Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Identification and origin of volatile compounds responsible for important wine sensory attributes

FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWRI 3.1.1**

Principal Investigator: Dr Leigh Francis

Research Organisation:

The Australian Wine Research Institute

Date: **22 September 2017**

Project 3.1.1 – Identification and origin of volatile compounds responsible for important sensory attributes

Abstract

Knowledge of the flavour compounds that are responsible for the sensory characteristics of wines is of great importance to be able to control and adjust wine aroma properties in production. Compounds not previously recognised as active flavorants in specific varieties have been identified in this project for the first time, including several compounds that give Viognier wines their distinctive 'apricot' flavour, and a 'green' flavour compound in Shiraz. In addition, numerous compounds not previously understood adequately have been revealed as significant in wine flavour, such as sulfur compounds in Chardonnay being found to confer fruit attributes. Ways of adjusting the contribution of various compounds have also been examined. For example, absorption onto food grade plastic film was shown to be effective in removing 'green' character from a red ferment, while avoidance of material from specific wind-break vegetation planted near vineyards reduced 'green' flavour in wine. Another outcome of this research has been the development of analytical tools for measurement of key wine flavour compounds, and improved understanding of winemakers' concept of 'green' tannin and flavour with reference to consumer preferences. Studies of Chinese consumer red wine preferences and behaviour have helped shed light on this rapidly growing and important market for Australian wine.

Executive summary

A good understanding of the relationship between wine composition and wine flavour is important to being able to control grape and wine quality. This research project was carried out to improve knowledge of:

- compounds responsible for key flavours, including the identity of previously unrecognised compounds, especially for the varieties Shiraz and Chardonnay
- the relationships between wine composition and wine sensory properties;
- the effect of viticultural and oenological techniques on the formation of compounds, such as 'green' flavour

and to develop routine analytical methods for flavour compounds that can be applied in research and industry trials.

Using sensory-guided chemical methods, gas chromatography-mass spectrometry, in combination with olfactometry (use of the human nose as a detector), liquid chromatography –mass spectrometry, and formal sensory studies, compounds responsible for flavour attributes in wine were identified. Analytical methods using isotopically labelled standards were adopted where possible, to ensure accurate, precise and sensitive analyses of compounds at or below ppb levels. Surveys of commercial wines were used to confirm the importance of compounds, while detailed chemical studies shed light on the formation reactions occurring in winemaking. Use of replicated viticultural and winemaking studies enabled the effect of different production practices to be determined, and formal sensory and consumer preference data were obtained.

Analytical methods were established for a number of key flavour compounds, providing enhanced ability to measure levels of compounds with excellent context regarding their sensory significance, including detection thresholds and flavour contributions. The compounds included 3-mercaptohexanol, 3-mercaptohexyl acetate, 4-methyl-4-mercaptopentan-2-one, benzyl mercaptan, furfuryl thiol and numerous aldehydes and other compounds related to oxidative effects. Being able to measure such compounds allowed greatly improved understanding of the effect of yeast and fermentation, oxygen, nutrients and many other variables through various AWRI research projects, and the methods were used by numerous wine industry personnel, through collaborative studies or in resolution of issues between third parties.

The compounds responsible for the 'apricot' flavour in Viognier wine were identified as the monoterpenes, geraniol, linalool and nerol. These are compounds not previously associated with this flavour, and their sensory interactions with other aroma compounds were established. While a series of lactone compounds were found to be generally unimportant to wine flavour, their contribution in combination with monoterpenes to 'stone fruit' flavour was indicated. Several esters were shown to contribute to 'peach-like' aroma in Chardonnay.

Several potent thiol compounds, previously known in Sauvignon Blanc wines, were determined to be major contributors to Chardonnay fruit flavour, including at high concentrations a Sauvignon Blanc-like 'tropical fruit' character. This opens a new area of control of flavour of this important variety. Similarly, the volatile thiol, benzyl mercaptan, was found to be related to 'flint'/'struck match' aroma in Chardonnay, providing a basis of control of this 'Burgundian' character in Australian wines.

Regarding 'green' flavour in red wines, grapevine proximity to Monterey Cypress trees was shown to be a cause, with grape rachis included in Shiraz ferments also a major influence. 'Green tannin' was elucidated to be related to both 'green' volatiles and elevated bitterness, probably related to specific tannins.

Studies investigating consumer preference and behaviour of Chinese consumers of red wines gave insight into Chinese-based language to describe wines, appropriate for marketing and communication purposes, with recognition of sensory attributes that relate to preference.

Overall, the results of this research project have increased knowledge of the main volatile compounds involved in wine flavour attributes, so that the causative compounds for many of the most important sensory attributes of wines are now established and many of the influences on their formation understood. While there are several key sensory attributes of wines where the cause is still not known, this research stream has given producers new knowledge to avoid negative flavours and enhance positive flavours.

Background

While many sensory attributes of wines have a known cause, there are major flavour characteristics where the source compounds are not known. The measurement of characteristics of grapes and wines that determine quality from a consumer perspective relies on knowledge of the constituents that give rise to these characteristics. Measurement of key flavour compounds to assess viticultural effects and outcomes from winemaking or processing options is of great importance; knowledge developed in this project will be applied in associated projects.

For attributes such as 'green' flavour in Shiraz and 'stone fruit' flavour in Chardonnay there are no clear target compounds known. For 'green' flavour, it is not clear if this attribute, which is used rather loosely across the wine sector, involves: volatile compounds such as methoxypyrazines; C6 compounds such as cis-3 hexanol; dimethyl sulfide; a low level of fruit flavour compounds; any relationship with tannin ('green tannin'); or links to higher acidity/lower alcohol matrix effects. There is also the possibility of an in-mouth effect of a compound such as isobutyl methoxypyrazine (IBMP) suppressing desirable mouth-feel characteristics.

For 'stone fruit' flavour, several γ-lactones important to peach/apricot fruits have been found in wines, however at levels well below aroma threshold (Cooke et al. 2009). There is some evidence that these lactones can act additively (Jarauta et al. 2006). While 'tropical' thiols are well-known key flavour compounds for Sauvignon Blanc, there is little knowledge of their importance to Chardonnay. However, unpublished work at the AWRI has provided evidence that they can contribute to the

flavour of this variety, and wines from some cooler regions (Margaret River and Great Southern in WA, and from Tasmania) can have a distinct 'tropical fruit'/'passionfruit' flavour.

For 'green' aroma, while methoxypyrazines are important to Bordeaux varieties, they are not found in Shiraz or Pinot Noir except potentially through extraction from green stalks. C6 alcohols, notably cis-3 hexanol, give 'grassy' aromas and have been implicated in greener wine flavour, but the evidence is not strong. There is very little evidence regarding 'green' tannins,

For volatile aroma and flavours from extraneous sources, the effect of cover crops on wine flavour has been studied only to a very limited extent (Xi et al. 2011), while a small quantum of work has been conducted on the effect of grapevine leaves, mainly on 'green' C6 compounds (Wildenradt et al. 1975). A recent paper outlined more than 600 volatile compounds in grapevine leaves (Weingart et al. 2012), while glycoconjugates of volatiles are known to be much more abundant in grapevine leaves than berries (Wirth et al. 2001). Recent work on eucalyptol at the AWRI showed that stalks and leaves mixed in the crusher with grapes can be a source of this compound in the final wine, greater than that of direct transfer from nearby eucalypt trees (Capone et al. 2012; Black et al. 2015).

Many Australian wine producers seek to achieve specific wine styles to suit specific markets, a task reliant on sourcing a suitable supply of grapes and selecting production methods to achieve the target style. Markers for measuring style have long been sought to inform production. However, relatively few objective measures of grape and wine compounds which can be directly related to style are currently available. Aroma compounds are of great importance in determining style, and building understanding of those compounds which influence consumers' perception of wine quality is an industry priority. Measures that can be applied in viticultural and winemaking studies are badly needed for evaluating industry trials and research studies, and potentially for streaming fruit.

Highlights

- The main compound known to be responsible for 'green' flavour in Cabernet Sauvignon, isobutyl methoxypyrazine, which was previously thought not to be biosynthesised in Shiraz grapevines. was found to contribute 'green' aroma in Shiraz wine made with stems, notably in whole bunch fermentations
- Nearby windbreak trees were found to contribute 'green' flavours to harvested grapes and thus wines
- The monoterpenes geraniol, linalool and nerol were found to give Viognier wines their distinctive 'apricot-like' flavour
- Thiols were confirmed as major contributors to Chardonnay flavour, with 3-mercaptohexanol and 3-mercaptohexyl acetate contributing, at moderate levels, 'citrus fruit' character and at higher levels 'tropical' aromas, and benzyl mercaptan adding 'flint'/'struck match' aromas.

Objectives

The project has an overall objective of identifying, and developing analytical methods for volatile compounds causing key flavour attributes of wines, and acquiring sufficient knowledge regarding their sensory significance, consumer response and origin to allow industry-relevant specifications to be developed.

There are three main objectives:

- To identify compounds responsible for specific important flavour characteristics in wine, and acquiring information about levels required for desirable flavour:

    a. Stone fruit in white wine
    b. ‘Green’ in red wine
    c. ‘Tropical fruit’ in Chardonnay
- To develop analytical methods for key volatile aroma compounds.
- To determine factors that affect the concentration of specific aroma compounds, including work on *Botrytis cinerea* and rotundone, leaves and stems and their effect on ‘green’ aroma, and volatiles from the local environment.

In addition, the interaction of wine composition, information and wine sensory properties on Chinese consumers’ preference, purchase intent and choice was studied.

Method

The project used several procedures to meet the objectives. For identification of key compounds, sensory-guided chemical analysis was used, with gas chromatography-mass spectrometry (GC-MS)-olfactometry, sensory-based aroma detection threshold measures and bench-style sensory assessments applied to identify probable compounds responsible for key sensory attributes of wines (Mayr et al. 2014). The identity of the volatile compounds responsible for aroma attributes in wine was established through synthesis of compounds for use as analytical standards, and through GC-MS studies and addition sensory studies, to confirm the identity and the importance of the compounds (Mayr et al 2014).

Targeted, specific analytical methods using GC-MS or LC-MS were developed for a range of compounds using stable isotope dilution analysis, with synthesised standards as required. The compounds included (E)-2-alkenals ((E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal and (E)-2-nonenal), several Strecker aldehydes (methional, 2-phenylacetaldehyde, 3-methylbutanal and 2-methylpropanal), aldehydes (furfural, 5-methylfurfural, hexanal, and benzaldehyde), furans (sotolon, furaneol, and homofuraneol), as well as alcohols (methionol, eugenol, and maltol), in the same analysis, with the aldehydes derivitised using O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride. These compounds were analysed by GC-MS/MS (Mayr et al. 2015). The thiol compounds 3-mercaptohexan-1-ol, 3-mercaptohexyl acetate, 4-mercapto-4-methylpentan-2-one, 2-furfurylthiol, and benzyl mercaptan were quantified using 4,4'-dithiodipyridine (DTDP) as a derivatising agent with HPLC-MS/MS (Capone et al. 2015). A new GC-MS/MS method for analysis of dairy lactone ((Z)-6-dodeceno-γ-lactone) was also developed (Siebert et al. submitted) using SPME, together with a chiral analysis olfactometry method using a chiral capillary column.

The methods were validated and simplified where possible to allow routine, relatively rapid analysis for high throughput as well as transfer of methods to commercial laboratories, with appropriate limits of quantification. Quantitative analytical methods for the major known aroma compounds in wines were applied to support other research programs and for analysis of industry samples (Mayr et al. 2015; Capone et al. 2015).

These quantitative analysis methods were used to survey commercial wines (e.g. Capone et al. 2017; Siebert et al. submitted), with the numbers of wines where a compound was above sensory detection threshold providing evidence that the compound was of importance to wine.

The formation of compounds was established through kinetic and mechanistic studies and ways of adjusting their levels through viticultural and winemaking practices were assessed (Capone et al. 2016). The project team worked closely with multiple industry partners to assess commercial options for adjusting levels of aroma compounds.

For many compounds, the sensory significance was further determined through collection of sensory descriptive and consumer preference data with consumers rating liking on a nine-point hedonic scale (e.g. Capone et al. 2017), with rapid sensory methods also applied (Pearson et al. 2015). Partial least squares regression and principal component analysis were commonly applied to assess the multivariate datasets obtained. Consumer preference information in China was approached using a discrete choice experiment methodology (Williamson et al. 2016), with preference mapping of identified clusters (Williamson et al. in press 2017)

Results and discussion

The research project concentrated on identifying and studying volatile compounds responsible for several important sensory attributes, attributes that were identified from previous consumer studies as important to wine quality. Knowledge of Shiraz and Chardonnay flavour was considered high priority as these varieties are of greatest commercial importance to the Australian industry.

'Stone fruit' flavour in white wine

The compounds that cause 'stone fruit' flavour in Chardonnay and Viognier have been identified. Over the initial period of the project, by studying commercial wines showing high and negligible 'stone fruit' aroma, a set of n-alkyl lactones was indicated as important, including a little understood lactone (gamma-dodecalactone, known as 'dairy lactone'), together with the monoterpenes linalool, geraniol and nerol (Siebert et al. 2016). From further extensive investigations, it was shown that for Chardonnay, the relatively subtle 'peach-like' aroma of wines of this variety was closely linked to several specific esters, notably 3-methylbutyl acetate, hexyl acetate and ethyl hexadecanoate. For Viognier, which was investigated as this variety is little studied and commonly shows a clear 'apricot-like' aroma, linalool, geraniol and nerol were notably important to the 'apricot' character (Figure 1, Siebert et al. submitted). There remains a possible sensory contribution of the lactones, but they are substantially less important than the other compounds identified, except for *Botrytis*-affected sweet wines, where gamma-nonalactone was identified as a major aroma contributor. This compound may also be a contributor to the 'apricot' character in other white wines. Consumers did not overall prefer wines with high 'apricot' flavour, although this was likely to be related to mouth-feel/taste attributes such as bitterness which correlated with the aroma character. The recognition of the monoterpenes linalool, geraniol and nerol as key compounds for the 'apricot' flavour attribute is novel, opening up viticultural and winemaking options to enhance this character in finished wines.

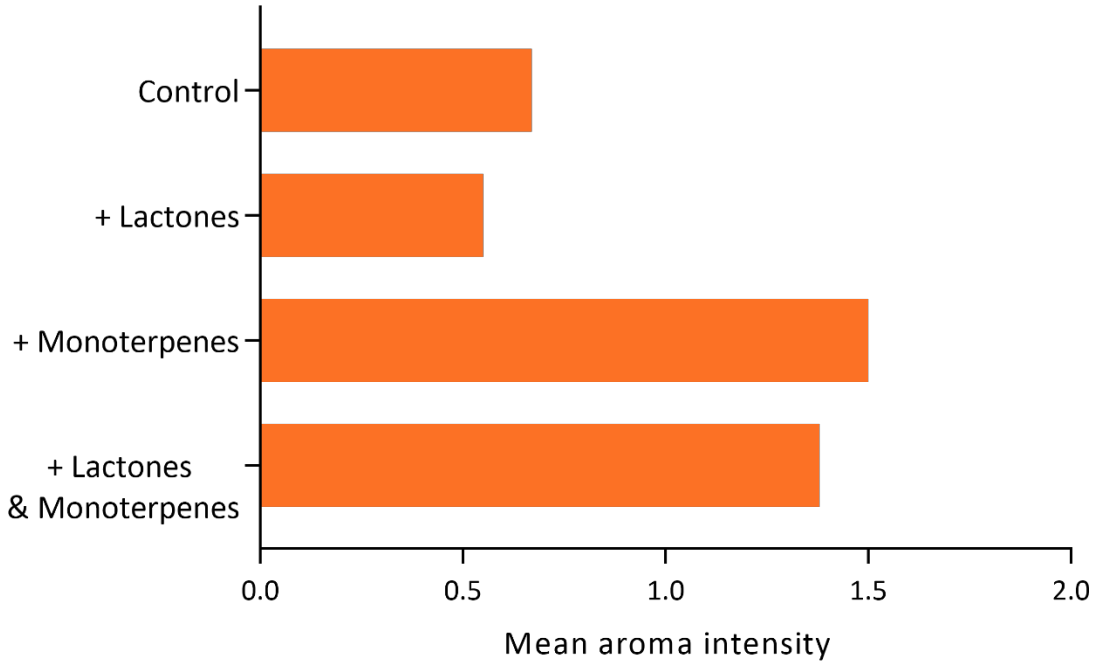

Figure 1. Mean 'apricot' aroma rating from a sensory panel for a model wine control, with added wine aroma compounds such as esters, acids and alcohols, compared to the model wine with aliphatic lactones and monoterpenes, added at the concentrations found in a white wine with distinct 'apricot' flavour.

Overall, this aspect of the project was completed largely as planned, although the publication of refereed manuscripts was not achieved to the final timeline. Three manuscripts describing the work have been drafted at the time of writing, with one submitted. One paper was published with collaborators from the University of Bordeaux describing work conducted on 'cooked orange' aroma in sweet white wines. A poster was presented at the 16th Australian Wine Industry Technical Conference in July 2016, and was awarded a best poster prize.

'Green' flavour in red wine

Studying commercially available Shiraz and Cabernet Sauvignon wines, the strong relationship of isobutyl methoxypyrazine to 'capsicum'/'bell pepper'/'stalky' character was confirmed. A major advance in knowledge from this project was the identification of this compound at a sensorially important concentration in 'green' Shiraz wines, a variety that previously was not considered to not be able to biosynthesise this compound. Working with CSIRO scientists, the compound was demonstrated to be present at high concentrations in research-scale wines made from warm inland grapes from vigorous rootstocks. From a winemaking study, it was clearly shown that the inclusion of stems in a Shiraz ferment gave an elevated concentration of the compound (8 ng/L), together with the closely related isopropyl methoxypyrazine (10 ng/L), and a clear sensory effect (Figure 2). Interestingly, grape leaves present in the ferment did not give any 'green' flavour but enhanced 'red berry' fruit, most likely due to conversion of leaf alcohols to hexyl acetate (Capone et al. 2016). This study shone new light into the practice of whole bunch fermentation, where compounds from the rachis will be extracted.

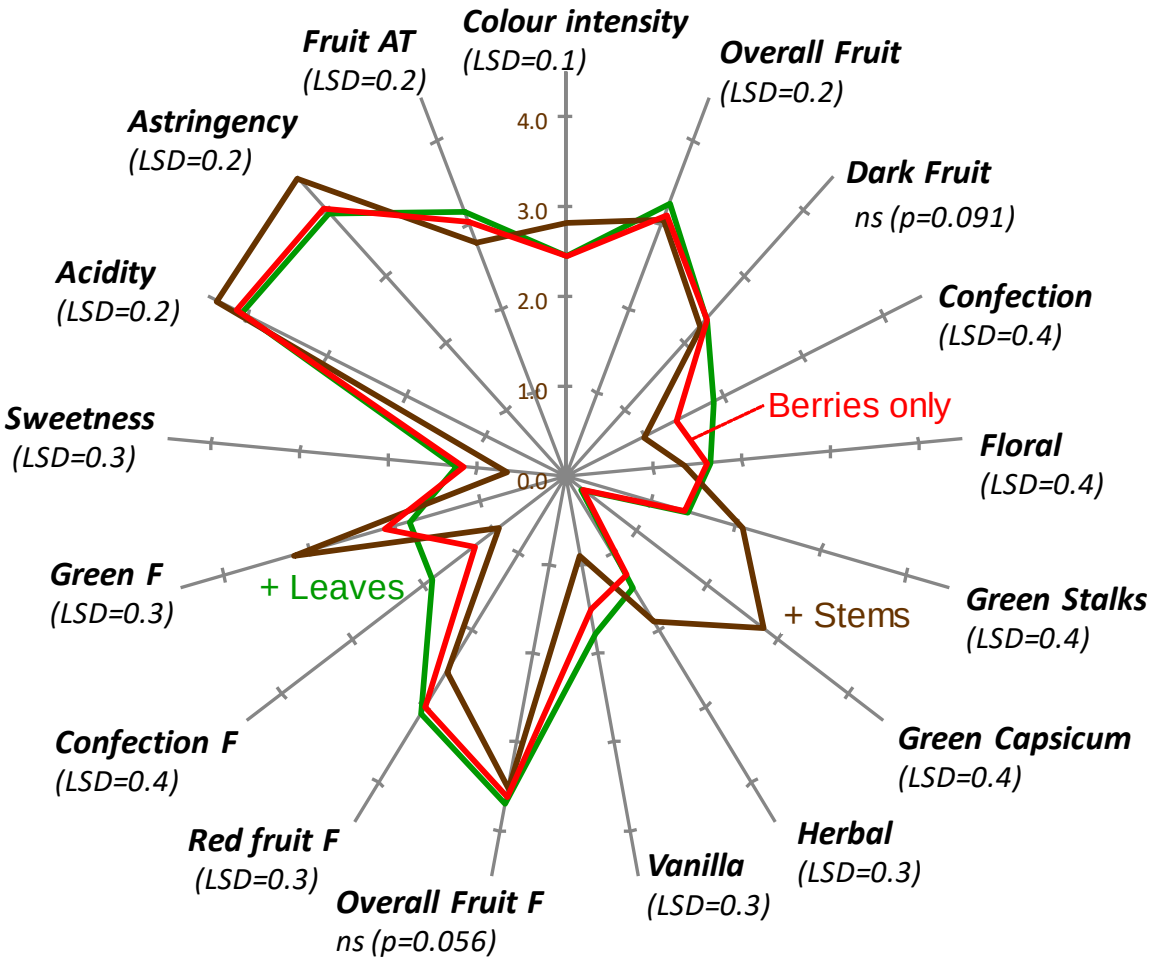

Figure 2. Mean sensory panel ratings for a Shiraz wine made from grape berries only (Berries only), berries plus stems (+ Stems), and berries plus grape leaves (+Leaves). The least significant difference (LSD, P=0.05) is also shown. Duplicate or triplicate ferments were assessed in triplicate by nine trained judges.

From a sensory point of view, improved understanding of winemaker concepts of 'green' character was achieved by using the technique of projective mapping with winemaker groups and comparing the data to that from trained sensory descriptive analysis panels and to compositional data (Pearson et al. 2015). For the commercially available wines studied, a winemaker description of 'green' or 'green tannin', through a projective mapping sensory task, related to the trained sensory panel attributes of bitterness and 'green stalks'/'vegetal' aroma and flavour attributes (see Figure 3 for the Cabernet Sauvignon sample set). Importantly, the proportion of catechin and percentage galloylation in the tannin was associated with bitterness, and the 'green stalks' attribute was linked to IBMP, as well as dimethyl sulfide concentration for both the Cabernet Sauvignon and Shiraz datasets. Consumer preference was also assessed and those wines with 'green' characters were found not to be liked by most consumers. Thus, this study has shown for the first time that the common, but hitherto poorly defined character of 'green tannin', can be related to chemical markers.

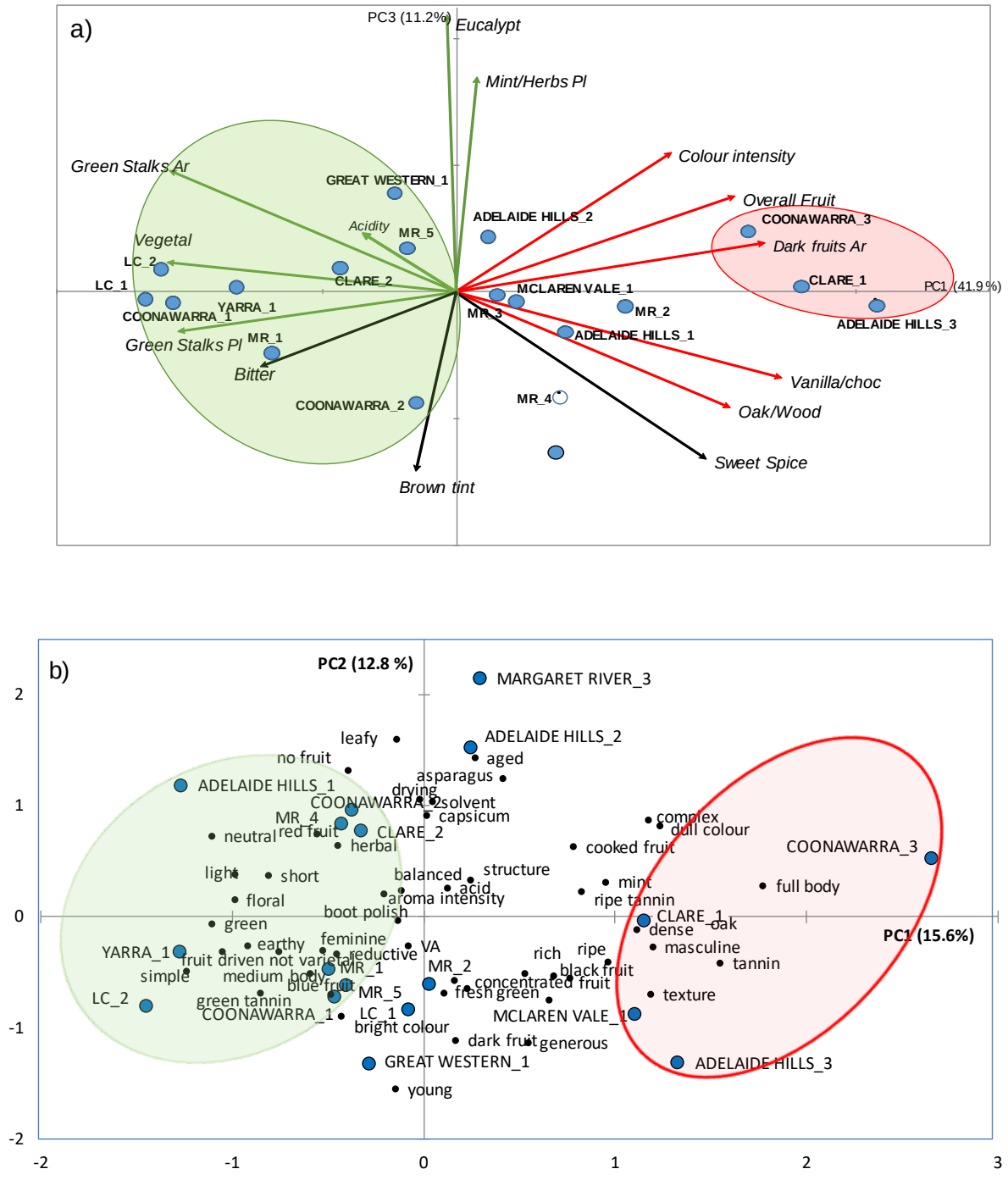

Figure 3. a) Principal component biplot of trained sensory panel descriptive analysis data and b) Mutiple factor analysis biplot from projective mapping data from a group of winemakers; for a set of commercial Cabernet Sauvignon wines.

A small-scale fermentation study was completed that showed that IBMP concentration was dramatically reduced if a food grade PVC-based cling wrap material was added to ferments, with the effect greater than for previously studied silicone or polyethylene materials (Figure 4). Some fermentation-derived esters and acids were reduced in concentration as a result of the treatment, but generally there was no significant effect of the plastic on most of the positive volatile compounds.

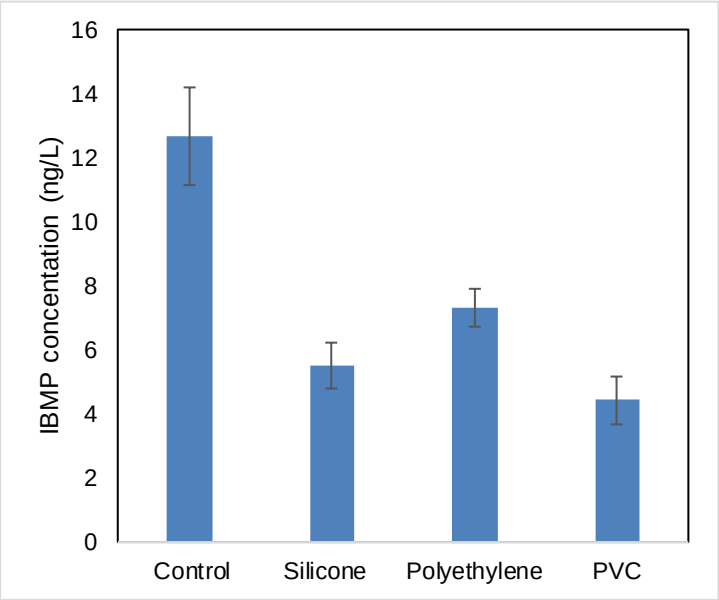

Figure 4. Mean isobutyl methoxypyrazine (IBMP) concentration in a Shiraz wine made from must with added IBMP and treated with three plastic materials, compared to the control, untreated must.

'Tropical fruit' in Chardonnay

The compounds responsible for 'grapefruit'/'tropical fruit' aroma in Chardonnay wines have been shown to be the polyfunctional thiols, 3-mercaptohexanol (3-MH), 3-mercaptohexyl acetate (3-MHA), and 4-methyl-4-mercaptopentan-2-one (4-MMP).

In data from 106 commercially available wines from multiple price points and multiple regions, each of these compounds was well above the reported sensory detection threshold in all wines, and the concentration for some wines approached that of wines from varieties such as Sauvignon Blanc, where these compounds are key varietal contributors. From data reported previously, the concentrations of these thiols in most of the wines examined was in the range where they would enhance fruit freshness but not confer 'tropical fruit'/Sauvignon Blanc-like flavour (Figure 5). Trends were observed that cooler climate regions produced wines with higher concentration of the potent 3-MHA, and younger Chardonnays were also generally higher in this compound.

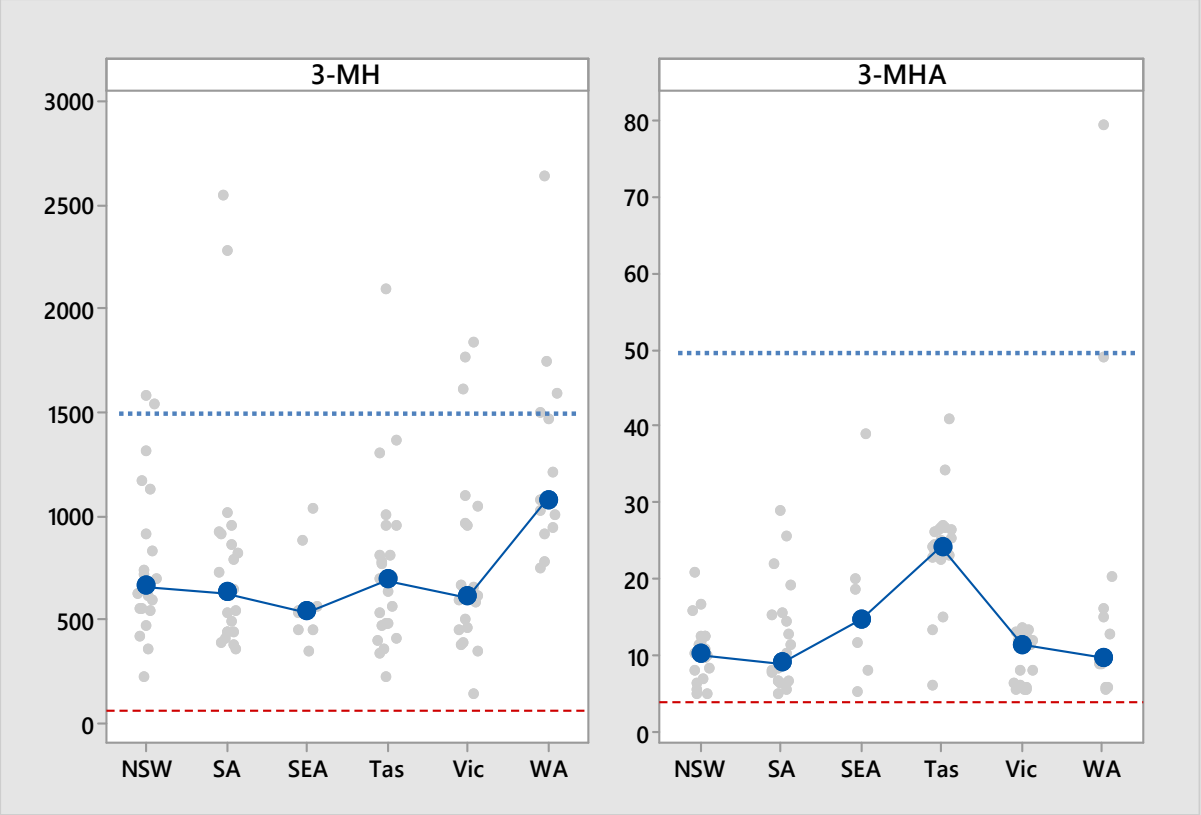

Figure 5. Concentration of two thiols for 106 commercial Chardonnay wines by Australia state, with the blue symbols indicating the median values and the grey symbols the individual data points. The broken red line denotes the aroma detection threshold, while the broken blue line the concentration previously reported to be the level above which the compounds give a clear 'tropical fruit' aroma.

A further sulfur aroma compound quantified was found to be related to 'struck flint'/match'/'smoky' aroma, namely benzyl mercaptan. This compound was also determined to be at higher concentration than the aroma detection threshold for all 106 commercially available Chardonnay wines, and from regression analysis those wines that had a higher retail price, that were from cooler regions and were somewhat older were more likely to have higher concentrations of benzyl mercaptan, most likely reflecting lees contact and barrel fermentation, which are associated with formation of this compound. Wines from Tasmania were also generally higher in this compound.

An experiment was completed where a set of 16 Chardonnay juices were obtained from across multiple viticultural regions, including the main premium-Chardonnay producing regions, and wines were produced using standardised winemaking with no oak influence. The thiol composition, as well as that of other volatile aroma compounds, and sensory properties were related. All of the wines had above threshold concentrations of 3-MH, with many above the level likely to give 'tropical fruit'-like flavour. Similarly, almost all wines had surprisingly high concentrations of 3-MHA, and benzyl mercaptan. 'Tropical fruit' character in the wines was found to be linked to the concentration of 3-MH and 3-MHA (Figure 6), while a 'box tree'/'cat pee' flavour was related to the presence of 4-MMP. The compound benzyl mercaptan was confirmed to be associated with 'struck flint'-like aroma. 'Citrus' flavour was also related to thiol concentration, in a non-linear fashion (Figure 6), indicating that higher concentration of 3-MHA/3-MH gives 'citrus'/'grapefruit' aroma, but at very high concentration the aroma changes to 'tropical fruit', confirming previous reports that fruit intensity is enhanced at moderate concentrations, and 'tropical fruit' is contributed at high concentration. Consumer preference was related to wines with relatively high concentration of the thiols, showing

that these compounds can be desirable and positive for wine quality for a large proportion of consumers. Interestingly, benzyl mercaptan was determined to above its aroma threshold concentration in these unoaked wines. This compound's mechanism of formation is not known but both oxidative and reductive conditions are probably required.

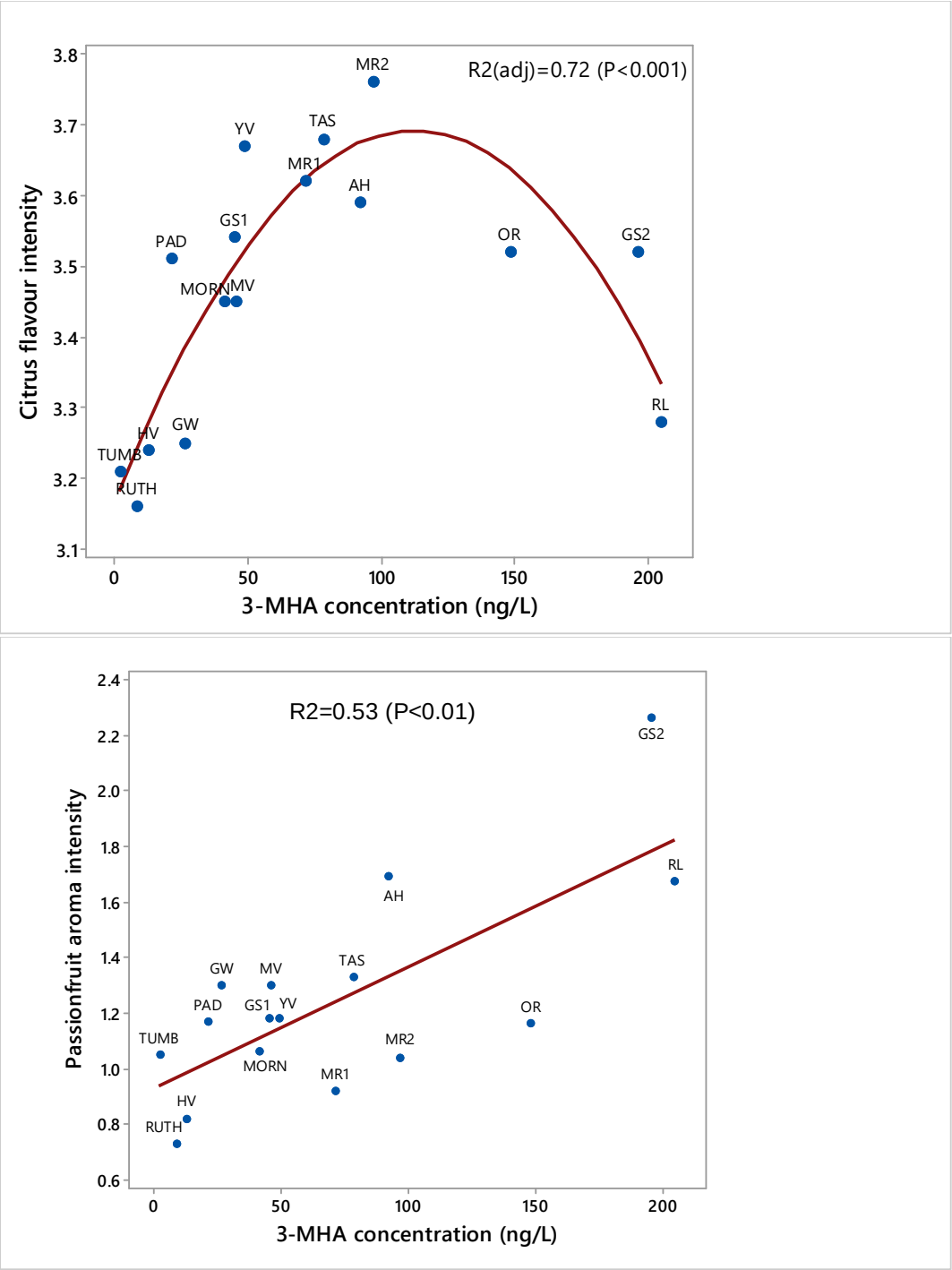

Figure 6. Regression plots showing the relationship for 'citrus' flavour and 'passionfruit' aroma scores from a trained sensory panel, with 3-MHA concentration for 16 Chardonnay wines made from juices sourced from multiple viticultural regions (RUTH: Rutherglen, TUM: Tumbarumba, PAD: Padthaway, GW: Great Western, MORN: Mornington, GS: Great Southern, MV: McLaren Vale, YV: Yarra Valley, MR: Margaret River, TAS: Tasmania, AH: Adelaide Hills, OR: Orange, RL: Riverland). Note that the juices were not necessarily typical of the respective regions.

This study also found that thiol precursors present in wine could act as a reserve to be released in-mouth upon consumption, as indicated by the importance of these non-volatile compounds to the fruit aftertaste sensory attribute.

It was of interest that for both the commercially available wines studied, as well as the wines made using standardised winemaking methods, that some warm inland irrigated regions such as the Riverina and the Riverland produced wines with thiol concentration at the upper range. It is possible that apart from yeast choice, which is well known to be influential in releasing these compounds from their precursors during fermentation, there may be other strong influences on thiol concentration, such as the nitrogen or sulfur fertilisation regime used. This has been previously shown from research done in France, and in discussing with some wine producers, it is a potential variable that warrants further study.

This sub-project was completed to the deadlines originally set, with a paper published (Capone et al. 2017), an industry article (Capone et al. 2016) and several AWITC posters and industry workshops/seminars completed.

Development of analytical methods for key volatile aroma compounds.
A method to quantify the main potent thiols that give 'tropical fruit', 'cat pee' and 'struck flint'-like aromas was developed with colleagues from the University of Adelaide. The method is much simpler than previous methods and is relatively rapid. A research paper describing the method was published (Capone et al. 2015).

A GC/MS method for quantification of a number of aldehydes and related compounds, including those most important to oxidative off-flavour and aged character, and some that are contributors to 'red berry' flavour, was optimised and the method published during the life of this project (Mayr et al. 2015), following from earlier development work.

Analytical methods have also been generated for the compound 'dairy lactone' (Siebert et al., in preparation) implicated in 'stone fruit' aroma, and a modification of the rotundone analytical method has been made using multidimensional GC to allow greater specificity and throughput (Scarlett et al. 2014).

Determining factors that affect the concentration of specific aroma compounds: Botrytis cinerea *and rotundone*
It has been previously shown that rotundone concentrations in grapes are elevated on the shaded back sides of bunches (Zhang et al. 2015). It had been speculated that this observation could be related to a relatively higher probability of fungal infections under shaded conditions, leading in turn to oxidation of sesquiterpene precursors to yield rotundone. After several experiments, it was determined that *Botrytis cinerea* infection of Shiraz berries had no obvious effect on production of the 'spicy'/'pepper' compound rotundone. However, in collaborative work with a French research group, powdery mildew infection of grapes was associated with the rotundone concentration of the subsequent wines (Geoffroy et al. 2015).

Substantial advances in knowledge of the influences on rotundone formation in the vineyard and winery were made over the life of this project. A study conducted with CSIRO and Mount Langi Ghiran wines, designed to confirm within-vineyard variability of rotundone in a premium Shiraz vineyard in the Grampians (Scarlett et al. 2014, Bramley et al. 2017), showed that independent of seasonal factors that alter rotundone in berries, spatial variation was clearly and consistently evident across seasons for a single site. While there were major differences in concentration of rotundone between the three seasons studied (2012, 2013 and 2015), the areas of high or low rotundone

concentration in the vineyard were highly consistent (Figure 7). This major study, the first of its kind to target a key grape-derived flavour compound, confirmed that rotundone concentration was related to temperature of the growing season, notably highlighting the veraison to harvest period. Within the vineyard, topographic variables were indicated as important. The work provided evidence that selective harvesting of vineyards, especially in cooler seasons, can give producers the ability to adjust wine style.

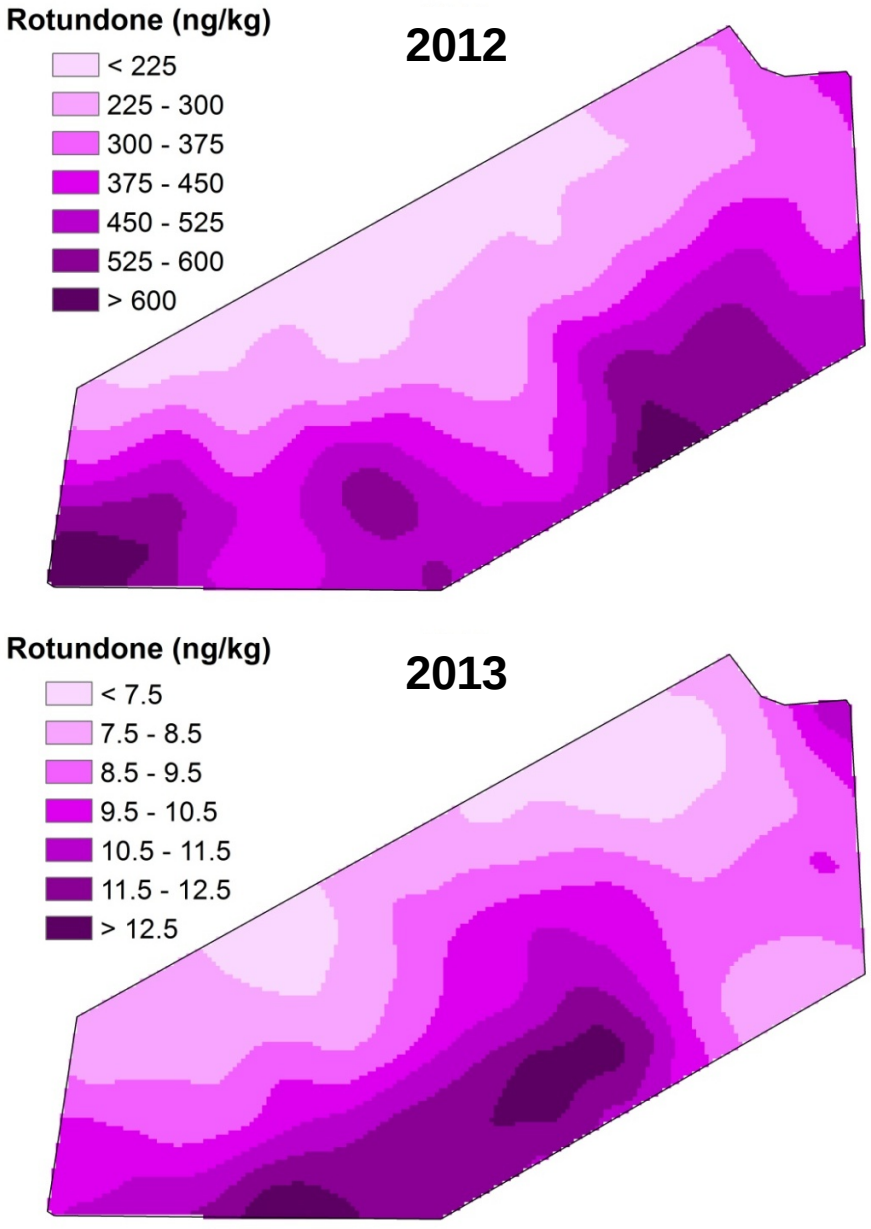

Figure 7. Spatial distribution of rotundone in Shiraz grape berries sampled, close to commercial harvest, across a vineyard from two seasons.

Investigations into the influence of sunlight on formation of rotundone showed that low sunlight conditions, mimicking 50% shade in the vineyard, resulted in a greater formation of rotundone than under full sunlight. This experiment was conducted in a model system using the major precursor of rotundone, α-guaiene, in a plant growth chamber or greenhouse environment. Similarly, under no light conditions, maximal concentration of rotundone was produced. This work showed that low light conditions in shaded bunches are likely to be favourable to the accumulation of rotundone and

might lead to being able to manipulate rotundone levels in the vineyard. Further research is required to include a number of vineyards comparing vine row aspect and shading or trellising of vines or application of physical sunscreen agents to confirm these results. The work was presented as a poster at the International Cool Climate Wine Symposium in 2016 and was awarded a best poster prize.

Studies have been completed into the effect of several viticultural and winemaking factors on rotundone concentration in grapes and wines. These projects have been carried out with international collaborators to increase data collection during both the northern hemisphere and southern hemisphere growing seasons. The ripeness of grapes was found to be a major influence (Geffroy et al. 2014), with grapes harvested 44 days post-veraison, that is at late stage ripening, having the highest concentration of rotundone. Leaf removal around the bunch zone had a large effect, resulting in strongly reduced rotundone concentration. An irrigation treatment was also found to increase rotundone, with veraison to harvest water status being a key factor (Geffroy et al. 2014). In a subsequent study, measurements of bunch surface temperature suggested that the effect of irrigation may be due to a cooler bunch microclimate through an increase in leaf area (Geffroy et al. 2016).

In a study on the variety Duras, which consistently has relatively high rotundone concentration, clonal effects were observed, with wines made from specific clones being widely varied in rotundone concentration (Geffroy et al. 2015), pointing to planting material being of importance to rotundone in other varieties. Assessing several winemaking variables, skin removal pre-ferment resulted in lower rotundone concentration, while use of macerating enzymes, increased fermentation temperature or longer skin contact time during fermentation did not result in elevated rotundone concentrations. Post-fermentation extended maceration or semi-carbonic fermentation resulted in lower rotundone concentrations.

Volatiles from the local environment.
The effect on wine flavour of vine proximity to windbreak trees was investigated. Wine made from Cabernet Sauvignon vines close to Monterey cypress trees had a notable eucalypt/pine-like aroma and flavour (Figure 8), while there was no clear sensory influence of she-oak trees or radiata pine trees on wines made from fruit from other vineyards studied (Capone et al. 2017). Wine producers can consider techniques to remove ‘matter other than grapes’ (MOG) from harvest bins or separate hand-harvesting of grapes from vines close to Monterey cypress trees if they wish to avoid their flavour influence.

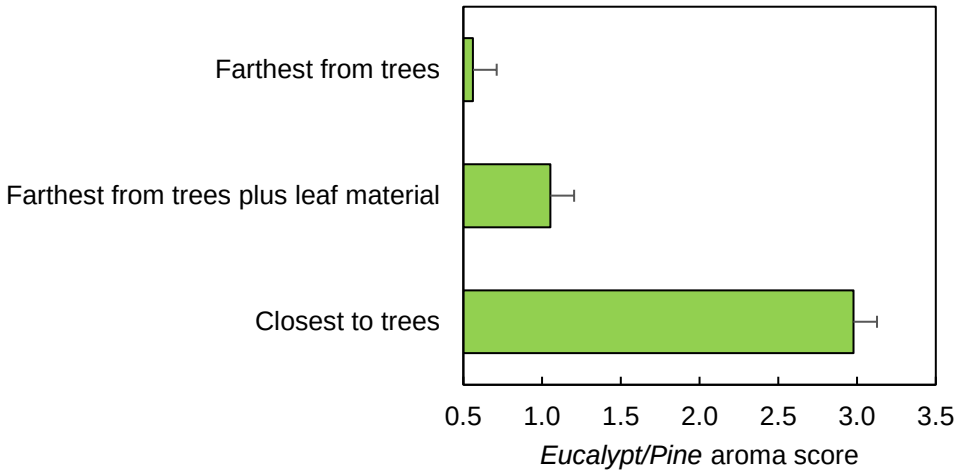

Figure 8. Mean sensory rating for the 'eucalypt'/'pine' aroma attribute for a Yarra Valley Cabernet Sauvignon wine made from grapes picked furthest from Monterey cypress trees, wine made from grapes picked furthest from the trees with the addition of Monterey cypress leaf material and wine made from grapes picked closest to the trees.

The interaction of wine composition, information and wine sensory properties on Chinese consumers' preference, purchase intent and choice

This study used sensory evaluation techniques to test the relative influence of country of origin, price and sensory attributes on consumer preferences in China, an example of a new wine market, and a key market for Australian producers.

From a methodological point of view, a study assessing a choice procedure with Chinese consumers provided the conclusion that in an emerging market with consumers less familiar with a product category, a discrete choice experiment approach with actual products is not sufficiently discriminating (Williamson et al. 2017).

Red wines from France, Australia and China were tasted by Chinese consumers under blind or informed conditions (Williamson et al. 2017, Figure 9). Sensory descriptive data were collected from a Chinese-trained panel using Chinese language attributes. A higher price and being from France were the strongest predictors of liking under informed conditions, while being from China had a negative influence for most of the consumers. Some consistency was found in sensory preferences between blind and informed tastings, indicating that sensory aspects of the wine are important, with one consumer cluster not influenced by price or country information. Australian wines were well-liked under both blind and informed conditions. Well-liked wines were generally high in 'fermented bean curd' (related to oak flavour), 'alcohol' flavour, 'hawthorn' ('ripe blackberry-like') and 'woody' characters, and low in 'dried longan' ('prune', 'raisin') attributes. French wines in contrast were not well-liked under blind conditions. Price and country of origin generally were more important than the sensory aspects. A significant proportion of consumers, however, were less concerned about origin and price and were more influenced by the sensory characteristics.

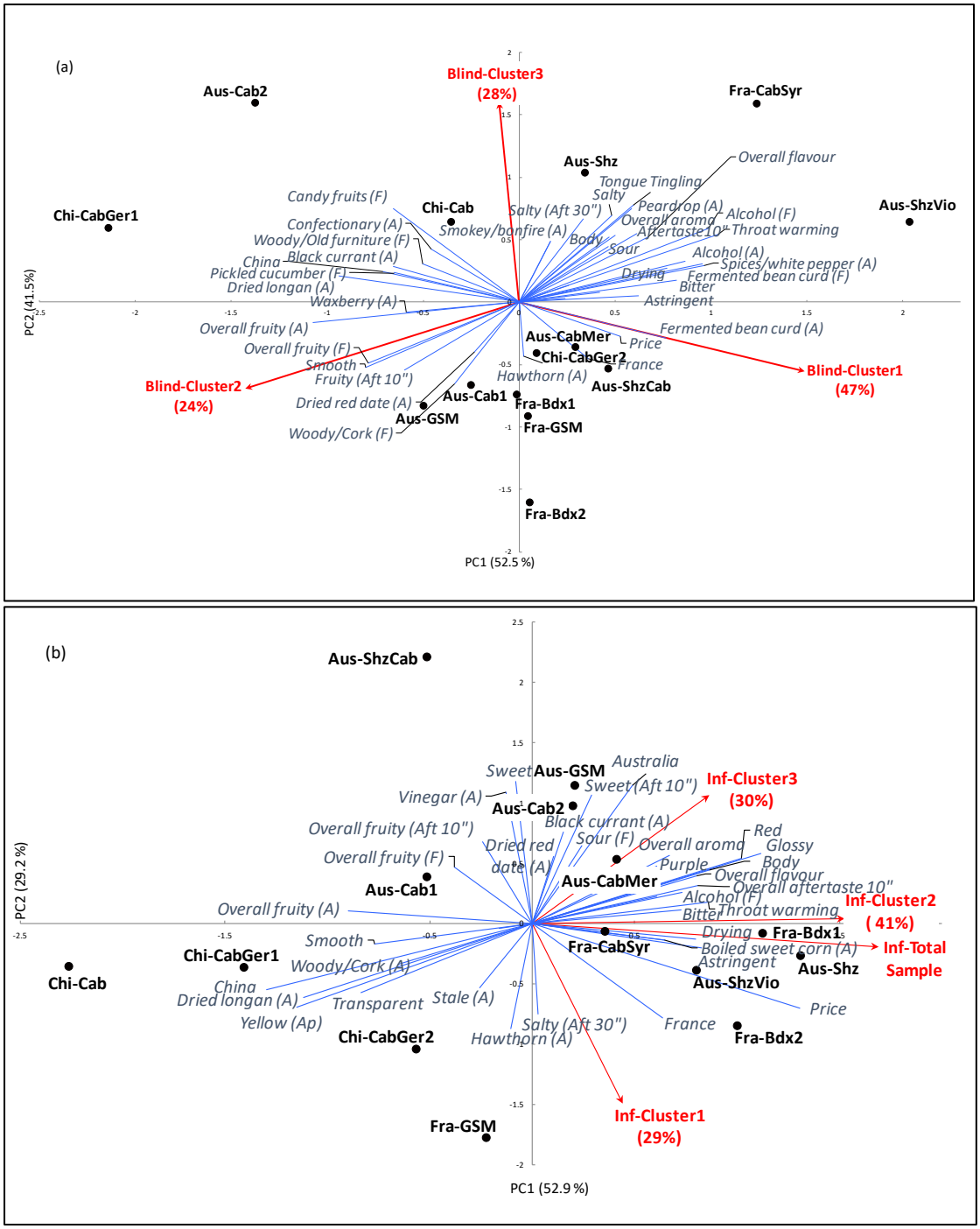

Figure 9. Principal component analysis biplot of the mean liking scores of three Chinese consumer clusters for (a) blind liking and (b) informed liking shown as vectors, together with the liking scores for the total Informed sample. The proportion of consumers in each cluster is shown in parentheses, and the 14 wines are shown as solid circles. The sensory attributes from a Chinese sensory descriptive analysis panel are indicated in italics.

In a further study, the effect of information about Australia on initial choice of Australian wines compared to that of other countries was investigated, and the results showed that information about Australia increased likelihood of choice of Australian wines, even after more than ten days, with specific messages such as Australia being a clean environment; Australian wines being great tasting to Chinese consumers, and Australia having strong regulation being strongest, while Australia being an

attractive tourism destination or with a long tradition of prestigious wines being less strong (Williamson et al. 2014; Williamson et al. 2016; Lockshin et al. 2017).

Outcome and conclusion

The project has advanced knowledge of volatile compounds that give rise to important flavour in varieties of commercial importance to the Australian wine industry. The recognition of isobutyl methoxypyrazine as a key contributor to 'green' aroma in Shiraz, especially when whole bunch fermentation is practiced, is a step forward in controlling 'green' characters in this variety. The defining of 'green tannin' as related to both 'leafy'/'stalky' flavour and bitterness, will also help to focus efforts to avoid these specific characteristics. Simple treatments such as food grade plastic material included in a red ferment were shown to have significant potential in removing this flavour.

In related work, 'green' aroma was shown to be able to be transferred to a wine from wind-break trees planted near a vineyard. Avoiding MOG in grapes near to the trees or harvesting separately are straightforward steps producers can take to minimise this character. A producer in the Yarra Valley has already taken advantage of this information to control this character.

As demonstrated by this project, the role of monoterpenes in 'apricot' flavour of white wines; specific esters in 'peach' aroma; thiols in both 'fruity'/'citrus' flavour and more overt 'tropical fruit' flavour in Chardonnay opens up avenues for producers to be able to control and adjust these flavours in their products. For the thiol compounds in Chardonnay, the effect of production variables such as skin contact post-harvesting, or choice of yeast strain, are well studied and allow straightforward optimisation of these flavours by winemakers in their processes. The importance of monoterpenes in Viognier has not been previously shown, and as these compounds are relatively easy to measure in grapes, this new knowledge gives the ability to understand the impact of different planting material and viticultural options on the attractive varietal flavour of this variety. A major Viognier producer has taken steps to use this information in their production systems.

Thiol compounds that contribute to general 'fruity' flavour in Chardonnay, as well as distinct 'grapefruit' or 'tropical fruit' flavour, can be adjusted in concentration through various options previously identified. The confirmation of benzyl mercaptan as having a role in 'struck flint'/'match' character has generated interest among winemakers at seminars as this is considered a positive 'funky' element for ultra-premium Chardonnay.

The studies on behaviour and sensory preferences of Chinese wine purchasers and consumers have clearly shown that messages about Australia will increase initial purchase of Australian wine compared to French or local Chinese wines, with information about the clean environment of Australia and the attractive flavour of Australian red wines to Chinese consumers being the most influential. This work allows Australian wine companies and Wine Australia to focus their efforts in specific approaches to improve sales. Studying the sensory preferences of Chinese consumers when tasting a range of wines informed with knowledge of country of origin, price and brand/packaging, provided insight into sensory properties and showed that there are specific Chinese language sensory attributes related to preference, which can be used not only to consider the most appropriate wine styles for the market but also with communication strategies alongside the complementary Chinese wine lexicon outcomes.

Recommendations

Further investigations into the contribution of thiols to the positive flavour of other wine types should be conducted. The enhancement of fruit intensity of Chardonnay wines may be a feature in other varieties, notably red wines, where these compounds have been little studied. While they can be susceptible to hydrolysis and oxidative degradation, their role may be significant. The unusually high levels of these thiols in a few warm climate wines may be related to viticultural factors such as fertiliser use, as previously indicated, and this would be a fruitful area to assess. The mechanism of formation of benzyl mercaptan should be identified, as it is likely formed from a yeast-derived compound with further transformation during barrel fermentation and ageing in oak.

Investigating the viticultural factors influencing monoterpene concentration in Viognier would also be an avenue for further work, with little known about aroma biosynthesis, clonal and ripening effects in this variety.

Whole bunch fermentation is widely practised in red wine production, and variables that affect 'green' aroma and flavour from this process should be further examined. The efficacy of food-grade fining materials in selectively reducing green character should be confirmed.

The role of specific volatiles in other key sensory attributes that are not well understood should be determined, with attributes previously shown to be important in influencing consumer response including 'raisin'/ 'jammy' flavour in Shiraz, 'musk' and 'chocolate' aroma and compounds other than rotundone contributing to 'spicy' flavour in red wine.

References cited

Black, C.A., Parker, M., Siebert, T.E., Capone, D.L., Francis, I.L. 2015. Terpenoids and their role in wine flavour: Recent advances. *Aust. J. Grape Wine Res.* 21: 582-600.

Bramley, R.G.V., Siebert, T.E., Herderich, M.J., Krstic, M.P. 2017. Patterns of within-vineyard spatial variation in the 'pepper' compound rotundone are temporally stable from year to year. *Aust. J. Grape Wine Res.* 23(1): 42-47.

Capone, D.L., Jeffery, D.W., Sefton, M.A. 2012. Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian red wine. *J. Agric. Food Chem.* 60(9): 2281-2287.

Capone, D., Barker, A., Pearson, W., Francis, L. 2016. Complexity, texture and flavour ... or green, hard and herbal? Incorporation of stems and leaves in cool climate Shiraz fermentation. *AWRI Tech. Rev.* 223: 6-8.

Capone, D.L., Ristic, R., Pardon, K.H., Jeffery, D.W. 2015. Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. *Anaytl. Chem.* 87(2): 1226-1231.

Capone, D.L., Barker, A., Williamson, P.O., Francis, I.L. 2017. The role of potent thiols in Chardonnay wine aroma. *Aust. J. Grape Wine Res.* 10.1111/ajgw.12294.

Capone, D., Bey, L., Barker, A., Espinase Nandorfy, D., Williamson, P., Solomon, M., Francis, L. 2017. Trees and vines: can different types of local vegetation contribute to wine flavour? *AWRI Tech. Rev.* 229: 7-10.

Capone, D., Francis, L., Barker, A., Williamson, P. 2016. 'Tropical' thiols are important contributors to the flavour of Chardonnay wines. *AWRI Tech. Rev.* 222: 12-15.

Cooke, R.C., Van Leeuwen, K.A., Capone, D.L., Gawel, R., Elsey, G.M., Sefton, M.A. 2009. Odor detection thresholds and enantiomeric distributions of several 4-alkyl substituted γ-lactones in Australian red wine. *J. Agric. Food Chem.* 57(6): 2462-2467.

Francis, I.L., Williamson, P.O. 2015. Application of consumer sensory science in wine research. *Aust. J. Grape Wine Res.* 21: 554-567.

Geffroy, O., Dufourcq, T., Carcenac, D., Siebert, T., Herderich, M., Serrano, E. 2014. Effect of ripeness and viticultural techniques on the rotundone concentration in red wine made from *Vitis vinifera* L. cv. Duras. *Aust. J. Grape Wine Res.* 20(3): 401-408.

Geffroy, O., Yobrégat, O., Dufourcq, T., Siebert, T., Serrano, É. 2015. Certified clone and powdery mildew impact Rotundone in red wine from *Vitis vinifera* L. cv. Duras N. *J. Int. Sci. Vigne Vin* 49(4): 231-240.

Geffroy, O., Siebert, T., Herderich, M., Mille, B., Serrano, E. 2016. On-vine grape drying combined with irrigation allows to produce red wines with enhanced phenolic and rotundone concentrations. *Sci. Hortic.* 207: 208-217.

Geffroy, O., Siebert, T., Silvano, A., Herderich, M. 2017. Impact of winemaking techniques on classical enological parameters and rotundone in red wine at the laboratory scale. *Am. J. Enol. Vitic.* 68(1): 141-146.

Herderich, M., Barter, S., Black, C.A., Bramley, R., Capone, D., Dry, P., Siebert, T., Zhang, P. 2015. Terroir Effects on Grape and Wine Aroma Compounds. Ebeler, S.B., Sacks, G., Vidal, S., Winterhalter, P. (eds.) *Advances in Wine Research.* Washington: ACS: 131-146.

Jarauta, I., Ferreira, V., Cacho, J.F. 2006. Synergic, additive and antagonistic effects between odorants with similar odour properties. *Devel. Food Sci.* 205-208 p.

Lockshin, L., Corsi, A.M., Cohen, J., Lee, R., Williamson, P. 2017. West versus East: Measuring the development of Chinese wine preferences. *Food Qual. Pref.* 56: 256-265.

Mayr, C.M., Geue, J.P., Holt, H.E., Pearson, W.P., Jeffery, D.W., Francis, I.L. 2014. Characterization of the key aroma compounds in shiraz wine by quantitation, aroma reconstitution, and omission studies. *J. Agric. Food Chem.* 62(20): 4528-4536.

Mayr, C.M., Capone, D.L., Pardon, K.H., Black, C.A., Pomeroy, D., Francis, I.L. 2015. Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines. *J. Agric. Food Chem.* 63(13): 3394-3401.

Pearson, W., Barker, A., Capone, D., Bindon, K., Williamson, P., Francis, L. 2015. Defining 'green' flavour in Cabernet Sauvignon wines. *AWRI Tech. Rev.* 219: 6-10.

Scarlett, N.J., Bramley, R.G.V., Siebert, T.E. 2014. Within-vineyard variation in the 'pepper' compound rotundone is spatially structured and related to variation in the land underlying the vineyard. *Aust. J. Grape Wine Res.* 20(2): 14-222.

Siebert, T., Pearson, W., Barker, A., Barter, S., Lopes, M.d.B., Darriet, P., Herderich, M., Francis, L. 2016. Volatile aroma compounds related to 'stone fruit' aroma in Viognier and Chardonnay wines. Poster presented at the 16th Australian Wine Industry Technical Conference, Adelaide. Available at: https://awitc.com.au/wp-content/uploads/2016/07/45_Siebert.pdf

Siebert, T.E., Solomon, M.R., Pollnitz, A.P., Jeffery, D.W. 2010. Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. *J. Agric. Food Chem.* 58(17): 9454-9462.

Staniatopoulos, P., Brohan, E., Prevost, C., Siebert, T.E., Herderich, M., Darriet, P. 2016. Influence of chirality of lactones on the perception of some typical fruity notes through perceptual interaction phenomena in Bordeaux dessert wines. *J. Agric. Food Chem.* 64(43): 8160-8167.

Williamson, P., Mueller-Loose, S., Lockshin, L., Francis, I.L. 2014. Thinking outside the bottle: information about Australia can increase the choice of Australian wines by Chinese consumers. *Wine Vitic. J.* 29(3): 71-74.

Williamson, P.O., Lockshin, L., Francis, I.L., Mueller Loose, S. 2016. Influencing consumer choice: Short and medium term effect of country of origin information on wine choice. *Food Qual. Pref.* 51: 89-99.

Williamson, P.O., Loose, S.M., Lockshin, L., Francis, L. 2017. Predicting wine repurchase: a case of low test-retest reliability in China. *Int. J. Market Res.* 59(4): 471-494.

Williamson, P. O., Mueller Loose, S. Lockshin, L., Francis, I. L. 2017. More hawthorn and less dried longan: the role of information and taste on red wine consumer preferences in China. *Aust. J. Grape Wine Res.*, accepted 5 June 2017.

Weingart, G., Kluger, B., Forneck, A., Krska, R., Schuhmacher, R. 2012. Establishment and application of a metabolomics workflow for identification and profiling of volatiles from leaves of *Vitis vinifera* by HS-SPME-GC-MS. *Phytochem. Anal.* 23(4): 345-358.

Wildenradt, H.L., Christensen, E.N., Stackler, B., Caputi, A., Slinkard, K., Scutt, K. 1975. Volatile Constituents of Grape Leaves. I. *Vitis vinifera* Variety 'Chenin Blanc'. *Am. J. Enol. Vitic.* 26(3): 148-153.

Xi, Z.M., Tao, Y.S., Zhang, L., Li, H. 2011. Impact of cover crops in vineyard on the aroma compounds of *Vitis vinifera* L. cv Cabernet Sauvignon wine. *Food Chem.* 127(2): 516-522.