 NS
	1 vs. 3	8	15	0.500 NS
	2 vs. 3	9	15	0.304 NS
C medium	1 vs. 2	7	14	0.605 NS
	1 vs. 3	7	14	0.605 NS
	2 vs. 3	5	14	0.910 NS
C high	1 vs. 2	7	14	0.605 NS
	1 vs. 3	8	14	0.395 NS
	2 vs. 3	7	14	0.605 NS
Y low	1 vs. 2	8	15	0.500 NS
	1 vs. 3	9	15	0.304 NS
	2 vs. 3	6	15	0.849 NS
Y medium	1 vs. 2	9	15	0.304 NS
	1 vs. 3	12	15	0.018 *
	2 vs. 3	7	15	0.696 NS
Y high	1 vs. 2	8	15	0.500 NS
	1 vs. 3	N/A		
	2 vs. 3	N/A		
H low	1 vs. 2	6	13	0.709 NS
	1 vs. 3	8	13	0.291 NS
	2 vs. 3	5	13	0.867 NS
H medium	1 vs. 2	9	15	0.304 NS
	1 vs. 3	8	15	0.500 NS
	2 vs. 3	11	15	0.059 NS
H high	1 vs. 2	8	15	0.500 NS
	1 vs. 3	10	15	0.151 NS
	2 vs. 3	9	15	0.304 NS

Table 5.46 Difference test between blocks for 2008 vintage (NS: non significant; * p<0.05; ** p<0.01; *** p<0.001)

Block	Yield comparison	Same (correct)	Total no. of people	p-value
C	High vs. Low	12	14	0.006**
H	High vs. Low	10	14	0.090NS
Y	High vs. Low	9	14	0.212NS

Table 5.47 Difference test between treatments for 2009 vintage (NS: non significant; * p<0.05; ** p<0.01; *** p<0.001)

Block	Yield comparison	Same (correct)	Total no. of people	p-value
C	High vs. Low	12	15	0.018*
	High vs. Medium	8	15	0.500NS
	Medium vs. Low	9	15	0.304NS
Y	High vs. Low	10	15	0.151NS
	High vs. Medium	8	15	0.500NS
	Medium vs. Low	10	15	0.151NS
H	High vs. Low	7	15	0.696NS

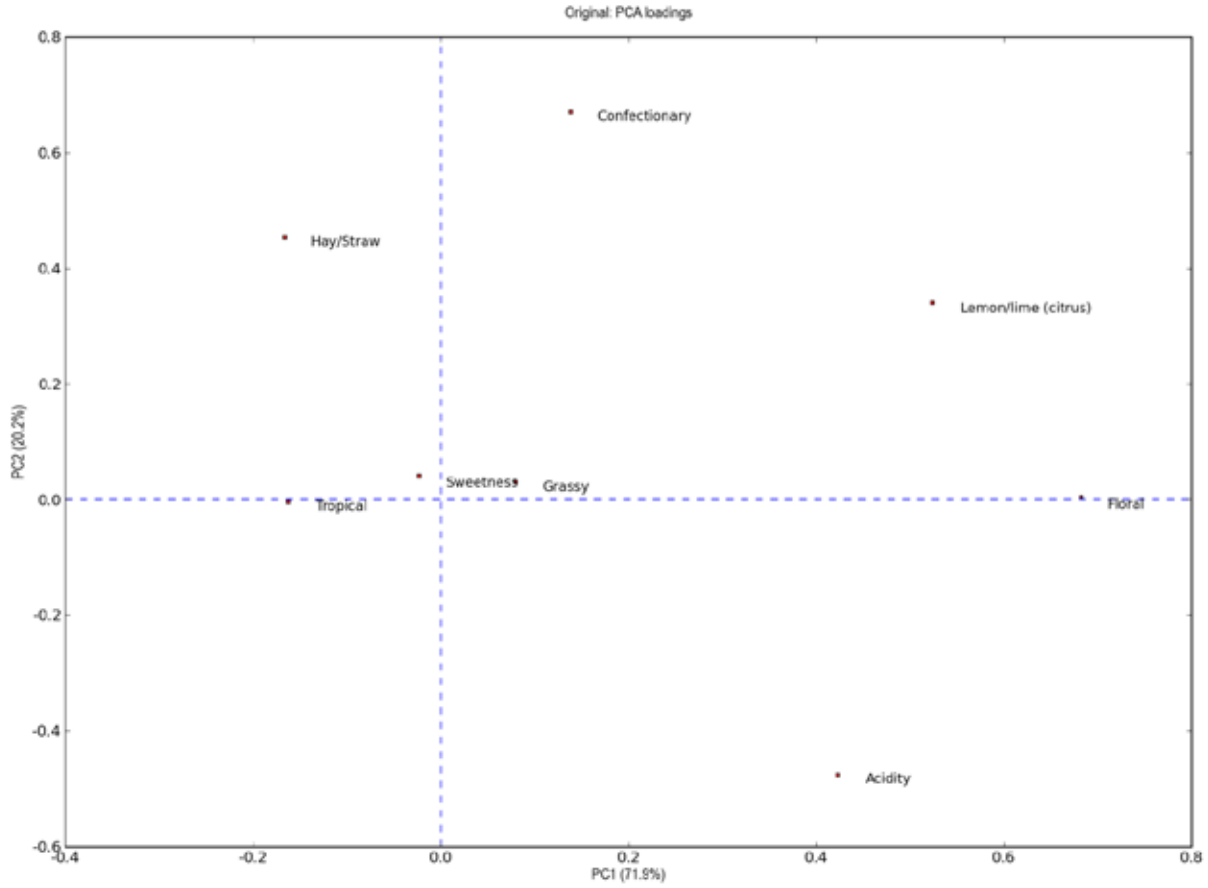


Figure 5.82 PCA attributes of Griffith wines 2009.

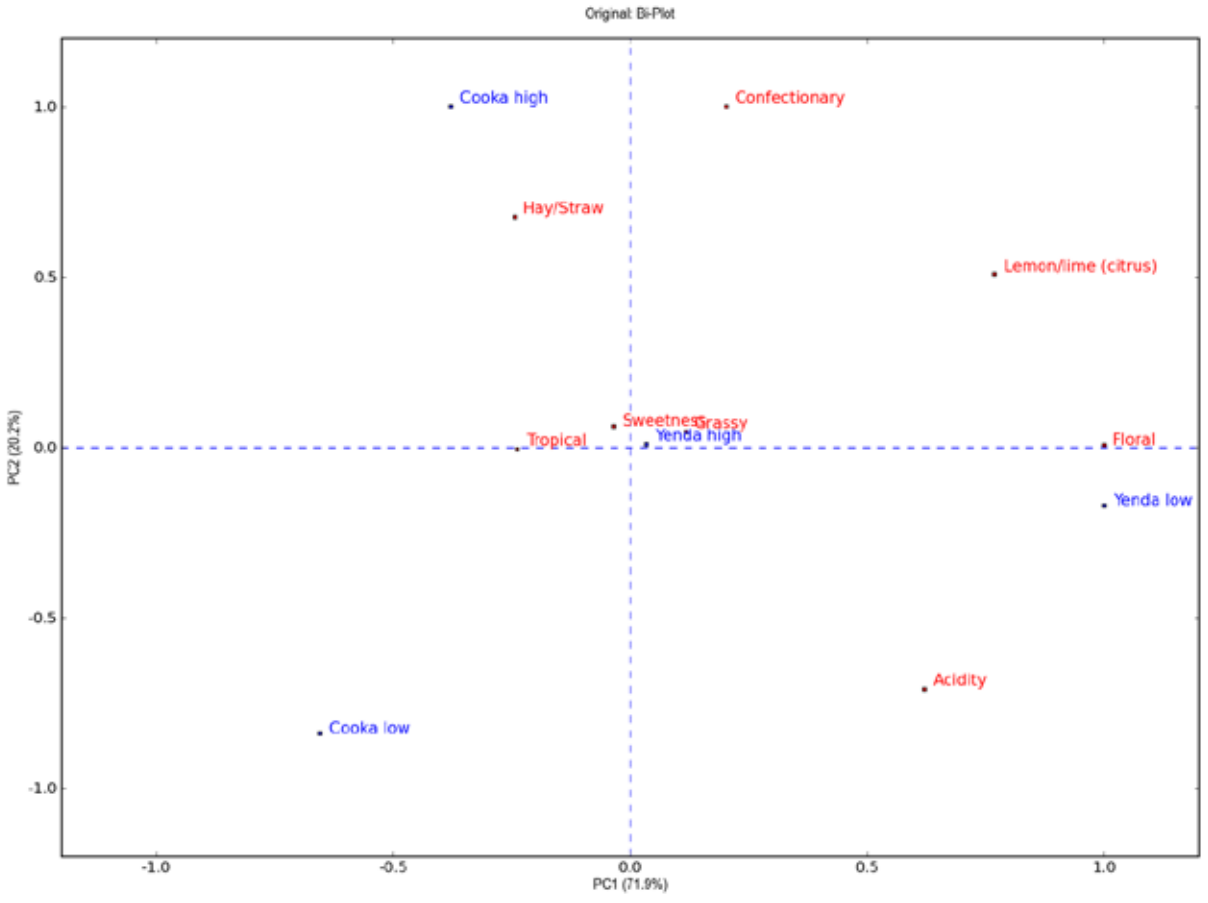


Figure 5.83 Biplot for the Griffith wines 2009

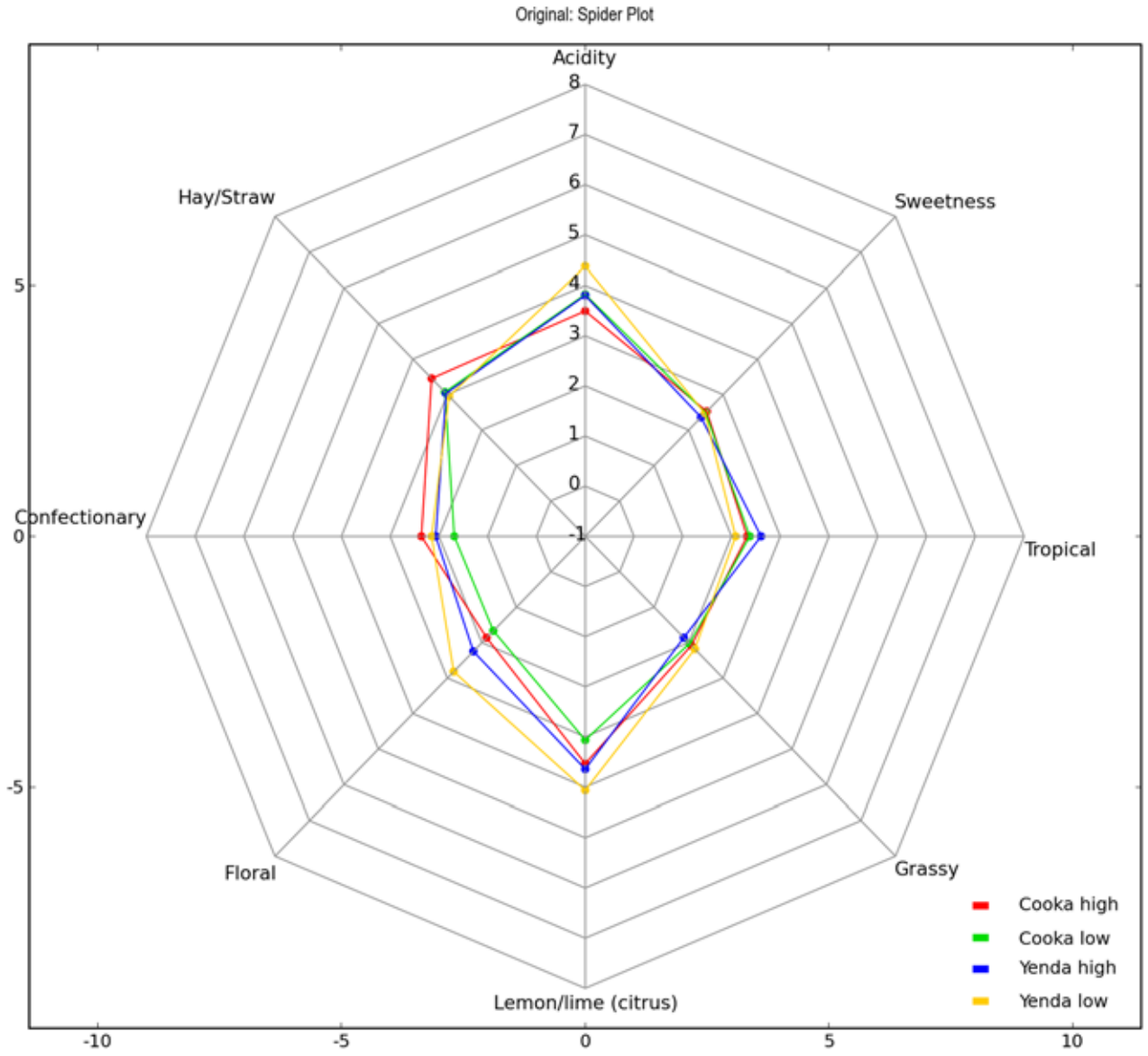


Figure 5.84 Spiderplot of the Griffith wines 2009

Experiment 5.13
Manipulating Semillon wine aroma profile with different strains of yeasts

The aroma profile of the wine is often thought to be strongly influenced by a range of local or site factors known as terroir. These include the climate, the soil, microclimate and, also, human factors including vineyard and cellar practices. However different yeasts can also influence the final aroma profile of wine. This experiment investigated how the flavour of Riverina Semillon could be manipulated with different yeasts to satisfy different market segments.

Material and methods

Amongst the three vineyards selected for the crop load trial (*Experiment 5.12*), vineyard H was also used for this yeast trial, over the same period of time from 2007 to 2010. Riverina Semillon traditionally has been sold as a blend, except for the premium Botrytis Semillon. This trial was hoping to create a market for Semillon as a varietal. In order to improve these flavours, neutral and flavoured yeasts were compared, as well as the effect of enzyme addition.

Selection of wine additives

Lallzyme Cuvée Blanc was selected as it is one of the most commonly used macerating enzymes in the white winemaking. It consists of a blend of pectinases and glycosidases. It is used to enhance aromatic complexity, provide gentle juice extraction and fast clarification after pressing. The recommended dose is 20 g t^{-1}.

DV10 and EC1118, neutral yeasts that do not add extra flavour, are renowned to be robust, efficient at low temperature and under stressful conditions, such as low nutrient level and high alcohol. QA23 is also a robust yeast with low nutrient requirements that is recommended for the production of fresh, fruity white wines. It enhances aromas of terpenic varietals and can develop the varietal passionfruit character typical in Sauvignon Blanc. Similarly, VIN13 is recommended for the production of aromatic white wines, enhancing volatile thiol aromas and producing esters to enhance the fruity characters of the wine.

Harvest and winemaking

About 1600 kg of fruit was harvested, from the control (67%) and from the low cropping (33%). At the experimental winery, high and low treatments were mixed together, and two batches of grapes were separated. To one batch, the enzyme was added at 0.02 g kg^{-1} before crushing; with no addition applied to the second batch. Each batch was split again into eight, two per yeast investigated (*Figure 5.87*). The rest of the winemaking followed the protocol outlined in *Figure 5.80*.

Table 5.48 Choice of yeasts used

Yeast	DV 10	EC 118	VIN 13	QA 23
Characteristics	neutral	neutral	aromatic	aromatic
Amount added (g L^{-1})	0.3	0.3	0.3	0.3

Wine tasting

Duo-trio tests or difference tests: each panellist is presented with a series of three glasses. One glass is labelled as the reference, the other two glasses, labelled with random numbers, contain different wines to be tested. The reference is actually a repeat of one of the two wines tested. The panellist is asked to determine which wine is the same as the reference. A statistical test is then applied to the whole panel to check if the panel can pick the difference between the wines.

Preference test: after the difference test, another simple question is asked as which wine each panellist prefers.

Descriptive analysis (DA): for this test, a series of descriptors typical for Semillon wines was determined by a panel of experts and winemakers. The NWGIC panel was then trained on typical wines with either low or high occurrence of a certain descriptor to rate each aroma on a scale from 0 (absent) to 9 (intense). The wines from the trial were then tested the same way and rated for each of the descriptors. Principal component (PC) analysis was then applied to the results to identify the descriptors that separated the best the different treatments.

Statistical analysis

The data were compiled and manipulated in the data base Excel 2002 SP3 and further analyzed using the graphic program Sigma plot (version 8.0). The statistical analysis was conducted by using Genstat release 11.1 (Rothhamsted Experimental Station, Harpenden, Herts, United Kingdom). Analysis of variance (ANOVA) and restricted maximum likelihood (REML), for unbalanced data set, were used as statistical tests; Fisher's LSD test was used to interpret differences in means at $P<0.05$.) Principal components test (PC) was used for the sensory analysis.

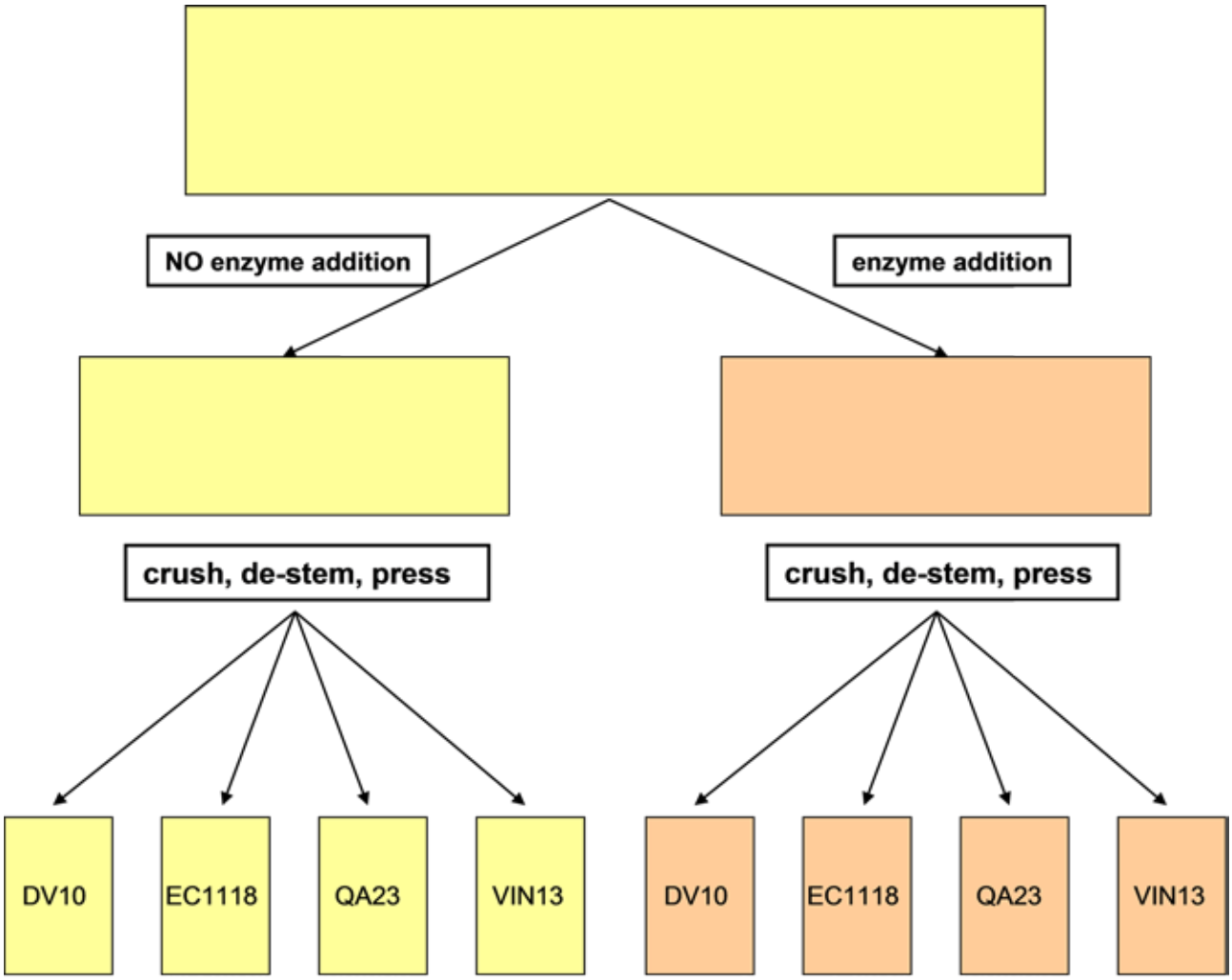


Figure 5.85 Protocol before fermentation

Results and discussion

Difference test

We checked the repeatability of the wines first by comparing the two replicates. The wine replicates could not be differentiated (*Table 5.49*), which means that either replicate could be used for the rest of the tasting without bias.

Treatment analysis

The difference between the treatments was tested over two seasons. In the first season (2008), there was no difference due to the enzyme use. Furthermore, only DV10 and VIN13 could be differentiated (*Table 5.50*).

However, in 2009, differences were significant (*Table 5.51*). An effect of the enzyme addition was observed with the yeast VIN13. DV10 was found to be different from QA23; while VIN13 was strongly different from both EC1118 and QA23.

This small study, when taken in conjunction with results from *Experiment 5.12,* confirms that the ability

Table 5.49 Difference test between wine replicates (example of 2009 vintage). NS: non significant; * p<0.05; ** p<0.01; *** p<0.001

Yeast	Comparison (reps)	Same (correct)	Total no. of people	p-value
EC1118	1 vs. 2	10	15	0.151^{NS}
EC1118 + E	1 vs. 2	8	15	0.500^{NS}
DV10	1 vs. 2	8	15	0.500^{NS}
DV10 + E	1 vs. 2	9	15	0.304^{NS}
QA23	1 vs. 2	8	16	0.598^{NS}
QA23 + E	1 vs. 2	8	16	0.598^{NS}
VIN13	1 vs. 2	5	16	0.962^{NS}
VIN13 + E	1 vs. 2	9	16	0.402^{NS}

Table 5.50 Difference test between treatments for 2008 vintage (NS: non significant; * p<0.05; ** p<0.01; *** p<0.001)

Yeast	Same (correct)	Total no. of people	p-value
DV10 and DV10 + E	9	15	0.304^{NS}
EC1118 and EC1118 + E	7	15	0.696^{NS}
QA23 and QA23 + E	10	15	0.151^{NS}
DV10 and EC118	10	14	0.090^{NS}
DV10 and QA23	7	14	0.605^{NS}
DV10 and VIN13	11	14	0.029*
EC1118 and QA23	9	14	0.212^{NS}
EC1118 and VIN13	8	13	0.291^{NS}
QA23 and VIN13	7	13	0.500^{NS}

Table 5.51 Difference test between treatments for 2009 vintage (NS: non significant; * p<0.05; ** p<0.01; *** p<0.001)

Yeast	Same (correct)	Total no. of people	p-value
DV10 and DV10 + E	11	15	0.059^{NS}
EC1118 and EC1118 + E	11	15	0.059^{NS}
QA23 and QA23 + E	5	14	0.910^{NS}
VIN13 and VIN13 + E	11	14	0.029*
DV10 and EC118	8	15	0.500^{NS}
DV10 and QA23	12	15	0.018*
DV10 and VIN13	10	15	0.151^{NS}
EC1118 and QA23	11	15	0.059^{NS}
EC1118 and VIN13	13	15	0.004**
QA23 and VIN13	11	14	0.029*
DV10 + E and EC1118 + E	9	15	0.304^{NS}
QA23 + E and VIN13 + E	13	14	0.001***

of some yeasts, such as QA23 and VIN13, to modify the aroma profile of Riverina Semillon is greater than any effect due to 33% or even 50% reduction in crop load. The addition of enzyme before pressing mostly did not affect the final wine profile.

Experiment 5.14
Vineyard and Semillon wine styles in the Hunter Valley

Material and methods

Vineyard selection

Semillon wines from across the Hunter Valley region were assessed by the winemakers and then by a trained panel from the NWGIC. Four types of wines were described and a typical wine from each type was kept as a reference. The fruit came from seven Semillon blocks across five vineyards surveyed. All blocks were colour coded for anonymity. Three (3) replicates were randomly spread in the blocks, with one replicate including twenty vines of similar size and vigour over four rows. No vineyard treatments were applied at any stage.

The blocks were planted from 1908 to 1982 (*Table 5.52*), so all vines were well established and mature. They were own rooted, and, for most of the vineyards, the cuttings were sourced around the region, but clone were not known. The trellis system consisted of VSP, single cordon on five (5) blocks and double cordon on the other two (2). The row orientation was mostly east-west.

Five of the blocks had drip irrigation, mainly used during dry seasons; however, the other two blocks were dry grown. Harvest and pruning were mainly done by hand, with the exception of one block. Cane and spur pruning were performed equally across the blocks (*Table 5.52*).

Three weather stations across the Hunter Valley collected climatic data for all seven blocks. Rainfall was recorded daily. Minimum and maximum air temperatures, as well as air pressure, solar radiation, and wind speed and direction were collected hourly. Relative humidity was recorded every hour whereas evapotranspiration (ETP) was calculated daily.

The soil was also assessed through soil coring to about 150 cm deep, in the middle of each trial. The samples were sent to CSBP Ltd in Perth (WA) for analysis. The colour and texture of the soils, including the amount of gravel, were assessed. The composition of the soil was also measured: total nitrogen, organic carbon using the Walkley-Black method, nitrate and ammonium, phosphorus via two methods (Olsen and Colwell), exchangeable cations, conductance and pH (in water and calcium chloride). Finally, soil moisture was monitored throughout the experiment.

Vine measurements

Measurements were made over three (3) growing seasons, from 2007 to 2010.

Vegetative growth and vigour were followed from budburst to leaf fall, whereas berry growth and composition were measured from veraison to harvest.

To measure shoot growth one shoot from every second vine of each replicate was randomly selected and tagged after budburst. Shoot length was measured weekly from budburst to veraison, and then monthly until leaf fall.

Vigour: pruning weight was assessed after leaf fall, around June.

To assess berry growth and composition, 100 berries were collected from each replicate at veraison and at harvest. Berries were collected randomly from top, middle and bottom of the bunches and from both sides (Iland). After collection, they were placed in an esky for transport back to the lab. In the laboratory berries were weighed, and then juiced for further

Table 5.52

Colour	Red	Green	Blue	Orange	Pink	Brown	Black
Location	Lower Hunter	Lower Hunter	Lower Hunter	Lower Hunter	Lower Hunter	Upper Hunter	Upper Hunter
Year of plantation	1982	1976	1946	1923	1908	1960s	1960s
Clone	Own roots, unknown clone (mainly local mass selection)						
Trellis system	VSP						
	single cordon	double cordon	single cordon	single cordon	single cordon	single cordon	double cordon
Irrigation	drip	drip	drip	none	none	drip	drip
Row orientation	E-W	N-S	E-W	E-W	NE-SW		
Row / vine spacing	4.6m 1.5 m						
Pruning	cane	spur	spur	cane	cane	spur	cane
Harvest / pruning	hand-pruning						

composition analysis. The sugar content (TSS) was measured with a bench refractometer (ATAGO PR-101, Tokyo, Japan) and juice pH and titratable acidity (TA) were measured with an automatic titrator (Titrando System LMWI 40-15, Metrohm Ltd, Herisau, Switzerland).

After filtering the juice, the phenolic composition was also assessed by spectrometry (UV-1700 PharmaSpec, Shimadzu Scientific Instruments) at 280 nm, 320 nm and 420 nm, after Illand:

- total phenolics (a.u.) $=A_{280}-4$
- total hydroxycinnamates (a.u.) $=A_{320}-.4$
- total flavonoids (a.u.) $=(A_{280}-4)-0.66\times(A_{320}-1.4)$
- estimate of brown pigments (a.u.) $=A_{420}$

Extra measurements were taken at particular stages. Active buds were counted at budburst; shoot number was assessed at flowering and bunch number was assessed at harvest. The leaf area of all tagged shoots was measured at flowering, using standardised leaf sizes collected from a typical plant of Semillon (see Appendix 1). The selected leaves were scanned through a special scanner (Leaf Area Meter, Li 3100C, LI-COR Environmental) that delivered each of their area in cm^2. The method consists of stripping the leaves of a two shoots from buffer vines. The leaves are then ranked by sizes, with a minimum of 8 levels usually required. The leaves are also scanned to get the precise leaf area of each one. Adding the area of all the leaves will give an estimation of the leaf area of a shoot. This area is then multiplied by the shoot number determined previously to get an estimation of the leaf area per vine.

At veraison, the canopy density was assessed using the point quadrat method. Furthermore, petioles were collected at both flowering and veraison, then dried and ground for nutrient analysis.

All three replicates were harvested on the same date. The sugar levels at harvest may differ, but to avoid introducing an extra parameter into the winemaking, sugar and acidity levels were adjusted to similar values for all treatments in the winery.

At harvest, as well as counting the bunches per vine, weight of fruit per vine was measured. Finally, the pruning wood from each vine was weighed. Pruning was performed manually after leaf fall (around June) on the same vines as per vigour measurements.

Winemaking

At harvest, experimental wines were made separately from the three replicates on each vineyard. Fruit was hand picked and put in 20 kg foam boxes with SO_2 for transport back to the experimental winery of TAFE in Kurri Kurri. It was pressed on arrival and then the must was stored in the cool room until transport back to Wagga Wagga where it was inoculated for fermentation. 40 kg of fruit per replicate were used. At arrival at the experimental winery in Wagga Wagga, the wines were adjusted in sugar and acidity to similar level to avoid confounding effects during fermentation.

Experimental wines were made following the protocol (*Figure 5.80*). The neutral yeast DV10 was chosen, as it is appropriate for white winemaking from fruit from this region, including Semillon.

Wine tasting

Duo-trio tests or difference tests: each panellist is presented with a series of 3 glasses. One glass is labelled as the reference, the other two glasses, labelled with random numbers, contain different wines that are tested. The reference is actually a repeat of one of the two wines tested. The panellist is asked to determine which wine is the same as the reference. A statistical test is then applied to the whole panel to check if the panel can pick the difference between the wines.

Preference test: after the difference test, another simple question is asked as which wine each panellist prefers.

Descriptive analysis (DA): for this test, a series of descriptors typical for Semillon wines was determined by a panel of experts and winemakers. The NWGIC panel was then trained on typical wines with either low or high occurrence of a certain descriptor to rate each aroma on a scale from 0 (absent) to 9 (intense). The wines from the trial were then tested the same way and rated for each of the descriptors. Principal component analysis (PC) was then applied to the results to identify the descriptors that separated the best the different treatments.

Statistical analysis

The data were compiled and manipulated in the data base Excel 2002 SP3 and further analyzed using the graphic program Sigma plot (version 8.0). The statistical analysis was conducted by using Genstat release 11.1 (Rothhamsted Experimental Station, Harpenden, Herts, United Kingdom). Analysis of variance (ANOVA) and restricted maximum likelihood (REML), for unbalanced dataset, were used as statistical tests; Fisher's LSD test was used to interpret differences in means at $P<0.05$.) Principal

components test (PC) was used for the sensory analysis.

Results

Wine tasting

Previous research by The National Wine and Grape Industry Centre into Hunter Valley Semillon has sought to differentiate the sensory characteristics of wines made between seven experimental sites. There has been little work linking anything other than basic winemaking and microbiology (especially yeast) to flavour, so this has been one of the first attempts to determine how site might influence flavour in Semillon wines.

Higher mean floral scores were associated with the pink block for both the 2008-09 and 2009-10 seasons. For the 2008-09 season, they were significantly higher than those for the green block, but no other statistically significant difference in floral scores between specific sites was detected for either season.

Higher mean hay/straw scores were associated with the green block for the 2008-09 season. These scores were significantly higher than those for the black block. No other statistically significant differences in hay/straw scores between specific sites were detected.

There were no statistically significant differences in mean acidity, confectionary, citrus, grassy or sweetness scores between vineyards for either of the seasons.

The experimental wine from the red block was not included in the 2008-09 data, as not enough fruit could be harvested. Similarly, the experimental wine from the green block was not included in the 2009-10 data.

For the rest of this report, we will mainly focus on three blocks that showed the most different aroma profiles: pink, green and black.

Berry growth and ripening

Berry weight

Berry weight increases from veraison to harvest are shown in *Figure 5.86*. The pink block showed the most linear increase. It also had the second heaviest berries throughout the sample collection, behind orange that is located nearby. On the other hand, the berries on the green block were the lightest throughout the study. Finally, berries of the black block were heavier than the average from mid-January on.

Berry composition

Here again, the sugar content increased constantly throughout berry development for all seven blocks, with very similar patterns (*Figure 5.87*). Berries on the pink block were the sweetest throughout the sampling, except for the first date where the ones of

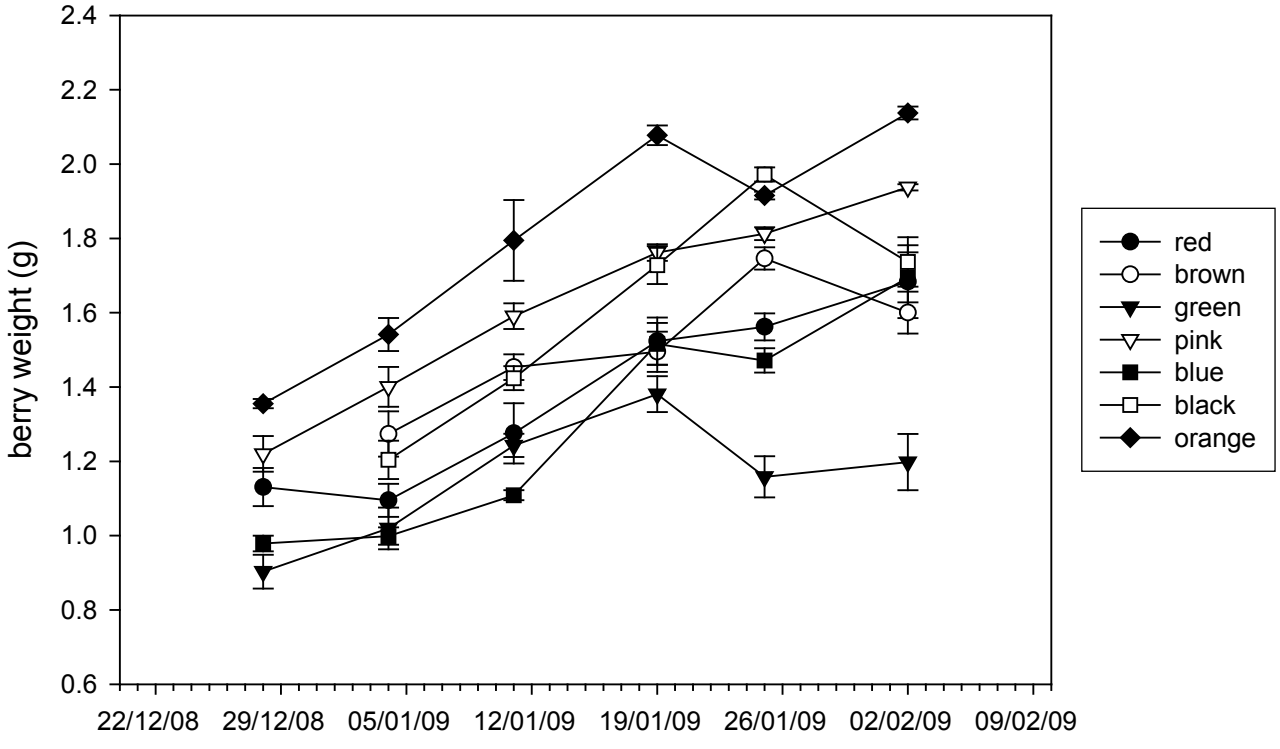


Figure 5.86 Effect of site on berry development.

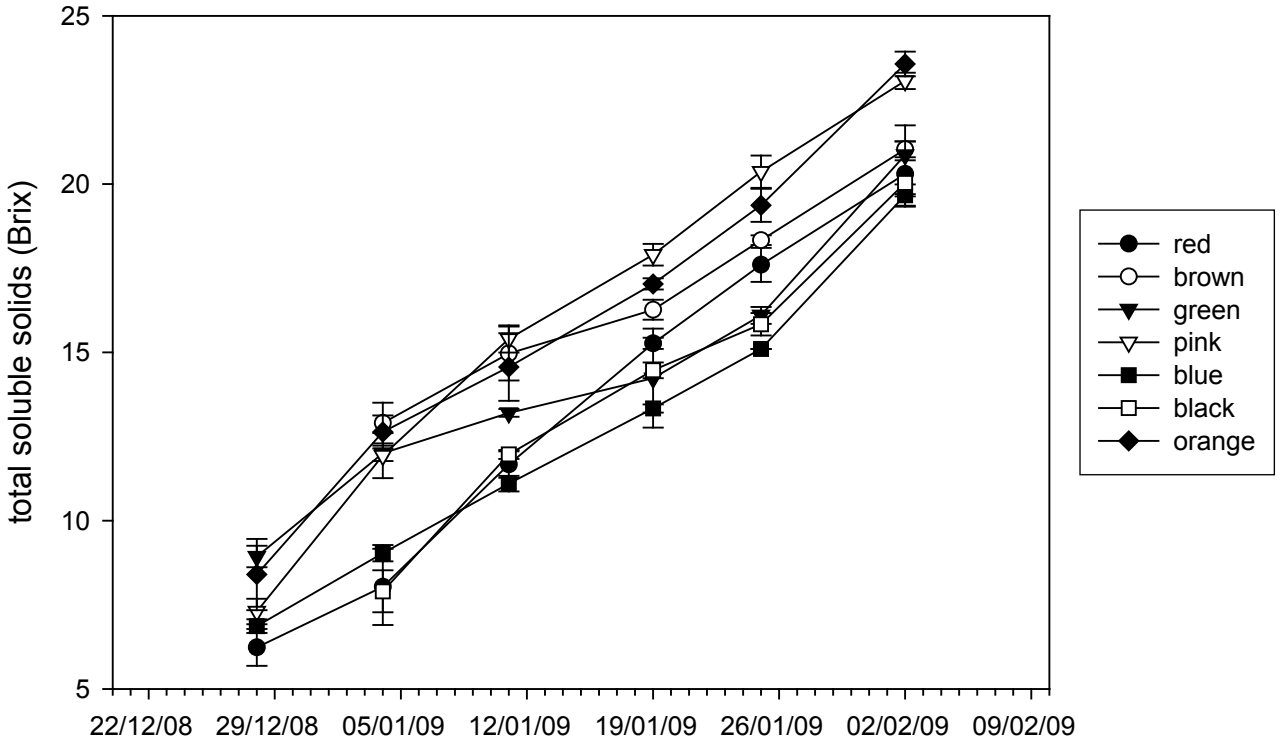


Figure 5.87 Effect of site on berry sugar content.

the green block had higher sugar content. However, the increase in sugar for the green block slowed down over the next month of berry collection. Finally, berries of the black block seemed less sweet than the average, but that would be due to their later ripening season.

The pH of the berries also increased throughout the development and across the blocks (*Figure 5.89*). The green and pink block had the highest pH throughout the study. On the other hand, berries of the black block showed the lowest pH throughout.

In contrast pH, titratable acidity (TA) decreased throughout berry development and across the seven blocks (*Figure 5.89*). Berries of the green blocks had the lowest TA throughout the study; on the other hand, berries of the black block had the highest TA. The pink block also had low TA, the decrease being slower than most of the other blocks.

Phenolic composition

The variability in the phenolic composition was very important between dates. This could be partly due to the sample storage.

The variability in brown pigments was very important, except for the brown and black blocks, both located in the Upper Hunter (*Figure 5.90*). Both showed low results, which could be due to a later ripening season. For both the green and the pink blocks, there was an unexpectedly high variation between sampling dates.

Across the blocks, the phenolic composition of the berries decreased dramatically early in the ripening, and then plateaued (*Figure 5.91*). The black block had the highest total phenolic composition, followed by the brown one. Here again, the later ripening season would have a strong impact. The pink block also had a high phenolic content; whereas the green block started at a lower point and its rate of decrease was much faster.

Total flavonoids followed an exact same trend as the total phenolic compounds (*Figure 5.92*). The black and brown blocks had the highest content, followed by the pink block. The green block was much lower with an initial sharper decrease.

The variability in berry hydroxycinnamates the results was much higher between sampling dates (*Figure 5.93*). The relation between the blocks remained similar to the one for total phenolics and flavonoids.

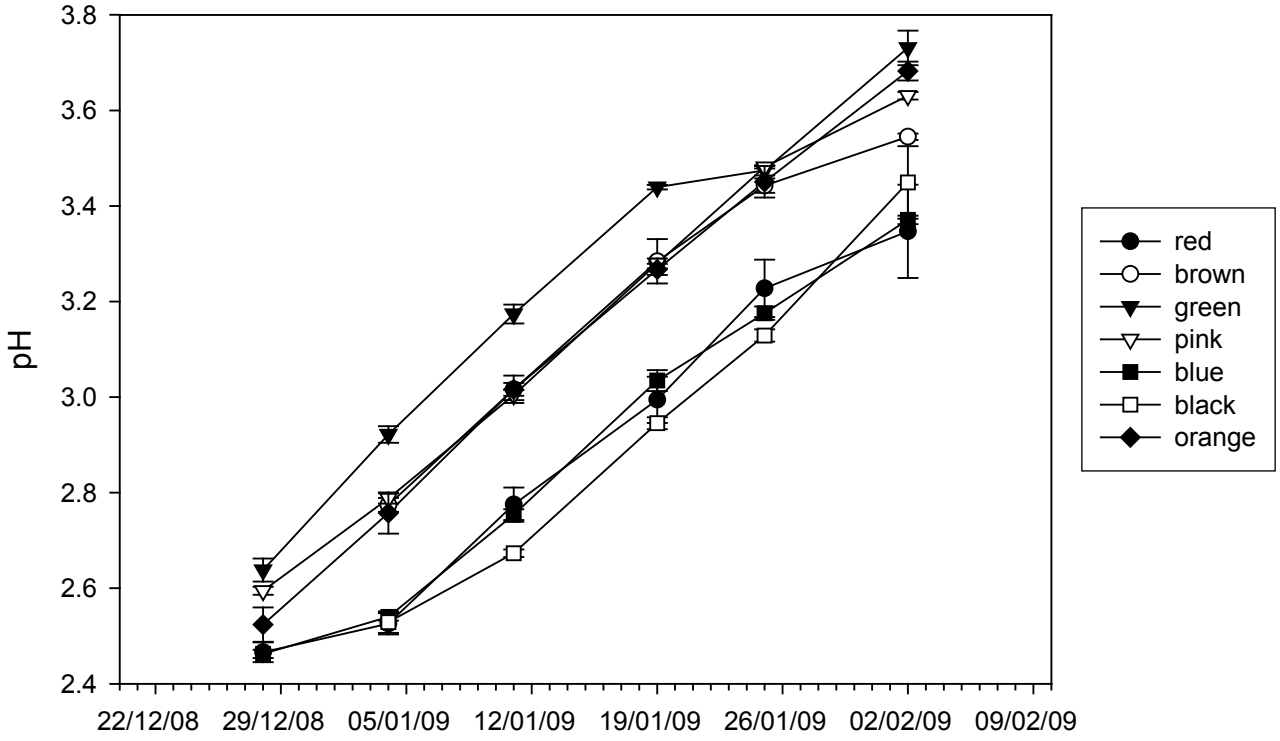


Figure 5.88 Effect of site on berry pH.

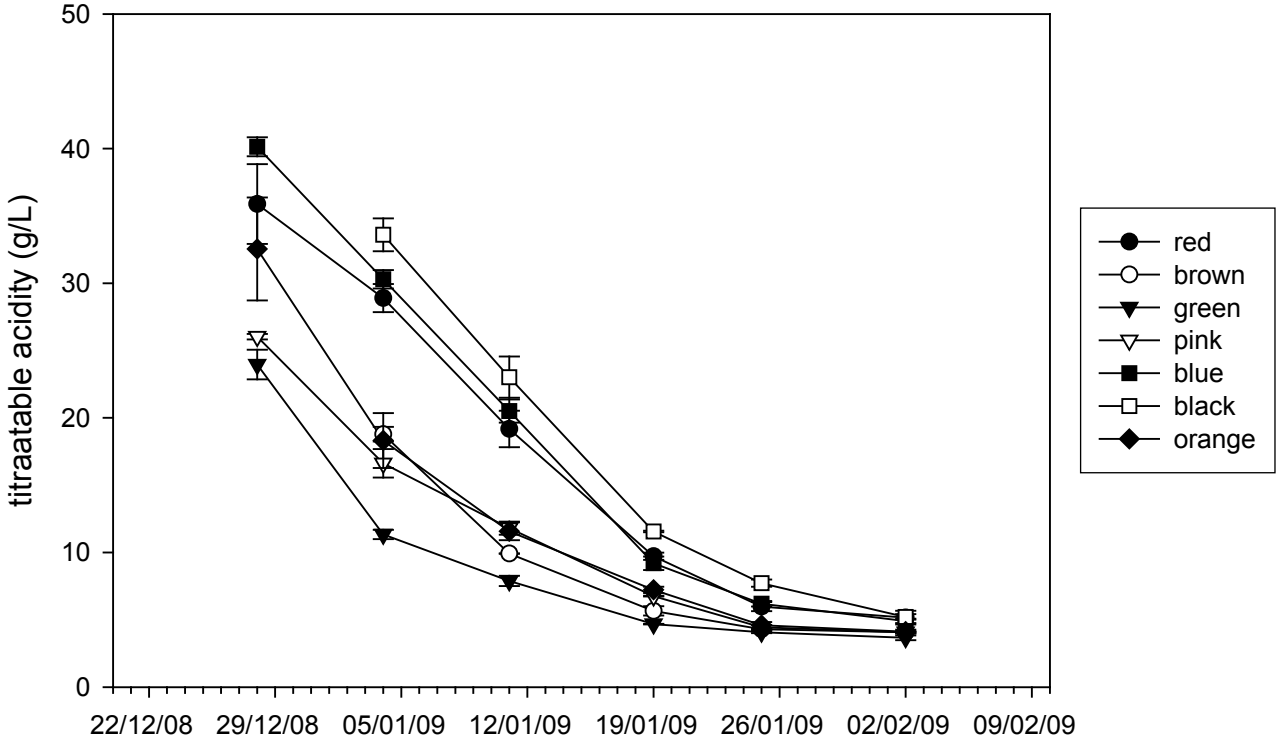


Figure 5.89 Effect of site on berry titratable acidity.

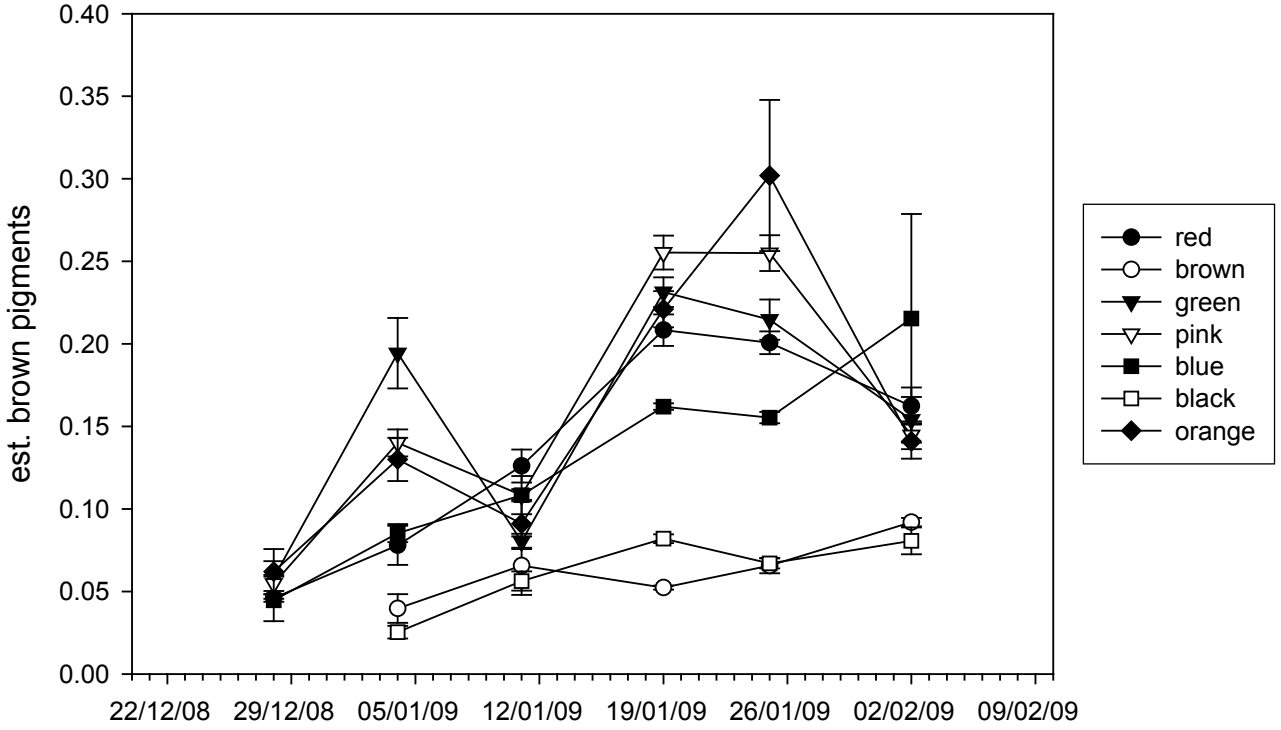


Figure 5.90 Effect of site on berry brown pigments.

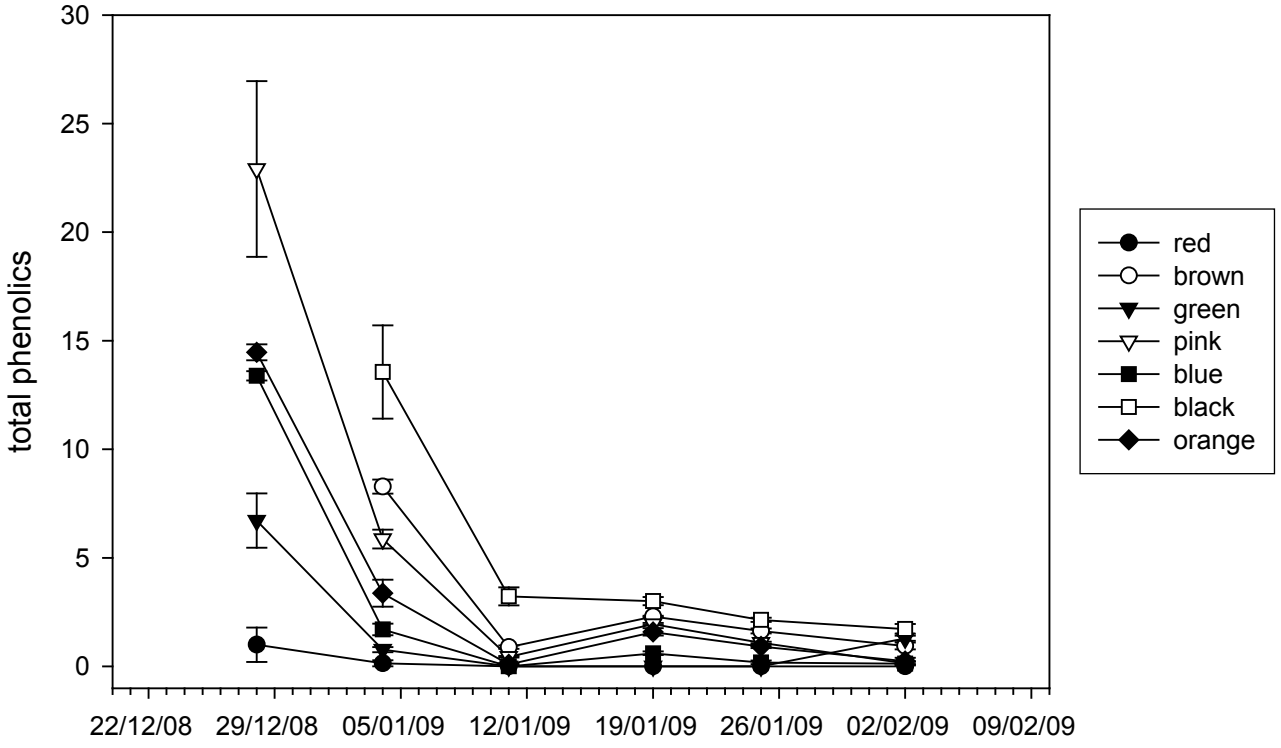


Figure 5.91 Effect of site on berry phenolic composition.

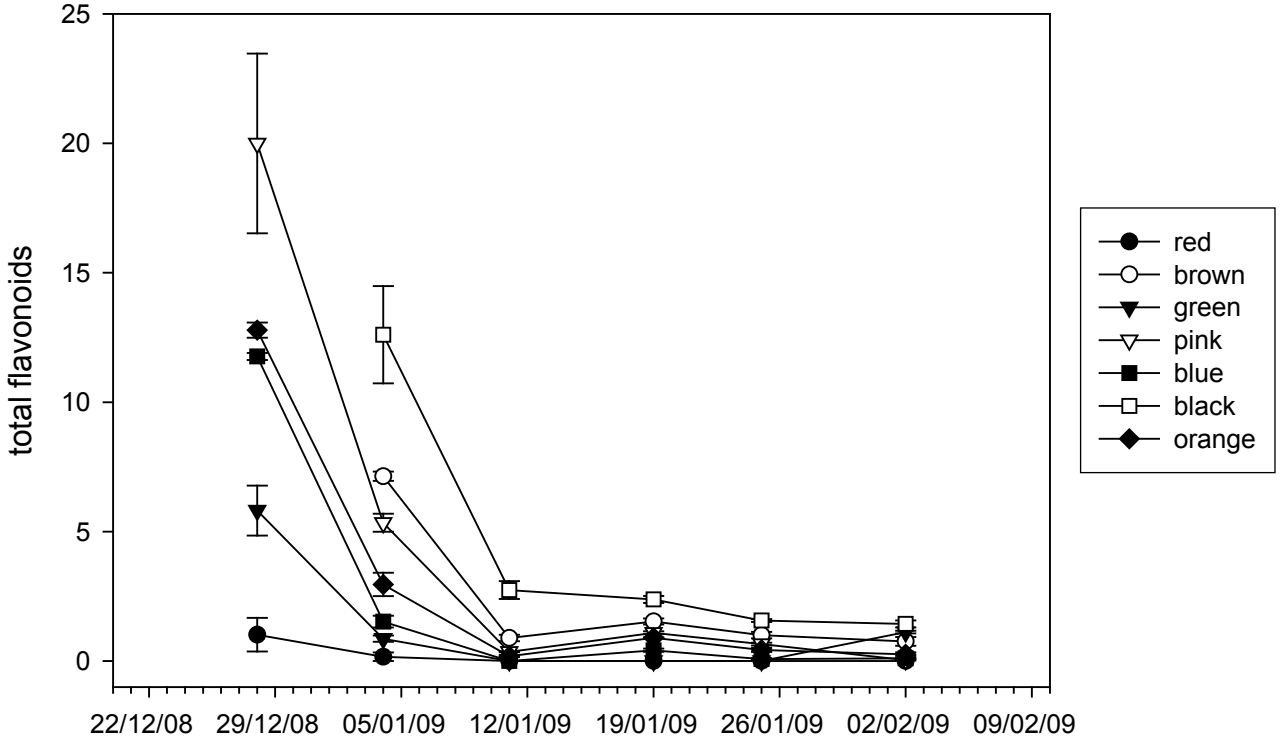


Figure 5.92 Effect of site on berry flavonoids.

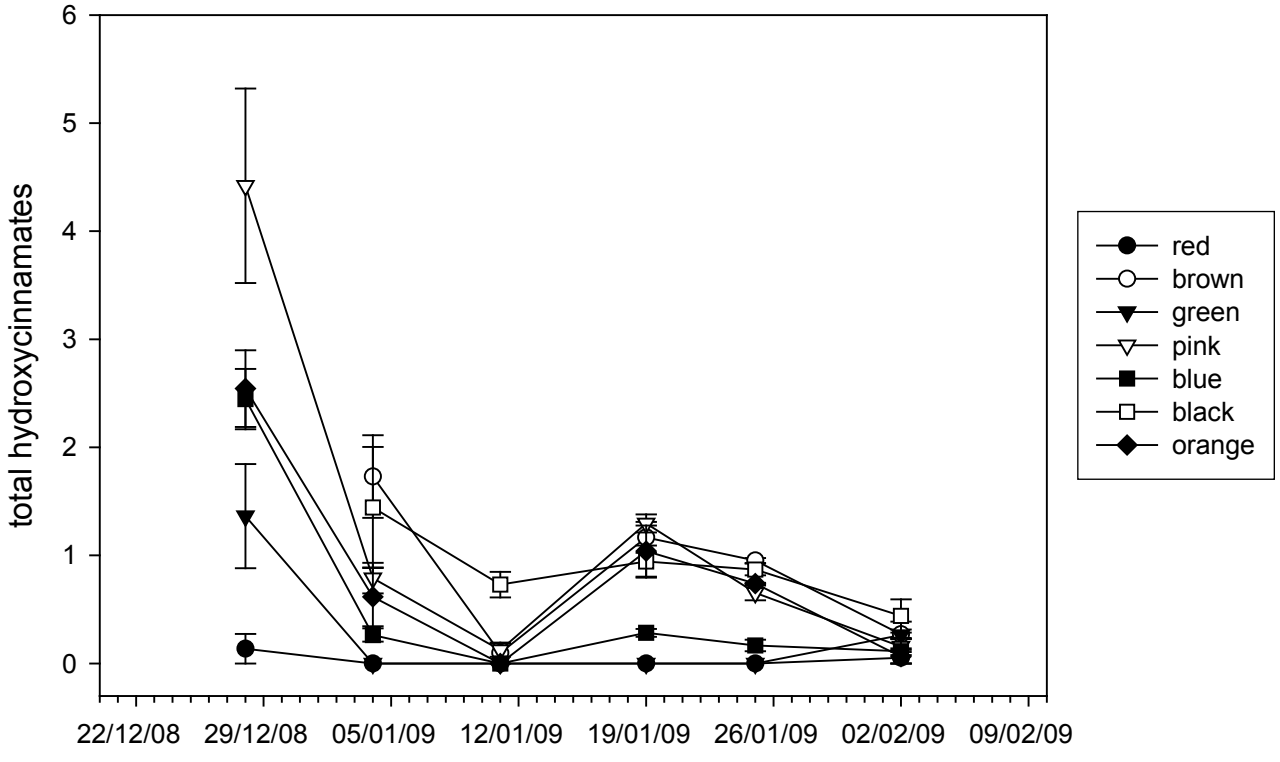


Figure 5.93 Effect of site on berry hydroxycinnamates.

Physiology

The variability between the seven blocks was very significant for all components measured (*Table 5.53*). The red block showed the lowest results for most parameters measured, whereas brown showed high yield components.

Regarding the three blocks that showed difference in their aroma profile, black had high results throughout; similarly green had high results, especially in bud and reproductive organs at both flowering and harvest. On the other hand, the pink block had the fewest buds and, therefore, shoots.

Environmental conditions

Soil

Soil cores have been taken in each of the blocks studied. On most of the blocks, the content of sand was largely predominant (*Table 5.54*): about twice the content of clay and four times the one of silt; however, two blocks showed a different pattern. On the black block, the content of the three components were very similar. On the other hand, on the green block, the content of clay was slightly superior to the one of sand, leaving about 20% silt. These two blocks have heavier soil and more capacity to retain water, due to their amount of clay. The orange and brown blocks also had heavier clay contents in their top layers of soil, whereas deeper, a layer of loam was found in both of them. For the other blocks, the variation in soil texture was not as pronounced.

Here again, two blocks had significantly higher conductivity compared to the average 0.18 dS m^{-1}, namely red and green, 0.29 dS m^{-1} and 0.40 dS m^{-1} respectively (*Table 5.55*).

Brown and green blocks have the highest soil pH, over 7.2 in calcium chloride and over 8.0 in water. However, in the brown block, the pH increased with the depth; whereas in the green block, the pH was lower in the deeper soil. On the other hand, pink and orange blocks had the lowest pH of the lot, below 6.0 in calcium chloride and below 7.0 in water. For both blocks, the top 50 cm had the lowest pH, between 4.0 and 4.8 in calcium chloride.

There was no difference in the aluminium content through the blocks (*Table 5.56*). Three blocks had very high total exchangeable cations, namely green, brown and black. The green block was the highest for magnesium, potassium and sodium. Both brown and black were high in calcium, but brown was high in magnesium and low in potassium; whereas black was high in potassium and low in sodium. Orange on the other hand had the lowest total exchangeable cations, being the lowest in calcium, magnesium and sodium. The other blocks were intermediate, except for relatively high sodium in red, low sodium in blue and low calcium in pink.

Table 5.53 Effect of site on yield components (BB: Budburst, F: Flowering, H: harvest, LF: leaf fall)

	Red	Green	Blue	Pink	Orange	Brown	Black	p value
Bud number (BB)	16.11^{c}	41.91^{f}	24.51^{d}	13.46^{a}	14.92^{b}	36.84^{e}	53.03^{g}	<0.001
Shoot number (F)	16.39^{b}	31.90^{d}	26.92^{c}	14.09^{a}	16.48^{b}	39.70^{f}	35.13^{e}	<0.001
Inflorescence number (F)	8.97^{a}	43.22^{e}	31.73^{d}	11.34^{b}	17.94^{c}	46.97^{f}	30.85^{d}	<0.001
Bunch number (H)	12.34^{a}	48.85^{f}	40.03^{d}	17.54^{b}	22.75^{c}	51.95^{g}	45.35^{e}	<0.001
Yield, kg (H)	1.64^{a}	7.32^{e}	6.54^{d}	2.82^{b}	5.02^{c}	8.72^{g}	8.40^{f}	<0.001
Bunch weight, g (H)	122.4^{a}	160.2^{c}	162.4^{c}	151.5^{b}	222.1^{f}	172.0^{d}	182.9^{e}	<0.001
Pruning weight, g (LF)	104.6^{a}	464.8^{c}	472.1^{c}	256.4^{b}	671.1de	690.9^{e}	648.0^{d}	<0.001

Table 5.54 Soil texture (in %) and colour (BR: brown, GR: grey, DK: dark, LT: light)

	Red	Green	Blue	Pink	Orange	Brown	Black	p value
Main colour	BR	BRGR	BRGR	BRGR	BR	DKBR	DKBR	
Clay	28.4^{b}	42.6^{d}	23.3^{a}	30.3^{bc}	24.6^{a}	32.6^{bc}	34.3^{c}	<0.001
Silt	9.5^{a}	19.3^{e}	12.2^{bc}	13.8^{c}	11.7^{b}	16.1^{d}	30.2^{f}	<0.001
Coarse Sand	25.0^{cd}	18.1^{b}	32.3^{e}	21.1^{bc}	30.9^{e}	29.4^{de}	4.4^{a}	<0.001
Fine Sand	37.1^{d}	20.0^{a}	32.2^{bc}	34.9^{cd}	32.8^{bc}	21.9^{a}	31.2^{b}	<0.001

Table 5.55 Soil properties of studied blocks

	Red	Green	Blue	Pink	Orange	Brown	Black	p value
Conductivity	0.29^{d}	0.40^{e}	0.10^{b}	0.16^{c}	0.07^{a}	0.14^{c}	0.10^{b}	<0.001
pH H2O	7.54^{cd}	8.02^{e}	7.27^{c}	6.89^{b}	6.23^{a}	8.72^{f}	7.69^{d}	<0.001
pH CaCl2	6.52^{c}	7.29^{d}	6.52^{c}	5.89^{b}	5.36^{a}	7.54^{d}	6.79^{c}	<0.001

Total of nitrogen was relatively low, less than 0.1% all through the blocks, the brown block only counting for 0.04% (*Table 5.57*). The organic carbon content was very similar for the red, blue, orange and brown blocks, around 0.4%. The black block showed the highest amount with more than 1%, which may be explained by its location on a flood plain.

The green block showed the highest content of ammonium and potassium, as well as the second highest amount of phosphorus and nitrate, respectively 5.08 mg kg^{-1}, 371.2 mg kg^{-1}, 33.1 mg kg^{-1} and 28.1 mg kg^{-1}. The black block was also showing high content of potassium, phosphorus and nitrate, 237.2 mg kg^{-1}, 54.1 mg kg^{-1} and 77.1 mg kg^{-1} respectively, but one the second lowest content of ammonium with 2.00 mg kg^{-1}.

The brown block, on the other hand, had the lowest amount of ammonium, potassium and phosphorus, 1.34 mg kg^{-1}, 96.8 mg kg^{-1} and 5.7 mg kg^{-1} respectively, as well as the second lowest amount of nitrate with 12.2 mg kg^{-1}. The orange and red blocks were also low in most of the elements analysed. The other blocks were intermediate: low in phosphorus, medium in ammonium, and high in nitrate for the pink block.

Weather

The Hunter Valley has temperate, coastal climatic conditions (*Figure 5.94*). Rainfall is spread across the entire year with higher fall in the summer months (January–March), winter (July–August) being relatively dry. The average temperature remained moderate between 10 and 25°C.

The second growing season was overall a lot drier (*Figure 5.95*) than the long-term average (*Figure 5.94*) by about 25%, except for the month of February,

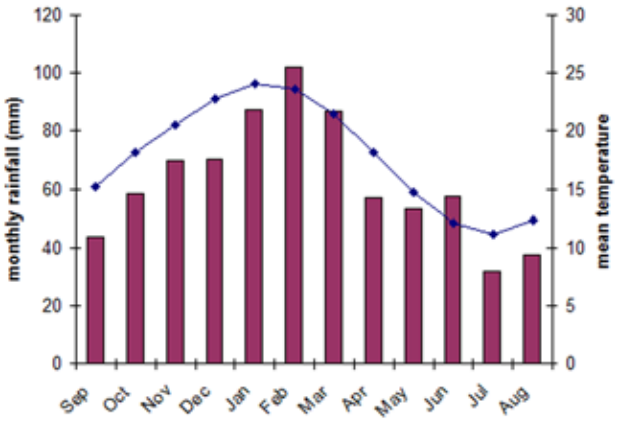


Figure 5.94 Average temperature and rainfall for the Hunter Valley region (long term)

the month at the beginning of which the grape was harvested. The temperature, however, was very similar to the long-term average.

Variability in rainfall was relatively high across the Hunter Valley. The rainfall measured in Cessnock was usually higher than the other two stations. On the

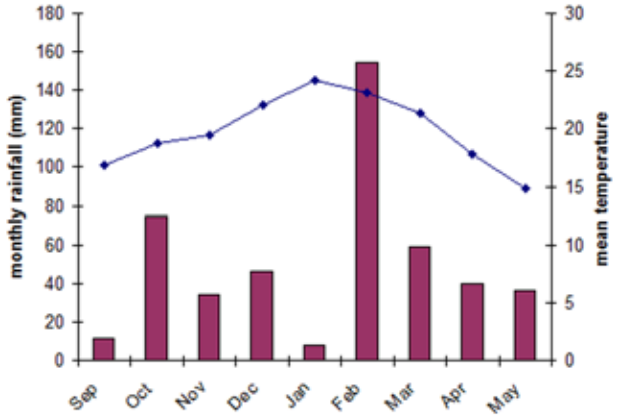


Figure 5.95 Temperature and rainfall for the Hunter Valley region (2008-09).

Table 5.56 Exchangeable cation contents for different sites (meq 100 g-1)

	Red	Green	Blue	Pink	Orange	Brown	Black	p value
Aluminium	0.01	0.00	0.00	0.00	0.19	0.00	0.05	0.068
Calcium	4.33^{b}	12.16^{d}	5.40^{c}	3.02^{a}	2.94^{a}	15.83^{e}	23.98^{f}	<0.001
Magnesium	5.47^{c}	11.62^{f}	3.49^{b}	6.87^{d}	1.88^{a}	10.56^{e}	6.57^{d}	<0.001
Potassium	0.39^{b}	0.84^{e}	0.45^{c}	0.46^{c}	0.42^{bc}	0.26^{a}	0.53^{d}	<0.001
Sodium	2.87^{d}	4.89^{e}	0.77^{b}	2.06^{c}	0.17^{a}	1.98^{c}	0.29^{ab}	<0.001

Table 5.57 Chemical composition of soils from the different sites

	Red	Green	Blue	Pink	Orange	Brown	Black	p value
Organic carbon (%)	0.39^{a}	0.55^{b}	0.40^{a}	0.68^{c}	0.39^{a}	0.42^{a}	1.12^{d}	<0.001
Total nitrogen (%)	0.07^{b}	0.10^{c}	0.07^{b}	0.08^{b}	0.07^{b}	0.04^{a}	0.10^{c}	<0.001
Ammonium (mg kg^{-1})	2.82^{c}	5.08^{e}	3.11^{cd}	3.62^{d}	2.62^{bc}	1.34^{a}	2.00^{b}	<0.001
Potassium (mg kg^{-1})	115.4^{ab}	371.2^{f}	134.1^{bc}	149.5^{cd}	168.5^{d}	96.8^{a}	237.2^{e}	<0.001
Phosphorus (mg kg^{-1})	6.1^{a}	33.1^{c}	11.0^{ab}	20.8^{b}	10.6^{ab}	5.7^{a}	54.1^{d}	<0.001
Nitrate N (mg kg^{-1})	3.4^{a}	28.1^{d}	17.0^{c}	31.1^{d}	3.5^{ab}	12.2^{bc}	77.1^{e}	<0.001

other hand, the average monthly temperature was a lot more constant between the three weather stations (*Figure 5.97*).

Similarly, looking at minimum and maximum temperature across the valley, follow the same pattern. High temperatures are also very consistent between stations; however, the minimum temperature recorded at Cessnock was regularly lower than the one at the other two locations. Pokolbin and Glenbawn had very similar pattern after November.

The weather dataset is incomplete for two reasons. The first one is that the project started mid October 2007 around flowering. We thus missed the beginning of the first season of data collection. Furthermore, in the Upper Hunter, the weather station was installed during the following winter, so that we only have, in theory, two seasons recorded. There were also some complications with the aerial and we only have very little data for the two blocks of the Upper Hunter it covered.

Towards the end of the third growing season, there was some lightening activity and a couple of weather stations were badly damaged; some data were not recorded and some others were lost.

Discussion

Despite there being large differences between site characteristics and, even vineyard management, the experimental wines were only perceived to differ slightly. Differences were too small to be detected by the tasting panel. However, it is possible that some differences may have been masked by the winemaking protocol used. Furthermore, the season had a stronger effect on the final aroma profile than any of the site characteristics.

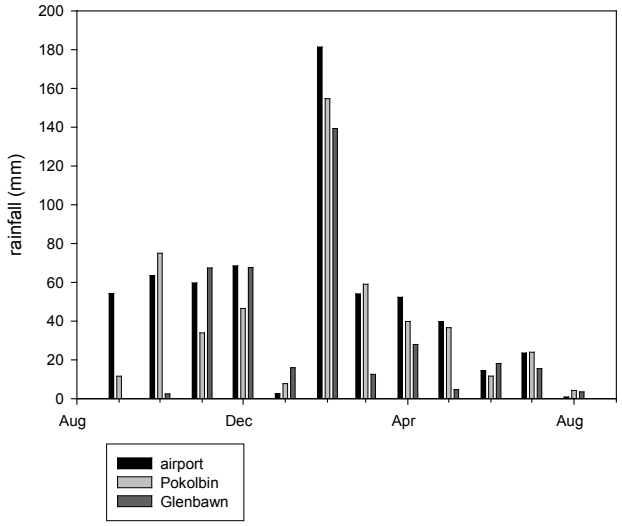


Figure 5.96 Rainfall at 3 weather stations around the Hunter Valley (2008-09).

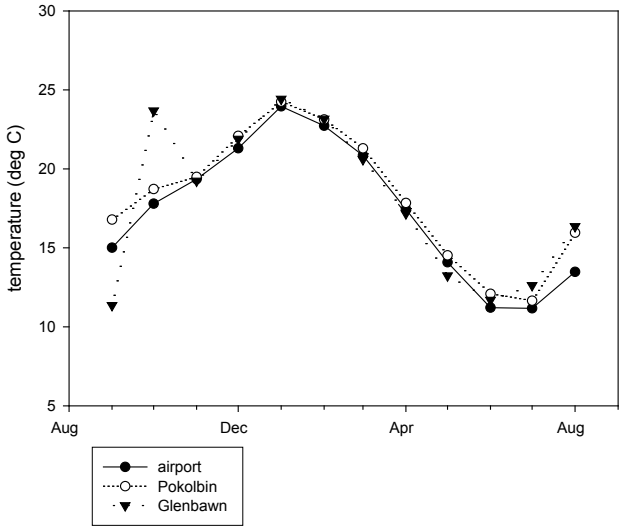


Figure 5.97 Average temperature at 3 weather stations around the Hunter Valley (2008-09).

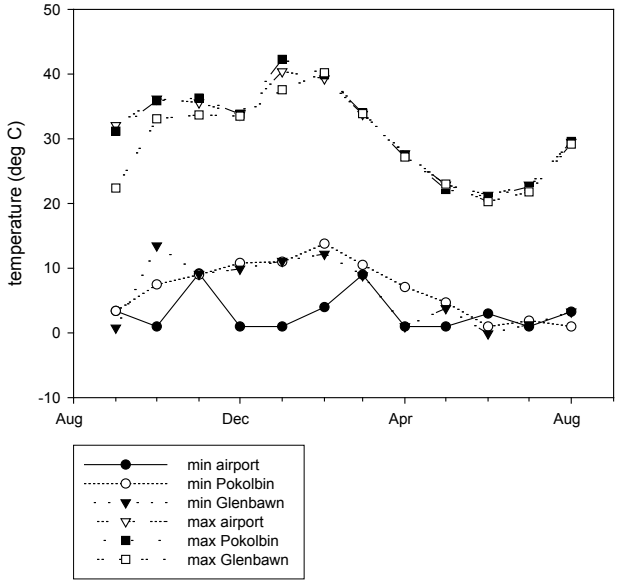


Figure 5.98 Minimum and maximum temperatures at three weather stations in the Hunter Valley (2008-09).

Outcomes/conclusions

Experiment 1: Characterisation of Hunter Valley Semillon

The current study has defined four distinct HVS styles, two of which have been reported previously (Ryan 1992; Clark and Spurrier 2001). Style 4 is the quintessential aged HVS style showing developed characters balanced with lemon-lime freshness. Style 3 is the group of wines that some refer to as lean, grassy with prominent acidity. Wine 15 from this group was the same label, made using similar viticultural and winemaking practices as Wine 10 from Style 4, although four vintages earlier. This provides some validation that wine 15 and others from Style 3 will likely develop into quintessential HVS (Style 4) after several years of cellaring. The identification of Styles 3 and 4 confirm that aging is important to flavour development; further, that claims of austerity when young and richness when aged are supported to some extent.

However, Style 1 had obvious fruit flavour with good acidity balanced with some sweetness. This style clearly illustrates a substantial flavour profile, demonstrating that HVS is not necessarily austere when young. Style 2 also demonstrates a substantial flavour profile based on expressive fruit characters with prominent acidity. Two of the wines from this group were the same labels as represented in style four only four years younger. As was the case with Style 3 wines, this suggests that wines from Style 2 have the potential to develop into typical aged HVS with bottle development. There was no evidence that Style 1 would develop into a Style 4 with age. None of the style four wines had significant levels of residual sugar and all had higher acid levels than Style 1. Style 1 appears to be the category suited exclusively for early-drinking market.

The detection of Style 2, a style of HVS that presents both a substantial sensory profile when young along with the prospect of developing into a typical aged style is significant. Firstly, this discovery refutes the general conception that HVS is austere when young as suggested by some (Robinson 2008). Secondly, the finding indicates that an early-drinking style can be achieved and at the same time be expected to develop into a typical developed profile.

In summary, the current study has shown that a substantial sensory profile can be produced for young HVS which would be suited for immediate consumption. Further, one style was identified that possessed both early drinking appeal and an apparent aging potential. Further research is needed to discover the winemaking and viticultural practices that lead to each of the four styles defined, as well as consumer preference research to determine whether all styles appeal to consumers.

Experiment 2: Consumer preference of Hunter Valley Semillon

Overall the study indicated that fresh fruit characters and acid-sugar balance were important drivers of preference for HVS. To a lesser extent, developed characters were important to a specialised segment of the market. An avenue for Hunter Valley Semillon to become even more approachable to consumers, with a new focus on an early drinking style, has also been discussed. It is hoped that quintessential HVS, "one of Australia's great gifts to the world of wine", will become more popular with consumers in the long term, as they are introduced to the early drinking style and graduate to other styles.

Experiment 3: Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines

The influence of the level of critical tasting and industry experience on sweet preference for consumer categories has not been previously reported in the scientific literature. This investigation demonstrated that each consumer category based on knowledge, experience and involvement in the wine industry, preferred different levels of residual sugar and sweetness in HVS. Experienced consumers preferred wines with less added glucose than did the novice group and significant differences existed at high glucose additions (32.0 g L^{-1}). The current experiment has confirmed the necessity to control for level of experience within a general consumer group, not just between consumers and 'experts'. Results from this investigation suggest that a higher than typical residual sugar level may increase consumer acceptance of HVS for novice and expert consumers alike, but may also alter winemaker's acceptance of specific styles of this wine. The use of paired comparison tests has also enabled a quantitative basis for winemakers to produce HVS with a range of residual sugars appropriate to the acceptance of the consumer. By choosing test wines that were not typically associated with overt sweetness, the most likely conditions to find differences between 'experts', 'novices' and winemaker consumers have been fulfilled. Having established differences do

Theme outcomes

	Outcome	Achieved
1	Sustaining and improving Semillon grape and wine quality by utilising vine reserves during grape maturation..	Y
2	Clarifying the link between viticultural and winemaking practices and economic value of the product..	Y
3	Knowledge of the impact of vineyard modulation or winery modulation on critical aroma and flavour compounds..	Y
4	Enhanced understanding of consumer awareness of the Semillon wine styles leading to increased market acceptance of the varietal wine.	Y
5	Improving commercial premium wine quality of Semillon while sustainably maintaining or increasing grape yields.	N
6	Managing seasonal consistency and optimising site selection and yield levels in the production of super premium, regional 'signature' Semillon wines.	N
7	Definition of the environmental conditions that favour the occurrence of noble rot.	Partial

Theme outputs

	Output	Achievement
1	Eight refereed publications across the lifetime of the theme	Exceeded: 17 plus one book chapter and two book chapters in press
2	Seven industry/Technical publications on Semillon cropping and wine flavours across lifetime of theme	Exceeded: 8 publications
3	Industry presentations at AWITC in 2007 and 2010; and at NWGIC Annual Symposiums (2007 to 2011).	2 presentations and 6 posters
4	Presentations to industry associations in the theme regions	12 presentations
5	Undergraduate training of students enrolled in CSU's wine science and viticulture courses	8 members of staff incorporated research findings into lectures and subjects
6	Short course and certificate programs offered as part of training programs offered by the NWGIC and its partner organizations in various wine regions.	None developed
7	Postgraduate training of students enrolled in higher degrees at CSU (coursework Masters and research degrees)	12 PhD students, 2 visiting Masters students and 9 honours students
8	Three PhD completions including one member of NWGIC st	Will be met by 2012

Theme milestones

	Milestone	Achieved
1	Semillon wine flavours from 20 Hunter and Riverina vineyards profiled (June 2007)	Y
2	Vinification protocols for small and medium lot winemaking of experimental Semillon wines defined (Sep 2007)	Y
3	Impact of site and crop yield, including maximised yields in the Riverina, on Semillon vine physiology and carbohydrate partitioning known (June 2009)	Y
4	Impact of site and crop yield on Semillon fruit composition and occurrence of noble rot in the Riverina region known (June 2010)	Partial*
5	Semillon flavour chemistry determined (June 2010)	Y
6	Consumer assessment of Semillon wines from the Hunter region related to their origin and composition (June 2010)	Y
7	Impact of region, site and crop yield on Semillon vine physiology and wine sensory attributes known (June 2011)	Y
8	Technical manual on Semillon growing/winemaking released (June 2011)	Not done**

* A PhD student began this project but did not complete. Another PhD student continued the project and is planning to complete the project within 18 months.

** The target audience (Hunter Valley grape growers and winemakers) decided that they preferred this information to be delivered via workshops rather than through a manual.

exist between specific consumer groups for sweet preference in HSV, future research is now required to extend this investigation to other wine styles, and broader consumer segments such as specific export markets of importance to the wine industry.

Experiment 5.4: Sensory characterisation of bottle age using Hunter Valley Semillon Wine

The effect of 3 years bottle age on HVS has been investigated using sensory descriptive analysis. Different HVS wine styles have been shown to develop at different rates. The ability of many HVS wines to retain their original primary fruit character whilst also developing desirable aged attributes has also been demonstrated.

Experiment 5.7: Wine chemistry

This section has investigated the ranking of perceived aroma compounds and the relative potency of aroma compounds as extracted from Semillon wine. Of the compounds identified, those stemming from yeast-fermentation processes would appear the most common group of compounds contributing to the aroma of the extracts. A range of unknown compounds were found to be of high aroma potency and are potential targets for future identification and recombination experiments.

SPME-GCMS: Conclusion.

This work allowed the comprehensive optimisation and validation of a robust and automated HS-SPME–GC–MS methodology for the determination of volatile compounds in Semillon wines. The fibre used was DVB/CAR/PDMS fibre as this allowed acceptable extraction and desorption of volatile compounds with widely different polarities. The addition of an I.S. mixture with four internal standards was the only sample pre-treatment required. The SPME fibre performance to be monitored during the lifetime of the analysis with one fibre. It also allowed a comparison of results between different fibres, an essential requirement for longitudinal studies assessing changes in aroma profiles of wine over time.

A chemometric approach to linking GCMS analysis of volatile compounds to wine sensory data: Conclusion.

This section has showed that PCA analysis of the aligned GCMS data (i.e. both SPE and derivatised-SPE) versus samples showed trends for vintage and wine style. Associated loadings will be used to identify specific compounds associated with these experimental factors. The use of PLS analysis allowed significant regression of aroma scores with certain regions of the GCMS chromatograms. In particular, aged sensory parameters (i.e., honey, toast, orange-marmalade), provided the highest regression scores and enabled the identification of marker regions within the GCMS chromatograms. Work is continuing to link these marker regions of the GCMS analysis to actual compounds, and markers of toast compounds would appear promising even at this early stage. Further multiblock-chemometric analysis was conducted using the CCSW technique in an attempt to provide directionality to the correlations within the GCMS chromatograms for sensory parameters. However, no strong grouping of samples was evident for the samples, indicating that most of the captured variance in these dimensions represented sensory or analytical noise.

Identification of aroma active compounds in Semillon wines – GCO analysis: conclusions

Whilst the identification of compounds responsible for the high regression in PLS matching between GCMS data and specific sensory parameters is flagged as future work, the following section outlines the use of GCOMS to identify the volatile compounds that when alone (i.e., as detected by a sensory panellist from the olfactory detector of a gas chromatographic condition) have a high odour impact.

Chemistry of aged and/or oxidised Semillon flavours: phenylacetaldehyde and methional.

This section showed the ability of sensory aroma compounds, relevant to 'honey' and 'potato' notes, to be generated from parent amino acids in wine-like conditions. Under high oxygen, and low sulfur dioxide conditions, higher temperatures favoured the formation of the aldehydes, as did additives that accelerated oxygen consumption and/or carbonyl compounds production. After consumption of 15 mg L^{-1} oxygen, in high sulfur dioxide conditions, minimal aldehydes were detected suggesting that sulfur dioxide is efficient in protecting against the aldehyde production regardless of the rate of oxygen consumption. The dissociation for the binding of sulfur dioxide to the aldehydes was determined and they were found to have similar binding strength as keto acids and certain oxidised sugars. The release of the bound forms of the aldehydes was also confirmed and found to occur in the order of days at room temperature. The results show that once formed, the aroma impact of these particular aldehyde compounds is difficult to remove with typical wine-like sulfur dioxide concentrations.

Examination of the potential for using 'simple' chemical analysis (i.e., non-volatile measures) as a surrogate for sensory analysis: conclusions

In summary, the multi-block analysis, ComDim, has provided considerable insight into the connections between analytical data and sensory descriptors for Hunter Valley Semillon. Zones for Sweetness and Acidity describing the connections between various analytical and sensory parameters were readily identified. The orthogonal relationship between the analytical measurement for malic acid and the sensory score for acidity opens up the possibility of further research on the factors contributing to acid taste. One group of aroma sensory attributes did not show any connection with the analytical data used in this analysis.

Overall Conclusions

The overall approach taken was to firstly identify the critical aroma and flavour compounds of Semillon wine that related to the wine styles identified in the sensory evaluation of the wines. Once identified, the source of the aroma compounds as well as the impact of vineyard and/or winery modulation was to be considered. Given the majority of compounds identified were related to yeast-fermentation, this limited the ability to provide comments on vineyard modulation. However, a variety of unknown compounds have be highlighted as potential areas of future work that may provide more insights into the effects of both viticulture and winery modulation on the final Semillon aroma.

In the first instance a range of gas chromatographic techniques were both designed as new, and employed from existing methods, for analysis of Semillon. This included the development of a robust SPME GCMS technique for the quantification of a range of aroma compounds in Semillon. Subsequent work utilised multi-block GCMS analysis (i.e. with both SPE and SPE-derivisation extraction methods) to maximise the range of volatile compounds detected and hence allow chemometric comparison to the sensory data of the wines. Using chemometric tools, the GCMS analysis data was able to show trends for separating the Semillon wine samples based on vintage and also on wine styles. Furthermore, it highlighted the portions of the GCMS chromatograms that in combination gave the best regression for particular sensory attributes. The attributes of orange/marmalade and toast provided the best regressions with the GCMS data. In this sense, the highlighted regions of the GCMS chromatograms are in combination, marker compounds for the specific attribute. Tentative identification of some of the marker compounds has been made but the comprehensive identification of all these compounds would be an area of further work. Consequently, it was not certain at this stage whether the marker compounds themselves were critical aroma compounds or rather behaved in a similar manner to key aroma compounds. In any case, the application of these chromatographic marker regions will be a useful tool in future investigations into winery/vineyard modulation on impacts on wine styles.

For further information on the critical aroma compounds in Semillon, GCO was conducted. This allowed the identification of the most potent aroma compounds in a solvent extract from Semillon. The results of this work again showed a variety of yeast-derived aroma compounds as being amongst the most potent aroma compounds in extracts. A variety of 16 unknown compounds were also highlighted and their identification would require further work. Once identified, recombination experiments in model wine solutions would be worthwhile for an assessment whether these range of aroma compounds themselves can simulate the different wine styles of Semillon described in this report.

Other work was conducted on aldehyde aroma compounds relevant to the aging/oxidation of wines, particularly the development of a 'honey' aroma compound (phenylacetaldehyde). Dissociation constants were obtained for the binding of these aldehydes to sulfur dioxide in model wine systems, and demonstrated that once generated they are very difficult to eliminate below threshold levels with typical sulfur dioxide levels. It is also consistent with the emergence of honey aromas during the bottle aging of wines whereby sulfur dioxide concentrations may fall gradually with age and release some previously bound 'honey' aldehydes. In a low oxygen environment, the production of these aldehyde compounds was adequately prevented by the presence of sulfur dioxide, and no significant difference (P=0.05) was observed between sulfur dioxide alone and sulfur dioxide with complementary antioxidant additive. The precursors to the aldehyde aroma compounds in these experiments were the parent amino acids, and it is described in another section of this report how vineyard operations can impact on amino acid profiles in both juice and wine. In the experiments conducted, negligible change in the amino acid concentration (i.e. 50–90 mg L^{-1})

occurred for the production of μg L^{-1} quantities of the aldehyde, but it remains to be seen what the effect of the initial amino acid concentration would have on the yield of aldehyde.

To allow wineries to classify their particular Semillon wines into a given style, an attempt was made to link simple chemical measures to wine sensory descriptors of Semillon. This would provide wineries with the ability to classify a wine without the application of a expensive and time-consuming sensory panels. By 'simple' measures it is meant the measures of wine chemistry that are routinely utilised in commercial wineries of all sizes, that is, measurements that do not require the more research orientated techniques of gas chromatography or liquid chromatography, but instead only use wet chemical techniques. Zones for Sweetness and Acidity describing the connections between various analysis and sensory parameters were readily identified. The results appear promising and further analytical measurements in conjunction with this technique are worthy of further investigation.

Experiment 5.8: Grape and wine composition in Semillon influenced by vine resources under different environmental conditions

The work led to an improved understanding of the role of carbohydrate and nutrient reserves during grape maturation under different environmental conditions, particularly with regard to fruit and wine composition. The understanding of different vineyard management practices on critical aroma and flavour compounds assists in optimizing grape production.

Different vineyard management practices altered nutrient and carbohydrate reserves and ultimately vine performance. Understanding the impact of these management practices on fruit N compounds assists in optimizing grape and wine production. This information suggests further benefits from optimisation of N management in the vineyard. A complete amino acid profile of berry and wine samples was analysed to investigate more detailed nitrogen storage and mobilisation processes during berry ripening and fermentation. The analysis of this data is currently in process.

Further research in yeast nutrient requirements is still necessary.

Experiment 5.9: Consumer wine show days and what they offer: Do the right consumers attend?

The purpose of this study was to determine if consumers who attend public tastings are representative of the Australian wine drinking population or a particular group within it and therefore able to provide valid consumer feedback on wine for exhibitors at wine shows. All the indices measured in this survey suggest that consumers who attended wine show public tasting days in 2010 are suitable consumers to use for wine consumer studies as they are enthusiastic, product users with average level of subjective knowledge of wine. They are also representative of the wine drinking population in Australia as their age demographics suggests. These consumers can therefore be used in a validated test method to provide useful consumer information for wine exhibitors to help them make wines that consumers like. A follow up survey to determine the change in consumer demographics over a few years is being carried out in 2011. Also a validated method to use within this context is being tested at selected wine shows.

Experiment 5.12: Vineyard management and Semillon wine styles in the Riverina

There was only a small impact on the final aroma profile of Semillon wines with 33% and, even, 50% bunch removal at pea size (EL stage 31). Any differences were too small for trained panellists to describe and, thus, probably too small for the average wine consumer to differentiate.

Thus, reducing crop load of Semillon in the Riverina below what is normal seems to have little effects on wine flavour profile. The flavour components still need to be analysed chemically in the grape and in the wine at different stages of ageing to understand their synthesis and degradation.

The vineyard itself seems to be more important than the actual crop removal. The wine of vineyard C showed a relatively strong change when the fruit was removed; on the other hand, no effect could be observed on the wines from vineyard Y.

As with sites, the effect of season was more important for wine aroma profile than the fruit removal.

Outputs

The next section details the specific deliverables relating to each planned output. Each of the publications detailed can be found in full in the appendix. Publications in bold relate specifically to the planned outcomes, while other listed publications show the additional work that the Winegrowing Future's program lead to. A brief summary of how each publication relates to the overall theme is provided below.

Refereed publications across lifetime of theme

Ryan, D., Prenzler, P., Saliba, A. and Scollary, G. (2008). The significance of low impact odorants in global odour perception. *Trends in Food Science and Technology,* **19**: 383–389.

It became apparent early in the project that low impact odorants may play an important role in the quality and consumer preference for Semillon. No methodology exists to identify low impact odorants that play an important role. This paper was the first step to developing such a technique.

Holzapfel, B., Field, S., and Mueller, M. (2008). Nitrogen and water management implications on grape production in the Riverina wine grape regions of South Eastern Australia. *Le Bulletin de l'OIV* **81:** 17–25.

Rudnitskaya, A., Schmidtke, L.M., Delgadillo, I., Legin, A. and Scollary, G. (2009). Study of the influence of micro-oxygenation and oak chip maceration on wine composition using an electronic tongue and chemical analysis. *Analytica Chimica Acta* **642:** 294.

The complex chemometric analysis that needed to be conducted within Theme 5 cannot be taken from existing methodologies alone. One project participant, Mr Leigh Schmidtke, built a collaboration and gained hands-on experience to develop skill in this area. A number of publications relate to this goal.

Saliba, A., Bullock, J. and Hardie, J. (2009). Consumer Rejection Threshold for 1,8-cineole (eucalyptol) in Australian Red Wine. *Food Quality and Preference* **20**: 500–504.

It was thought that a key-compound would be discovered that related to consumer preference. If that were the case, we would need to determine the ideal levels of that compound, as well as when it is too little or too much. The CRT technique adapted in this paper would allow such an investigation. Eucalyptol was focused on first, as a good deal of industry were contacting us asking the question: how much is too much.

Blackman, J. and Saliba, A. (2009). Sensory characterisation of Hunter Valley Semillon using descriptive analysis. *Flavour and Fragrance Journal* **24**: 238–244.

This paper presents the results of the sensory descriptive analysis performed on Hunter Valley Semillon, including the definition of four distinct 'styles' for HVS.

Mendes-Pinto, M. (2009). Carotenoid breakdown products the-norisoprenoids-in wine aroma. *Archives of Biochemistry and Biophysics*, **483**: 236–245.

This paper was written by the first PhD student assigned to the Botrytis Semillon project. That student had thought that carotenoids might play an important role in Semillon, though this turned out not to be the case.

Saliba, A., Wragg, K. and Richardson, P. (2009). Sweet taste preference and personality traits using a white wine. *Food Quality and Preference* **20**: 572–575.

Mainstream market segmentation has traditionally been based on factors that are either too complicated, or so simple that they are not helpful in a real setting like a cellar door. This paper discovered a world first, in showing that personality type relates to personality type. It is far easier to determine someone's personality type at cellar door, than it is to determine their point in a lifecycle, or other complicated segmentation. This paper also led to substantial press, including international recognition that allowed promotion of the Australian wine industry. It relates specifically to the consumer discovery in Theme 5 that consumers preferred sweeter wines, and this paper offers guidance on what sort of consumers prefer sweeter wines (and which do not).

Bouveresse, D.J.-R., Pinto, R.C., Schmidtke, L.M., Locquet, N. and Rutledge, D.N. (2009). Identification of significant factors by an extension of ANOVA-PCA based on multiblock analysis. *Chemometrics and Intelligent Laboratory Systems* **106:** 173–182.

As described above, Mr Schmidtke participated in collaborative research to gain training in the complex chemometric analysis required in Theme 5.

Barril, C., Clark, A. C., Prenzler, P. D., Karuso, P. and Scollary, G. R (2009). Formation of pigment precursor (+)-1"-methylene-6"-hydroxy-2H-furan-5"-one-catechin isomers from (+)-catechin and a degradation product of ascorbic acid in a model wine system. *Journal of Agricultural and Food Chemistry* **57:** 9539–9546.

It became clear that wine bottle-age was an important variable with Hunter Valley Semillon. Oxidation was therefore an important area to study,

and ascorbic acid may be a potential solution to any oxidation issues that are discovered by Theme 5.

Climaca P.R., Barros, A.S., Locquet, N., Schmidtke, L.M. and Rutledge, D.N. (2009). Improving the detection of significant factors using ANOVA-PCA by selective reduction of residual variability. *Analytica Chimica Acta* **653**(2): 131–142.

Schmidtke, L.M., Rudnitskaya, A., Saliba, A.J., Blackman, J.W., Scollary, G.R., Clark, A.C., Rutledge, D.N., Delgadillo, I. and Legin, A. (2010). Sensory, Chemical, and Electronic Tongue Assessment of Micro-oxygenated Wines and Oak Chip Maceration: Assessing the Commonality of Analytical Techniques. *Journal of Agricultural and Food Chemistry* **58**(8): 5026–5033.

As described above, Mr Schmidtke participated in collaborative research to gain training in the complex chemometric analysis required in Theme 5. The above two papers related to this activity.

Yoo, Y., Saliba, A.J. and Prenzler, P. (2010). Should red wine be considered a functional food? *Critical Reviews in Food Science and Food Safety* **9**(5): 530–551.

Theme 5 contained no specific health related milestones. However, in an initial survey, it was found that a high proportion of respondents thought that health was an important factor in wine choice and consumption frequency. A PhD was therefore setup, with no Winegrowing Future's funding, to address this issue. The student was from a functional foods background, and developed the project in that direction.

Saliba, A.J. and Moran, C.C. (2010). The influence of perceived healthiness on wine consumption patterns. *Food Quality and Preference* **21**: 692–696.

From an initial survey, it was found that consumers of wine were influenced by their health perception. Furthermore, an opinion was developing in the media that wine cannot be spoken about in terms of health affordance, as this might lead to problematic drinking patterns. This paper established that this slippery slope argument is not valid. That is, where people consider wine to be healthy, they are more likely to follow healthy drinking patterns than people who do not consider it healthy. Indirectly, this related to a core component of Theme 5–understanding consumer drivers of wine consumption.

Blackman, J., Saliba, A.J. and Schmidtke, L. (2010). Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon. *Food Quality and Preference* **21**(7): 679–683.

All of the sensory work in Theme 5 was pointing to the importance of acid-sugar balance to consumer taste preference. Further, differences were being noticed between the preferences of novices and other drinkers. A possible third group was winemakers – this group may be so familiar with the product that their preferences do not accord with everyday drinkers. This paper determined the differences between different levels of experience, so that this information can be taken into account when wine is developed.

Blackman, J., Rutledge, D.N., Tesic, D., Saliba, A. and Scollary, G. (2010). "Examination of the potential for using chemical analysis as a surrogate for sensory analysis". *Analytica Chimica Acta* **660**(1/2): 2–7.

One of the issues that kept presenting in Theme 5 was the cost of performing consumer sensory-chemistry and viticultural research together. Therefore, at several points, specific attempts were made to investigate opportunities to perform the work for reduced cost. This paper is one such example, where chemical analysis was investigated as a way of replacing the need to perform expensive and time consuming sensory analysis. There may be promise in this approach for some flavours, though much further work is needed.

Rebière, L., Clark, A.C., Schmidtke, L.M., Prenzler, P.D. and Scollary, G.R. (2010). A robust method for quantification of volatile compounds within and between vintages using headspace solid phase micro-extraction coupled with GC-MS – Application on Semillon wines. *Analytica Chimica Acta* **660:** 149–157.

In order to complete the chemical analysis of Semillon wines planned in Theme 5, a method needed to be developed and validated. A part of that validation was to publish in a top-class chemistry Journal, which was done here.

Bruwer, J., Saliba, A.J. and Miller, B. (2011). Consumer behaviour and sensory preference differences: implications for wine product marketing. *Journal of Consumer Marketing* **28**: 5–18.

A core component of Theme 5 was consumer taste preference. A collaborative opportunity presented to write a paper based on what was known from Theme 5 work, and existing dataset owned by A/Pro Johan Bruwer. This paper outlines generational differences in taste preference and outlines implications to wine product marketing.

Book Chapters

Laurie, F. and Clark, A.C. (2010). Wine Oxidation. In: Oxidation in food and beverages and antioxidant applications, Decker, E. and Elias, R. (Eds.). Cambridge UK: Woodhead Publishing.

Moran, C.C. and Saliba, A.J. (2011). Chapter 190: Anxiety and Self Medication with Alcohol. In: V. R. Preedy, R. R.

Watson, and C. R. Martin (Eds.), International Handbook of Behavior, Diet, and Nutrition. New York: Springer. Due Jan 2012. pp 3061–3076.

Richardson, P. and Saliba, A.J. (2011). Chapter 7: Personality traits in the context of sensory preference: a focus on sweetness. In: V. R. Preedy, R. R. Watson, and C. R. Martin (Eds.), International Handbook of Behavior, Diet, and Nutrition. New York: Springer. pp 85–98.

When an author is asked to write a book chapter, it is an indication that they are considered a world expert in that area. The three book chapters produced by Theme 5 researchers are therefore objective validation of their standing. This does relate to one goal of Theme 5, and that was to build capacity and expertise in new areas, where previously the NWGIC had only been strong in Viticulture. The above chapters related to wine oxidation, including mitigation strategies, the fact that wine can be used to lower transient levels of anxiety without concern in the short-term, and an extension of the discovery that taste preference for wine relates to personality type.

Industry/technical publications on Semillon cropping and wine flavours across lifetime of theme

Blackman, J., Saliba, A., and Scollary, G. (2007). Sensory characterisation and consumer preference of Hunter Valley Semillon - preliminary sensory characterisation findings. *Australian and New Zealand Grapegrower and Winemaker*. October edition.

Yoo, Y., Saliba, A. and Prenzler, P. (2008). Does wine have real opportunities in the functional food market? *Australian and New Zealand Grapegrower and Winemaker* December edition.

Saliba, A. J., Bruwer, J., and Blackman, J. (2009). Can consumers be convinced that a wine is a regional hero? *Australian and New Zealand Grapegrower and Winemaker* **541:** 42–48.

Saliba, A. J., Bullock, J. and Hardie, W. J. (2009). Answering the Great Debate: How much Eucalyptus Flavor is too much? *Australian and New Zealand Grapegrower and Winemaker* **547**:

Schmidtke, L. M., Blackman, J. W., Saliba, A. J. (2010). Red wine composition and sensory analysis with different inoculation times for maloactic fermentation. *GrapeGrowers and Vignerons* 28–32.

Moran, C.C., Saliba, A.J. and Blackman, J. (2010). What do consumers think about Semillon? *The Australian and New Zealand Wine Industry Journal* **25:** 71–77.

Crozier, A., Borges, G. and Ryan, D. (2010). The glass that cheers. *The Biochemist*. **32**: 4–9.

Saliba, A.J., Thomas, J.B. and Moran, C.C. (2010). Differences in wine preferences among Australian states–testing the stereotypes. *The Australian and New Zealand Wine Industry Journal* **25**: 66–70.

Industry presentations at AWITC in 2007 and 2010; and at NWGIC Annual Symposiums (2007 to 2011)

The NWGIC Annual Symposium program was only run in two years of the Winegrowing Future's Program, 2007 and 2011. In those years the following presentations were delivered:

Saliba, A. (2007). Consumer sensory preference: why and how we capture it. Presentation at the NWGIC Industry Conference, held in Wagga Wagga.

Saliba, A., Blackman, J. and Heymann, H. (2011). Consumer preferred wine styles. Presentation at the NWGIC Industry Conference, held in Wagga Wagga.

The AWITC in 2007 was attended by all Theme 5 team members. The following posters and talks were delivered at AWITC in 2007 and 2010:

Saliba, A., Blackman, J., and Scollary, G. (2007). Consumer preferences for Hunter Valley Semillon (poster). Thirteenth Australian Wine Industry Technical Conference, Adelaide, 28th July – 2nd August 2007.

Mueller, M. (2007). Grape and wine composition in *Vitis vinifera* L. cv. Semillon influenced by vine resources under different environmental conditions (poster). Thirteenth Australian Wine Industry Technical Conference, Adelaide, 28 July – 2 August 2007.

Hofmann, C., Clark, A., Hardie, W.J.M, Steele, C. and Saliba, A.J. (2010). Flavour characteristics of botrytis wines (poster). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Saliba, A.J., Blackman, J. Gourieroux, A. and Rebiere, L. (2010). Targeting wine styles that consumers want (poster). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Blay, M., Saliba, A.J., Hardie, W.J.M., Heymann, H. and Carr, T. (2010). A method to collect consumer sensory data at Australian wine shows (poster). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Saliba, A.J., Blackman, J., Gourieroux, A. and Rebiere , L. (2010). Targeting wine styles that consumers want (invited presentation). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Presentations to industry associations in the theme regions

Anthony Saliba, John Blackman and Geoff Scollary. "Semillon Seminar" to Hunter Valley Vineyard Association, 26th March, 2007.

Theme 5 Presentation to Mudgee Grape-growers and Winemakers, April, 2008.

John Blackman, Laure Rebiere, Aude Gourieroux, Tony Somers and Anthony Saliba. Results and outcomes from Theme 5 so far. Workshop presented through the HVVA, October 28th, 2008, Hunter Valley, NSW.

Markus Mueller. Nitrogen and water management implications on grape and wine production in the Riverina. Presentation at a workshop organised by the Riverina Wine Grape Marketing Board, 12 November, 2008, Griffith, NSW.

Anthony Saliba. Wine quality as judged by consumers. Presentation at a workshop organised by the Yarra Valley Wine Growers Association, November 27th, 2008, Yarra Valley, Victoria.

Markus Mueller.Workshop presentation in Griffith 8th June 2010. Irrigation and nutrition science for Riverina Wine production.

Peter Torley, Anthony Saliba and Leo Quirk. Consumer preference and mouthfeel workshop, Cowra, 12th May 2010.

Austrian Wine Delegation – Visit to CSU, 22nd February 2007, 26 attendees (Floor Management Vine Response, Dejan Tesic).

Winegrowing Futures – Update to Riverina Region, 23rd April 2007, 15 attendees (Dejan Tesic).

Food and Wine Matching workshop in Wagga, 22nd February 2008, 14 attendees (Anthony Saliba).

Industry presentation of PhD project (Markus Mueller) at McWilliams, Griffith, with experimental wines. Wine evaluation was done by McWilliams winemakers. 24th June, 2009, 10 attendees.

Carmen Moran and Anthony Saliba. CSU Psychology Colloquium in Wagga on "Why do people drink"? 29th September2010, 30 attendees

Undergraduate training of students enrolled in CSU's wine science and viticultural courses

The following staff on Theme 5 delivered content into the wine science and viticultural course programs at CSU between 2006 and 2011:

Dr Andrew Clark

Mr John Blackman

Dr Peter Torley

A/Pro Anthony Saliba

Dr Dejan Tesic

Prof Geoff Scollary

Mr Leigh Schmidtke

Prof Chris Steele

Postgraduate training of students enrolled in higher degrees at CSU (coursework Masters and research degrees)

The following Theme 5 staff supervised post-graduate students (PhD, Masters) or Honours in wine science students between 2006 and 2011:

Dr Andrew Clark (1 PhD)

Mr John Blackman (1 Honours)

Dr Peter Torley (2 visiting Masters students)

A/Pro Anthony Saliba (6 Honours, 8 PhD)

Prof Geoff Scollary (2 PhD)

Mr Leigh Schmidtke (1 Honours)

Prof Chris Steele (1 Honours, 1 PhD)

Three PhD completions including one member of the NWGIC staff.

There was a total of 5 PhDs started under Theme 5. One was not directly funded by Theme 5, one was part-funded and the remaining three were fully funded. The projects, participants and expected submission dates are listed below.

Fully funded within Theme 5

Student: Markus Mueller. Grape and wine composition influenced by vine resources under different environmental conditions. Supervisors: Bruno Holzapfel, Andrew Clark and Hans-Reiner Schultz (Geisenheim Research Centre). Expected submission date March 2012.

Student: John Blackman. Sensory assessment and consumer preference for Hunter Valley Semillon wines. Supervisors: Anthony Saliba, Andrew Clark and Geoff Scollary. Expected submission date end 2012 (this PhD was part-time, and this date does submit 'on time'). Note: Stipend and fees were paid by CSU.

Flavour characterisation of Botrytis Semillon from Griffith. Supervisors: Anthony Saliba, Chris Steele, Andrew Clark and Jim Hardie. Expected submission date end 2012. Note: this PhD effectively replaced a previous candidate who withdrew.

Part-funded

Student: Maame Blay. Consumer preference mapping using wine show derived data. Supervisors: Anthony Saliba, Jim Hardie, Hildegarde Heymann (UC Davis) and Tom Carr (Carr Statistical Consulting). Expected submission date mid 2012.

Not funded

Student: Yung Yoo. Can wine be considered a functional food? Supervisors: Anthony Saliba, Paul Prenzler and Danielle Ryan. Expected submission date start 2012. Note: this project is fully funded by a CSU PhD scholarship awarded to Yung Yoo and Anthony Saliba. The only 'cost' to Theme 5 has been in the supervisory time provided by Anthony Saliba.

Appendix 1
Communications

Refereed articles

Barril, C., Clark, A. C., Prenzler, P. D., Karuso, P. and Scollary, G. R (2009). Formation of pigment precursor (+)-1"-methylene-6"-hydroxy-2H-furan-5"-one-catechin isomers from (+)-catechin and a degradation product of ascorbic acid in a model wine system. Journal of Agricultural and Food Chemistry, 57 (20), 9539–9546.

Blackman, J. and Saliba, A. (2009). Sensory Characterisation of Hunter Valley Semillon using Descriptive Analysis. Flavour and Fragrance Journal, 24(5), 238-244.

Blackman, J., Rutledge, D. N., Tesic, D., Saliba, A. and Scollary, G. (2010). "Examination of the potential for using chemical analysis as a surrogate for sensory analysis". Analytica Chimica Acta, 660(1/2), 2-7.

Blackman, J., Saliba, A.J. and Schmidtke, L. (2010). Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon. Food Quality and Preference, 21(7), 679-683.

Bouveresse, D. J.-R.; Pinto, R. C.; Schmidtke, L. M.; Locquet, N.; Rutledge, D. N. (2009). Identification of significant factors by an extension of ANOVA-PCA based on multiblock analysis. Chemometrics and Intelligent Laboratory Systems, 106(2), 173-182.

Bruwer, J., Saliba, A.J. and Miller, B. (2011). Consumer behaviour and sensory preference differences: implications for wine product marketing. Journal of Consumer Marketing, 28(1), 5-18.

Climaca P. R., Barros, A.S., Locquet, N., Schmidtke, L. M. and Rutledge, D. N. (2009). Improving the detection of significant factors using ANOVA-PCA by selective reduction of residual variability. Analytica Chimica Acta, 653(2), 131-142.

Holzapfel, B., Field, S., and Mueller, M. (2008). Nitrogen and water management implications on grape production in the Riverina wine grape regions of South Eastern Australia. Le Bulletin de l'OIV, 81 (923-925), 17-25.

Mendes-Pinto, M. (2009). Carotenoid breakdown products the-norisoprenoids-in wine aroma. Archives of Biochemistry and Biophysics, 483(2), 236-245.

Rebière, L., Clark, A. C., Schmidtke, L. M., Prenzler, P. D., and Scollary, G. R. (2010). A robust method for quantification of volatile compounds within and between vintages using headspace solid phase micro-extraction coupled with GC-MS – Application on Semillon wines. Analytica Chimica Acta, 660(1-2), 149-157.

Rudnitskaya, A., Schmidtke, L.M., Delgadillo, I., Legin, A. and Scollary, G. (2009). Study of the influence of micro-oxygenation and oak chip maceration on wine composition using an electronic tongue and chemical analysis. Analytica Chimica Acta, 642(1-2), 294.

Ryan, D., Prenzler, P., Saliba, A. and Scollary, G. (2008). The significance of low impact odorants in global odour perception. Trends in Food Science and Technology, 19(7), 383-389.

Saliba, A., Bullock, J. and Hardie, J. (2009). Consumer Rejection Threshold for 1,8-cineole (eucalyptol) in Australian Red Wine. Food Quality and Preference, 20(7), 500-504.

Saliba, A., Wragg, K. and Richardson, P. (2009). Sweet taste preference and personality traits using a white wine. Food Quality and Preference, 20(9), 572-575.

Saliba, A.J. and Moran, C.C. (2010). The Influence of Perceived Healthiness on Wine Consumption Patterns. Food Quality and Preference, 21(7), 692-696.

Schmidtke, L. M., Rudnitskaya, A., Saliba, A. J., Blackman, J. W., Scollary, G. R., Clark, A. C., Rutledge, D. N., Delgadillo, I., Legin, A. (2010). Sensory, Chemical, and Electronic Tongue Assessment of Micro-oxygenated Wines and Oak Chip Maceration: Assessing the Commonality of Analytical Techniques. Journal of Agricultural and Food Chemistry, 58(8), 5026–5033.

Yoo, Y., Saliba, A.J., and Prenzler, P. (2010). Should red wine be considered a functional food? Critical Reviews in Food Science and Food Safety, 9(5), 530-551.

Book chapters

Laurie, F. and Clark, A. C. (2010). Wine Oxidation. In: Oxidation in food and beverages and antioxidant applications, Decker, E. and Elias, R. (Eds.). Cambridge UK: Woodhead Publishing.

Moran, C.C. and Saliba, A.J. (in press). Anxiety and Self Medication with Alcohol. In: V. R. Preedy, R. R. Watson, and C. R. Martin (Eds.), International Handbook of Behavior, Diet, and Nutrition. New York: Springer. Due Jan 2012.

Richardson, P. and Saliba, A.J. (in press). Personality traits in the context of sensory preference: a focus on sweetness. In: V. R. Preedy, R. R. Watson, and C. R. Martin (Eds.), International Handbook of Behavior, Diet, and Nutrition. New York: Springer. Due Jan 2012.

Conference proceedings and industry articles

Blackman, J., Saliba, A., and Scollary, G. (2007). Sensory characterisation and consumer preference of Hunter Valley Semillon - preliminary sensory characterisation findings. Australian and New Zealand Grapegrower and Winemaker. October edition.

Blay, Saliba, Hardie, Heymann and Carr (2010). A method to collect consumer sensory data at Australian wine shows (poster). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Crozier, A., Borges, G. and Ryan, D. The glass that cheers. The Biochemist. Accepted October 2010.

Hofmann, C., Clark, A., Hardie, W.J.M., Steele, C. and Saliba, A.J. (2010). Flavour characteristics of botrytis wines (poster). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Moran, C.C., Saliba, A.J. and Blackman, J. (2010). What do consumers think about Semillon? *The Australian and New Zealand Wine Industry Journal* **25**(3): 71–77.

Mueller, M. (2007). Grape and wine composition in Vitis vinifera L. cv. Semillon influenced by vine resources under different environmental conditions (poster). Thirteenth Australian Wine Industry Technical Conference, Adelaide, 28 July – 2 August 2007.

Saliba, A.J. (2007) Consumer sensory preference: why and how we capture it. Presentation at the NWGIC Industry Conference, held in Wagga Wagga.

Saliba, A.J., Bruwer, J. and Blackman, J. (2009). Can consumers be convinced that a wine is a regional hero? *Australian and New Zealand Grapegrower and Winemaker* **541:** 42–48.

Saliba, A.J., Bullock, J. and Hardie, W.J. (2009). Answering the Great Debate: How much Eucalyptus Flavor is too much? *Australian and New Zealand Grapegrower and Winemaker* 547.

Saliba, A., Blackman, J. and Scollary, G. (2007). Consumer preferences for Hunter Valley Semillon (poster). Thirteenth Australian Wine Industry Technical Conference, Adelaide, 28th July – 2nd August 2007.

Saliba, A.J., Thomas, J.B. and Moran, C.C. (2010). State differences and wine preferences – Testing the stereotypes. The Australian and New Zealand Wine Industry Journal, March/April.

Saliba, A.J., Blackman, J. and Heymann, H. (2011). Consumer preferred wine styles. Presentation at the NWGIC Industry Conference, held in Wagga Wagga.

Saliba, A.J., Blackman, J., Gourieroux, A. and Rebiere, L.(2010). Targeting wine styles that consumers want (invited presentation). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Saliba, A.J., Blackman, J., Gourieroux, A. and Rebiere, L. (2010). Targeting wine styles that consumers want (poster). Fourteenth Australian Wine Industry Technical Conference, Adelaide, 3 – 8 July 2010.

Schmidtke, L.M., Blackman, J.W. and Saliba, A.J. (2010). Red wine composition and sensory analysis with different inoculation times for maloactic fermentation. *Grape Growers and Vignerons,* 28–32.

Yoo, Y., Saliba, A. and Prenzler, P. (2008). Does wine have real opportunities in the functional food market? *Australian and New Zealand Grapegrower and Winemaker* December edition.

Seminars, workshops and lectures

Anthony Saliba, John Blackman and Geoff Scollary. "Semillon Seminar" to Hunter Valley Vineyard Association, 26th March, 2007.

Anthony Saliba. Wine quality as judged by consumers. Presentation at a workshop organised by the Yarra Valley Wine Growers Association, November 27th, 2008, Yarra Valley, Victoria.

Austrian Wine Delegation – Visit to CSU, 22nd February 2007, 26 attendees (Floor Management Vine Response, Dejan Tesic).

Carmen Moran and Anthony Saliba. CSU Psychology Colloquium in Wagga on "Why do people drink"? 29th September2010, 30 attendees

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John Blackman, Laure Rebiere, Aude Gourieroux, Tony Somers and Anthony Saliba. Results and outcomes from Theme 5 so far. Workshop presented through the HVVA, October 28th, 2008, Hunter Valley, NSW.

Markus Mueller. Nitrogen and water management implications on grape and wine production in the Riverina. Presentation at a workshop organised by the Riverina Wine Grape Marketing Board, 12 November, 2008, Griffith, NSW.

Markus Mueller. Workshop presentation in Griffith 8th June 2010. Irrigation and nutrition science for Riverina Wine production.

Peter Torley, Anthony Saliba and Leo Quirk. Consumer preference and mouthfeel workshop, Cowra, 12th May 2010.

Theme 5 Presentation to Mudgee Grape-growers and Winemakers, April, 2008.

Winegrowing Futures – Update to Riverina Region, 23rd April 2007, 15 attendees (Dejan Tesic).

Appendix 2
Intellectual property

Not applicable

Appendix 3
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Appendix 4
Staff

Dr Dejan Tesic
Prof Geoff Scollary
Dr Danielle Ryan
Mr John Blackman
Dr Andrew Clark
Dr Bruno Holzapfel
Mr Michelle Meunier
Mr Chris O'Connell
A/Prof Anthony Saliba
Dr Laure Rebiere
Ms Aude Gourieroux
Mr Markus Mueller
Ms Maame Blay
Ms Conny Hofmann
Ms Jennifer Bullock
Ms Jasmine Thomas
Dr Paul Prenzler
Mr Leigh Schmidtke
Prof Carmen Moran
Dr Peter Torley
Dr. Hans Rainer Schultz
Mr Ned Sharkey
Mr Peter Carey
Mr Rob Lamont
Ms Emily Rouse

Appendix 5
Other relevant material
Small lot winemaking procedure

INTELLECTUAL PROPERTY OF NWGIC

Small Lot Winemaking Procedure

Method No.: 40-1a

Document Type Laboratory Method

Version 4.0

Adoption Date 20-02-2007

Prepared by E. Rouse

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Revised by H. Pan & E. Rouse

Approved by

Small Lot Winemaking Procedure

1. Introduction

The National Wine and Grape Industry Centre (NWGIC) has established protocols for the effective and efficient winemaking of red and white wines. Emphasis has been the establishment of procedures that ensure minimal winemaking influence on the finished wine. This ensures that the chemical and sensory properties of the finished wine reflect the impact of the viticulture trials that are being investigated.

This procedure presents an overview of the small lot winemaking practice used in our Experimental Winery. The procedure from harvest through to finished wine is described. The protocols for red and white winemaking and the equipment used for grape processing and winemaking are presented.

2. Principle of Procedure

Small lot winemaking is carried out as per the procedure shown in section 6 of this report. Grape and wine analysis methods are performed as described in Iland *et al* (2004) Chemical analysis of grapes and wine: techniques and concepts.

3. Scope and Application

This procedure will apply to all aspects of wine making and analysis conducted in the experimental winery at the NWGIC. Small lot red and white wines are produced for viticulture and oenology research trials including analysis on juice and wine to test for the impact of viticultural management on wine quality. The number of wines to be made each vintage depends upon the requirements of the research projects running at the time. Data reports are produced for the wines made and sensory analysis is carried out (see laboratory method No 40-10).

4. Safety Procedures

Refer to the following SOPs:

LMWI 20-3 Laboratory Safety Rules

LMOP 10-35 Use of Compressed Air

LMWI 40-1 Manual Handling of Ferments and Barrels

LMWI 40-2	Cleaning and Sterilisation
LMWI 40-3	CO2 Build up in Confined Spaces
LMWI 40-4	VELO Pneumatic Press
LMWI 40-5	Crusher-Destemmer
LMWI 40-6	Small Bag Press
LMWI 40-7	Crown Walkie Stacker
LMWI 40-8	Pressure Cleaner – NILFISK Gerni
LMWI 40-9	TEM Filtering/Filling Machine
LMWI 40-16	TENCO Semiautomatic capping machine
LMWI 120-3	Glassware Cleaning
LMWI 120-4	Gas Cylinders
LMWI 120-10	Homogeniser

5. Apparatus and Reagents

5.1 ***Apparatus***

5.1.1 Small lot winemaking

- 100L variable capacity stainless steel tanks
  Algor, Via Aldo Moro, 5-35010 Villa Del Conte ITALY

- Glass demijohns – 5L, 10L, 15L, 20L, 25L
- Crusher-Destemmer (LMWI 40-5)
  Zambelli, Via dell' Artigianato, 36043 Camisano Vicentino ITALY

- Small bag press (LMWI 40-6)
- VELO Pneumatic press (LMWI 40-4)
  VELO s.p.a, Via Piave 55 – 31030 Altivole ITALY

- Zambelli 70L electric stainless steel basket press (LMWI 40-11)
  Zambelli, Via dell' Artigianato, 36043 Camisano Vicentino ITALY

- Zambelli Tifone T25 flexible impeller variable speed pump (LMWI 40-12)
  Zambelli, Via dell' Artigianato, 36043 Camisano Vicentino ITALY

- TEM filtering/filling machine (LMWI 40-9)
  Toscana Enologica Mori, 50028 Tavarnelle Val di Pesa ITALY

- TENCO Semiautomatic capping machine (LMWI 40-16)
  Tenco, Via Arbora 1, 16030 Avegno ITALY
- Mettler Toledo PB3002-S/FACT top pan balance
  Mettler-Toledo AG, CH-8606 Greifensee SWITZERLAND

- Yeast and GoFerm
  Danstar Ferment Ag, Alpenstrasse 12, CH 6304, ZUG SWITZERLAND
  In Australia imported by: Lallemand Australia Pty Ltd
  Address: 131 Mooringe Avenue, Camden Park, South Australia, 5038

5.1.2 Chemical analysis on grapes and wine

- Anton Paar DMA 35N portable density meter
  Anton Paar, Str. 20, 8054 Graz AUSTRIA
- Atago pocket refractometer
  Atago Ltd., 32-10, Honcho, Itabashi-ku, Tokyo 173-0001 JAPAN

- Denver Instrument pH/mV meter
  Denver Instrument GmbH, Robert-Bosch-Briete 10, 37079 Gottingen GERMANY

- Metrohm Fully Automated 59 Place Titrando System (LMWI 40-15)
  Metrohm Ltd., CH-9101 Herisau SWITZERLAND
- FOSS WineScan™ 79000 Auto (FTIR)
  FOSS Analytical AB, Box 70, SE-263 21 Höganäs SWEDEN

- FOSS FIAstar™ 5000 (LMWI 40-14)
  FOSS Analytical AB, Box 70, SE-263 21 Höganäs SWEDEN

- Rankine apparatus for sulfur determination (LMLM 40-6)
- Shimadzu UV 1700 PharmaSpec
  Shimadzu Corporation, Kyoto JAPAN

- Anton Paar Alcolyser, DMA 4500 density meter – NIR (LMWI 40-10)
  Anton Paar, Str. 20, 8054 Graz AUSTRIA
- TKA deioniser – Type I & Type II water
  TKA Water Purifications Systems GmbH, Stockland 3, D-56412 Niederelbert GERMANY

- Eppendorf refrigerated centrifuge 5810R (LMWI 40-13)
  Eppendorf AG, 22331 Hamburg GERMANY
- Ultra-Turrax T25 Homogeniser (LMWI 120-10)
  IKA® Werke GmbH & Co. KG, Janke & Kunkel-Str. 10, D-79219 Staufen GERMANY
- Panasonic Juice Extractor
  Matsushita Electric Industrial Co., Ltd., Osaka 542-8588 JAPAN

- Eppendorf research variable pipettes
  Eppendorf AG, 22331 Hamburg GERMANY
- Hotplate/stirrer
  Industrial Equipment & Control Pty. Ltd.

61-65 McClure St, Thornbury, Melbourne 3071 AUSTRALIA

- Schilling burette – 25mL & 50mL
- Hirschmann ceramus & Jencons variable 10mL dispenser
- Heidolph Reax vortex
  Heidolph Instruments GmbH & Co., KG · Walpersdorfer Str. 12, 91126 Schwabach GERMANY

- Sartorius BL1500S top pan balance
  Sartorius AG, Weender Landstrasse 94-108, D-37075 Goettingen GERMANY

- Mettler AE260 analytical balance
  Mettler-Toledo AG, CH-8606 Greifensee SWITZERLAND

- Glassware
- Fumehood
- Enzymatic kits as required (Glucose/Fructose, Malic acid)
  Megazyme International Ireland Limited, Bray Business Park, Bray, Co. Wicklow IRELAND

5.2 ***Reagents***

5.2.1 Small lot winemaking procedure

A list of chemicals used in the Experimental Winery for analysis on juice and wine can be found on the Charles Sturt University shared drive at the following link:

S:\Centres\Research Centres\NWGIC\QMS Viticulture Laboratory\Chemical Storage & Disposal\Chemical Register – Laboratory.xls

5.3 ***Stock Solutions***

5.3.1 Name of Stock Solution and Concentration for the Small lot winemaking procedure

- 1M sodium hydroxide (NaOH)
- 3M potassium chloride (KCl)
- 1000mg/L sulfur dioxide (SO_2) – 0.1483% sodium metabisulfite, 10% ethanol

5.4 ***Working Solutions***

5.4.1 Name of Working Solution and Concentration for the Small lot winemaking procedure

- 0.1 NaOH
- 0.01 NaOH
- 10% tartaric acid (H_2T)
- 0.1M hydrochloric acid (HCl)
- 25% orthophosphoric acid (H_3PO_4)
- 1M HCl
- 50% ethanol (EtOH)
- 16% EtOH
- 0.3% hydrogen peroxide (H_2O_2)
- Mixed indicator - 0.1% methyl red, 0.05% methylene blue
- 0.5% phenolphthalein indicator
- 10, 20, 40, 80, 140, 200, 250 mg/L SO_2 via 1000mg/L SO_2 stock solution
- Phosphate buffer, pH 8.4 – 0.05% monosodium phosphate monohydrate, 3.3% di-sodium phosphate heptahydrate
- DTNB reagent – 0.076% nitrobenzoic acid, 10% ethanol, 90% phosphate buffer
- 25% sodium metabisulfite
- 10% acetaldehyde
- 0.2% copper sulfate

Reducing sugars by the Rebelein method requires the following solutions:

- Z1 solution – 4.2% copper sulfate, 0.1% sulfuric acid
- Z2 solution – 25% sodium potassium tartrate, 8% sodium hydroxide

- Z3 solution – 30% potassium iodide, 0.1M sodium hydroxide
- Z4 solution – 17.5% sulfuric acid
- Z5 solution – 2% potassium iodide, 0.01M sodium hydroxide, 1% starch
- Z6 solution – 1.4% sodium thiosulfate, 0.05M sodium hydroxide

6. Preparation of work solutions and Reagent Indicator Solutions

6.1 1 M NaOH solution

- Weigh 40g of AR grade NaOH.
- Measure approximately 800mL of cold purified water into a large beaker.
- Carefully and slowly add the NaOH, with immediate stirring, to the purified water.
- When all the NaOH is dissolved, allow the solution to cool.
- Quantitatively transfer the NaOH to a 1L volumetric flask with purified water.
- Make to the mark with purified water.
- Mix thoroughly and carefully.

Storage: Glass or plastic container (if storing in glass use a plastic not a glass top to seal the container).

NB: When the NaOH is dissolving a large amount of heat will be evolved and it may be necessary to stand the beaker in a cold water bath.

6.2 0.1 M NaOH solution

- Weigh 4.00g of AR grade NaOH.
- Dissolve in purified water with constant stirring.
- Quantitatively transfer the NaOH to a 1L volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Glass or plastic container (if storing in glass use a plastic not a glass top to seal the container).

6.3 0.333 M NaOH solution

- Weigh 13.32g of AR grade NaOH.
- Dissolve in purified water with constant stirring.
- Quantitatively transfer to a 1L volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Glass or plastic container (if storing in glass use a plastic not a glass top to seal the container).

6.4 0.01 M NaOH solution

- Measure 100mL of 0.1 M NaOH using a 100mL volumetric flask.
- Quantitatively transfer to a 1L volumetric flask with purified water.

- Make to the mark with purified water and mix thoroughly.

Storage: Glass or plastic container (if storing in glass use a plastic not a glass top to seal the container).

Stability: Approximately seven days.

6.5 Reagent Preparation for Indicator Solutions

6.5.1 Methyl red / Methylene blue mixed indicator

- Weigh 0.1g of methyl red (sodium salt) indicator.
- Weigh 0.05g of methylene blue indicator.
- Add to a 100mL beaker.
- Prepare 100mL of 50% aqueous ethanol (50mL ethanol plus 50mL purified water).
- Add some of the 50% aqueous ethanol to the beaker to dissolve the indicators.
- Make to final volume of 100mL with the aqueous ethanol.
- Mix thoroughly.

Storage: Indicator dropping bottle.

6.5.2 Phenolphthalein indicator solution

- Weigh 0.5g of phenolphthalein indicator.
- Prepare 100mL of a 50% aqueous ethanol (EtOH) solution (mix 50mL of AR grade EtOH with 50mL of purified water).
- Dissolve the phenolphthalein indicator in the 100mL of 50% aqueous ethanol.
- Mix thoroughly.

Storage: Glass or plastic container.

Stability: Solution can deteriorate over time – if not being used regularly, prepare smaller volumes.

6.6 Reagent Preparation for Acid Solutions

6.6.1 1 M Hydrochloric acid (HCl)

- Carefully measure 100mL of concentrated HCl.
- Measure approximately 700mL of cold purified water into a large beaker.
- Carefully and slowly add the 100mL HCl, with stirring, to the purified water.
- Quantitatively transfer to a 1L volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Glass container.

6.6.2 25% v/v (approx. 18% w/v) Orthophosphoric acid (H3 PO4)

- Measure 294mL of 85% w/w H3 PO4.
- Measure approximately 700mL of cold purified water into a large beaker.
- Carefully and slowly add the H3 PO4, with stirring, to the purified water.
- Quantitatively transfer to a 1L volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Sealed container at room temperature.

Stability: Stable for at least six months.

NB: It may be necessary to stand the beaker in an ice bath during the mixing operation.

6.7 Reagent Preparation for Other Solutions

6.7.1 0.3% w/v Hydrogen peroxide (H_2O_2)

- Accurately measure 10mL of 100 volume (30%w/v) H_2O2.
- Add to a 1L volumetric flask.
- Make to volume with purified water and mix thoroughly.

Storage: Store in a dark reagent bottle in a refrigerator.

Stability: Advisable to prepare the solution fresh each week although has been shown to be stable for at least 2 months in a refrigerator. Hydrogen peroxide will deteriorate more rapidly if stored where it may be exposed to sunlight.

NB: Allow to warm to room temperature before use.

6.7.2 25% w/v Sodium metabisulfite (Na2S2O5)

- Weigh 6.25g of sodium metabisulfite.
- Dissolve in purified water.
- Quantitatively transfer to a 25mL volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Glass or plastic container in a refrigerator.

Stability: Prepare fresh monthly.

6.7.3 10% w/v Acetaldehyde (CH_3CHO)

- Measure 3.2mL of AR grade acetaldehyde.
- Add to 10mL of purified water.
- Quantitatively transfer to a 25mL volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Glass or plastic container in a refrigerator (this solution can also be stored in a freezer dedicated to the storage of chemicals).

Stability: Prepare fresh monthly.

6.8 Reagent preparation for Reducing sugars by the Rebelein method

6.8.1 Z1 solution – 4.2% Copper sulfate, 0.1% Sulfuric acid.

- Measure approximately 600mL of purified water into a 1L beaker.
- Carefully add 1mL of concentrated sulfuric acid to the 600 mL of water and mix thoroughly.
- Accurately weigh 41.92g of AR grade copper (cupric) sulfate.
- Dissolve the copper sulfate in a portion of the sulfuric acid solution.
- Quantitatively transfer (using the sulfuric acid solution) to a 1L volumetric flask.
- Add remaining sulfuric acid solution to the volumetric flask and quantitatively transfer with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Store in a sealed container.

6.8.2 Z2 solution – 25% Sodium potassium tartrate, 8% Sodium hydroxide

- Measure approximately 600mL of purified water into a 1L beaker.
- Accurately weigh 250g of AR grade sodium potassium tartrate and dissolve in purified water.
- Weigh 80g of AR grade sodium hydroxide and carefully add to the sodium potassium tartrate solution.
- Allow to cool and quantitatively transfer the solution to a 1L volumetric flask with purified water.
- Make to the mark with purified water and mix thoroughly.

Storage: Glass or plastic container (if storing in glass use a plastic not a glass top to seal the container).

NB: When the NaOH is dissolving heat will be generated and it may be necessary to stand the beaker in a cold water bath.

6.8.3 Z3 solution – 30% Potassium Iodide, 0.1M Sodium hydroxide.

- Measure approximately 600mL of purified water into a 1L beaker.
- Measure 100mL of 1M NaOH and add, with mixing, to the purified water.
- Accurately weigh 300g of AR grade potassium iodide (KI).
- Dissolve the KI in a portion of the 600mL NaOH solution.
- Quantitatively transfer (using the NaOH solution) the KI solution to a 1L volumetric flask.
- Quantitatively transfer any remaining NaOH solution to the volumetric flask.
- Make to the mark with purified water and mix thoroughly.

Storage: Plastic container.

6.8.4 Z4 solution – 17.5% Sulfuric Acid

- Measure approximately 825mL of purified water into a large beaker.
- Measure (using a measuring cylinder) 175mL of concentrated sulfuric acid.
- Carefully and slowly, with stirring, add the sulfuric acid to the purified water.
- Mix thoroughly.

Storage: Glass containter.

NB: It may be necessary to stand the beaker in an ice bath during the mixing operation.

6.8.5 Z5 solution – 2% Potassium Iodide, 0.01M NaOH, 1% starch solution.

- Measure 500mL of purified water and bring to the boil.
- Weigh 10g of soluble starch and with stirring, dissolve in the boiling purified water.
- Allow to cool.
- Weigh 20g of AR grade potassium iodide (KI).
- Measure (using a measuring cylinder) 10mL of 1M NaOH and add to approximately 500mL of purified water and mix.
- Dissolve, with stirring, the KI in the 500mL NaOH solution.
- Combine the starch solution and the KI solution.
- Mix thoroughly.

Storage: Glass or plastic container (if storing in a glass container, use a plastic stopper, as a glass stopper may fuse to the neck of the glass container due to the formation of sodium carbonate between the surfaces).

6.8.6 Z6 solution – 1.4% Sodium thiosulfate, 0.05M NaOH.

- Accurately weigh 13.78g of AR grade sodium thiosulfate.
- Dissolve in purified water.
- Quantitatively transfer to a 1L volumetric flask with purified water.
- Measure (using a measuring cylinder) 50mL of 1M NaOH and add to the volumetric flask.
- Make to the mark with purified water and mix thoroughly.

Storage: Plastic container.

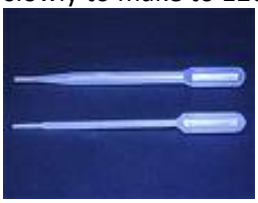
6.9 Ethanol standard (16.00%) preparation for Acolyser calibration

Weigh 126.72g/L Ethanol with density of 0.788-0.795 g/ml into a 250ml Volumetric flask using an analytic balance in lab 406/201, then make to final volume of 1Litre in a 1000ml Volumetric flask to get **16.00% Ethanol standard.**

Procedures:

- Check the density of ethanol on the label of the bottle
- If density is a range of 0.788-0.795 g/ml, then use the average of minimum and maximum, i.e. Ave density =(0.788+0.795)/2=0.792g/ml

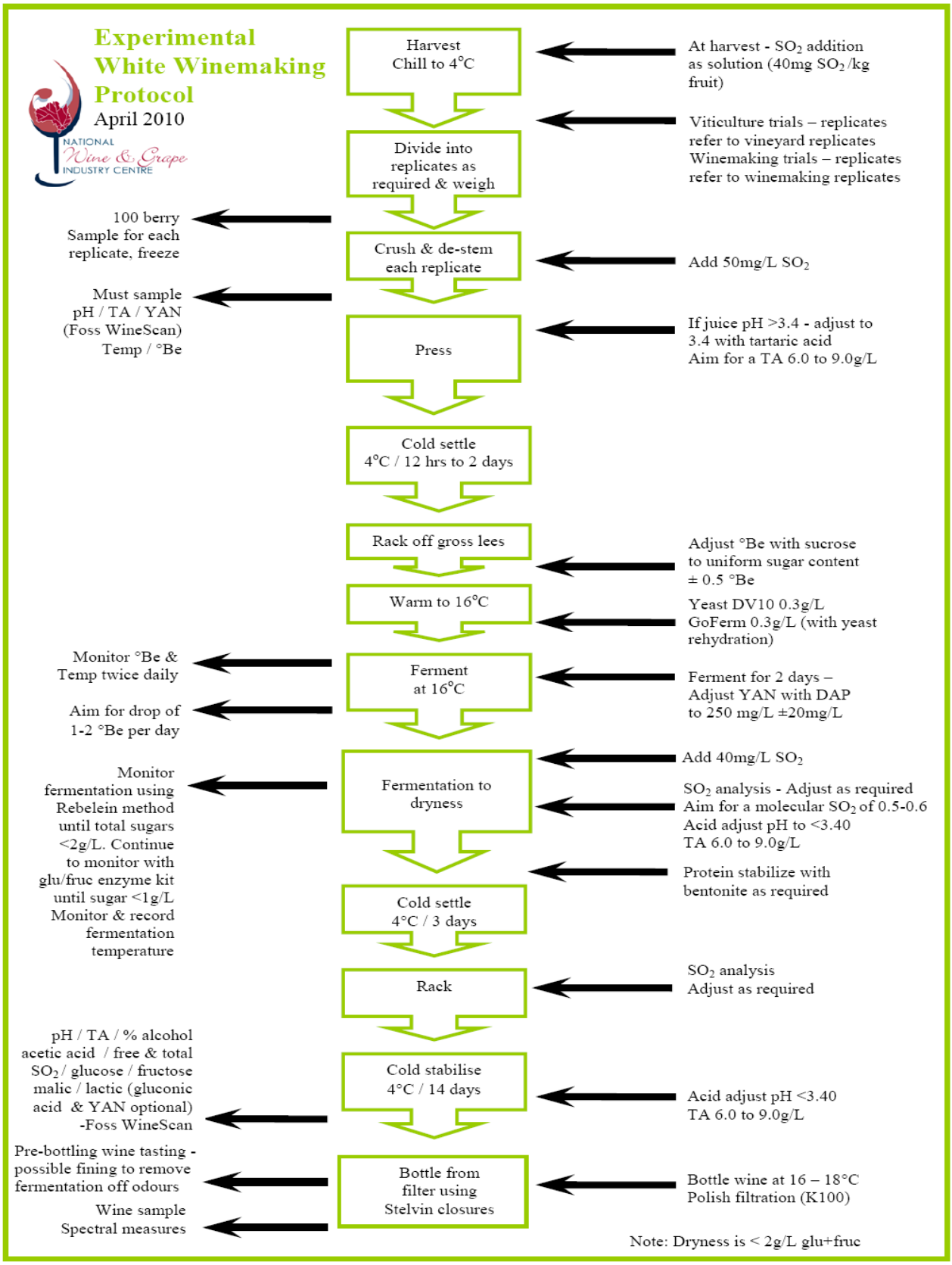
- For 1 litre solution the ethanol will be 0.792g/ml * 1000=792.0g/L
- We want to make 15% to 17% (V/V), saying 16.00% standard solution, therefore the required weight (X) of pure 100% ethanol to make 1 litre 16% solution is (792.0g/L:100%=X:16%)
- X=792.0g/L * 16%/100%=126.72g/L Ethanol
- Using 250 ml Volumetric flask (Wt of 90.3g) with labelled of Expt Alcohol
- Must using analytical balance No 16 with maximum weight of 220g in lab 406/201 because all other balance having no enough capacity to weigh 126.72g/L Ethanol in a 250 ml Volumetric flask
- Place the 250 ml Volumetric flask on balance No 16 and Press [Tare] to Zero the balance
- Transfer pure ethanol into a small beaker
- Holding a small glass funnel by hand and purring ethanol in the small beaker into the 250 ml Volumetric flask on the balance until the reading in really close to 126.5g
- Then using a plastic pipette to add it slowly to make to 126.72g



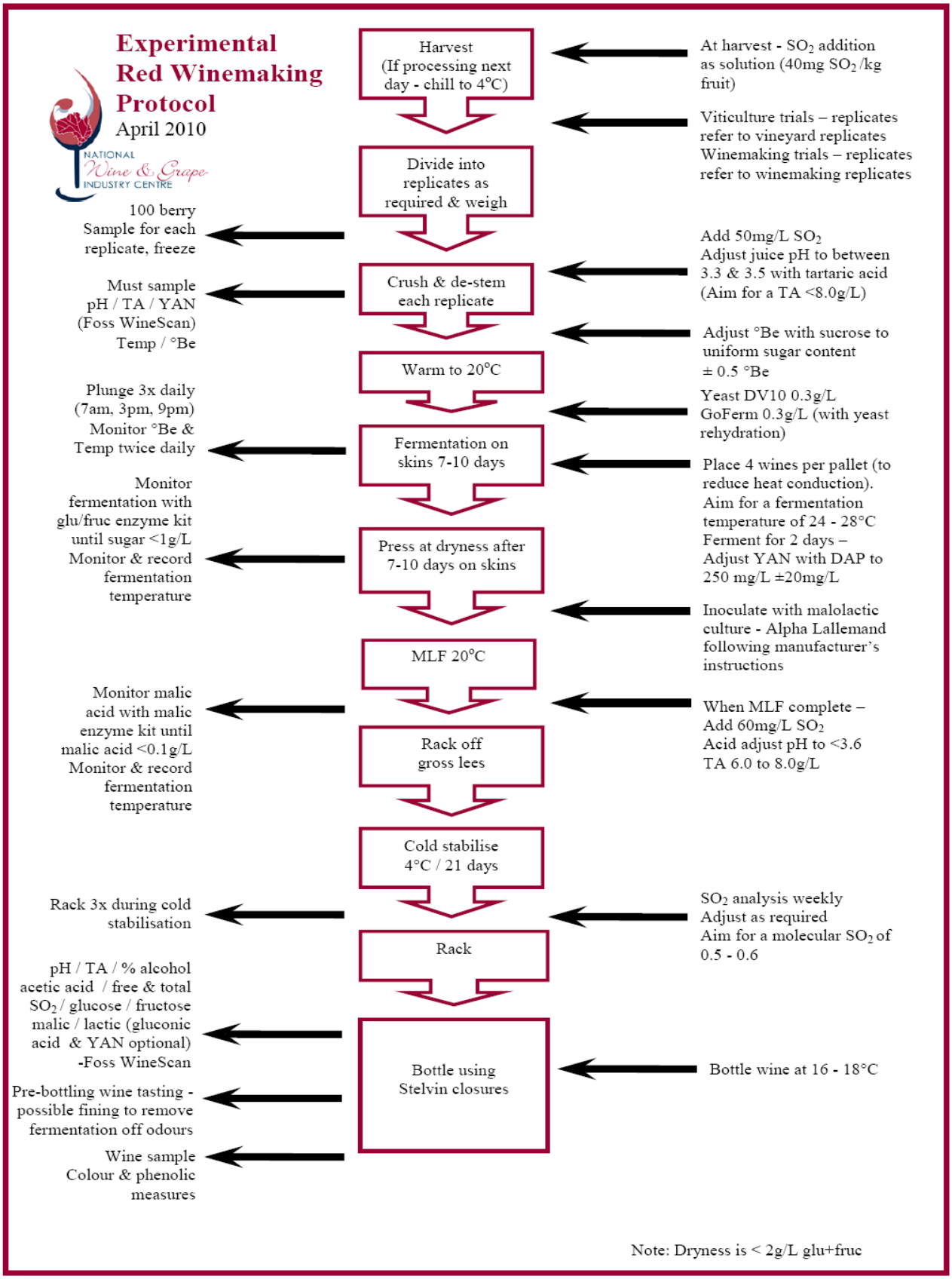
- If slightly more than 126.72g, eg 127.0534g, (DON'T PANIC), using the following formula to calculate your final concentration (X%) of the solution:
- %X=Actual reading of weight/792.0*100
- =127.0534/792.0*100
- =16.04%
-
- Record the concentration of 16.04% on the label of solution on the bottle
- When using the solution to calibrate the Acolyser, please REMEMBER to change to the concentration of 16.00% to the correct concentration (16.04%) of the ethanol solution used for calibration (using the up and down arrow keys and the side arrow key).

7. Procedure

7.1 NWGIC Small Lot White Winemaking Protocol



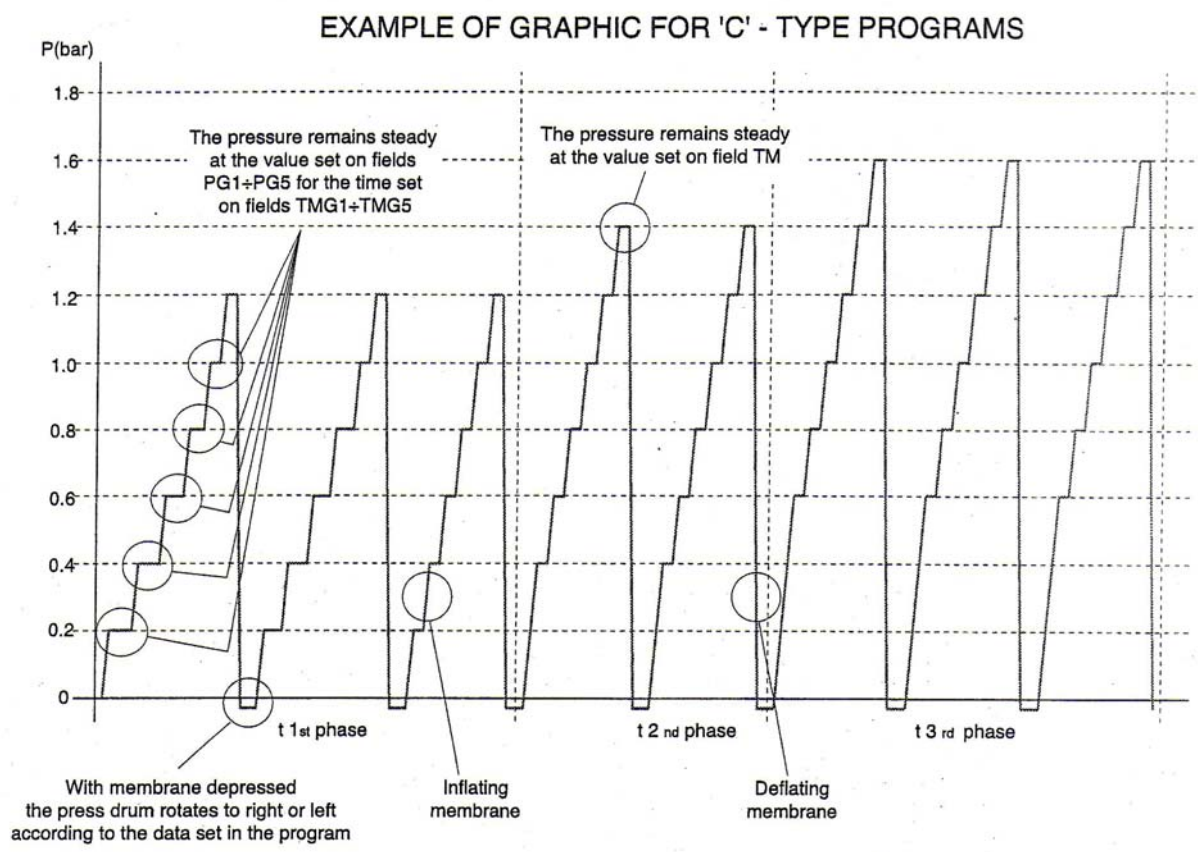
7.2 NWGIC Small Lot Red Winemaking Protocol



Temperature and Baumé are measured twice daily (7am and 3pm) using an Anton Paar DMA 35N portable density meter.

For viticulture trials and small scale batches (up to 50kg), a Zambelli 70 litre electric stainless steel basket press is used for pressing. The press enables a slow, constant pressing process where the press working pressure has a step-by-step trend. The operating pressure is adjusted to 1 bar, 1.5 bar followed by a maximum applied pressure of 2 bar. Pressure is applied gradually until the maximum pressure is reached. The pressure is increased steadily, until the required pressure for the particular phase is reached.

For winemaking trials and larger batches, a Velo one tonne air-bag press is used which contains three different pressing methods. The method used is the 'C'-type program. This program type has three phases, each having a maximum pressure level to reach. The pressure increases, remains steady, increases and so on until it reaches the required pressure for that particular phase and will then move on to the next phase (see figure below).



7.3 ***Analysis***

7.3.1 *Juice analysis*

- Baumé – Anton Paar DMA 35N portable density meter (LMWI 40-10)
- pH – Denver Instrument pH/mV meter or Metrohm Fully Automated 59 Place Titrando System – refer to LMLM 40-2 (10.1 Patrick Iland *et al* 2004, Pp32-35) and LMWI 40-15
- Titratable acidity (TA) – manual titration via Denver Instrument pH/mV meter or Metrohm Fully Automated 59 Place Titrando System – refer to LMLM 40-3 (10.1 Patrick Iland *et al* 2004, Pp39-43) and LMWI 40-15
- Yeast assimilable nitrogen (YAN) – FOSS WineScan™ 79000 Auto (FTIR)
- Must analysis via FOSS WineScan™ 79000 Auto – measurements of °brix, density, fructose, glucose, pH, total acidity, alpha amino nitrogen, ammonia, Yeast Assimilable Nitrogen (YAN), glycerol, acetic acid, tartaric acid, malic acid, lactic acid and gluconic acid.

Spectral measures on white grapes:

- Total phenolics
- Total hydroxycinnamates
- Total flavonoids
- Estimate of brown pigments
  – via Shimadzu UV 1700 PharmaSpec refer to LMLM 40-4 (10.1 Patrick Iland *et al* 2004, Pp92)

Spectral measures on red grapes:

- Colour
- Total phenolics
  – via Shimadzu UV 1700 PharmaSpec refer to LMLM 40-5 (10.1 Patrick Iland *et al* 2004, Pp44-48)

Berry samples are collected prior to processing and are stored at -20°C for three months before analysis is carried out. For spectral measures on white berries, the frozen berries are thawed at room temperature and juiced using a Panasonic Juice Extractor. For spectral measures on red berries, the frozen berries are partially thawed and are homogenised using an Ultra-Turrax T25 Homogeniser. Grape and wine analysis methods are performed as described in Iland *et al* (2004) Chemical analysis of grapes and wine: techniques and concepts.

7.3.2 *Fermentation/must analysis*

- pH – Denver Instrument pH/mV meter or Metrohm Fully Automated 59 Place Titrando System – refer to LMLM 40-2 and LMWI 40-15
- Titratable acidity (TA) – manual titration via Denver Instrument pH/mV meter or Metrohm Fully Automated 59 Place Titrando System – refer to LMLM 40-3 and LMWI 40-15
- Free and total sulfur dioxide – FOSS FIAstar™ 5000 (NIR) or the Aspiration method

– refer to LMWI 40-14 and LMLM 40-6 (10.1 Patrick Iland *et al* 2004, Pp54-58)

- Reducing sugars by the Rebelein method – refer to LMLM 40-7 (10.1 Patrick Iland *et al* 2004, Pp68-71)
- Enzymatic analysis of Glucose/fructose via Shimadzu UV 1700 PharmaSpec – refer to LMLM 40-8 (Megazyme D-Fructose and Glucose Assay Procedure - K-FRUGL 11/05)
- Enzymatic analysis of malic acid via Shimadzu UV 1700 PharmaSpec – refer to LMLM 40-9 (Megazyme L-Malic acid Assay Procedure - K-LMALR: 58 Assays per Kit, K-LMALL: 116 Assays per Kit, 11/05)
- Bentonite fining trial – refer to LMLM 40-10 (10.2 Patrick Iland *et al* 2004, Pp76-77)
- Protein stability/Heat stability test – refer to LMLM 40-11 (10.1 Patrick Iland *et al* 2004, Pp 96)
- Addition of copper sulfate for removal of hydrogen sulfide – refer to LMLM 40-12 (10.2 Patrick Iland *et al* 2004, Pp78-79)

7.3.3 *Wine analysis*

- pH – Denver Instrument pH/mV meter or Metrohm Fully Automated 59 Place Titrando System – refer to LMLM 40-2 and LMWI 40-15
- Titratable acidity (TA) – manual titration via Denver Instrument pH/mV meter or Metrohm Fully Automated 59 Place Titrando System – refer to LMLM 40-3 and LMWI 40-15
- Acetic acid – FOSS WineScan^{TM} 79000 Auto (FTIR) or Volatile acidity using a Markham Still – refer to LMLM 40-13 (10.1 Patrick Iland *et al* 2004, Pp72-75)
- Free and total sulfur dioxide – FOSS FIAstar^{TM} 5000 (NIR) or the Aspiration method – refer to LMWI 40-14 and LMLM 40-6

- Alcohol % v/v – Anton Paar Alcolyser, DMA 4500 density meter (NIR) LMWI 40-10
- Wine analysis via FOSS WineScan^{TM} 79000 Auto – measurements of pH, total acidity, acetic acid, volatile acidity, alcohol, density, specific gravity, fructose, glucose, fructose/glucose combined, glycerol, tartaric acid, malic acid, lactic acid and gluconic acid.

Spectral measures on white wine:

- Total phenolics
- Total hydroxycinnamates
- Total flavonoids
- Estimate of brown pigments – via Shimadzu UV 1700 PharmaSpec refer to LMLM 40-4

Spectral measures on red wine:

- Wine colour density
- Wine colour hue
- Degree of red pigment colouration
- Estimate of the concentration of sulfur resistant pigments
- Total red pigments

- Total phenolics
  – via Shimadzu UV 1700 PharmaSpec refer to LMLM 40-14 (10.1 Patrick Iland *et al* 2004, Pp88-91)

8. Calculations and Reporting

8.1 Calculation of additions during wine fermentation

The spreadsheet of 40-1a Calculation for Small Lot Winemaking.xls is used to calculate the additions of PMS, Sucrose, Tartaric Acid, Yeast & Goferm, and DAP during wine fermentation and winemaking process

8.2 Calculations of the parameters of wine and juice analyses

The following methods require the calculation of determination of parameters for wine and juice analyses:

- Titratable acidity via manual titration – refer to LMLM 40-3
- Anthocyanins (colour) and total phenolics of grape berries – refer to LMLM 40-5
- Sulfur dioxide by the Aspiration method – refer to LMLM 40-6
- Reducing sugars by the Rebelein method – refer to LMLM 40-7
- Volatile acidity using a Markham Still – refer to LMLM 40-13
- Red wine colour and phenolic measures – refer to LMLM 40-14
- White juice and wine spectral measures – refer to LMLM 40-4

Data reports are produced for each vintage and are sent to the project leader and Director of the NWGIC.

9. Quality Control

- Maintain quality assurance of wine analysis by participating in the Interwinery Analysis Group program. Statistical reports are sent back from the Interwinery Analysis Group giving our laboratory a z-score stating if our results for analysis are satisfactory, questionable or unsatisfactory. Procedures are set in place if questionable or unsatisfactory results occur to rectify the problem.
- The use of standards, maintenance and calibration of equipment (pipettes, balances, pH meter, Auto-titrator, sulfur analyser) to ensure running efficiency, longevity, result reliability and to minimise sample contamination. Log books for maintenance and quality assurance are kept up to date.

10. Method Precision

- Refer to LMLM 40-15 (10.1 Patrick Iland *et al* 2004, Pp10-13)

– Validating and checking analysis methods: Testing accuracy and precision.

11. References

11.1 Iland, P., Bruer, N., Edwards, G., Weeks, S. & Wilkes, E., 2004**.** Chemical analysis of grapes and wine: techniques and concepts. Patrickk Iland Wine Promotions, Campbelltown, South Australia.

11.2 Iland, P., Bruer, N., Ewart, A., Markides, A. & Sitters, J., 2004. Monitoring the winemaking process from grapes to wine: techniques and concepts. Patrickk Iland Wine Promotions, Campbelltown, South Australia
11.3 Megazyme D-Fructose and Glucose Assay Procedure (K-FRUGL 11/05)
11.4 Megazyme L-Malic acid (L-MALATE) Assay Procedure [K-LMALR (58 Assays per Kit) K-LMALL (116 Assays per Kit), 11/05]

Appendix 6
Budget reconciliation

See Winegrowing Futures Final Report, Theme 1