Stream 1.1: Defining and controlling important volatile compounds in wine and their impact on wine aroma and flavour

1. Abstract:

Knowledge of the critical chemical compounds that are responsible for the sensory characteristics of wines is of great importance to be able to control and adjust wine flavour during wine production. Compounds not previously recognised as contributing to flavour have been identified for the first time through work in this stream, including the compound rotundone: giving Shiraz wines from many of Australia's wine regions their distinctive peppery or spicy flavour. In addition, numerous compounds not previously understood adequately have been revealed as significant, and ways of adjusting their contribution have been examined. For example, the *Eucalyptus*/mint compound eucalyptol was found as a consequence of nearby eucalypt trees, with aerial transfer an important element, but much less influential than the presence of grape-vine or eucalypt leaves in the harvested grapes. Studies into the origin, formation and chemical stability of many compounds have provided guidance for wine producers to dial up or down these compounds. For example, this research included the determination of the 'tropical fruit' thiols precursors, which allowed the recognition that post-harvest storage of crushed grapes gives greatly enhanced levels of these compounds. Further major outcomes of the activities under this stream have been the development of analytical tools for measurement of key wine flavour compounds, and knowledge of viticultural and winemaking options that alter the levels of these flavours. Another major aspect of the work concentrated on off-flavours. Winemakers now have improved ability to avoid unpleasant and economically damaging taints and ensure wine is suitable for sale. The work on off-flavours included compounds responsible for 'reductive' off-flavour, found to be largely due to hydrogen sulfide and methane thiol, and smoke taint originating from bushfires. For smoke taint, grape metabolites have been identified as precursor compounds that release the taint in-mouth during consumption.

2. Executive summary:

A thorough understanding of the relationship between wine composition and wine flavour is pivotal to being able to control grape and wine quality. Work in this stream was carried out to improve knowledge of compounds that are responsible for key flavours, especially for the variety Shiraz: the predominant and, in many respects, flagship red wine variety of Australia. Studies involved the identity of previously unrecognised compounds and compounds causing off-flavours and taints, the relationships between wine composition and wine sensory properties, the effect of viticultural and oenological techniques on the formation of compounds, such as '*Eucalyptus*' flavour, and development of routine analytical methods for flavour compounds which can be applied in research and practical applications.

New aroma compounds and some of their precursors were identified using sensory guided chemical analysis, gas chromatography-mass spectrometry, in combination with olfactometry (use of the human nose as a detector), liquid chromatography–mass spectrometry, and formal sensory studies. Synthesis of compounds was used to produce reference standards and for confirming the identity of compounds. To ensure accurate, precise and sensitive analyses of compounds that can be present at trace levels, state-of-the-art analytical methods using isotopically labeled standards were developed. Surveys of commercial wines confirmed the importance of these compounds, while detailed chemical studies shed light on the formation reactions occurring in winemaking. Use of replicated viticultural and winemaking studies enabled the effects of different production practices to be determined, and formal sensory and consumer preference data were also obtained.

The compound causing the pepper flavour in Shiraz was identified as rotundone, a compound not previously known in grapes or wine, and the sensory significance and influences on its concentration in wine were established. The compound arises very late in the ripening process, it is easily extracted from grape skins, and is very stable in wine. Surprisingly, approximately 20% of the population is unable to perceive the compound. Dimethyl sulfide and beta-damascenone were also found to be important to Shiraz flavour, giving fruity flavour.

Grape metabolites of bushfire-derived phenolic compounds were identified as important to smoke taint. They were found to act as a direct source of flavour, breaking down in-mouth due to enzymatic action of saliva components. This opened up a new area of research applicable to wine flavour and aftertaste generally. The key smoke taint compounds were identified, diagnostic tests were successfully validated and the abundance of key indicators for smoke exposure was established in ‘clean’ and ‘problem’ grape and wine samples, this knowledge was used actively by grape growers and wine producers following smoke events in their regions.

For ‘reductive’ off-flavour, two sulfur compounds were found to be the most significant. Analytical methods were established enabling the robust quantification of a range of undesirable sulfur compounds for shelf life studies and by wine companies seeking to avoid this flavour. Compounds causing a plastic/chemical taint were also identified as chlorophenols, and the information developed was applied to several major taint issues in the industry.

Grapevine proximity to *Eucalyptus* trees was shown to be the primary cause of the minty/eucalypt flavour found in some wines. Small numbers of eucalypt leaves entrained in the must were the primary source, with grape vine leaves also being a surprising origin. The potent thiols responsible for ‘tropical fruit’ flavour in white wines, especially in Sauvignon Blanc, were found to be derived from a complex group of precursors. These precursors were greatly enhanced in crushed grapes after a period of storage following harvest, whether from mechanical harvesting and transport or by deliberate post-harvest cool storage. These findings provided producers with a straightforward opportunity to increase this flavour in white wines.

Analytical methods for a large number of key compounds were established, providing unprecedented ability for robust measurement. The quantitative data generated provided essential context regarding the compounds’ sensory significance, detection thresholds and contribution to wine flavour. These methods allowed greatly improved understanding of the effect of yeast and fermentation, oxygen, bottle storage and closures, nutrients, and many other variables on these compounds through work undertaken on various AWRI streams. The results of the work were utilised by numerous wine industry personnel for viticultural and oenological improvement work or in resolution of issues with third parties.

The results of this research stream have greatly increased our knowledge of the main volatile compounds involved in wine flavour. 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, the work in this stream has given producers unprecedented ability to avoid off-flavours and taints while controlling positive flavours.

Affiliation	**Area of support/contribution**
Symrise, Germany	Identification of rotundone
University of Adelaide, School of Agriculture, Food and Wine	Smoke taint, thiols, eucalyptol
Flinders University	Chemical synthesis, mechanistic studies, lactone research
University of Auckland	Rotundone studies
CSIRO Ecosystem Sciences	Rotundone studies
University of Melbourne	Rotundone studies
Institut Francais de la Vigne et du Vin Pole Sud-Ouest, France	Rotundone studies
University of Bonn, Germany	Rotundone and sesquiterpene studies
Shaw and Smith	Oenological studies

Affiliation	Area of support/contribution
Casella	Oenological studies
Mt Langi Ghiran	Oenological studies
Orlando	Oenological studies
Curtin University	Thiols

3. Background:

A thorough understanding of the relationship between wine composition and aroma/flavour is pivotal to being able to control grape and wine quality. The body of research conducted at the AWRI and overseas prior to the initiation of this stream, has resulted in an appreciation of how many volatile compounds contribute to wine flavour, how they are formed in grapes and wine, and how they are degraded. Nevertheless, many important flavours, both desirable and undesirable, were poorly or not at all explained, and the factors affecting their concentration were also poorly understood.

At the time work in this stream commenced, the compound causing pepper/spice flavour in Shiraz was not known, nor was the identity of sulfur compounds that were most important to 'reductive' flavour, or the cause of the eucalypt/mint flavour in red wines. Indeed, there were few studies investigating Shiraz flavour generally, with little known about the differences between Shiraz flavour and Cabernet Sauvignon flavour, for example. Yet knowledge of what factors are responsible for particular sensory properties and the reasons for variability in concentration of wine components is necessary to devise methods to enable producers to manage these sensory characteristics. Additionally, how the vine nitrogen status influences the quality and composition of secondary metabolites in grapes and wine flavour was poorly understood.

This stream had the expected outcomes of achieving knowledge to guide industry's ability to improve viticultural and oenological practices to achieve desired flavour in wines. Knowledge was also sought to avoid incidences of taints and other off-flavours. The stream also aimed to provide economic benefits to industry as a result of quality improvements and the capability to produce wines that respond to market demands.

4. Stream objectives:

The stream had the following objectives:

- Determine the chemical nature of hitherto unrecognised important volatile wine aroma and flavour compounds. The initial focus was on the Shiraz variety, and on characters associated with oak and oxidation, as well as taints, off-flavours, 'reduced' and 'green' characters. As a result of budget constraints from the onset of the research program, the emphasis on oak and 'green' flavour was reduced, and planned studies on glycoside precursors were deferred. From industry feedback and knowledge of the research area, it was considered that oak flavour was relatively well understood while previous work on glycoside precursors was already quite extensive and unlikely to achieve major results in the short-term. Examination of 'green' flavour, while reduced in scope, was continued through collaboration with other research projects which required compositional analysis to characterise grape maturity.
- Enhance understanding of the relationship between wine composition and wine sensory properties, including what combinations of known volatile and non-volatile constituents have the greatest effect on 'in mouth' sensory properties. The details of this component of the stream will be reported in Stream 1.2 (Phenolics and their contribution to wine composition and sensory properties).
- Determine the effect of viticultural and oenological techniques on the formation and fate of volatile wine aroma/flavour compounds, notably for control of compounds such as those causing 'eucalypt', 'tropical fruit' and 'pepper' flavour.

- Develop analytical methods for volatile aromas in grapes and wine to enable industry to produce wines that respond to market demand.

5. Methodology:

The stream used multiple approaches to meet the objectives, with several steps including establishing the identity of target compounds, producing a reliable analytical method, determining their sensory significance and importance to wine flavour, and investigating influences on concentrations found in wines.

For identification of new compounds, sensory guided chemical analysis was used, with gas chromatography-mass spectrometry (GC-MS)-olfactometry, aroma threshold measures, and informal sensory assessments applied to identify probable compounds responsible for key sensory attributes of wines (Wood et al. 2008). The identity of the volatile compounds responsible for the flavour attributes in wines was established through synthesis of compounds for use as analytical standards, and through GC-MS studies and additional sensory studies, to confirm the identity and the importance of the compounds.

Targeted, specific analytical methods using GC-MS or LC-MS were developed for the identified compounds using stable isotope dilution analysis (Siebert et al. 2008), with synthesised standards as required. The methods were validated and simplified where possible to allow routine, relatively rapid analysis, as well as transfer of methods between instruments, with appropriate limits of quantification. Quantitative analytical methods for the major known aroma compounds in wines were applied to support other research programs and industry trials.

For the relatively reactive compounds involved in ‘reductive’ aroma, sample preparation needed to be minimised where possible and a highly selective method was required, hence a sulfur specific detector was adopted (Siebert et al. 2010).

Accurate, precise quantitative analyses were used to survey commercial wines. Data was collected on the numbers of wines where an aroma compound was above sensory detection threshold. These data provided evidence that this compound was of importance to wine. For many compounds, the sensory significance was further determined through comprehensive threshold testing using wine as the matrix, reconstitution experiments, and collection of sensory descriptive and consumer preference data (with Stream 3.1).

The formation of aroma compounds was established through kinetic and mechanistic studies, ways of adjusting their levels through viticultural and winemaking practices were assessed, stability of compounds was characterised during extended storage, and propensity of key aroma compounds to be absorbed by wine closures was examined. Work was conducted in close collaboration with multiple industry partners, including Casella Wines, Orlando Wines, Mt Langi Ghiran, and Shaw and Smith, to assess commercially viable options for adjusting levels of aroma compounds. The program linked very closely with other streams, notably 1.2, 1.3, 2.1, 2.2, 2.4, 3.1 and 4.1. For example, off-flavour investigations of smoke taint were undertaken with Stream 4.1 (knowledge transfer) and 3.1 (sensory and consumer).

6. Results and discussion:

The wine aroma stream concentrated on compounds that result in important sensory attributes, attributes that were identified from consumer studies as important to wine quality, notably kerosene flavour, oxidation, ‘reductive’ flavour, pepper, eucalypt, oak flavour, ‘green’ flavour, fruit flavour in red wines, as well as taints such as smoke and plastic/chemical taints. Knowledge of Shiraz flavour was considered as high priority because very limited understanding of the flavour of this important variety was previously available.

The identification of key flavour compounds

The compound causing black pepper/spicy flavour in some Shiraz wines, especially those from cooler climates, was successfully identified as the grape-derived sesquiterpene rotundone (Siebert et al. 2008, Wood et al. 2008, Pollnitz et al. 2008, Herderich et al. 2012). The identification of the chemical structure of the peppery aroma compound proved to be a large challenge (Parker et al. 2007), requiring several years of effort. Following sourcing of an authentic reference standard and sensory detection threshold testing, it was found that rotundone not only had a very low threshold (8 ng/L in water), but that approximately 20% of the subjects tested were insensitive, being unable to smell it even at extremely high concentrations (Wood et al. 2008). The compound was previously known as a constituent in nut grass and some other plants, but was not recognised as an important aroma compound and had not previously been found in wine. Following the identification of the compound responsible (Wood et al. 2008), a quantitative method was developed using a synthesised labeled analogue as an analytical standard (Siebert et al. 2008). A survey of 137 red wines showed that while more than 80% had no detectable rotundone, of those that had appreciable levels above the sensory threshold, most were Shiraz, with lower levels of the compound also present in Durif and Pinot Noir, most were from cooler regions (Jeffery 2009, Herderich et al. 2012).

The role of glycoside precursors of volatile phenols as releasers of flavour, by breakdown in-mouth, was established during research into the chemical basis of bushfire-derived smoke taint (Parker et al. 2012). Sensory experiments showed that glycoside conjugates of volatile phenols, while odourless, release flavour in mouth (Stream 3.1).This is a previously unrecognised source of flavour, and with many glycosides of fruity flavour compounds found in wine, this could also be a major source of positive flavour in wine.

The compounds 2,6-dichlorophenol and 6-chlorocresol were found to be of particular significance in taint studies, due to very low sensory thresholds (Coulter et al. 2008, Capone et al. 2010b).
Indole was found to be a key compound involved in mothball-like fermentation off-flavour developed by yeast under stress conditions (Coulter et al. 2008, Capone et al. 2010a).

Wine composition and sensory studies

Methanethiol and hydrogen sulfide were found to be of greatest importance in ‘reductive’ off-flavours (Siebert et al. 2010). This result is based on sensory threshold measures, quantitative data from a range of studies with sensory data, as well as additional studies. Dimethyl sulfide was also found to be important to wine flavour, although this compound was not invariably a negative influence, being involved in varietal flavour especially of red wines as discussed below.

Detailed studies were conducted on premium commercial Shiraz wines from the Barossa Valley, and a cooler climate (Margaret River). Sensory differences between the wines related to rotundone concentration, oak volatiles, and dimethyl sulfide (DMS), with beta-damascenone and several fatty acid ethyl esters also being very important. These results were obtained by GC-olfactometry, including aroma extract dilution analysis, followed by quantitative analysis and reconstitution/omission sensory studies (Herderich et al. 2012). The key role of DMS, previously indicated as important to fruit flavour, is of great interest as a marker compound of wine quality that can be traced to precursors in grapes. The presence of acids and salts had a large effect on increasing the perception of rotundone, a relatively polar compound, while reducing the effect of DMS.

Investigations of relationships of a wide range of volatile aroma compounds with sensory data showed that relatively small numbers of key volatiles are needed to predict important sensory attributes (Francis et al. 2010b). The sensory thresholds of four isomers of oak lactone were determined (Brown et al. 2006), giving guidance of the sensory significance of these compounds.

The concentration of a number of gamma-lactone enantiomers in a wide range of wine types were measured and their odour detection thresholds determined (Brown et al. 2007, Cooke et al. 2009a, Cooke et al. 2009b). Previous studies have suggested that these compounds might be important to wine flavour, with some having aromas of stone fruit. The results from experiments conducted under this stream indicated that gamma-lactones are not likely to contribute individually to wine aroma, although they might in combination have an effect for red wines.

Effects of winemaking and viticultural variables

It was shown that the key peppery compound, rotundone, was very stable in wine and was not removed through absorption by closures of various types to any extent (Jeffery et al. 2009). Rotundone was found to be present in grape skins rather than pulp or seeds and is a relatively water soluble compound. Studies on extraction during fermentation on skins showed that fairly high levels were present in must early in fermentation, and that it was readily extracted from skins as fermentation progressed (Herderich et al. 2012). The compound was formed in grape berries very late in the ripening process, and rotundone concentrations at harvest were found to vary significantly from season to season (cooler seasons resulted in higher levels; Wood et al., 2008). Additional effects were found to be due to vine vigour and degree of bunch shading. Fermentation had no effect on rotundone in wine, with no yeast-related differences observed for the *S. cerevisiae* strains studied (Herderich et al. 2012).

The compound known to be the cause of *Eucalyptus*/mint flavour, eucalyptol (also known as 1,8 cineole, a monoterpene compound) was previously found to be present in wine as an end-product of grape derived monoterpene hydrolysis and rearrangement reactions, as well as due to transfer from *Eucalyptus* trees grown near vineyards (summarised in Capone et al. 2011a). Studies were conducted to find the major source of the compound. The formation of eucalyptol in wines from grape-derived monoterpene precursors was found to be of negligible significance, showing that other sources of the compound must be the cause of *Eucalyptus* flavour (Capone et al. 2011a).

The origin of eucalyptol was clearly identified as a result of quantifying eucalyptol amongst airborne volatile organic compounds in vineyards, kinetic model studies, measurement of eucalyptol concentrations in grapes, stems, and leaves near to *Eucalyptus* trees, and fermentation investigations (Capone et al. 2011a, 2012b). Grapevine proximity to *Eucalyptus* trees was shown to be the primary cause. While aerial transfer directly to the skin of grape berries was an important source, eucalyptol in wine was found to be derived mainly from matter other than grapes (MOG), most notably the presence of eucalypt leaves or woody material, which increased levels in wine dramatically (Capone et al. 2011a, 2012b). Eucalyptol was demonstrated to be very stable in wine over extended storage and was not removed through absorption by wine closures (Capone et al. 2011a). Survey data showed that the compound was only important to red wines, and that some regions can have very high concentrations in some wines. The *Eucalyptus*/minty flavour is not necessarily undesirable, with many consumers appreciating the character (Herderich et al. 2012). Finally, GC-olfactometry studies provided little evidence for other compounds contributing to this flavour.

Changes involved in the ‘C6’ compounds involved in ‘green/grassy’ aroma were investigated in tropical thiol precursor studies (Capone et al. 2011e, 2012c), and in a study on Cabernet Sauvignon maturity (Bindon et al. 2013, discussed in Stream 1.2). These compounds are involved in the production of tropical thiols during fermentation. In riper grapes, their concentration in wines diminishes dramatically compared to grapes picked earlier, which is related to a disappearance of ‘green’ flavour, together with isobutyl methoxypyrazine, also involved in ‘green’ flavour.

A key step in the formation of the ‘kerosene’ aroma compound TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) was made clear for the first time. This provided the ability to monitor the precursor to this compound to predict how an aged wine might be affected by this character (Daniel et al. 2008a). A survey of commercial Riesling wines showed clearly that aged wines under screw cap can have higher levels of TDN than cork sealed wines, and also showed that some regions had a higher number of wines with elevated levels of TDN (Black et al. 2012).

A number of new precursors to the potent varietal thiol compounds 3-MH and 4MMP (‘tropical fruit’) were identified. The studies showed that the glutathione form of 3-MH was much more abundant than the cysteine conjugate, and provided understanding of the various reactions in grape must giving rise to these important compounds (Pardon et al. 2008, Fedrizzi et al. 2009, Capone et al. 2010a, Grant-Preece et al. 2010, Capone et al. 2011b, Capone et al. 2011c). Storage of crushed grapes post-harvest, whether through transport in harvester bins or cold storage (Capone and Jeffrey 2011d, Capone et al. 2012a), was found to give a very large increase in concentrations of the precursors, providing a practical means of enhancing levels in wines, with smaller differences related to level of sulfur dioxide or ascorbic acid addition.

The compound beta-damascenone, a strong fruit flavour enhancer compound in most wines, was found to be derived from several precursor systems (Daniel et al. 2008b, Lloyd et al. 2011a, b), laying the foundation for better control of the level of this compound in wines.

In work conducted with the University of Adelaide, wine lactone, a potent monoterpene, was found to be formed slowly at wine pH from two precursor compounds, a monoterpene acid and the corresponding glucose ester (Giaccio et al. 2011), reinforcing the idea that it is likely to be important to the aged flavour of white wines, notably Riesling, where it may contribute to the 'lime' flavour of older wines (Francis 2010a).

A glycoside precursor of the potent oak derived compound *cis*-oak lactone (coconut flavour), was synthesised and an analytical method developed, allowing measurement of the oak lactone potential of oak wood and the effect of malolactic fermentation on this precursor (Hayasaka 2007, Fudge et al. 2008).

Investigations of smoke affected grapes and wines showed clearly that volatile phenols from smoke are extensively metabolised by vines, resulting in a broad range of non-volatile glycosides, these sugar-bound phenols act as flavour precursors and were proven to be effective markers of smoke exposure of grapes and wine (Hayasaka et al. 2010).

The causes of the formation of indole during problematic fermentations were investigated, notably the role of the amino acid tryptophan (Capone et al. 2010b, Arevalo-Villena et al. 2010, Stream 2.1).

Analytical methods

A large number of highly sensitive, selective, accurate and precise analytical methods for quantification of volatile aroma compounds and their precursors were developed. The new analytical methods greatly expanded knowledge of wine flavour, with methods applied across multiple projects in microbiology, sensory, and processing studies.

An analytical method, using isotope-dilution GC-MS, for quantification of the newly identified 'pepper' compound rotundone was developed (Siebert et al. 2008), capable of measuring levels in wines and grapes at ng/L concentrations. The method was later improved to achieve greater throughput, being particularly useful for analyses of grape berry samples.

Method development and validation was completed for:

- the potent varietal thiol compounds 3-MH (Capone et al. 2011c), 4-MMP and 3-MHA, involved in 'tropical fruit' flavour in white wines, notably Sauvignon Blanc, and
- precursors to 3-MH (Capone et al. 2010a), involving synthesis of standards and optimisation of GC-MS and LC-MS/MS methods in collaboration with the University of Adelaide, including a novel derivatisation method.

The methods were applied to yeast strain studies discussed in Stream 1.3 and oxidation studies of Stream 2.2. The derivatisation procedure also resulted in the capacity to quantify benzenemethanethiol thought to be responsible for 'struck flint' aroma, and furfuryl thiol (coffee aroma).

An analytical method for the 'cut grass/green' compound cis-3-hexanol and other 'C6' compounds was established (Capone et al. 2012a). A quantitative analytical method for 1,8 cineole (*Eucalyptus* flavour) was also developed using a synthetic isotope-labelled analytical standard (Capone et al. 2011a).

A highly sensitive analytical method was developed and applied for the determination of halophenol compounds to allow diagnosis of industry taint problems (Capone et al. 2010b).

A method for quantification of indole ('plastic, moth-ball'), a yeast derived off-flavour compound that can be produced during sluggish fermentations, was developed (Capone et al. 2010b).

A highly sensitive method to determine 10 volatile sulfur-containing off-flavour compounds in wine was developed, validated (Siebert et al. 2010) and applied to wines with 'reductive flavour', as well as in studies discussed in Stream 2.2 and 1.3, and in investigations and projects with wine companies.

A method for quantifying compounds related to oxidation off-flavour was adopted and validated (Mayr et al. 2013). This greatly improved the ability to directly assess chemical changes due to oxidation, rather than indirectly as previously undertaken, using sulfur dioxide or brown colour as markers.

An analytical method for the quantification of a series of pairs of enantiomers (compounds with the same chemical structure but mirror images of each other) of alkyl gamma-lactones was achieved, using synthesised labelled standards (Cooke et al. 2009 b).

In order to better understand smoke taint, highly challenging syntheses of numerous phenol glycosides allowed analytical methods to be developed for these precursors (Hayasaka et al. 2013). With the recognition that cresols were important to smoke tainted wines, the routine volatile phenol analytical method was expanded to include these compounds (Hayasaka et al. 2010).

7. Outcome and Conclusion:

The results of this stream have greatly increased knowledge of many key volatile compounds involved in wine flavour, so that the causative compounds for many of the most important sensory attributes of wines are now established.

A suite of analytical methods for quantifying a range of key volatile compounds important to wine aroma have been developed, with many able to be used by producers through the AWRI Commercial Service and other service laboratories. The analyses provide a much needed resource for evaluating the results of viticultural and winemaking studies, including the major off-flavours of oxidative or reductive characters, as well as more positive flavours such as damascenone and dimethyl sulfide.

Regarding pepper flavour in Shiraz, tools have now been developed which allow producers to adjust the causative compound, rotundone, in wines, through skin contact and harvest time. Information regarding differences in sensitivities among people, including winemakers, to rotundone has been elucidated. This enables industry trials to be supported which aim to improve viticultural management of rotundone concentrations by targeting individual vineyards, sections of vineyards and clonal selection.

The important issue of *Eucalyptus*/mint flavour, which is particularly relevant to Australian producers but is also a wine sensory attribute in many other countries where eucalypt trees are grown, has been another priority target of this research. The fact that grape leaves act as a source was not previously recognised, nor the very strong effect of the inclusion of even a small amount of eucalypt material as MOG. Producers now have the option of simple practices to modulate this aroma in their wines, by ensuring, for example, that rows close to *Eucalyptus* trees are harvested separately, and that those grape-lots, and those with MOG, are kept separate during processing. Harvester settings can also be adjusted to minimise MOG, or rows closest to trees can be hand-harvested in order to reduce MOG.

'Tropical fruit' thiols and their precursors are now better understood, with post-harvest storage of crushed white grapes a straightforward practical means of enhancing precursors of these thiols in must.

Knowledge of the key sulfur compounds involved in reductive off-flavour, and the ability to measure them accurately, gives the basis for solving problems through fermentation management or other interventions.

The knowledge generated regarding a wide range of taint and off-flavour compounds, including their sensory effects and relative potency, provides a strong basis for diagnostic investigations which reduce the risks of further taint and fault issues arising, as well as evidence that can be used for insurance claims and in legal disputes. Detailed chemical knowledge regarding taints in grapes and wines, such as smoke or halophenol taint, can save the industry significant financial loss through avoidance of processing tainted grapes, by showing that wine in tank is not tainted and can be sold, as well as protecting Brand Australia and individual producers from damage resulting from tainted wine being released on to the market.

8. Recommendations:

Investigations of the fundamentals of grape berry biosynthesis of rotundone would have great value to explain variations that have been observed across sites and sections of vineyards, this might lead to the recognition of key environmental switches required for the formation of grape and wine aroma compounds; this will be addressed in Project 3.1.1 of the AWRI’s 2013-2018 R,D&E plan.

Several sensory attributes that are not well understood should be investigated, including ‘stone fruit/apricot’ flavour in white wines, ‘raisin/jammy’ flavour in red wines, especially in warmer vintages, and ‘slow/stuck fermentation’ flavour (to be addressed in Project 3.1.1 of the AWRI’s 2013-2018 R,D&E plan).

The recognition that grape leaves can act as a source of flavour derived from nearby native vegetation is also worthy of further study, as this may cause some ‘terroir’ differences, as well as desirable or undesirable flavour variations in vineyards (to be addressed in Project 3.1.1 of the AWRI’s 2013-2018 R,D&E plan).

There are several outcomes from the studies carried out over the last seven years that require further investigation. While it has been demonstrated that varietal thiols are of great importance to Sauvignon Blanc flavour, their relevance to other varieties is still not clear. In addition, the link between the concentration of thiol precursors in grapes and the levels of precursors and aroma-active thiols found in wines needs to be corroborated, to allow assessment of the feasibility for targeting thiol precursors as a quality measure of the ‘tropical’ flavour potential of grapes. If such relationship is established, especially for varieties such as Riesling or Chardonnay, measurement of these compounds of grapes will help companies to select parcels of fruit for appropriate wine styles (to be addressed in Project 3.1.2 of the AWRI’s 2013-2018 R,D&E plan).

Related to this, analysis of damascenone and DMS precursors in grapes would have great potential as simple markers of red wine flavour potential. Further research into wine lactone, to assess its role in lime/aged flavour in Riesling, would be valuable, to have as a target for viticultural and winemaking control of this desirable attribute (to be addressed in Project 3.1.2 of the AWRI’s 2013-2018 R,D&E plan).

Studies to assess in-mouth release of glycosides and other precursors would be productive, potentially confirming the source of fruity aftertaste and length of flavour, and once again to provide a measure of this aspect of wine quality (to be addressed in Project 3.1.3 of the AWRI’s 2013-2018 R,D&E plan).

Following the identification of specific compounds responsible for off-flavours and taints and subsequent preventative strategies being implemented, tools are required to remove these compounds from wine as prevention will not always be possible (to be addressed in Project 3.5.1 of the AWRI’s 2013-2018 R,D&E plan).

The compound benzenemethanethiol is likely to be the key to the reductive ‘struck flint’ attribute that can be undesirable when present at too high a level in bottle aged wines and further investigations regarding it and other sulfur compound formation and control are warranted (to be addressed in Project 3.5.3 of the AWRI’s 2013-2018 R,D&E plan).

9. Budget reconciliation:

Financial Year	Receipts / Income ❶	Outgoings / Expenditure ❷
Year 1: 2006/2007	$1,411,348	$1,411,348
Year 2: 2007/2008	$1,619,303	$1,619,303
Year 3: 2008/2009	$1,547,153	$1,547,153
Year 4: 2009/2010	$1,623,376	$1,623,376
Year 5: 2010/2011	$1,541,669	$1,541,669
Year 6: 2011/2012	$1,243,252	$1,243,252
Year 7: 2012/2013	$1,274,937	$1,274,937
TOTAL	$10,261,038	$10,261,038

❶ Note that the GWRDC – AWRI Investment Agreement budget was established and approved at an aggregate level, with variances to budget (i.e. annual overspends and underspends) reported and considered at that same aggregate (i.e. whole of agreement) level. The receipts / income relating to a Stream for any year therefore equate to the outgoings / expenditure within that Stream for that year, as any variances between total Investment Agreement funding received and total funds expended were considered at the whole of Agreement rather than individual Stream level.

❷ Includes a pro-rated share of Theme 5 *Executive management and administration*.

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative...

Chris Day	Group Manager – Corporate Services	29/11/2013
Name:	*Title:*	*Date:*

10. References:

Arevalo-Villena, M., Bartowsky, E.J., Capone, D., Sefton, M.A. (2010) Production of indole by wine-associated microorganisms under oenological conditions. Food Microbiology 27 (5): 685-690.

AWRI's R,D&E plan 2013-2018, http://www.awri.com.au/about_the_awri/rde-plan/

Bindon, K., Varela, C.A., Kennedy, J., Holt, H.E., Herderich, M.J. (2013) Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry Food Chemistry 138: 1696-1705.

Black, C., Francis, I.L., Henschke, P., Capone, D.L., Anderson, S., Day, M., Holt, H., Pearson, W., Herderich, M.J., Johnson, D.L. (2012) Aged Riesling and the development of TDN. Wine and Viticulture Journal Sept/Oct: 20-26.

Brown, R.C., Sefton, M.A., Taylor, D.K., Elsey, G.M. (2006) An odour detection threshold determination of all four possible stereoisomers of oak lactone in a white and a red wine. Australian Journal of Grape and Wine Research 12 (2): 115-118.

Brown, R.C., Sefton, M.A., Taylor, D.K., Elsey, G.M. (2007) The quantification and chiral distribution of γ-lactones in Australian red wine. Recent Highlights in Flavour Chemistry & Biology, Proceedings of the Wartburg Symposium on Flavour Chemistry and Biology, 8th, Eisenach, Germany, 27 Feb-2 March:, 256-260.

Capone, D.L., van Leeuwen, K.A., Pardon, K.H., Daniel, M.A., Elsey, G.M., Coulter, A.D., Sefton, M.A. (2010b) Identification and analysis of 2-chloro-6-methylphenol, 2,6-dichlorophenol and indole: Causes of taints and off-flavours in wines. Australian Journal of Grape and Wine Research 16 (1): 210-217.

Capone, D.L., Sefton, M.A., Hayasaka, Y., Jeffery, D.W. (2010a) Analysis of precursors to wine odorant 3-mercaptohexan-1-ol using HPLC-MS/MS: resolution and quantitation of diastereomers of 3-S-cysteinylhexan- 1-ol and 3-S-glutathionylhexan-1-ol. Journal of Agricultural and Food Chemistry 58(3): 1390-1395.

Capone, D.L., Jeffery, D.W. (2011d) Effects of transporting and processing Sauvignon Blanc grapes on 3-mercaptohexan-1-ol precursor concentrations. Journal of Agricultural and Food Chemistry 59(9): 4659-4667.

Capone, D.L., Leeuwen, K.V., Taylor, D.K., Jeffery, D.W., Pardon, K.H., Elsey, G.M., Sefton, M.A. (2011a) Evolution and occurence of 1,8-cineole (Eucalyptol) in Australian wine. Journal of Agricultural and Food Chemistry 59: 953-959.

Capone, D.L., Herderich, M., Pardon, K.H., Hayasaka, Y., Cordente, A.G., Grant-Preece, P.A., Sefton, M.A., Elsey, G.M., Jeffery, D.W. (2011e) Formation of varietal thiol aroma compounds in wine: synthetic and analytical studies of grape and wine thiol conjugates. *Hoffman, T., Meyerhof, W., Schieberle, P. (eds)* In Proceedings of 9th Wartburg Symposium on Flavour Chemistry and Biology, Eisenach, Germany, 13-16 April 2010, Deutsche Forchungsanstalt fur Lebensmittelchemie: Freising, Germany, pp 242-248.

Capone, D.L., Pardon, K.H., Cordente, A.G., Jeffery, D.W. (2011b) Identification and quantitation of 3- S -cysteinylglycinehexan-1-ol (Cysgly-3-MH) in Sauvignon Blanc grape juice by HPLC-MS/MS. Journal of Agricultural and Food Chemistry 59(20): 11204-11210.

Capone, D.L., Sefton, M.A., Jeffery, D.W. (2011c) Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. Journal of Agricultural and Food Chemistry 59(9): 4649-4658.

Capone, D.L., Jeffery, D.W., Sefton, M.A. (2012c) Analytical investigations of wine odorant 3-mercaptohexan-1-ol and its precursors. Qian, M.C., Shellhammer, T.H. (eds) In ‘Flavor Chemistry of Wine and Other Alcoholic Beverages’. ACS Symposium Series, Vol. 1104 American Chemical Society: Washington, DC. 15-35

Capone, D.L., Jeffery, D.W., Sefton, M.A. (2012b) Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian Wine. Journal of Agricultural and Food Chemistry 60: 2281-2287.

Capone, D.L., Black, C.A., Jeffery, D.W. (2012a) Effects on 3-Mercaptohexan-1-ol precursor concentrations from prolonged storage of Sauvignon Blanc grapes prior to crushing and pressing. Journal of Agricultural and Food Chemistry 60(13): 3515-3523.

Cooke, R.C., Capone, D.L., van Leeuwen, K.A., Elsey, G.M., Sefton, M.A. (2009b) Quantification of several 4-alkyl substituted γ--lactones in Australian wines. Journal of Agricultural and Food Chemistry 57: 348-352.

Cooke, R.C., van Leeuwen, K.A., Capone, D.L., Gawel, R., Elsey, G.M., Sefton, M.A. (2009a) Odour detection thresholds and enantiomeric distributions of several 4-alkyl substituted γ-lactones in Australian red wine. Journal of Agricultural and Food Chemistry 57(6): 2462-2467.

Coulter, A.D., Capone, D.L., Baldock, G.A., Cowey, G.D., Francis, I.L., Hayasaka, Y., Holdstock, M.G., Sefton, M.A., Simos, C.A., Travis, B. (2008) Taints and off-flavours in wine - case studies of recent industry problems.Blair, R.J., Williams, P.J., Pretorius, I.S. (eds.) The 13th Australian Wine Industry Technical Conference, Adelaide, South Australia. The Australian Wine Industry Technical Conference Inc., Adelaide, South Australia: 73-80.

Daniel, M.A., Puglisi, C.J., Capone, D.L., Elsey, G.M., Sefton, M.A. (2008b) Rationalizing the formation of damascenone: Synthesis and hydrolysis of damascenone precursors and their analogues, in both aglycone and glycoconjugate forms. Journal of Agricultural and Food Chemistry 56(19): 9183-9189.

Daniel, M.A., Capone, D.L., Sefton, M.A., Elsey, G.M. (2008a) Riesling acetal is a precursor to 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in wine. Australian Journal of Grape and Wine Research 15 (1): 93-96.

Fedrizzi, B., Pardon, K.H., Sefton, M.A., Elsey, G.M., Jeffery, D.W. (2009) First identification of 4-S-glutathionyl-4-methylpentan-2-one, a potential precursor of 4-mercapto-4-methylpentan-2-one, in Sauvignon Blanc juice. Journal of Agricultural and Food Chemistry 57: 991-995.

Francis, I.L. (2010a) Riesling Flavour. Helm, K., Burgess, T. (eds.) Riesling in Australia. Winetitles, Adelaide, South Australia: 40-43.

Francis, L., Osidacz, P., Bramley, B., King, E., O’Brien, V., Curtin, C., Waters, E., Jeffery, D., Herderich, M.J., Pretorius, I.S. (2010b) Linking wine flavour components, sensory properties and consumer quality perceptions. Australian and New Zealand Wine Industry Journal 25 (5): 18-23.

Fudge, A.L., Elsey, G.M., Hayasaka, Y., Wilkinson, K.L. (2008) Quantitative analysis of Î²-d-glucopyranoside of 3-methyl-4-hydroxyoctanoic acid, a potential precursor to cis-oak lactone, in oak extracts using liquid chromatography-tandem mass spectrometry based stable isotope dilution analysis. Journal of Chromatography A 1215 (1-2): 51-56.

Giaccio, J., Capone, D.L., Hakansson, A.E., Smyth, H.E., Elsey, G.M., Sefton, M.A., Taylor, D.K., (2011) The formation of wine lactone from grape-derived secondary metabolites. Journal of Agricultural and Food Chemistry 59: 660-664.

Grant-Preece, P.A., Pardon, K.H., Capone, D.L., Cordente, A.G., Sefton, M.A., Jeffery, D.W., Elsey, G.M. (2010) Synthesis of wine thiol conjugates and labeled analogues: Fermentation of the glutathione conjugate of 3-mercaptohexan-1-ol yields the corresponding cysteine conjugate and free thiol. Journal of Agricultural and Food Chemistry 58(3): 1383-1389.

Hayasaka, Y., Wilkinson, K.L., Elsey, G.M., Raunkjaer, M., Sefton, M.A. (2007) Identification of natural oak lactone precursors in extracts of American and French oak woods by liquid chromatography-tandem mass spectrometry. Journal of Agricultural and Food Chemistry 55(22): 9195-9201.

Hayasaka, Y., Baldock, G.A., Parker, M., Pardon, K.H., Black, C.A., Herderich, M.J., Jeffery, D.W. (2010) Glycosylation of smoke-derived volatile phenols in grapes as a consequence of grapevine exposure to bushfire smoke. Journal of Agricultural and Food Chemistry 58(20): 10989-10998.

Hayasaka, Y., Parker, M., Baldock, G.A., Pardon, K.H., Black, C.A., Jeffery, D.W. Herderich, M.J. (2013) Assessing the Impact of Smoke Exposure in Grapes: Development and Validation of a HPLC-MS/MS Method for the Quantitative Analysis of Smoke-Derived Phenolic Glycosides in Grapes and Wine. J. Agric. Food Chem. Journal of Agricultural and Food Chemistry, 61, 25−33.

Herderich, M.J., Siebert, T.E., Parker, M., Capone, D.L., Jeffery, D.W., Osidacz, P., Francis, I.L. (2012) Spice up your life: analysis of key aroma compounds in Shiraz. Qian, M., Shellhammer, T.H. (eds.) Flavour Chemistry of Wine and Other Alcoholic Beverages. Washington, D.C.: American Chemical Society: 3-13.

Jeffery, D.J., Siebert, T.E., Capone, D., Pardon, K., van Leeuwen, K.A., Solomon, M. (2009) Grape and wine pepper aroma – analytically challenging but we sniff it out in the end. AWRI Technical Review (180): 11-16.

Lloyd, N.D.R., Capone, D.L., Ugliano, M., Taylor, D.K., Skouroumounis, G.K., Sefton, M.A., Elsey, G.M. (2011a) Formation of beta-damascenone under both commercial and model fermentation conditions. Journal of Agricultural and Food Chemistry 59: 1338-1343.

Lloyd, N.D.R., Capone, D.L., Ugliano, M., Taylor, D.K., Sefton, M.A., Elsey, G.M. (2011b) The role of yeast in the generation of the odorant damascenone in wine. *Hoffman, T., Meyerhof, W., Schieberle, P. (eds)* In Proceedings of 9th Wartburg Symposium on Flavour Chemistry and Biology, Eisenach, Germany, 13-16 April 2010, Deutsche Forchungsanstalt fur Lebensmittelchemie: Freising, Germany: 447-451.

Mayr, C.M., Capone, D.L., Francis, I.L. (2013) Development of a comprehensive GC- MS/MS method to quantify 18 volatile aroma compounds related to oxidative off-flavour in wines. (manuscript in preparation – further information available on request).

Pardon, K.H., Graney, S.D., Capone, D.L., Swiegers, J.H., Sefton, M.A., Elsey, G.M. (2008) Synthesis of the individual diastereomers of the cysteine conjugate of 3-mercaptohexanol (3-MH). Journal of Agricultural and Food Chemistry 56(10): 3758-3763.

Parker, M., Pollnitz, A.P., Cozzolino, D., Francis, I.L., Herderich, M.J., (2007) Identification and quantification of a marker compound for ‘pepper’ aroma and flavor in Shiraz grape berries by combination of chemometrics and gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry 55(15): 5948-5955.

Parker, M., Osidacz, P., Baldock, G.A., Hayasaka, Y., Black, C.A., Pardon, K.H., Jeffery, D.W., Geue, J.P., Herderich, M.J., Francis, I.L. (2012) Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine. Journal of Agricultural and Food Chemistry 60(10): 2629-2637.

Pollnitz, A.P., Wood, C., Siebert, T.E., Parker, M., Capone, D.L., et al. (2008) Pepper aroma in Shiraz. Blair, R.J., Williams, P.J., Pretorius, I.S. (eds.) The 13th Australian Wine Industry Technical Conference, Adelaide, South Australia. Australian Wine Industry Technical Conference Inc., Adelaide, South Australia: 157-159.

Siebert, T.E., Wood, C., Elsey, G.M., Pollnitz, A.P. (2008) Determination of rotundone, the pepper aroma impact compound, in grapes and wine. Journal of Agricultural and Food Chemistry 56(10): 3745-3748.

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. Journal of Agricultural and Food Chemistry 58(17): 9454-9462.

Wood, C., Siebert, T.E., Parker, M., Capone, D.L., Elsey, G.M., et al. (2008) From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. Journal of Agricultural and Food Chemistry 56(10): 3738-3744.

Contact:
Communication Manager
Tel: (08) 8313 6600