Analysis and Amelioration
of Smoke Taint in Wine

A thesis presented in fulfilment of the requirements

for the degree of Doctor of Philosophy

Anthea L. Fudge

BSc, BSc (Hons)

THE UNIVERSITY
of ADELAIDE

The University of Adelaide

School of Agriculture, Food and Wine

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Abstract

The occurrence of smoke taint in wine is of worldwide concern for grape growers, winemakers and the wine industry. An improved understanding of the impact of bushfire smoke on grapes and wine is important to enable industry to overcome this challenge. Smoke taint occurs when vineyards are exposed to smoke from bushfires or prescribed burns and can result in wine which exhibits undesirable smoky and ashy aromas and flavours. The prior research summarised in Chapter 1 addresses many of the early knowledge gaps concerning the impact of smoke on grape and wine quality such as: the composition of smoke taint, including identification of several of the volatile compounds involved, and the complex sensory properties imparted to wine that cause the detrimental effects to wine quality. However, these studies do not offer industry many practical solutions to mitigate the incidence or severity of smoke taint.

This research reported in Chapter 2 describes several methods for ameliorating smoke taint in wine; i.e. the efficacy of (i) reverse osmosis (RO) and solid phase adsorption, and (ii) commercial fining agents, as treatments for smoke tainted wine. The ability of these methods to reduce the perception of smoke-related sensory attributes and concentrations of smoke-derived volatile phenols are described in two papers, which form the basis for this chapter on the amelioration of smoke taint in wine.

The studies reported in Chapter 3 describe the use of spectroscopy as a rapid analytical method for screening wines for smoke taint. The capability of spectroscopic techniques, in combination with chemometrics, to be used for the classification and discrimination of wine are also introduced. The need for rapid detection of smoke taint in grapes and wines was identified as a priority for industry following the demand for analysis of grapes and wine after a fire event. Winemakers operate under considerable time and resource constraints during vintage, thus rapid determinations of fruit quality, including the assessment of smoke exposure by fruit, are required. A predictive method employing mid-infrared (MIR) spectroscopy was developed by scanning control and smoke-affected wines, and applying multivariate data analysis techniques to the resulting data, to generate a calibration model for the classification of smoke tainted wines. Two-dimensional correlation spectroscopy (2D-COS) was also investigated as a novel method for characterising smoke taint in wines. Again, two papers are presented as the basis for a chapter concerning the spectroscopic analysis of smoke taint in wine.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Publications

This thesis is in part a collection of manuscripts that were published in various refereed journals during candidature. The Journal of Agricultural and Food Chemistry; and Food Chemistry are two journals that research was published in. Other manuscripts were published in the Australian Journal of Grape and Wine Research.

A statement of authorship, signed by all of the authors listing individual contributions to the work is included at the beginning of each chapter.

Chapter 2.

i)   *Amelioration of Smoke Taint in Wine by Reverse Osmosis and Solid Phase Adsorption, Australian Journal of Grape and Wine Research, 2011, 17 (2), pp S41–S48*

ii)   *Amelioration of Smoke Taint in Wine by Treatment with Commercial Fining Agents, Australian Journal of Grape and Wine Research, 2012, 18 (3), pp 302-307*

Chapter 3.

iii) *Classification of Smoke Tainted Wines Using Mid-Infrared Spectroscopy and Chemometrics, Journal of Agricultural and Food Chemistry, 2012, 60 (1), pp 52–59*

iv)   *Synchronous Two-Dimensional Correlation Spectroscopy (2D-COS) as a Novel Method for Screening Smoke Tainted Wine, Food Chemistry, 2013, 139 (1-4), pp 115-119*

An additional 3 related publications co-authored by the candidate are provided in the appendices.

Symposia

2011 Crush Grape and Wine Symposium, Adelaide, Australia, September 2011

Reverse osmosis-solid phase adsorption as an amelioration technique for reducing smoke taint in wine (oral presentation).

<u>Fudge, A.L.</u>, Ristic, R., Wollan, D. and Wilkinson, K.L.

34th World Congress of Vine and Wine, Porto, Portugal, July 2011

Impact of smoke exposure on different grape varieties (poster presentation).

<u>Ristic, R.</u>, Pinchbeck, K., **Fudge, A.,** Hayasaka, Y. and Wilkinson, K.

The effect of leaf removal and smoke exposure on Chardonnay wines (poster presentation). <u>Ristic, R.</u>, Pinchbeck, K., **Fudge, A.,** Hayasaka, Y. and Wilkinson, K.

9th Wartburg Symposium on Flavour Chemistry and Biology, Eisenach, Germany, April 2010

Identification of smoke derived volatiles in grapes and wine by GC-Olfactory (poster presentation).

<u>Fudge, A.</u>, Boss, P. and Wilkinson, K.

23rd RACI 'Organic 08' Conference, Hobart, Australia, December 2008

Quantitative analysis of a potential precursor to cis-oak lactone, in oak extracts using liquid chromatography-tandem mass spectrometry based stable isotope dilution analysis (poster presentation).

<u>Fudge, A.L.</u>, Elsey, G.M., Hayasaka, Y., and Wilkinson K.L.

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Chapter 1: Introduction

Chapter 1

Introduction

The issue of smoke taint

Smoke taint was first reported following the 2003 bushfires in Victoria and Southern New South Wales and then again in the summer of 2007 when fires ravaged Eastern Victoria in Australia. In December 2006, the King and Alpine Valleys in Victoria experienced a prolonged smoke haze due to bushfires in the region. Thus, the 2007 Victorian vintage was greatly damaged with most of the smoke affected grapes unable to be used, without significant blending to produce relatively cheap wine. An estimated value of $75-90m was the loss of wine not produced in this region (Jones 2008). In 2007 in the King and Alpine Valleys of Victoria, thousands of tonnes of grapes were lost as a direct result of smoke exposure (Vine Finders 2008). Parts of South Australia, Tasmania and North-East Victoria were also affected by drifting smoke from the 2007 bushfires (Port 2007). Concurrently, vineyards in WA were affected by smoke taint due to burn-offs conducted by the Department of Agriculture (Port 2007). The Black Saturday bushfires that occurred on the 7th of February 2009 burned over 450,000 hectares of south-eastern Australia (Cruz et al. 2012). The proximity of fire and subsequent smoke caused damage to vineyards in many wine regions in the area. In particular, the Yarra Valley wine region was significantly affected (Scarlett et al. 2011). Recently in January 2015 fires again occurred in grape-growing regions at Sampson Flat in the Adelaide Hills of South Australia causing problems for growers (Peddie 2015). Smoke taint is therefore a national problem that has occurred and continues in wine regions throughout Australia.

Smoke taint is a widespread problem but it is not unique to Australia. Fire events occur all over the world and they have occurred in close proximity to wine regions elsewhere in the world. Cape Town in South Africa has also reported 'smoky', 'burnt' and 'singed' characters in some red wines (James 2008); while forest fires in Greece in 2007 also burned near vineyards (Manessis & Furer 2007), with more fires continuing to sporadically occur throughout Europe. Bushfires in California, United States of America, caused significant problems during the 2008 vintage (Chong & Cleary 2012). Many wildfires,

some in premium wine producing vineyards caused a negative impact due to smoke exposure at 50 wineries in the Mendocino County region (Chong & Cleary 2012). These areas of the United States and also some parts of Western Canada have encountered more frequent fires and this has caused problems as further expansion of vineyards into these wildfire prone regions becomes a far-reaching concern (Sheppard et al. 2009).

Wine and smoke

Wine is a complex mixture and its aroma is a culmination of many different natural and processing variables. These include: grape maturity and composition at harvest; the amount of solids in the fermented grape juice; winemaking techniques; maturation or ageing; and the yeast strain utilised during fermentation (Rankine 2004; Swiegers et al. 2005). The aroma of wine is driven by hundreds of volatile constituents that are present at varying concentrations. Volatile compounds are present in wine at concentrations ranging from 0.1 ng/L to 100 mg/L, and their odour detection thresholds can range from 1 pg/L to 0.1 mg/L (Rapp & Mandery 1986). In addition, aromas can be pleasant or unpleasant; at varying concentrations and combinations, volatile compounds can also influence the overall aroma and bouquet (Rapp & Mandery 1986; Francis & Newton 2005). For example, ethyl acetate can be perceived to contribute a positive aroma at lower concentrations but is considered a fault in wine at higher concentrations (Regodón Mateos, Pérez-Nevado & Ramírez Fernández 2006).

Taints are defined as "unpleasant odours or tastes resulting from contamination of a food by some foreign chemical with which it accidentally comes into contact" (Baigrie 2003). Smoke taint is considered an undesirable characteristic in wine that occurs following vineyard exposure to smoke from bushfires or prescribed burning. The 'smoky', 'ashtray', 'burnt' and 'bitter' characters associated with smoke taint are not palatable (Kennison et al. 2007; Kennison et al. 2008). One compound present in smoke and both smoke-affected and oak-aged wines is guaiacol, but it is desirable only in low concentrations. For example, guaiacol-derived from oak barrels can add complexity and for this reason, toasted oak barrels are often used for maturation or storage of wine. Oak-derived volatiles such as

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guaiacol are extracted into the wine, and can impart pleasant smoky notes, that are not considered to be a taint. However, guaiacol has also been identified as a smoke-derived volatile compound and used as a marker of smoke taint in grapes and wine (Kennison et al. 2007).

Smoke-derived volatile compounds

The characteristic smoky flavour does not appear to be limited to one class of compound; rather it is a combination of a number of different compounds such as phenols and carbonyls (Fiddler, Wasserman & Doerr 1970). This was confirmed by Kostyra and Barylko-Pikielna (2006), who reported that smoke flavouring is a mixture of those compounds and derivatives of phenol, including syringol, guaiacol and carbonyls.

Volatile phenols, including guaiacol and 4-methylguaiacol (**Figure 1**) have been identified as indicator compounds or markers of smoke taint in wine; with guaiacol found to be the most abundant volatile phenol in smoke tainted wine (Kennison et al. 2007). Following bushfires in North Eastern Victoria in the alpine regions during 2003, routine analyses of these marker compounds were established at the Australian Wine Research Institute's (AWRI) Commercial Services laboratory. Gas chromatography-mass spectrometry (GC-MS) analysis of wines, combined with sensory evaluation, was employed to assess the extent of smoke taint. These compounds are not likely to be solely responsible for smoke taint, since they can be found at higher levels in oak-aged wines without an apparent taint, than in smoke tainted wines. Nonetheless, they remain useful indicator compounds.
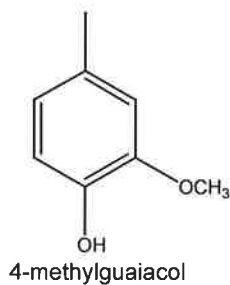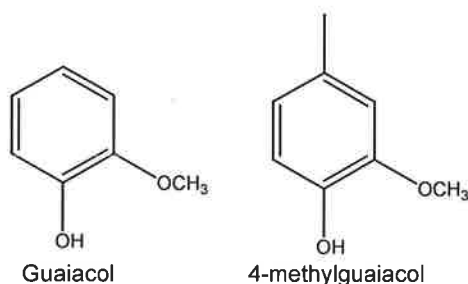

Guaiacol   4-methylguaiacol

Figure 1: *Major marker compounds for smoke taint*

Guaiacol and 4-methylguaiacol were detected at high concentrations in wine made from Verdehlo grapes exposed to smoke post-harvest (Kennison et al. 2007). They are formed during the combustion process from thermal degradation of lignin; however other compounds are also thought to contribute to the smoky aroma. Guaiacol has been described as 'phenolic', 'smoky', 'aromatic', 'sweet' and 'somewhat pungent'; whereas, 4-methylguaiacol is described as 'toasted', 'ash', 'cinnamon' and 'smoky' (Boidron et al. 1988; Pollnitz et al. 2004).

Smoke generation and flavour components

Liquid smoke flavouring is now widely used to impart smoky aromas and flavours to food instead of traditional methods of smoking (Guillén, Sopelana & Partearroyo 2000). Smoke and liquid smoke flavourings (i.e. condensed smoke in a water solution) are highly complex mixtures containing various compounds and are readily used by the food industry. Smoking was traditionally used to preserve foods such as meat and fish (Wittkowski et al. 1992; Suñen, Aristimuño & Fernandez-Galian 2003), but it also afforded desirable colour, aroma and flavour (Gilbert & Knowles 1975). Previous research has investigated the impact of smoking on texture and firmness of meat (Martinez et al. 2004). Smoking has been used in foods, not only as a preservative, but also for its unique flavour (Wittkowski, Baltes, & Jennings 1990; Wittkowski et al. 1992). Although it is widely used, the composition of smoke flavourings remains unclear (Wittkowski, Baltes, & Jennings 1990). Smoke is produced from the combustion of plant materials, typically wood. During combustion, thermal degradation of lignin, cellulose, hemicellulose and tannin, liberates volatile compounds including those responsible for the aroma of smoke.

The chemistry profile varies in different woods as different types of fuels result in variations in the compounds present. Consequently, different wood smokes have distinct sensory properties (Manga & Chen 1985). A detailed review was published in 1975 that looked at the chemistry of smoked foods including their flavour, colour, preservative effect

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and food safety, and the formation of several smoke compounds is also described (Gilbert & Knowles 1975). Pyrolysis of lignin, one of the major constituents of wood, produces volatile phenols such as guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and syringol (Gilbert & Knowles 1975). The aromatic rings in the lignin structure are stable and fission is likely to occur at the heterocyclic furan, pyran rings and the ether linkage. The substitution on aromatic rings determines whether guaiacol, cresols, phenols or syringol are formed (Gilbert & Knowles 1975).

A study by Guillén and co-workers (Guillén & Ibargoitia 1996) investigated how temperature affects the composition of aqueous smoke flavour preparations obtained from *Vitis vinifera L.* shoot sawdust. This research found that an increase in maximum temperature during smoke generation resulted in increased acidity and yield of flavour components as long as the temperature did not exceed nominal values. They were able to identify various compounds in the smoke flavouring preparation, including aldehydes, ketones, diketones, furan derivatives, esters, pyran and phenol derivatives. At lower combustion temperatures the smoke flavour preparation had more carbonyl derivatives resulting in a sweet, burnt caramel flavour and less phenol derivatives which afford pungent, cresolic and smoky flavours. The concentrations of the flavour components were not influenced by the rate and length of heating but were instead dependent on the maximum temperature reached during smoke generation (Guillén & Ibargoitia 1996).

Guaiacol has also been implicated in a smoky/phenolic taint identified in chocolate milk, where the formation of guaiacol was linked to the presence of bacterium (Jensen, Varelis & Whitfield 2001). Guaiacol was also identified as an odorant in medium-roasted Arabica coffee and its concentration increased when roasting was increased (Mayer, Czerny & Grosch 1999). The occurrence of guaiacol in smoked food or in some whiskies has a positive impact, but in vanilla ice-cream, apple juice and of course in wine it can be a taint (Baigrie 2003). Wasserman (1966) determined taste and odour thresholds of guaiacol, 4-methylguaiacol and syringol in an oil and water system. Odour thresholds in water ranged from 1.7×10^{-7} M at the lowest concentration for guaiacol and up to 1.2×10^{-5} M for syringol (almost 100 times higher), with 4-methylguaiacol around 5 times higher than guaiacol at a concentration of 6.5×10^{-7} M. Mean taste thresholds in water for all three

compounds again exhibited a similar trend, with guaiacol more potent than 4-methlyguaiacol, followed by syringol. The values were: 1 x 10^{-7} M, 4.7 x 10^{-7} M and 1 x 10^{-5} M for guaiacol, 4-methylguaiacol and syringol, respectively (Wasserman 1966). Subsequent odour threshold levels are shown in **Table 1** for guaiacol and 4-methylguaiacol (Boidron et al. 1988; Ferreira et al. 2000; Francis & Newton 2005).

Table 1: Wine odour thresholds and sensory descriptors for important phenols

Phenols	Odour Quality	Odour Threshold (μg/L)
Guaiacol	smoke, sweet, medicine	9.5
4-Methylguaiacol	toasted, ash	65

Boidron et al. 1988; Ferreira et al. 2000; Francis & Newton 2005

Industry concern for Australia

Smoke taint has become an increasingly costly problem for both grape growers and winemakers. It is a particular concern in Australia, given the number of wine regions that are located in or near to 'smoke prone' regions. Large financial losses were reported as a consequence of reduced wine quality and/or loss of product placement and reputation, following the 2007 Victorian bushfires (Whiting 2012).

Several issues have arisen from the smoke taint problem, including: back-burning and prescribed burns being undertaken to reduce severity of fires, but these can also cause concern for nearby growers; government and council bodies being at odds with wineries over the need for prescribed burning; and the need for more research to demonstrate the consequences of grapevine exposure to smoke and to identify solutions to the problem. As of May 2008, Tasmania's wine industry and Forestry Tasmania had worked together to develop a deal to help stop smoke damage to wine (ABC News 2008). There could have been a problem with grape harvests and annual forestry burn-offs occurring together. However, Forestry Tasmania now consults with vineyards before proceeding with burns. This cooperation has greatly improved communication between the two industries and has enabled development of a good working relationship to prevent smoke taint from occurring in the future.

Prescribed burning remains an ongoing issue in Western Australia (WA). To date, at least four lawsuits have been filed against Conservation and Land Management. In South-West WA, the Pemberton region has had ongoing problems with the Department of Environment and Conservation's prescribed burn regimes. Lightning strikes have also been a major problem in WA, causing numerous fires.

Inquiries have been made into the impact of public land practices on bushfires in Victoria. Grape growers in the region want recognition that their assets are their vineyards. They are trying to protect their grapevines (and wines subsequently produced from those vines) from smoke exposure. Prior to the 2003 fires, this was a non-issue, but now it has become a significant issue, especially after the 2007 and 2009 fires, and given the likelihood of more fires to come. Within Victoria's Department of Sustainability and Environment (DSE), there have been positive steps in regards to controlled burns and smoke taint (Vine Finders 2008). The DSE now works more closely with growers to control the timing of burns and this has ensured that the risk of smoke taint associated with the burn-offs is minimised.

A timeline of smoke taint research

Over the past decade, considerable research has been undertaken to understand the impact of vineyards exposure to bushfire smoke. The AWRI conducted preliminary studies following the 2003 NSW bushfires; for example they investigated various washing treatments of smoke-affected grape berries, but found none to be terribly effective in reducing the concentration of guaiacol. Activated carbon treatments were found to give only a small decrease (~5%) in guaiacol levels (Høj, Pretorious & Blair 2003).

Early research by Kennison and colleagues established the correlation between smoke exposure and 'smoke taint' in grapes and the subsequent wine produced, as no definitive link had been previously demonstrated and published. Following harvest, Verdehlo grapes were exposed to straw-derived smoke using a purpose-built smokehouse (Kennison et al. 2007). The results of this study demonstrated that the volatile phenols guaiacol and 4-methylguaiacol were found in the smoked wine samples at elevated levels, but were absent in wines made from control (unsmoked) fruit. Sensory studies confirmed the smoked wines

exhibited unpleasant 'smoky', 'dirty', 'earthy', 'burnt' and 'smoked meat' characters (Kennison et al. 2007). Thus, the 'smoke taint' character was attributed to the application of smoke to grapes and provided evidence of smoke influencing wine sensory characteristics.

In addition, this work also looked at the impact of fermentation with and without skins, to mimic different winemaking treatments; i.e. white and red winemaking, without skin contact and with skin contact, respectively (Kennison et al. 2007). It was initially postulated that there may be a higher accumulation of smoke taint in the grape skins and therefore differences in skin contact were investigated. An increase in smoke compounds was found in the wine produced from juice of smoke-affected fruit without skin contact. Therefore, the smoke compounds were considered to have penetrated the grape berry and/or the differences in winemaking conditions (e.g. temperature) during fermentation of smoked fruit on skins was also considered to be influencing final levels of smoke compounds (Kennison et al. 2007).

Kennison and co-workers then considered grapevine exposure to smoke, in a study involving the application of smoke to Merlot grapevines at 8 different time points between veraison and harvest (Kennison et al. 2008). Wines were made from fruit harvested from control (unsmoked) and smoked grapevines, with samples collected at different stages of fermentation to compare the evolution of taint during winemaking. Only trace levels of volatile phenols were detected in control wines, but volatile phenol concentrations increased progressively during both alcoholic and malolactic fermentations. Observing the phenol levels continuing to increase after pressing (i.e. after skins were removed) provided the first evidence to suggest the presence of non-volatile precursor compounds.

The presence of precursor compounds was further validated by a series of acid and enzyme hydrolysis experiments (Kennison et al. 2008). It was envisaged that after enzyme or acid hydrolysis of smoked grape juice, increased volatile phenol levels would indicate the presence of bound precursor compounds. Free run juice from smoke-affected grapes was hydrolysed via either acid hydrolysis at a pH of 1.0, or enzyme hydrolysis with β-glucosidase (Kennison et al. 2008). These hydrolytic studies confirmed that under the hydrolytic conditions employed, the concentrations of volatile phenols increased,

demonstrating the accumulation of smoke-derived volatile compounds in glycoconjugate precursor forms.

Once it was confirmed that guaiacol and 4-methylguaiacol were found to have an impact on the smokiness of wines, further research investigated the influence of timing of smoke exposure on taint levels in resulting wines (Sheppard et al. 2009). Grapevines were exposed to smoke from combustion of Ponderosa pine chips, both pre- and post-veraison for one hour. Grapes were then analysed to determine guaiacol and 4-methylguaiacol levels using SIDA (stable isotope dilution analysis) at different times of maturity. Each time point showed that the compounds had been absorbed by grapes; showing that the smoke could potentially have a significant impact on the sensory characteristics of a resulting wine.

Research continued trying to pinpoint crucial times where smoke might have the most impact on the resultant wine quality and if multiple smoke effects had any additional influence (Kennison et al. 2009). Experiments involved a single application of smoke to Merlot grapevines at veraison, and a range of time points post-veraison; as well as multiple applications of smoke at each of these time points, to investigate any cumulative effect. The results from these phenology experiments indicated guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol were present at increased levels in all wines corresponding to smoke treatments, but levels were influenced by the timing and duration of smoke exposure. Sensory analysis confirmed that smoke-affected wines again exhibited characteristic aromas of 'smoked meat' and 'burnt rubber', which were not detected in control wines. Repeated smoke applications had a cumulative effect on the levels of smoke taint compounds and for single smoke applications; grapevines were particularly susceptible to smoke exposure at 7 days after veraison, (Kennison et al. 2009). Further research into chemical and sensory impacts over multiple seasons and the entire growing seasons were also considered (Kennison et al. 2011), to determine whether or not there was any carry-over of smoke taint between seasons and to further confirm crucial smoke timing stages. The taint had the greatest effect when smoke was applied between 7 days post-veraison and harvest. One important outcome from this investigation was that the vines did not seem to cause any further smoke taint in the grape berry at the next growing season as wines produced from fruit harvested from grapevines exposed to smoke in the previous

season were unaffected. However, crop yields were lower in the subsequent season for vines that had been exposed to smoke, and so smoke exposure was thought to be having an impact on vine physiology. Another important finding was the direct relationship between the chemical analysis of key smoke taint compounds and smoke aromas detected via sensory analysis; i.e. higher concentrations of both guaiacol and 4-methlyguaiacol produced increased smoky aromas and decreased fruit aromas, as determined by trained sensory panelists (Kennison et al. 2011).

As the presence of precursor compounds had previously been inferred (Kennison et al. 2008), research by Hayasaka et al. (2010a) later identified a β-D-glucopyranoside precursor to one of the major smoke taint compounds, i.e. guaiacol. This provided confirmation that the glucoside was present in smoke-affected grapes. As the glucoconjugate precursor of guaiacol was able to be confirmed, other potential glycosides of guaiacol were also thought to occur in the grape berry (Hayasaka et al. 2010a). The occurrence of other glycoconjugate precursors to guaiacol was investigated via extensive applications of labelled forms of guaiacol to both leaves and berries of potted grapevines, and 35 days after treatments were applied, leaf and fruit samples were collected for analysis. Screening for other glycoconjugate forms was achieved via intensive monitoring using high performance liquid chromatography tandem mass spectrometry (HPLC-MS and MS/MS) analysis. This spectroscopic analysis found 7 alternative guaiacol conjugates –identified from mass spectra and product ion scans (Hayasaka et al. 2010b). Development of a method for quantification of these glucosides followed, using liquid chromatography-tandem mass spectrometry based stable isotope dilution analysis (Dungey, Hayasaka & Wilkinson 2011). The ability to quantify the potential amount of precursor to guaiacol provided the understanding that a certain amount of guaiacol could be produced from its glucoside precursor form. This method was subsequently used for both grape and wine analysis to study the potential for smoke taint to intensify in the bottle due not only to the smoky aroma compounds themselves, but also precursor forms of these compounds (i.e. that were later released in the bottle over time via enzymatic or alternate reactions). This was a great step forward in knowledge.

Identification of a guaiacol precursor was important to be able to assess the impact smoke taint may have on wine and the further work of Hayasaka et al. (2012) identified and quantified even more conjugated glycosides. Various glycosidic grape metabolites were used in the analysis and the assay was developed to run the suite of compounds using HPLC-MS/MS to evaluate the level of smoke taint precursors present and whether they are above or below the determined thresholds. This method used a mixture of phenolic glycosides (as opposed to the major volatile phenols) to determine if the concentration of smoke taint could be determined; and if exposed, to what extent. A labeled syringol gentiboside was used as the internal standard for the quantification. A total of 12 glycosides were assessed as part of this method; these are now used as additional smoke taint markers to assess the extent of taint (Hayasaka et al. 2012). This broad range is able to be used as a diagnostic marker for smoke taint in comparison with simply using guaiacol; which is not always indicative of smoke, but in this research the glycoside analysis was more definitive than just guaiacol alone (Hayasaka et al. 2012). Precursors can be released following fermentation or after maturation over time and glycosides, found in smoke affected grapes and wine, are thought to better relate to the amount of smoke taint.

An alternate method for quantifying smoke taint was developed by Singh et al. (2011). Guaiacol and 4-methlyguaiacol are bound in the grape berries as glycoconjugates (Kennison et al. 2008; Hayasaka et al. 2010a; Hayasaka et al. 2010b). To determine if there could be pools of further smoke taint from these glycoconjugate precursors that could cause guaiacol and other volatile phenols to be released during bottle aging, an acid hydrolysis method to release these potential pools (of volatile smoke compounds) and then assess it was explored (Singh et al. 2011). This method used GC-MS to measure guaiacol concentrations after acid hydrolysis of either juice or wine to release the precursors. In addition, analysis of smoke affected wines was performed after bottle aging to confirm that bound guaiacol was released with aging, thereby confirming that detection of precursor forms of taint volatiles is important.

Multiple publications using various methods of quantification of the main compounds responsible for smoke taint all provide suitable methods to detect and/or quantify the extent of taint. A method comparison paper was published in which the different analytical tools

available to measure smoke taint compounds were evaluated (Wilkinson et al. 2011). Key findings were that it may be better to look at detection of both free and bound forms of smoke taint markers, which may then allow better predictive models to be investigated. However, it is also a necessity to consider and optimise the quantification, to minimise the time and sample preparation involved. Industry needs rapid methods to enable detection/quantification of smoke taint during vintage (Wilkinson et al. 2011); especially so as to enable timely decisions to be made by grape-growers and winemakers leading up to vintage.

More recent studies have looked at different analytical methods for detection of smoke taint compounds and because of this the AWRI have further expanded the range of volatile phenols that they measure, i.e. to include cresols and syringol (Parker et al. 2012). Sensory analysis has also been undertaken to look at other volatile phenols and their glycoconjugates; i.e. in addition to guaiacol and 4-methylguaiacol (Parker et al. 2012).

Aims

Smoke taint has the potential to cause significant losses not only to Australian grape and wine producers, but to the global wine industry, so further research is of upmost importance to address this issue. Smoke events will likely occur again due to environmental conditions associated with climate change including prolonged drought and increased temperatures during the growing season, and thus, the consequences for industry cannot be readily predicted. The viability of growers and sustainability of industry is threatened by smoke events. Further research is needed to minimise future losses; in particular, to develop rapid methods for detecting and quantifying the smoke taint in grapes and wine; as well as methods for the amelioration of smoke tainted wine.

The aims of this research are therefore:

- To evaluate UV/Vis/NIR/MIR spectroscopy as a rapid analytical tool for assessing smoke taint in grapes and wine; and

- To evaluate the capacity of reverse-osmosis and fining agent treatments to ameliorate smoke taint in wine.

The outcomes of the proposed research will enable the wine industry to more readily identify smoke tainted grapes and wine. It will also give winemakers options for treating smoke tainted wine.

Chapter 2

Amelioration of Smoke Taint in Wine

Amelioration of Smoke Taint in Wine by Reverse Osmosis and Solid Phase Adsorption

Anthea L. Fudge[1], Renata Ristic[1],
David Wollan[2] and Kerry L. Wilkinson[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide,
PMB 1, Glen Osmond, SA 5064, Australia
[2]Memstar Pty. Ltd, 29 Dalgety Street, Oakleigh, Vic. 3166, Australia

Statement of Authorship

Amelioration of Smoke Taint in Wine by Reverse Osmosis and Solid Phase Adsorption

Australian Journal of Grape and Wine Research – 2011, 17 (2), pp S41–S48

Anthea Fudge (Candidate)

Performed pilot-scale amelioration experiments and chemical and sensory analysis of wines, analysed and interpreted chemical and sensory data, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate.

Signed.. ....*Date.*25/9/15

Renata Ristic

Assisted with sensory analysis of wines, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed. *Date.*25.9.15

David Wollan

Performed commercial scale amelioration experiments, provided access to pilot-scale equipment, and revised the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed.. ....*Date.*................

Kerry Wilkinson

Supervised experimental work, analysed and interpreted chemical and sensory data, drafted and revised the manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis

Signed.. ....Date..25/9/15

Chapter 2
Amelioration of Smoke Taint in Wine

Amelioration of Smoke Taint in Wine by Treatment with Commercial Fining Agents

Anthea L. Fudge[1], Mathilde Schiettecatte[1], Renata Ristic[1],
Yoji Hayasaka[2] and Kerry L. Wilkinson[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide,
PMB 1, Glen Osmond, SA 5064, Australia
[2]The Australian Wine Research Institute, P.O. Box 197,
Glen Osmond, SA 5064, Australia

Statement of Authorship

Amelioration of Smoke Taint in Wine by Treatment with Commercial Fining Agents

Australian Journal of Grape and Wine Research – 2012, 18 (3), pp 302–307

Anthea Fudge (Candidate)

Performed fining experiments and chemical and sensory analysis of wines, analysed and interpreted chemical and sensory data, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate.

Signed..   ....*Date.*.25/9/15

Mathilde Schiettecatte

Assisted with fining experiments and chemical and sensory analysis of wines, and revised the manuscript. I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed..........   ....*Date....*.25/9/15

Renata Ristic

Assisted with sensory analysis of wines, and drafted and revised the manuscript. I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed...   ....*Date...*.25-9,15

Yoji Hayasaka

Performed LC-MS/MS analysis of wines, and drafted and revised the manuscript. I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed. ....Date 25/9/2015

Kerry Wilkinson

Supervised experimental work, analysed and interpreted chemical and sensory data, drafted and revised the manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed.... ....Date. 25/9/15

Chapter 2

Amelioration of Smoke Taint in Wine

The content of this chapter is based on the following two publications:

i) *Amelioration of Smoke Taint in Wine by Reverse Osmosis and Solid Phase Adsorption, Australian Journal of Grape and Wine Research, 2011, 17 (2), pp S41– S48*

ii) *Amelioration of Smoke Taint in Wine by Treatment with Commercial Fining Agents, Australian Journal of Grape and Wine Research, 2012, 18 (3), pp 302-307*

The smoke-taint related research described in Chapter 1 has addressed many of the early knowledge gaps concerning the impact of smoke on grape and wine quality such as: the composition of smoke taint, including identification of several of the volatile compounds involved and the complex sensory properties imparted into the wine that cause the detrimental effects to wine quality. However, these studies do not offer industry any practical solutions to mitigate the incidence or severity of smoke taint.

Two recent studies have investigated the potential for amelioration of smoke-affected grapes. One is focused on the viticultural practice of defoliation, to investigate whether or not leaf removal (applied pre- or post-smoke exposure) can minimise smoke taint and therefore improve the outcome of wine produced (Ristic et al. 2013). The other approach involved the addition of oak or tannins, or the use of different yeast strains, to reduce the intensity of smoke taint during fermentation (Ristic et al. 2011).

Defoliation has been applied to grapes in the vineyard to help remove some additional taint at this initial stage of the winemaking process. Treatments involved removing leaves pre- and post-smoke application from the grapevine. After winemaking using grapes picked from the defoliated vines was completed, the wine was analysed to determine any impacts on colour, composition of smoke taint markers and sensory analysis (Ristic et al. 2013). The wine composition presented increased levels of smoke taint marker compounds and guaiacol precursors in wines that had leaves removed before smoke exposure. This

coincided with a decrease in these compounds in the wines produced when leaf removal was performed after smoke exposure, indicating that leaf removal after a smoke event might help to lower smoke taint effects in the vineyard prior to winemaking. The sensory data aligned with compositional analysis and revealed reduced intensity scores for 'cold ash' and 'ashy' descriptors in wine derived from treatments involving defoliation post-smoke exposure, together with enhanced 'fruity' attributes; thus demonstrating smoke taint can be partially reduced by employing this viticultural practice (Ristic et al. 2013).

Winemaking trials involving various additions of oak chips or tannin, and the use of different yeast strains during fermentation were also performed, and found to influence the level of smokiness in a tainted wine (Renata et al. 2011). The treatments included: different skin contact times and different yeast strains for fermentation, and the addition of either oak chips or tannins to fermentations. Minimising skin contact can offer an alternative winemaking practice to minimise the amount of smoke taint precursor compounds and thus the amount of potential taint that can occur with the finished wine. The addition of oak chips or tannin was able to mask some of the objectionable smoke attributed with added complexity in the wine and therefore improve the overall wine sensory profile of the treated wines. Differing yeast strains were able to exhibit diverse sensory attributes and a selection were able to reduce some of the smoke effect by reducing 'smoke' attributes without altering desirable 'fruit' characters. These treatments had varying impacts on wine composition and sensory properties, but offer winemaking techniques that might increase acceptance of wines made from smoke affected fruit (Renata et al. 2011).

In this chapter several methods for ameliorating smoke taint in wine are described; i.e. the efficacy of reverse osmosis (RO) and solid phase adsorption, and fining agents as treatment methods for smoke tainted wines was evaluated. The ability of these methods to reduce the perception of smoke-related sensory attributes and concentrations of smoke-derived volatile phenols are described.

Introduction

Taints, off-odours and/or various deposits can be removed during winemaking via fining. Fining is commonly used to reduce or remove particular compounds from wine in order to improve sensory properties, including colour and/or clarity (Iland 2004); i.e. to overcome certain wine faults. Different fining agents are able to act on different wine compounds or classes of compounds. Given fining agents are not selective, they have to be tested in a fining trial to ensure they do not negatively impact desirable wine components; i.e. so that the overall quality of wine is not compromised.

Clarity and haze formation, particularly in white wine styles, are common faults caused by proteins. Proteins can aggregate and cause wine stabilisation issues (Sauvage et al. 2010; Ferreira et al. 2001). This unattractive cloudy haze is undesirable and thus ways of removing proteins are important. Fining with clays such as bentonite can reduce the levels of proteins by adsorption of the agent. This treatment is effective, but a downside is that bentonite is not selective and can also remove other desirable wine aroma compounds (Ferreira et al. 2001). At high addition rates, bentonite can also cause loss of wine total volume (Sauvage et al. 2010).

Browning is another undesirable characteristic affecting wine colour and sensory attributes due to oxidation of polyphenols (Spagna, Barbagallo & Pifferi 2000). Phenolics are present in wine and contribute to sensory attributes, astringency and particularly bitterness and colour (Oberholster et al. 2009). Use of additives such as potassium caseinate (a protein) to act as a fining agent can help to reduce these polyphenols but can also cause haze, which in turn can be removed with bentonite as indicated earlier (Spagna, Barbagallo & Pifferi 2000).

RO/solid phase adsorption was investigated first to reduce the impact of treatment on desirable wine components. The fining agent study was then undertaken to investigate a less targeted amelioration approach.

Reverse osmosis processes work by membrane separation and osmotic pressure. A molecular weight cut-off occurs, such that different compounds either pass through the membrane or are retained, largely based on molecular weight. RO can therefore allow a

more selective treatment of different wine fractions following separation of permeate and retentate; i.e. permeate can be treated separately leaving the retentate unaffected, thus minimising other losses. The RO process has been used to treat wines with high volatile acidity (VA) and also Brettanomyces spoilage (Memstar viewed 2014). Volatile acidity is caused by high levels of acetic acid and ethyl acetate in wine which contributes to a fault in the wine with undesirable estery aromas or a sour acid finish. The VA can be reduced using RO in conjunction with a column comprising an ion exchange resin that treats the permeate containing high acid concentrations. Brettanomyces taint is another problem that can be effectively removed via RO. Brettanomyces produces disagreeable aromas akin to 'barnyard' or 'medicinal' traits but following separation of the permeate which contains the Brettanomyces compounds, 4-ethylguaiacol and 4-ethylphenol, treatment with an adsorption resin can remove these compounds, and thus the taint, whilst colour and desirable volatile compounds remain (Memstar viewed 2014).

It was therefore thought that if other taints and off-odours are able to be commonly removed via fining techniques, one or more of these agents could have an influence on smoke taint components. This is where common fining agents and other more specific agents thought to be potentially effective on smoke taint compounds were then trialed to investigate both efficacy and efficiency on smoke tainted and control wines. That experiment and those findings are herein presented in the following publications.

The studies described in this chapter demonstrate that smoke taint can be ameliorated by the addition of particular fining agents. Furthermore, any agents found to be good fining candidates could then be employed and applied more specifically to the wine components that have those smoke taint volatiles by separating the wine using a process of RO. By utilising RO and then solid phase adsorption (like a fining agent) it can be further selectively treated to remove an increasing amount of smoke taint and therefore dramatically improve the quality of treated wine.

Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption

A.L. FUDGE[1], R. RISTIC[1], D. WOLLAN[2] and K.L. WILKINSON[1]

[1] The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia
[2] Memstar Pty. Ltd, 29 Dalgety Street, Oakleigh, Vic. 3166, Australia
Corresponding author: Dr Kerry Wilkinson, fax +61 8 8303 7116, email kerry.wilkinson@adelaide.edu.au

Abstract

Background and Aims: Wines made from grapes harvested from vineyards exposed to bushfire smoke can exhibit objectionable 'smoky', 'cold ash', 'medicinal' and 'ashy' aroma and flavour characters. This study evaluated a combined reverse osmosis and solid phase adsorption process as a potential amelioration method for the treatment of smoke-tainted wines.

Methods and Results: Smoke-tainted wines were treated using either pilot or commercial scale reverse osmosis systems and the chemical composition and sensory properties of wine compared before and after treatment. The concentrations of smoke-derived volatile phenols, including marker compounds, guaiacol and 4-methylguaiacol, decreased significantly with treatment. As a consequence, diminished smoke-related sensory attributes enabled treated wines to be readily differentiated from untreated wines. However, the taint was found to slowly return with time, likely because of hydrolysis of glycoconjugate precursors, which were not removed during the treatment process.

Conclusions: Reverse osmosis and solid phase adsorption reduced the concentration of smoked-derived volatile phenols and improved the sensory attributes of smoke-tainted wines.

Significance of the Study: This is the first study to evaluate the amelioration of smoke taint in wine using reverse osmosis and solid phase adsorption.

Keywords: amelioration, guaiacol, reverse osmosis, smoke taint, wine

Introduction

Aroma is an important aspect of wine quality and has therefore been the subject of considerable research. Indeed, several hundred volatile compounds have been identified in wine to date. These compounds contribute to the complexity and varietal character of wine and can originate from grapes, the action of yeast during fermentation, oak wood during barrel maturation or in the bottle with aging (Williams et al. 1981, Günata et al. 1985, Winterhalter et al. 1990, Pollnitz et al. 2004). However, not all volatile compounds make a desirable contribution to wine aroma. Some volatiles are indicative of winemaking faults; e.g. the 'bruised apple' and 'vinegar' characters associated with excessive concentrations of acetaldehyde and acetic acid due to oxidation and lactic bacteria spoilage (volatile acidity), respectively (Ribéreau-Gayon et al. 2000). In other cases, contamination by exogenous volatiles can lead to taints in wine; 2,4,6-trichloroanisole, for example, is considered to contribute to the 'musty' attribute associated with cork taint (Buser et al. 1982).

In recent years, the potential for smoke to taint grapes and wine has been a concern for some winemakers, following the occurrence of significant bushfires in the vicinity of wine grape-growing regions. Kennison et al. (2007) demonstrated the presence of several smoke-derived volatile phenols, including guaiacol and 4-methylguaiacol, in wines made from smoke-affected grapes. These wines were found to exhibit objectionable 'smoky', 'cold ash', 'medicinal' and 'ashy' aroma and flavour characters (Kennison et al. 2007), with the intensity of smoke-related sensory attributes dependent on the timing and duration of grapevine smoke exposure (Kennison et al. 2009). Vineyard exposure to smoke cannot be readily predicted or prevented, but can have a significant financial impact on grape and wine production. As such, methods which reduce the concentration of smoke-derived volatile compounds in wine, thereby mitigating the effects of smoke exposure, would be of benefit to grape growers and winemakers. Ristic et al. (2011) investigated the effect of different winemaking techniques on the extent of smoke taint in wine and found the duration of skin contact, choice of yeast strain and addition of oak chips or tannins influenced smoke-related sensory properties. These techniques can be applied by winemakers when processing smoke-affected grapes, but do not address the issue of smoke taint in wine.

Reverse osmosis is a filtration process involving diffusion across a semi-permeable membrane against a concentration gradient (Paulsen et al. 1985), in which separation efficiency relies on both size exclusion and solution-diffusion mechanisms (Cuperus and Nijhuis 1993). Reverse osmosis is routinely used for water purification and desalination (Madaeni 1999), with an increasing number of applications being reported within food and beverage industries, e.g. the preparation of milk (Glover 1971) and fruit juice concentrates (Paulsen et al. 1985, Kane et al. 1995). Within the wine industry, reverse osmosis has been used to manipulate wine alcohol content, volatile acidity and acidification through concentration of grape must (Duitschaever et al. 1991) and wine (Bui et al. 1988, Massot et al. 2008). Reverse osmosis has also been coupled with solid
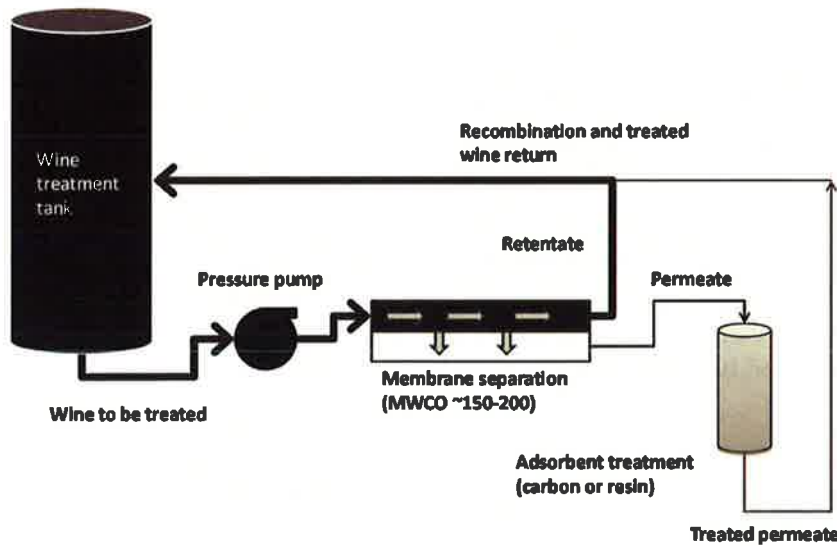
Figure 1. Schematic diagram of combined reverse osmosis and solid phase adsorption system.

phase adsorption to remove 4-ethylguaiacol and 4-ethylphenol from *Brettanomyces*-affected wine (Ugarte et al. 2005). Ugarte et al. (2005) demonstrated 4-ethylguaiacol and 4-ethylphenol could permeate the reverse osmosis membrane and be adsorbed by Amberlite XAD-16 HP resin, such that the 'animal', 'medicinal' and 'stable' aromas associated with *Brettanomyces* spoilage (Chatonnet et al. 1992) became less apparent with treatment. Given volatile phenols are implicated in both *Brettanomyces* spoilage and smoke taint, there is clearly potential for this treatment process to be used for the removal of smoke-derived volatile phenols from wine. This study was therefore undertaken to evaluate the capacity of a combined reverse osmosis and solid phase adsorption process to ameliorate smoke taint in wine.

Materials and methods

Smoke-affected wines

Three Pinot Noir wines were used in this study. Two commercial wines, hereafter referred to as Pinot Noir 1 and Pinot Noir 3, were sourced from wineries located in the Gippsland (37°45′S, 147°43′E) and Yarra Valley (37°37′S, 145°24′E) wine regions, respectively. These wines were made from grapes exposed to smoke following a series of bushfires that occurred in North Eastern Victoria between 1 December 2006 and 7 February 2007; however, specific details regarding the timing, duration and density of grapevine smoke exposure are not known. Pinot Noir 1 was aged in French oak for 19 months, whereas Pinot Noir 3 was un-oaked. A third wine, hereafter referred to as Pinot Noir 2, was made from smoke-affected grapes sourced from field trials involving the application of smoke to vines, using experimental conditions described previously (Kennison et al. 2008, Dungey et al. 2011). Pinot Noir vines growing in a vineyard located in the Adelaide Hills wine region (34°30′S, 138°59′E) were exposed to straw-derived smoke at approximately 7 days post-veraison, i.e. at a juice total soluble solids (TSS) concentration of approximately 13°Brix, as determined using a digital handheld refractometer (PAL-1, Atago, Tokyo, Japan). The duration of smoke exposure was 60 min. Smoke-affected grapes (120 kg) were harvested when juice TSS reached 16 ± 1°Brix (i.e. an early harvest, due to fruit from this vineyard being allocated to a commercial sparkling wine base) and processed according to small-lot winemaking procedures (Holt

et al. 2006). Bunches were de-stemmed and crushed, and tartaric acid added to adjust the pH to 3.5, prior to inoculation with Maurivin PDM yeast (200 ppm). Musts were fermented on skins for 7 days at 15°C, with the cap plunged at least twice per day. The wine was pressed at a TSS level of 1°Baume and transferred to stainless steel vessels and held at 20°C until the residual sugar approached 0 g/L. Wines were then racked from gross lees and cold stabilised (at 4°C for 3 weeks). Wine pH and free SO_2 were adjusted to 3.4 and between 25 to 30 ppm, respectively, before filtration and bottling (under screw cap closures).

Pilot scale reverse osmosis and solid phase adsorption treatment of wine

Reverse osmosis and solid phase adsorption treatment of Pinot Noir 1 and Pinot Noir 2 was performed (in triplicate) using a pilot scale Micro AA unit (Memstar Pty. Ltd, Oakleigh, Victoria, Australia), in accordance with the manufacturer's operating instructions. The unit was equipped with a proprietary spiral wound nanofiltration membrane ('1812' full fit ('sanitary') form factor; nominal molecular weight cut-off of 150–200 amu; filtering area: 0.3 m²), a polystyrene based adsorbent resin (MemTaint, Memstar Pty. Ltd, Oakleigh, Victoria, Australia; 200 mL) in a packed column and a heat exchanger (to ensure wine temperature remained below 20°C). Wine was pumped from the feed tank (6 L) across the membrane under 1700 kPa pressure to generate permeate and retentate fractions at flow rates of approximately 50 mL/min and 600 mL/min, respectively. Retentate flow was circulated back to the feed tank, while permeate flowed through the packed column and then back to the feed tank (Figure 1). Samples (50 mL) were collected before treatment (i.e. untreated wine) and then after $t = 0.5$, 1, 2 and 3 h of treatment, for chemical analyses. On completion of treatment (i.e. at $t = 3$ h), the resulting wine was bottled and stored at 15°C, until sensory analyses.

In a separate experiment, permeate and retentate samples (50 mL) were collected during the reverse osmosis treatment of Pinot Noir 1. Wine (6 L) was filtered as above, but with both retentate and permeate flow circulated back to the feed tank; i.e. without solid phase adsorption treatment of the permeate fraction. The system was allowed to circulate for 30 min before samples were collected for quantification of volatiles by gas

chromatography-mass spectrometry (GC-MS) and glycocon-jugates by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). This experiment was not replicated.

Commercial scale reverse osmosis and solid phase adsorption treatment of wine

Reverse osmosis and solid phase adsorption treatment of Pinot Noir 3 was performed by Memstar Pty. Ltd. using a commercial scale R80940 unit (Memstar Pty. Ltd, Oakleigh, Victoria, Australia). The unit was equipped with nine proprietary spiral wound nanofiltration membranes ('8040' full fit ('sanitary') form factor; nominal molecular weight cut-off of 150–200 amu; filtering area: 270 m^2), a polystyrene based adsorbent resin (MemTaint, Memstar Pty. Ltd, Oakleigh, Victoria, Victoria; 200 L) in a packed column and a heat exchanger (to ensure wine temperature remained below 20°C). Wine (5000 L) was pumped across the membranes under pressure of approximately 3000 kPa to generate permeate and retentate fractions. Retentate flow was circulated back to the feed tank, while permeate was passed through the packed column and then recombined with the retentate prior to being returned to the feed tank (Figure 1), as per pilot scale treatment. Wine was treated for 12 h during which time 16 400 L of permeate was generated (i.e. a permeate flow rate of approximately 1370 L/h). Untreated and treated wine was bottled (48 × 750 mL) and stored at 15°C, until required for chemical and sensory analyses. The commercial scale treatment was not repeated.

Wine analysis

Untreated and treated wines were analysed to determine pH and titratable acidity (TA), as tartaric acid equivalents to an endpoint of pH 8.2; residual sugar (glucose) using an enzymatic test kit (R-Biopharm AG, Darmstadt, Germany); alcohol content using an alcolyzer (Anton Paar, North Ryde, NSW Australia); and wine colour density and phenolics according to the methods of Iland et al. 2004. Analyses were performed within 1 week of treatment.

Quantification of volatiles by GC-MS

Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethyphenol, eugenol, vanillin, cis- and trans-oak lactone, and furfural were quantified by GC-MS using stable isotope dilution assay (SIDA) methods reported previously (Pollnitz 2000, Pollnitz et al. 2000, 2004). These publications describe the synthesis of internal standards used herein. Analyses were performed by the Australian Wine Research Institute's Commercial Services Laboratory (Adelaide, Australia) using an Agilent 6890 gas chromatograph (Agilent Technologies, Forest Hill, Vic, Australia), coupled to a 5973 mass selective detector. For Pinot Noir 1, both smoke- and oak-derived volatiles were quantified; whereas only the smoke-derived phenols, guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol, were measured for Pinot Noir 2 and Pinot Noir 3. A deuterated internal standards solution comprising known concentrations of d$_3$-guaiacol, d$_3$-4-methylguaiacol, d$_4$-4-ethylphenol, d$_3$-vanillin, d$_4$-cis-oak lactone, d$_4$-trans-oak lactone and d$_4$-furfural in ethanol (100 µL) was added to each sample (10 mL) in a screw cap vial. Organic solvent (n-pentane c. 2 mL) was added and the mixture shaken briefly. A portion of the organic layer (c. 1.5 mL) was then taken for instrumental analysis. GC-MS analyses were performed within 2 weeks of treatment. Analysis of untreated and treated Pinot Noir 3 wines was repeated at 6, 12 and 30 months post-bottling.

Quantification of guaiacol glycoconjugates by HPLC-MS/MS

Guaiacol glycoconjugates were quantified by HPLC-MS/MS using SIDA methods reported previously (Dungey et al. 2011, Wilkinson et al. 2011). Dungey et al. (2011) described the synthesis of the internal standard used herein. Analyses were performed using an Agilent 1200 HPLC system, coupled to a 4000 Q TRAP hybrid tandem mass spectrometer (Agilent Technologies, Forest Hill, Vic, Australia). Guaiacol glycoconjugate concentrations were determined for untreated and treated Pinot Noir 1 wine ($n = 3$) and for permeate and retentate fractions generated (as above) from Pinot Noir 1 ($n = 1$). d$_4$-Guaiacol β-D-glucopyranoside (100 µg/mL in water, 10 µL) was added to each sample (1 mL) as an internal standard, the mixture shaken briefly and filtered through a 0.45 µm GHP membrane (Acrodisc®, PALL Life Sciences, Cheltenham, South Australia, Australia) prior to instrumental analysis. HPLC-MS/MS analyses were performed within 2 weeks of treatment.

Sensory analysis of wine

Descriptive sensory analysis of Pinot Noir 1 and Pinot Noir 2 wines. Descriptive sensory analysis (DA) (Stone and Sidel 2004) was performed on Pinot Noir 1 and Pinot Noir 2, to determine any differences in the intensity of smoke-related attributes between untreated and treated wines. DA consisted of a series of training and formal evaluation sessions. Twelve panellists were recruited among staff and students from the University of Adelaide and the Australian Wine Research Institute (AWRI). Ten panellists had extensive experience in sensory analysis of smoke-tainted wines, while two less experienced panellists underwent additional training sessions prior to the panel training and formal evaluation sessions. Training sessions involved presentation of each wine replicate to the sensory panel to generate appropriate aroma and palate attributes. Six aroma attributes and eight palate attributes (Table 1) were rated during formal sessions, using an unstructured 15-cm line scale with indented anchor points of 'low' to 'high' placed at 10% and 90%, respectively. Evaluations were carried out using covered, three-digit coded International Organization for Standardization (ISO) tasting glasses. Each wine (30 mL) was presented at 22–24°C, in isolated and well-ventilated tasting booths under red-light illumination, to limit potential for bias based on wine colour. The presentation order was randomised and balanced across judges. Four wines were presented per session, with one session per day, run over three consecutive days. On each day, panellists assessed the different treatment wine replicates, as well as untreated wines. After assessing each sample, panellists rinsed with water and a 1 g/L citrus pectin solution (Sigma). Data was collected using FIZZ software (Version 2.40 E, Biosystemes, Couternon, France).

Difference testing of wines

Difference tests were conducted for each wine (i.e. before and after treatment), using the duo-trio method described by Meilgaard et al. (2007) and a panel of 48 judges. Panellists were aged between 18 and 55, with similar numbers of males and females. Wines were presented to the panel using a balanced, randomised presentation order, comprising all possible configurations (i.e. R$_A$AB, R$_A$BA, R$_B$AB, R$_B$BA, where A denotes untreated wine and B denotes treated wine) an equal number of times. Wines (20 mL) were served at 22–24°C, in covered ISO tasting glasses with randomly assigned three-digit codes. Panellists smelled and tasted the samples, and were asked to identify the sample which was the same as the reference.

Table 1. Sensory attributes used for descriptive analysis of wines before and after reverse osmosis and solid phase adsorption treatment.

Attribute	Description
Aroma	
Fruit aroma intensity	The intensity of the overall fruit aroma, includes red fruit, red berry, dark berry, capsicum, green, tropical fruit, honey.
Smoke	Perception of any type of smoke aroma, includes smoked meat/bacon, toasty, charry, cigar-box, estery.
Cold ash	Burnt aroma associated with ashes, includes ashtray, tarry, campfire.
Earthy	Any aroma associated with musty, dusty, wet-wood, barnyard, mushroom-like, dank, mouldy, stagnant, stale.
Burnt rubber	Perception of burnt rubber-like aromas.
Medicinal	Aromatic characteristic of band-aids, disinfectant-like, including cleaning products, solvents, chemicals.
Palate	
Fruit flavour intensity	The intensity of the overall fruit flavour; includes red fruit, red berry, dark berry, capsicum, green, tropical fruit, honey.
Smoky	Perception of smoke flavour, includes bacon and smoked meat.
Ashy aftertaste	Length of taste associated with residue of ashtray perceived in the mouth after expectorating, includes coal ash, ashtray, tarry, acrid, campfire.
Woody aftertaste	Length of taste associated with woody residue, includes wood, oak, pencil shavings.
Acidity	Intensity of sour/acid taste.
Metallic	The 'tinny' flavour associated with metals.
Bitter	Intensity of bitter taste, bitter aftertaste.
Drying	Drying, puckering mouth-feel after expectorating the wine.

Consumer acceptance testing of wines

Consumer acceptance tests were conducted using a hedonic rating method described by Meilgaard et al. (2007) using the same 48-judge panels described above. Untreated and treated wines were presented to the panel using a balanced, randomised presentation order. Wines (20 mL) were served at 22–24°C, in covered ISO tasting glasses with randomly assigned three-digit codes. Panellists were asked to rate their liking for each wine on a nine-point hedonic scale with anchors: 'don't like the wine at all' (1); 'neither like nor dislike the wine' (5); and 'like the wine very much' (9). The ratings for each wine were then measured in cm from the left hand anchor and subjected to statistical analysis.

Data analysis

Sensory descriptive data for each attribute was analysed using statistical software (JMP, Version 7, SAS Institute, Cary, NC, USA) and the analysis of variance (ANOVA), mixed model,

testing for the effects of treatment, fermentation replicate, presentation replicate and judge (considered as a random effect). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$. Chemical data were analysed by one-way ANOVA using GenStat (9th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by LSD multiple comparison test at $P < 0.05$.

Results and discussion

Since vineyard exposure to smoke cannot be readily predicted or prevented, the current study intended to evaluate reverse osmosis and solid phase adsorption as a potential method of ameliorating smoke-tainted wine. Pinot Noir 1 and Pinot Noir 2 were chosen as examples of wines affected by bushfire and experimental smoke, respectively, and were treated (in triplicate) using pilot scale equipment. Pinot Noir 3, which was also affected by bushfire smoke, was chosen as an example of a wine treated on a commercial scale, but without replication. The initial composition of each wine was determined by quantitative GC-MS analysis with guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol measured as markers of smoke taint (Table 2). Since guaiacol and 4-methylguaiacol are also oak-derived volatile compounds (Pollnitz 2000, Pollnitz et al. 2004), in the case of Pinot Noir 1, these compounds likely originated from grapevine exposure to smoke as well as oak barrel maturation. A number of additional oak-derived volatiles, i.e. eugenol, vanillin, *cis*- and *trans*-oak lactone, and furfural, were also measured in Pinot Noir 1, which received 19 months barrel maturation. Guaiacol and 4-methylguaiacol are typically the most abundant volatile phenols present in smoke-tainted wines (Kennison et al. 2007, 2008). The elevated levels of 4-ethylguaiacol and 4-ethylphenol observed in Pinot Noir 1 (Table 2) might therefore indicate some degree of *Brettanomyces* spoilage. Guaiacol and 4-methylguaiacol were detected in Pinot Noir 2 and Pinot Noir 3, albeit at lower levels than for Pinot Noir 1. Low levels of 4-ethylguaiacol and 4-ethylphenol were also measured in Pinot Noir 3 (Table 2). The capacity of reverse osmosis and solid phase adsorption to ameliorate smoke taint in wine was determined by monitoring the chemical composition and sensory properties of wines before and after treatment, in particular, the concentration of smoke-derived volatile phenols and smoke-related sensory attributes.

Effect of reverse osmosis and solid phase adsorption treatment on wine composition

The volatile phenol content of each wine decreased significantly with treatment (Table 2). Analysis of Pinot Noir 1 and Pinot Noir 2 throughout the treatment process demonstrated the progressive loss of phenols. After 3 h of treatment, more than 67% of volatile phenols initially present in Pinot Noir 1 had been removed and guaiacol and 4-methylguaiacol were almost completely removed from Pinot Noir 2. A progressive loss of oak-derived volatiles was also observed during Pinot Noir 1 treatment. Eugenol, vanillin, *cis*- and *trans*-oak lactone and furfural concentrations were significantly reduced after treatment (Table 2), with potential implications for the contribution of oak-related sensory attributes to overall wine aroma. Some loss of desirable wine aroma was to be expected, given the membrane's molecular weight cut-off of 150–200 amu would allow permeation of a range of volatile wine constituents besides the volatile phenols. Indeed, GC-MS analysis of retentate and permeate fractions derived from reverse osmosis treatment of Pinot Noir 1 demonstrated passage of each volatile compound across the membrane (Table 2). The pilot scale system could be used to

Table 2. Volatile composition (guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, vanillin, *cis*- and *trans*-oak lactone and furfural) of smoke-affected Pinot Noir wines before and after reverse osmosis and solid phase adsorption treatment, and permeate and retentate fractions derived from reverse osmosis treatment of Pinot Noir 1.

Sample		Concentration (µg/L)								
		Guaiacol	4-Methylguaiacol	4-Ethylguaiacol	4-Ethylphenol	Eugenol	Vanillin	cis-oak lactone	trans-oaklactone	Furfural
Pinot Noir 1†										
Untreated		49ᵃ	36ᵃ	294ᵃ	391ᵃ	16ᵃ	237ᵃ	137ᵃ	111ᵃ	154ᵃ
Treated	t = 0.5 h	35ᵇ	28ᵇ	225ᵇ	277ᵇ	12ᵇ	176ᵇ	114ᵃᵇ	91ᵃᵇ	130ᵇ
Treated	t = 1 h	27ᶜ	23ᶜ	190ᶜ	214ᶜ	n.d.	148ᶜ	102ᵇᶜ	85ᵃᵇ	117ᶜ
Treated	t = 2 h	18ᵈ	15ᵈ	134ᵈ	126ᵈ	n.d.	104ᵈ	79ᶜᵈ	64ᵇᶜ	109ᶜ
Treated	t = 3 h	13ᵉ	11ᵉ	94ᵉ	78ᵉ	n.d.	79ᵉ	61ᵈ	47ᶜ	109ᶜ
P		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.003	<0.001
Retentate		44	34	277	348	16	226	151	97	138
Permeate		39	26	193	323	11	154	68	47	112
Pinot Noir 2‡										
Untreated		7ᵃ	2	n.d.	n.d.	–	–	–	–	–
Treated	t = 0.5 h	5ᵇ	2	n.d.	n.d.	–	–	–	–	–
Treated	t = 1 h	3ᶜ	1	n.d.	n.d.	–	–	–	–	–
Treated	t = 2 h	2ᵈ	1	n.d.	n.d.	–	–	–	–	–
Treated	t = 3 h	1ᵉ	n.d.	n.d.	–	–	–	–	–	–
P		<0.001	–	–	–	–	–	–	–	–
Pinot Noir 3§										
Untreated	0 months post-bottling	12	5	1	4	–	–	–	–	–
Untreated	6 months post-bottling	12	3	n.d.	n.d.	–	–	–	–	–
Untreated	12 months post-bottling	15	4	n.d.	n.d.	–	–	–	–	–
Untreated	30 months post-bottling	16	4	n.d.	n.d.	–	–	–	–	–
Treated	0 months post-bottling	3	tr.	2	8	–	–	–	–	–
Treated	6 months post-bottling	5	2	n.d.	n.d.	–	–	–	–	–
Treated	12 months post-bottling	6	3	n.d.	n.d.	–	–	–	–	–
Treated	30 months post-bottling	9	4	n.d.	10	–	–	–	–	–

Values followed by a different letter are significantly different. †Pinot Noir affected by bushfire smoke and treated with pilot RO system (*n* = 3) and retentate and permeate fractions (*n* = 1); ‡Pinot Noir affected by experimental smoke and treated with pilot RO system (*n* = 3); §Pinot Noir affected by bushfire smoke and treated with commercial RO system (*n* = 1). n.d., not detected. tr, trace.

Table 3. Wine composition (pH, TA, glucose, alcohol, total phenolics, colour density and hue) of smoke-affected Pinot Noir wines before and after reverse osmosis and solid phase adsorption treatment ($n = 3$).

Sample		pH	TA (g/L)	Glucose (g/L)	Alcohol (%, v/v)	Total phenolics (au)	Colour density (au)	Colour hue
Pinot Noir 1†								
Untreated		3.31	5.6	<1.0	14.3	69.24	7.68[a]	0.83
Treated	$t = 0.5$ h	3.34	5.5	<1.0	14.4	70.51	7.82[ab]	0.84
Treated	$t = 1$ h	3.26	5.6	<1.0	14.4	71.98	7.98[b]	0.84
Treated	$t = 2$ h	3.22	5.6	<1.0	14.4	73.04	7.97[b]	0.84
Treated	$t = 3$ h	3.21	5.5	<1.0	14.4	75.39	8.09[b]	0.84
P		n.s.	n.s.	–	n.s.	n.s.	0.051	n.s.
Pinot Noir 2‡								
Untreated		3.20	5.8	<1.0	8.7	18.68	2.20[a]	1.04
Treated	$t = 0.5$ h	3.24	5.6	<1.0	8.5	16.82	1.91[b]	1.05
Treated	$t = 1$ h	3.18	5.7	<1.0	8.4	17.33	1.88[b]	1.04
Treated	$t = 2$ h	3.17	5.8	<1.0	8.5	17.14	1.79[b]	1.02
Treated	$t = 3$ h	3.18	5.9	<1.0	8.4	18.13	1.77[b]	1.01
P		n.s.	n.s.	–	n.s.	n.s.	<0.001	n.s.
Pinot Noir 3§								
Untreated		3.38	9.0	<1.0	14.1	39.13	5.19	0.80
Treated		3.38	7.8	<1.0	13.7	34.79	5.00	0.77

Values followed by a different letter are significantly different. †Pinot Noir affected by bushfire smoke and treated with pilot RO system ($n = 3$); ‡Pinot Noir affected by experimental smoke and treated with pilot RO system ($n = 3$); §Pinot Noir affected by bushfire smoke and treated with commercial RO system ($n = 1$). n.s., not significant; TA, titratable acidity.

optimise the treatment process, to achieve the required reduction in smoke-derived volatile compounds with minimal loss of desirable wine components, prior to upscaling.

Commercial scale treatment of Pinot Noir 3 yielded similar results. Guaiacol and 4-methylguaiacol concentrations were reduced from 12 to 3 µg/L and from 5 to less than 1 µg/L (i.e. trace), respectively (Table 2). It is unclear why 4-ethylguaiacol and 4-ethylphenol increased slightly with treatment; however, these concentrations are well below reported detection threshold values of 70 and 1100 µg/L, respectively (Boidron et al. 1988), so these compounds are unlikely to significantly impact the aroma attributes of either untreated or treated Pinot Noir 3. Determination of volatile phenols in treated Pinot Noir 3 was repeated 6, 12 and 30 months post-bottling to investigate the cellaring potential of treated wines. A gradual increase in guaiacol and 4-methylguaiacol concentrations was observed for both untreated and treated wines (Table 2), suggesting smoke taint might be enhanced with bottle age. That said, it should be noted that the volatile phenol content of treated wine 30 months post-bottling was lower than that of untreated wine at the time of bottling. The evolution of guaiacol and 4-methylguaiacol with time is likely attributable to the hydrolysis of glycoconjugate precursor forms of these compounds present in the wine, and is consistent with earlier studies (Kennison et al. 2008).

Hayasaka et al. (2010) employed stable isotope tracer experiments to identify glucoside and disaccharide conjugates of guaiacol in grapevine leaves and berries. The glycoconjugation of guaiacol following grapevine exposure to smoke has subsequently been demonstrated (Dungey et al. 2011) and hydrolysis of these glycoconjugates can explain the increased phenol concentrations reported by Kennison et al. (2008) during fermentation of smoke-affected grapes and ageing of subsequent wines. In the current study, guaiacol glycoconjugate concentrations of 479 ± 11 µg/L and 550 ± 49 µg/L, respectively, were measured in untreated and treated Pinot Noir 1 wine. These concentra-

tions were not statistically different, indicating glycoconjugate content was not affected by the treatment process. Analysis of retentate and permeate fractions of Pinot Noir 1 confirmed exclusion of the guaiacol glycoconjugate pool by the membrane. While a glycoconjugate concentration of 645 µg/L was measured in the retentate fraction, glycoconjugates were not detected in the permeate. This is not surprising, given glycoconjugate molecular weights range from 286 (glucoside) to 448 (glucose-glucose disaccharide) amu and the nominal membrane cut-off was 150–200 amu. These findings support the hypothesis suggested above, that the return of smoke taint in treated Pinot Noir 3 with time occurred as a consequence of the slow hydrolysis of glycoconjugates present in the bottle. This highlights a limitation of the treatment process; the removal of smoke-derived volatile compounds only and not their conjugate forms, which accordingly limits the cellaring potential of treated wines. For untreated wines, the intensity of smoke-related attributes would be further amplified with bottle age.

To ensure the treatment process did not significantly affect desirable wine attributes, a range of compositional measurements, i.e. pH, TA, sugar and alcohol contents, wine colour density and total phenolics, were made before and after treatment (Table 3). For pilot scale treatment, there was no significant effect on either pH or TA, but a small reduction in TA was observed during commercial scale treatment of Pinot Noir 3. It is unclear if this resulted from adsorption of organic acids by either the membrane or the adsorbent resin. Irrespective, the loss was minor and could be easily adjusted through acid additions, if deemed necessary. Neither residual sugar (i.e. glucose) nor alcohol content was affected by treatment and the process had no significant influence on total phenolics. However, wine colour density differed significantly between untreated and treated wines (Table 3). The molecular weights of red wine pigments (anthocyanins) far exceed the molecular weight cut-off of the reverse osmosis membrane used in the current study.
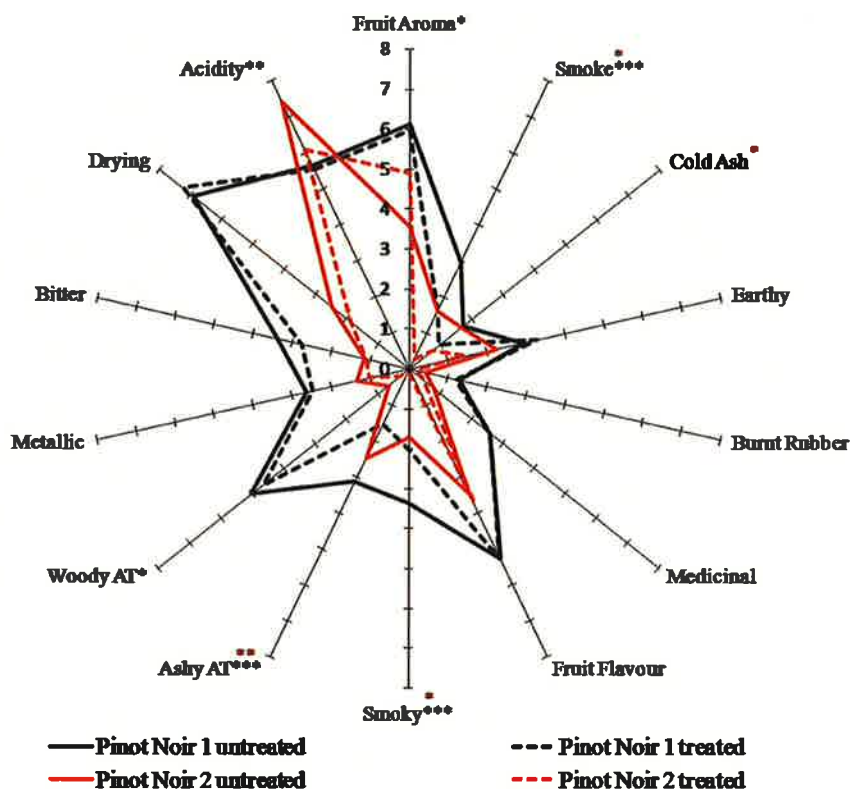
Figure 2. Mean ratings for sensory attributes in smoke-affected Pinot Noir wines before and after reverse osmosis and solid phase adsorption treatment. Values are mean scores from one (untreated) or three (treated, $t = 3$ h) fermentation replicate wines that were presented to 12 judges in three replicate sensory sessions. *, **, *** indicate significance between untreated and treated wines at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

As such, wine colour was preserved in the retentate, whereas permeate fractions were completely colourless (data not shown). While differences in colour density were statistically significant, wine hue was unaffected, and no meaningful difference in wine colour was apparent (Table 3).

Effect of reverse osmosis and solid phase adsorption treatment on wine sensory properties

Sensory analyses, comprising descriptive analysis, difference tests and acceptability ratings were undertaken to determine the impact of treatment on wine sensory properties. DA indicated significant differences were perceived between the aroma profiles of untreated and treated Pinot Noir 1 and Pinot Noir 2 wines (Figure 2). Pinot Noir 1 wines were characterised by intense 'fruit' aroma and flavour, 'woody aftertaste' and 'drying' finish. 'Fruit' aroma and 'woody aftertaste' diminished slightly with treatment ($P < 0.05$), but importantly, the intensity of 'smoke' aroma, 'smoky' flavour and 'ashy aftertaste' were significantly reduced ($P < 0.0001$), which is likely attributable to the significant reduction in smoke-derived volatile phenols (Table 2). Untreated Pinot Noir 2 exhibited considerably less intense aroma and flavour attributes compared with Pinot Noir 1 (Figure 2). The reduced 'fruit' intensity and higher 'acidity' rating were because of the early harvest date of this fruit (i.e. at TSS of $16 \pm 1°$Brix), whereas the relatively short duration of grapevine exposure to smoke, i.e. only 60 min, resulted in lower intensity smoke-related sensory characters. However, following treatment, Pinot Noir 2 wine displayed significantly reduced 'smoke' and 'cold ash' aromas, 'smoky' flavour ($P < 0.05$) and 'ashy aftertaste' ($P < 0.01$; Figure 2), again corresponding with the removal of smoke-derived volatile phenols (Table 2).

Duo-trio tests confirmed a perceptible difference in wine before and after treatment, attributable to the modification of chemical composition (Table 3) and sensory properties

(Figure 2). Untreated and treated Pinot Noir wines were readily differentiated (at the 99% confidence level), yielding 35, 33 and 33 (out of 48) correct responses for Pinot Noir 1, Pinot Noir 2 and Pinot Noir 3 wines, respectively. While there was no significant difference in acceptability ratings for the Pinot Noir 1 and Pinot Noir 2 wines, a significant difference was observed for Pinot Noir 3 wines, with treated wine (4.65) rated somewhat more favourably than untreated wine (3.74; Table 4). Interestingly, the acceptability ratings for Pinot Noir 1 and Pinot Noir 3 wines ranged from 1.0 to 9.0, and from 1.0 to 7.5 for Pinot Noir 2 wines (Table 4), indicating that the sensory panel comprised judges who clearly perceived the impact and acceptability of 'smoke' characters quite differently. In the case of Pinot Noir 1, smoke-related sensory attributes might have been masked by oak complexity arising from extraction of oak-derived volatiles during barrel maturation, as proposed by Ristic et al. (2011). The narrower range of acceptability ratings observed for Pinot Noir 2 probably reflected the aforementioned lack of fruit ripeness and intensity.

Conclusion

Reverse osmosis and solid phase adsorption proved a capable and effective process for reducing the concentration of smoke-derived volatile phenols and perception of sensory attributes of smoke-tainted wines, such that the acceptability of wine treated on a commercial scale was significantly improved. Compared with the direct addition of adsorptive resins to wine, the reverse osmosis fraction of wine involved in the process employed in this study should improve the selectivity of compounds targeted for removal, with less impact on desirable wine attributes. Some limitations were identified: the inherent removal of desirable wine volatiles and potential for smoke taint to slowly return with time. Nevertheless, the process offers the wine industry a method for amelioration of smoke taint in wine.

Table 4. Acceptability ratings for smoke-affected Pinot Noir wines before and after reverse osmosis and solid phase adsorption treatment.

Sample	Acceptability rating	
	Mean	Range
Pinot Noir 1†		
Untreated	3.72 ± 0.32 n.s.	1.0–9.0
Treated ($t = 3$ h)	4.18 ± 0.28 n.s.	1.0–9.0
Pinot Noir 2‡		
Untreated	3.99 ± 0.27 n.s.	1.0–7.4
Treated ($t = 3$ h)	4.10 ± 0.28 n.s.	0.6–7.5
Pinot Noir 3§		
Untreated	3.74 ± 0.34[a]	1.0–9.0
Treated	4.65 ± 0.37[b]	1.0–9.0

Values followed by a different letter are significantly different ($P < 0.05$). †Pinot Noir affected by bushfire smoke and treated with pilot RO system ($n = 48$); ‡Pinot Noir affected by experimental smoke and treated with pilot RO system ($n = 48$); §Pinot Noir affected by bushfire smoke and treated with commercial RO system ($n = 48$). n.s., not significant.

References
Boidron, J.N., Chatonnet, P. and Pons, M. (1988) Influence du bois sur certaines substances odorantes des vins. Connaissance de la Vigne et du Vin **22**, 275–294.

Bui, K., Dick, R., Moulin, G. and Galzy, P. (1988) Partial concentration of red wine by reverse osmosis. Journal of Food Science **53**, 647–648.

Buser, H.-R., Zanier, C. and Tanner, H. (1982) Identification of 2,4,6-trichloroanisole as a potent compound causing cork taint in wine. Journal of Agricultural and Food Chemistry **30**, 359–362.

Chatonnet, P., Dubourdieu, D., Boidron, J.-N. and Pons, M. (1992) The origin of ethylphenols in wines. Journal of the Science of Food and Agriculture **60**, 165–178.

Cuperus, F.P. and Nijhuis, H.H. (1993) Applications of membrane technology to food processing. Trends in Food Science and Technology **4**, 277–282.

Duitschaever, C.L., Alba, J., Buteau, C. and Allen, B. (1991) Riesling wines made from must concentrated by reverse osmosis. I. Experimental conditions and compositions of musts and wines. American Journal of Enology and Viticulture **42**, 19–25.

Dungey, K.A., Hayasaka, Y. and Wilkinson, K.L. (2011) Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. Food Chemistry **126**, 801–806.

Glover, F.A. (1971) Concentration of milk by ultrafiltration and reverse osmosis. Journal of Dairy Research **38**, 373–379.

Günata, Y.Z., Bayonove, C., Baumes, R. and Cordinnier, R. (1985) The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. Journal of Chromatography A **331**, 83–90.

Hayasaka, Y., Baldock, G.A., Pardon, K.H., Jeffrey, D.W. and Herderich, M.J. (2010) Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine Vitis vinifera L. cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. Journal of Agricultural and Food Chemistry **58**, 2076–2081.

Holt, H.E., Iland, P.G. and Ristic, R. (2006) A method for mini-lot fermentation for use in research and commercial viticultural and winemaking trials. The Australian and New Zealand Grapegrower and Winemaker **509a**, 74–81.

Iland, P.G., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004) Chemical analysis of grapes and wine: techniques and concepts (Patrick Iland Wine Promotions: Adelaide).

Kane, L., Braddock, R.J., Sims, C.A. and Matthews, R.F. (1995) Lemon juice aroma concentration by reverse osmosis. Journal of Food Science **60**, 190–194.

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith, J.H. and Gibberd, M.R. (2007) Smoke-derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry **55**, 10897–10901.

Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry **56**, 7379–7383.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research **15**, 228–237.

Madaeni, S.S. (1999) The application of membrane technology for water disinfection. Water Research **33**, 301–308.

Massot, A., Mietton-Peuchot, M., Peuchot, C. and Milisic, V. (2008) Nanofiltration and reverse osmosis in winemaking. Desalination **231**, 283–289.

Meilgaard, M., Civille, G.V. and Carr, B.T. (2007) Sensory evaluation techniques (CRC Press: Boca Raton).

Paulsen, D.J., Wilson, R.L. and Spatz, D.D. (1985) Osmosis and ultrafiltration applied to the processing of fruit juices. In: Reverse osmosis and ultrafiltration. Eds. S. Sourirajan and T. Matsura (ACS Symposium Series 281, American Chemical Society: Washington) pp. 325–344.

Pollnitz, A.P. (2000) The analysis of volatile wine components derived from oak products during winemaking and storage. PhD Thesis, The University of Adelaide, Australia.

Pollnitz, A.P., Pardon, K.H. and Sefton, M.A. (2000) Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine. Journal of Chromatography A **874**, 101–109.

Pollnitz, A.P., Pardon, K.H., Sykes, M. and Sefton, M.A. (2004) The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. Journal of Agricultural and Food Chemistry **52**, 3244–3252.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. (2000) Chemical nature, origins and consequences of the main organoleptic defects. In: The handbook of enology: the chemistry of wine stabilization and treatments. Ed. P. Ribéreau-Gayon, Y. Glories, A. Maujean and D. Dubourdieu (John Wiley and Sons Ltd: Chichester) pp. 209–253.

Ristic, R., Osidacz, P., Pinchbeck, K.A., Hayasaka, Y., Fudge, A.L. and Wilkinson, K.L. (2011) The effect of winemaking techniques on the intensity of smoke taint in wine. Australian Journal of Grape and Wine Research **17**, S29–S40.

Stone, H. and Sidel, J.L. (2004) Descriptive analysis. In: Sensory evaluation practices. 3rd edn. Ed. S.L. Taylor (Elsevier Academic Press: San Diego) pp. 201–245.

Ugarte, P., Agosin, E., Bordeu, E. and Villalobos, J.I. (2005) Reduction of 4-ethylphenol and 4-ethylguaiacol concentration in red wines using reverse osmosis and adsorption. American Journal of Enology and Viticulture **56**, 30–36.

Wilkinson, K.L., Ristic, R., Pinchbeck, K.A., Fudge, A.L., Singh, D.P., Pitt, K.M., Downey, M.O., Baldock, G.A., Hayasaka, Y., Parker, M. and Herderich, M.J. (2011) Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. Australian Journal of Grape and Wine Research **17**, S22–S28.

Williams, P.J., Strauss, C.R. and Wilson, B. (1981) Classification of the monoterpenoid composition of Muscat grapes. American Journal of Enology and Viticulture **32**, 230–235.

Winterhalter, P., Sefton, M.A. and Williams, P.J. (1990) Two-dimensional GC-DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoids, and shikimate-derived metabolites from Riesling wine. Journal of Agricultural and Food Chemistry **38**, 1041–1048.

Amelioration of smoke taint in wine by treatment with commercial fining agents

A.L. FUDGE[1], M. SCHIETTECATTE[1], R. RISTIC[1], Y. HAYASAKA[2] and K.L. WILKINSON[1]

[1] School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond, Adelaide, SA 5064, Australia
[2] The Australian Wine Research Institute, PO Box 197, Glen Osmond, Adelaide, SA 5064, Australia
Corresponding author: Dr Kerry L. Wilkinson, fax +61 8 8313 7116, email kerry.wilkinson@adelaide.edu.au

Abstract

Background and Aims: Fermentation of smoke-affected grapes can lead to wines that exhibit objectionable smoke-related sensory attributes, i.e. smoke taint. Fining agents are routinely used at different stages of the winemaking process to address constituents that are considered to adversely affect juice or wine quality. This study aimed to evaluate the efficacy of commercial fining agents in reducing the concentration of volatile phenols and the intensity of sensory attributes associated with smoke-tainted wine.
Methods and Results: Smoke-affected wines were treated with a range of fining agents, two of which, an activated carbon and a synthetic mineral, were found to appreciably ameliorate the taint. Treated wines contained a significantly lower level of smoke-derived volatile phenols and exhibited less intense 'smoke' and 'cold ash' aromas, 'smoky' flavour and 'ashy' aftertaste, compared with that of untreated (control) wines; with little or no impact on wine colour.
Conclusions: Selected fining agents can ameliorate smoke taint in wine. Whereas most fining agents showed poor specificity towards the wine components responsible for smoke taint, some, an activated carbon in particular, were highly effective.
Significance of the Study: This research identifies a treatment that can be used to mitigate the impact of grapevine exposure to smoke on wine composition and sensory properties.

Keywords: activated carbon, amelioration, fining agent, guaiacol, smoke taint, wine

Introduction

Fining agents are routinely used at different stages of winemaking to address constituents that are considered to adversely affect juice or wine quality. For example, gelatine, isinglass, silicon dioxide and bentonite have been used to facilitate the clarification of juice and wine, while egg albumin, casein, polyvinylpolypyrrolidone (PVPP) and activated carbon can remove the phenolic compounds associated with bitterness, astringency and browning (Iland et al. 2004b). Fining involves the addition of one or more adsorptive substrates to juice or wine to bind certain components, thus reducing their concentration (Castellari et al. 2001). Binding occurs as a result of complex van der Waals, resonance, electrostatic and hydrogen bonding interactions (Furuya et al. 1997) between the adsorbent (i.e. the fining agent) and the adsorbate (i.e. the component in juice or wine to be removed). Following treatment, fining agents will either settle or precipitate, allowing clear juice or wine to be separated by racking, filtration or centrifugation (Iland et al. 2004b).

Fining agents have also been used to remove volatile compounds responsible for the occurrence of various off-odours and flavours in wine. The remedial treatment of wine affected by *Harmonia axyridis* (multicoloured Asian lady beetle) taint was reported by Pickering and co-workers (2006). The concentration of 2-isopropyl-3-methoxypyrazine, derived from lady beetles present in grape bunches at the time of harvest, was reduced in white wine following the addition of activated carbon. Activated carbon and PVPP have also been shown to reduce the concentration of 4-ethylphenol and 4-ethylguaiacol in wine (Lisanti et al. 2008); i.e. the volatile phenols associated with *Brettanomyces/Dekkera* spoilage (Chatonnet et al. 1992). The

adsorptive properties of oenological fining agents have even been exploited for the removal of ochratoxin A from wines (Castellari et al. 2001, Gambuti et al. 2005, Kurtbay et al. 2008).

In the last 5 years, an increasing number of studies concerning grapevine exposure to smoke and the occurrence of smoke taint in grapes and wine have been published in the scientific literature. Several studies have described the compositional and sensory implications of grapevine smoke exposure for grapes and wine (Kennison et al. 2007, 2008, Sheppard et al. 2009, Hayasaka et al. 2010a,b,c) and the influence of grapevine phenology on the extent of smoke taint in wine (Kennison et al. 2009, 2011). Additionally, a range of chromatographic and spectroscopic methods has been developed for the identification and screening of smoke-tainted grapes and wine (Dungey et al. 2011, Singh et al. 2011, Wilkinson et al. 2011, Fudge et al. 2012). To date, however, only two studies have investigated methods by which the impact of grapevine exposure to smoke on the chemical and sensory profiles of wine can be mitigated. Ristic and co-workers reported the influence of winemaking techniques, such as the duration of skin contact, yeast selection and the use of oak and tannin additives, on the concentration of smoke-derived volatile phenols and intensity of smoke-related sensory attributes in wines made from smoke-affected grapes (Ristic et al. 2011). The capacity of reverse osmosis and solid-phase adsorption for the amelioration of smoke-tainted wine has also been demonstrated (Fudge et al. 2011); with treated wines shown to contain a reduced level of volatile phenols and diminished 'smoke' and 'ash' aromas and flavours, compared with that of untreated wines. While these studies give winemakers some options for managing smoke-affected grapes and wine, further

research is required to identify additional methods of ameliora-
tion. The objective of this study was to evaluate the efficacy
of commercial fining agents as remedial treatments for wine
affected by smoke taint. Given the affinity of many fining agents
for phenolic compounds (Iland et al. 2004b), fining offers a
potential method by which the concentration of smoke-derived
volatile phenols associated with smoke taint could be reduced. As
such, the effect of fining on the volatile phenol content, colour
and sensory attributes of wine was evaluated.

Materials and methods

Smoke-affected wines
Three smoke-affected wines were used in this study: a commer-
cial Pinot Noir made from grapes harvested from a vineyard
located in the Gippsland wine region (37°45′S, 147°43′E), which
was exposed to smoke from bush fires that occurred in North-
Eastern Victoria between 1 December 2006 and 7 February 2007;
and two experimental wines, made from Cabernet Sauvignon
and Merlot grapes harvested in 2010 from grapevines grown
at the University of Adelaide (UA)'s Waite campus in Adelaide,
South Australia (34°58′S, 138°38′E), which were exposed to
straw-derived smoke (for 60 min, at approximately 7 days post-
veraison) using experimental conditions described previously
(Kennison et al. 2008, Dungey et al. 2011, Ristic et al. 2011).
Wine selection was based on the availability of samples known to
exhibit smoke taint.

Treatment of wine
Thirteen commercial fining agents (Table 1) were sourced: egg
albumin (supermarket); potassium caseinate, activated carbon,
isinglass, sodium bentonite 1 (Vason, Verona, Italy); PVPP (ISP
Chemicals, Calvert City, Kentucky, USA); gelatine (Gelatine
Liquida, Verona, Italy); yeast cell walls 1 and 2, a silica sol/activated
carbon mixture, calcium and sodium bentonite 2 (Laffort, Bor-
deaux, France); and a synthetic mineral (Life Material Techno-
logies Ltd, Bangkok, Thailand). A preliminary screening was
undertaken (in duplicate) using all 13 fining agents. A single
addition of each fining agent was made to smoke-affected Pinot
Noir wine (250 mL) at a concentration approaching the high end
of recommended dosages (Table 1) prescribed by either the manu-
facturer or Iland et al. (2004b). Treated wines were then stored at
4°C for 3 days under an inert gas (carbon dioxide) atmosphere,
before being racked in preparation for chemical analysis. The

Table 1. Fining agents and addition rates.

Fining agent		Dose (g/L)
Active ingredient	**Trade name**	
Egg albumin	—	0.4
Potassium caseinate	Clarito® Spray Dry	0.3
Activated carbon	FPS	1.0
Isinglass	Premium® Fish	0.1
Sodium bentonite 1	Plusgran® gel	4.0
PVPP	Polyclar® 10	0.5
Gelatine	Instantgel 45	0.3
Yeast cell walls 1	Biolees	0.4
Silica sol/activated carbon	Toxicol	0.6
Calcium bentonite	Microcol-Cl	4.0
Sodium bentonite 2	Volclay	4.0
Yeast cell walls 2	Biocell	0.4
Synthetic mineral	BA/S-00-1A	2.0

—, no trade name; PVPP, polyvinylpolypyrrolidone.

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second experiment was performed (in triplicate) using only the
activated carbon and synthetic mineral. These fining agents were
selected on the basis of results obtained from the preliminary
screening and were added to smoke-affected Cabernet Sauvignon
and Merlot wines (1100 mL), as (i) a single addition (i.e. sepa-
rately) and as (ii) co-fining agents (i.e. together), at the same
concentration used in the first experiment (Table 1). Treated wines
were then stored at 15°C for 3 days under an inert gas (carbon
dioxide) atmosphere, before being racked in preparation for
chemical and sensory analysis.

Chemical analysis of wine
The pH and titratable acidity (TA), as tartaric acid equivalents
to an end-point of pH 8.2, of untreated and treated wines were
determined. Wine colour and phenolics were measured with
a Cary ultraviolet-visible spectrophotometer (Varian Inc., Palo
Alto, California, USA) according to the methods of Iland et al.
(2004a). The concentration of guaiacol, 4-methylguaiacol, o-,
p- and m-cresol (as total cresols) and syringol was measured by gas
chromatography-mass spectrometry (GC-MS) using stable isotope
dilution assay (SIDA) methods reported previously (Pollnitz et al.
2000, 2004, Hayasaka et al. 2010a). These publications describe
the preparation of internal standards and instrumental operating
conditions used herein. GC-MS analysis was performed by the
Australian Wine Research Institute (AWRI)'s Commercial Services
Laboratory (Adelaide, Australia) using an Agilent 6890 gas chro-
matograph (Agilent, Palo Alto, California, USA), coupled to a 5973
mass selective detector. Guaiacol glycoconjugates were quantified
by high performance-liquid chromatography (HPLC)-MS/MS
using SIDA methods reported previously (Dungey et al. 2011,
Wilkinson et al. 2011). Dungey et al. (2011) describes the synthe-
sis of the internal standard used herein. Analyses were performed
using an Agilent 1200 HPLC system, coupled to a 4000 Q TRAP
hybrid tandem mass spectrometer (Agilent).

Sensory analysis of wine
Descriptive sensory analysis (Stone and Sidel 2004) was per-
formed on Cabernet Sauvignon and Merlot wines to determine
any differences between the intensity of smoke-related attributes
of untreated and treated wines. Descriptive analysis consisted of
a series of training and formal evaluation sessions. Twelve pan-
ellists were recruited from staff and students of the UA and the
AWRI. Ten panellists had extensive experience in sensory analy-
sis of smoke-tainted wines, while two less-experienced panellists
underwent additional training sessions prior to the panel training
and formal evaluation sessions. Training sessions involved pre-
sentation of each wine replicate to the sensory panel to generate
appropriate aroma and palate attributes. During formal evalua-
tion sessions, six aroma attributes and eight palate attributes
(Table 2) were rated, using an unstructured 15-cm line scale with
anchor points of 'low' to 'high' placed at 10 and 90%, respec-
tively. Evaluations were carried out using covered, three-digit
coded International Standards Organization tasting glasses. Each
wine (30 mL) was presented at 22 to 24°C, in isolated and
well-ventilated tasting booths under red-light illumination, to
limit potential for bias based on wine colour. Twelve wines were
presented per session, with one session per day, over 2 consecu-
tive days. Panellists assessed untreated and treated Cabernet
Sauvignon wine replicates on 1 day and untreated and treated
Merlot wine replicates the following day. The presentation order
was randomised and balanced across judges. After assessing each
sample, panellists rinsed with water and a 1 g/L citrus pectin
solution (Sigma-Aldrich, St Louis, Missouri, USA). Data were
collected using FIZZ software (Version 2.40 E, Biosystemes,
Couternon, France).

Table 2. Sensory attributes used for descriptive analysis of wines.

Attribute	Description
AROMA	
Fruit	The intensity of the overall fruit aroma
Smoke	Any type of smoke aroma, includes smoked meat/bacon, charry, cigar-box
Cold ash	Burnt aroma associated with ashes, includes ash, ashtray, tarry, campfire
Earthy	Any aroma associated with earthy and stale character, includes earthy, musty, dusty, wet cardboard, barnyard, dank, stagnant, stale, leesy
Woody	Woody aromas, includes toasty
Medicinal	Aromatic characteristic of band-aids, cleaning products, solvents, phenolics
PALATE	
Fruit flavour	The intensity of the overall fruit flavour
Smoky flavour	Perception of smoke flavour, includes smoked meat/bacon
Ashy aftertaste	Length of aftertaste associated with residue of ashtray perceived in the mouth after expectorating, includes ash, acrid, ashtray, tarry, campfire
Woody aftertaste	Woody flavours, includes wood, oak, pencil shavings
Medicinal flavour	Aromatic characteristics of band-aids, cleaning products, solvent, phenolics
Metallic	The 'tinny' flavour associated with metals
Bitter	Intensity of bitter taste, bitter aftertaste
Drying	Drying, puckering mouthfeel after expectorating the wine
Acidity	Intensity of acid/sour taste

Data analysis

Sensory data were analysed using statistical software (SenPaq, Version 4.82, Qi Statistics Ltd, Reading, UK) and included analysis of variance (ANOVA), mixed model, testing for the effects of treatment, experimental replicate, presentation replicate and judge (considered as a random effect). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$. Chemical data were analysed by ANOVA using GenStat (10th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by LSD multiple comparison test at $P < 0.05$.

Results and discussion

Preliminary screening of commercial fining agents

A preliminary screening was conducted to evaluate the commercial fining agents (Table 1) as remedial treatments for wine affected by smoke taint. The performance of each fining agent was determined by comparing the volatile phenol composition of a smoke-affected Pinot Noir wine, before and after treatment (Table 3). Prior to treatment, a relatively high level of guaiacol and syringol was measured in the Pinot Noir, being 24 and 36 µg/L, respectively; a lower level of 4-methylguaiacol and cresols, 7 and 15 µg/L, respectively, was also present. Of the fining agents evaluated, the activated carbon and synthetic mineral treatments proved most effective at removing volatile phenols from wine. The activated carbon reduced the concentration of volatile phenols by between 58 and 71%. The synthetic mineral showed a similar affinity for syringol, removing 58% during fining, but 4-methylguaiacol was reduced by only 29% and guaiacol and total cresols by 13%. The bentonite and silica sol/activated carbon treatments showed limited efficacy, reducing phenol concentration by just 1 to 3 µg/L (or 3 to 14%). The remaining fining agents had no effect. Surprisingly, the addition of Biocell yeast cell walls gave a significantly higher concentration of total cresols. However, the reason for this remains unclear. The poor performance of these fining agents could be attributed to a combination of weak binding interactions with the smoke-derived volatile phenols and adsorption interference by other

wine components. Certainly the non-specific action of fining agents is well known, and the physical and chemical properties of individual fining agents will determine to what extent binding interactions occur with different wine components. It has also been well established that fining can adversely affect both wine colour and flavour (Castellari et al. 2001, Iland et al. 2004b, Gambuti et al. 2005, Lisanti et al. 2008). In the current study, all fining treatments significantly reduced TA and colour density, but pH and colour hue were unaffected (Table 3). Gelatine and bentonite treatments resulted in the greatest loss of colour density and also had the greatest impact on TA. Wine total phenolics were significantly reduced by the addition of activated carbon, PVPP and gelatine, suggesting the adsorption of wine polyphenols, as reported in previous studies (Sims et al. 1995, Castellari et al. 2001, Lisanti et al. 2008).

On the basis of the results obtained from the preliminary screening, the activated carbon and synthetic mineral adsorbents were considered to be capable of ameliorating smoke taint in wine. These fining agents achieved a significant reduction in volatile phenol concentration, albeit with a moderate effect on TA, phenolics and colour density. As such, they were selected for use in a subsequent experiment that incorporated descriptive sensory analysis to evaluate the effect of fining on the intensity of smoke-related sensory attributes.

Effect of activated carbon and/or synthetic mineral treatments on smoke-affected wines

In the second experiment, fining treatments comprised a single addition of activated carbon or synthetic mineral, as well as the addition of both fining agents (i.e. co-fining), to smoke-affected Cabernet Sauvignon and Merlot wines. The volatile phenol content, pH, TA, total phenolics, and colour density and hue were again measured to evaluate the performance of fining agents. Prior to treatment, the smoke-affected wines contained a similar level of guaiacol, 4-methylguaiacol, cresols and syringol (Table 4) and both exhibited 'smoke' and 'cold ash' aromas, 'smoky' flavour and an 'ashy' aftertaste (Figure 1); i.e. sensory attributes characteristic of smoke-tainted wines (Kennison et al.

Table 3. Concentration of volatile phenols, pH, titratable acidity (TA), total phenolics, colour density and hue of smoke-affected Pinot Noir wine before and after treatment with selected fining agents.

Fining treatment	Concentration (µg/L)				pH	TA (g/L)	Total phenolics (au)	Colour density (au)	Colour hue
	Guaiacol	4-Methyl guaiacol	Total cresols	Syringol					
Untreated (control)	24[a]	7[a]	15[c]	36[a]	3.5	5.0[a]	64.0[ab]	4.95[a]	1.2
Egg albumin	24[a]	7[a]	15[c]	35[ab]	3.4	4.6[cd]	60.1[abcde]	4.55[d]	1.2
Potassium caseinate	24[a]	7[a]	15[c]	35[ab]	3.4	4.8[b]	61.4[abcd]	4.80[b]	1.2
Activated carbon	10[d]	2[c]	5[f]	14[d]	3.4	4.5[d]	52.4[e]	4.50[d]	1.2
Isinglass	24[a]	7[a]	16[b]	35[ab]	3.4	4.2[ef]	61.8[abcd]	4.20[ef]	1.2
Sodium bentonite 1	23[ab]	7[a]	15[c]	33[c]	3.4	3.9[g]	62.5[abcd]	3.85[g]	1.3
PVPP	24[a]	7[a]	15[c]	35[ab]	3.4	4.2[ef]	53.6[de]	4.20[ef]	1.2
Gelatine	24[a]	7[a]	15[c]	36[a]	3.4	3.5[h]	55.5[cde]	3.50[h]	1.3
Yeast cell walls 1	24[a]	7[a]	16[b]	36[a]	3.4	4.3[e]	59.5[abcde]	4.30[e]	1.2
Silica sol/activated carbon	22[bc]	6[b]	14[d]	33[c]	3.4	4.5[d]	66.3[a]	4.50[d]	1.2
Calcium bentonite	22[bc]	6[b]	14[d]	33[c]	3.5	4.1[f]	58.1[bcde]	4.10[f]	1.3
Sodium bentonite 2	23[ab]	6[b]	15[c]	34[bc]	3.5	3.9[g]	64.4[ab]	3.85[g]	1.3
Yeast cell walls 2	24[a]	7[a]	23[a]	35[ab]	3.5	4.6[cd]	62.5[abcd]	4.60[cd]	1.2
Synthetic mineral	21[c]	5[c]	13[e]	15[d]	3.4	4.7[bc]	59.7[abcde]	4.70[bc]	1.2
P	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.05	<0.001	ns

Values are means from two experimental replicates (*n* = 2) and were in agreement to ca. 5%. Values followed by a different letter within columns are significantly different. ns, not significant; PVPP, polyvinylpolypyrrolidone.

Table 4. Concentration of volatile phenols, pH, titratable acidity (TA), total phenolics, colour density and hue of smoke-affected Cabernet Sauvignon and Merlot wines before and after treatment with selected fining agents.

Fining treatment	Concentration (µg/L)				pH	TA (g/L)	Total phenolics (au)	Colour density (au)	Colour hue
	Guaiacol	4-Methyl guaiacol	Total cresols	Syringol					
Cabernet Sauvignon									
Untreated (control)	18[a]	3[a]	7[a]	18[a]	3.5	7.1[a]	55.0[a]	8.8	0.7
Activated carbon	8[c]	1[c]	2[c]	7[c]	3.5	6.9[b]	51.9[b]	8.5	0.7
Synthetic mineral	15[b]	2[b]	6[b]	9[b]	3.5	6.9[b]	53.1[ab]	9.1	0.7
Carbon and mineral	7[d]	tr	2[c]	5[d]	3.5	6.8[c]	49.4[c]	9.1	0.7
P	<0.001	<0.001	<0.001	<0.001	ns	<0.001	<0.01	ns	ns
Merlot									
Untreated (control)	17[a]	3[a]	6[a]	15[a]	3.4	6.0[a]	48.1[a]	7.1[b]	0.7
Activated carbon	8[c]	1[b]	2[c]	6[c]	3.5	6.0[a]	44.1[b]	6.9[b]	0.7
Synthetic mineral	15[b]	3[a]	4[b]	7[b]	3.5	5.8[ab]	47.7[a]	7.6[a]	0.7
Carbon and mineral	7[d]	1[b]	1[d]	4[d]	3.5	5.7[b]	44.4[b]	7.0[b]	0.7
P	<0.001	<0.001	<0.001	<0.001	ns	<0.05	<0.001	<0.001	ns

Values are means from three experimental replicates (*n* = 3) and were in agreement to ca. 5%. Values followed by a different letter within columns are significantly different. ns, not significant; tr, trace (i.e. positive identification, but <1 µg/L).

2007, 2009, Ristic et al. 2011). As in the preliminary screening experiment, the activated carbon treatment significantly reduced the concentration of all volatile phenols; reductions of 56 to 71% and 53 to 67% were achieved for treatment of Cabernet Sauvignon and Merlot wines, respectively (Table 4). The high surface area to unit mass ratio of activated carbon, typically 500 to 1500 m²/g, makes it an effective adsorbent (Pickering et al. 2006). The chemical data suggest the activated carbon used in the current study also possesses a high affinity for low-molecular-weight phenols. Again, the synthetic mineral showed a greater affinity for syringol than the other phenols. The level of syringol was reduced by 50%, but only small amounts (less than 3 µg/L,

or 33%) of guaiacol, 4-methylguaiacol and cresols were adsorbed. Not surprisingly, wines treated with both fining agents contained the lowest volatile phenol concentration. Co-fining resulted in slightly improved adsorption than fining with activated carbon alone. Similar results, however, could probably be achieved with a higher dose of activated carbon.

Previous studies have demonstrated that smoke-derived volatile phenols can accumulate in grapes in glycoconjugate forms (Hayasaka et al. 2010b,c, Dungey et al. 2011, Wilkinson et al. 2011) and that a significant proportion of the glycoconjugate pool can remain in wine after fermentation (Wilkinson et al. 2011). Hydrolysis of these glycoconjugates can lead to the
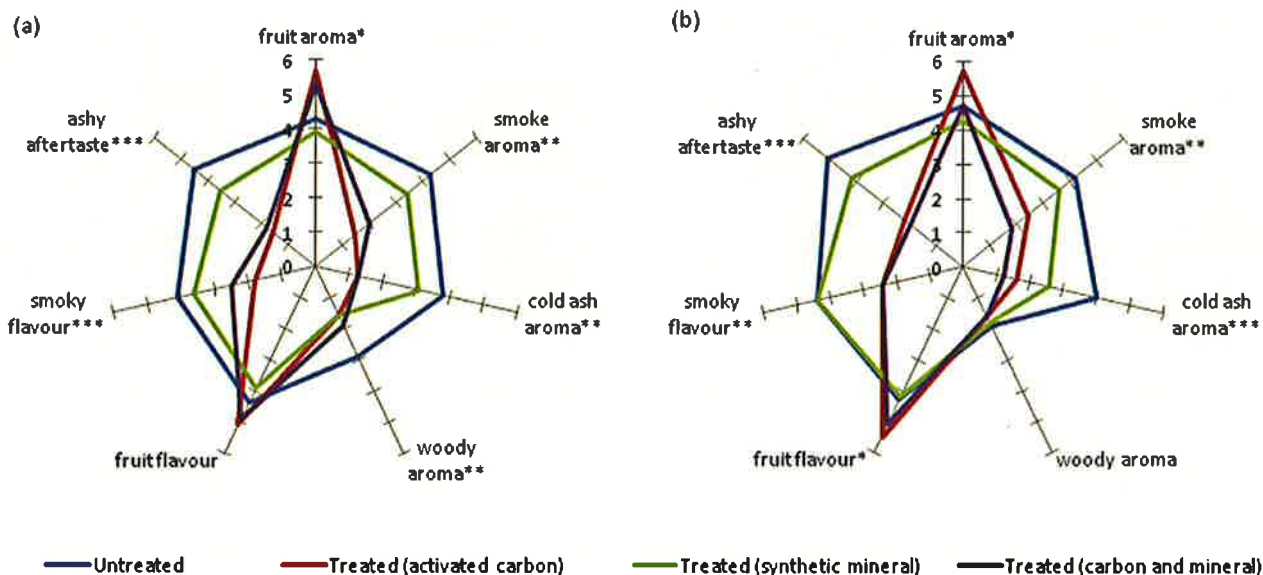
Figure 1. Mean ratings for sensory attributes of smoke-affected (a) Cabernet Sauvignon and (b) Merlot wines before and after treatment with activated carbon and/or synthetic mineral fining agents. Values are mean scores from three experimental replicates that were presented to 12 judges. *, **, *** indicate significance between (——) untreated and ((——) activated carbon; (——) synthetic mineral; (——) carbon and mineral) treated wines at $P < 0.05$, $P < 0.01$ and $P < 0.0001$, respectively.

intensification of smoke taint as wine ages (Kennison et al. 2008, Fudge et al. 2011). As such, the concentration of guaiacol glycoconjugates in Cabernet Sauvignon and Merlot wines was determined before and after fining, to evaluate the capacity of the activated carbon and synthetic mineral to adsorb glycosylated forms of guaiacol; i.e. to investigate the potential for smoke taint to recur in treated wines with time. No significant differences were observed in the guaiacol glycoconjugate concentrations of untreated and treated wines. The total glycoconjugate level ranged from 154 to 293 μg/L ($P = 0.468$) for Cabernet Sauvignon wines and from 484 to 599 μg/L ($P = 0.337$) for Merlot wines. These results indicate that the fining agents did not interact significantly with bound (glycosylated) forms of guaiacol. The reason for this remains unclear, but may be a function of size or polarity; i.e. the adsorption capacity of activated carbon may be influenced by differences in the molecular weight and/or polarity of glycoconjugates, relative to volatile phenols. Therefore, as with reverse osmosis-based amelioration of smoke-affected wine (Fudge et al. 2011), it is likely that the volatile phenol concentration and thus the intensity of 'smoky' attributes, will slowly increase with time, because of the gradual hydrolysis of guaiacol glycoconjugates; i.e. treated wines will have limited ageing potential.

The colour density of Cabernet Sauvignon and Merlot wines was higher than the Pinot Noir wine (Table 4), because of increased concentrations of red-coloured pigments, as determined by absorbance at 520 nm (Iland et al. 2004b). This enabled the impact of fining on wine colour to be better evaluated. Wine colour density decreased slightly, but not significantly, following treatment with activated carbon. In contrast, the synthetic mineral increased colour density, with a significant increase observed for treatment of Merlot. These results indicate that at the addition rates employed, there was little or no loss of red wine pigments. Colour hue was also unaffected. Wine pH remained constant, but again fining decreased TA. At wine pH, activated carbons have been shown to adsorb tartaric and malic acid (Robert et al. 1995), which could explain the reduction in TA observed in the current study. However, this is not problematic from a wine quality per-

spective, given TA can be easily adjusted through acid additions. Both fining agents adsorbed a small proportion, up to 10%, of wine polyphenols. The greater impact of activated carbon can again be attributed to its high adsorption surface.

The definitive evaluation of the efficacy of fining agents to ameliorate smoke taint in wine is the sensory outcome of fining. Descriptive analysis enabled perceptible differences in the sensory profiles of untreated and treated wines to be identified (Figure 1, Table S1). Cabernet Sauvignon and Merlot wines treated with activated carbon exhibited significantly reduced 'smoke' and 'cold ash' aromas, 'smoky' flavour and 'ashy' aftertaste. The synthetic mineral also reduced 'smoke' and 'cold ash' aromas, albeit to a lesser extent, but did not significantly affect 'smoky' flavour or 'ashy' aftertaste. The intensity of smoke-related sensory attributes in wines subjected to co-fining treatments was not dissimilar to that of wines treated with activated carbon. This suggests that for co-fining treatments, the diminished intensity of smoke-related sensory characters is largely attributable to the activated carbon. This conclusion is strongly supported by the wine compositional data (Table 4).

Previous studies have demonstrated a negative correlation between the intensity of 'fruit' and 'smoke' attributes (Kennison et al. 2009, Ristic et al. 2011); i.e. as the intensity of 'smoke' characters increase, 'fruit' characters decrease, likely because of suppression or masking effects. Similar results were observed in the current study for fining treatments involving the addition of activated carbon. A significant reduction in 'smoke' attributes was coupled with enhanced 'fruit' aroma and flavour, for both Cabernet Sauvignon and Merlot wines, and with the exception of Cabernet Sauvignon 'fruit' flavour, these results were statistically significant (Figure 1, Table S1). The activated carbon treatment removed a sufficient proportion of smoke-derived volatile compounds to counter suppression of 'fruit' attributes. In contrast, the synthetic mineral appeared to inhibit 'fruit' character slightly, suggesting adsorption of some desirable wine volatiles. On this basis, the activated carbon was considered to be the more suitable fining agent for the amelioration of smoke-tainted wine.

Conclusion

Most of the commercial fining agents evaluated as potential remedial treatments for smoke-affected wine showed poor specificity towards the volatile phenols associated with smoke taint. However, two adsorbents, an activated carbon in particular, were highly effective at reducing volatile phenol concentration and the intensity of smoke-related sensory attributes. As such, a treatment that can be used to mitigate the impact of grapevine exposure to smoke has been identified and winemakers have an additional tool for the amelioration of smoke-tainted wine.

Acknowledgements

References

Castellari, M., Versari, A., Fabiani, A., Parpinello, G.P. and Galassi, S. (2001) Removal of ochratoxin A in red wines by means of absorption treatments with commercial fining agents. Journal of Agricultural and Food Chemistry 49, 3917–3921.

Chatonnet, P., Dubourdieu, D., Boidron, J.-N. and Pons, M. (1992) The origin of ethylphenols in wines. Journal of the Science of Food and Agriculture 60, 165–178.

Dungey, K.A., Hayasaka, Y. and Wilkinson, K.L. (2011) Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. Food Chemistry 126, 801–806.

Fudge, A.L., Ristic, R., Wollan, D. and Wilkinson, K.L. (2011) Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption. Australian Journal of Grape and Wine Research 17, S41–S48.

Fudge, A.L., Wilkinson, K.L., Ristic, R. and Cozzolino, D. (2012) Classification of smoke tainted wines using Mid-Infrared spectroscopy and chemometrics. Journal of Agricultural and Food Chemistry 60, 52–59.

Furuya, E.G., Chang, H.T., Miura, Y. and Noll, K.E. (1997) A fundamental analysis of the isotherm for the adsorption of phenolic compounds on activated carbon. Separation and Purification Technology 11, 69–78.

Gambuti, A., Strollo, D., Genovese, A., Ugliano, M., Ritieni, A. and Moio, L. (2005) Influence of enological practices on ochratoxin A concentration of wine. American Journal of Enology and Viticulture 56, 155–162.

Hayasaka, Y., Baldock, G.A., Pardon, K.H., Jeffrey, D.W. and Herderich, M.J. (2010a) Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine Vitis vinifera L. Cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. Journal of Agricultural and Food Chemistry 58, 2076–2081.

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

Hayasaka, Y., Dungey, K.A., Baldock, G.A., Kennison, K.R. and Wilkinson, K.L. (2010c) Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. Analytica Chimica Acta 660, 143–148.

Iland, P.G., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004a) Chemical analysis of grapes and wine: techniques and concepts (Patrick Iland Wine Promotions: Adelaide, Australia).

Iland, P.G., Bruer, N., Ewart, A., Markides, A. and Sitters, J. (2004b) Monitoring the winemaking process from grapes to wine: techniques and concepts (Patrick Iland Wine Promotions: Adelaide, Australia).

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith, J.H. and Gibberd, M.R. (2007) Smoke-derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry 55, 10897–10901.

Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile

phenols during fermentation of Merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry 56, 7379–7383.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research 15, 228–237.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2011) Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. Australian Journal of Grape and Wine Research 17, S5–S12.

Kurtbay, H.M., Bekci, Z., Merdivan, M. and Yurdakoc, K. (2008) Reduction of ochratoxin A levels in red wine by bentonite, modified bentonite and chitosan. Journal of Agricultural and Food Chemistry 56, 2541–2545.

Lisanti, M.T., Piombino, P., Gambuti, A., Genovese, A., Siani, V.L. and Moio, L. (2008) Analytical evaluation of remedial treatments for red and white wines contaminated by volatile phenols. Bulletin de l'OIV (Office International de la Vigne et du Vin) 81, 45–55.

Pickering, G., Lin, J., Reynolds, A., Soleas, G. and Riesen, R. (2006) The evaluation of remedial treatments for wine affected by Harmonia axyridis. International Journal of Food Sciences and Technology 41, 77–86.

Pollnitz, A.P., Pardon, K.H. and Sefton, M.A. (2000) Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine. Journal of Chromatography A 874, 101–109.

Pollnitz, A.P., Pardon, K.H., Sykes, M. and Sefton, M.A. (2004) The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. Journal of Agricultural and Food Chemistry 52, 3244–3252.

Ristic, R., Osidacz, P., Pinchbeck, K.A., Hayasaka, Y., Fudge, A.L. and Wilkinson, K.L. (2011) The effect of winemaking techniques on the intensity of smoke taint in wine. Australian Journal of Grape and Wine Research 17, S29–S40.

Robert, L., Mourges, J., Pamar-Robert, A., Achour, D. and Molinier, J. (1995) Adsorption of tartaric and malic acid by active carbons. Journal International des Sciences de la Vigne et du Vin 29, 49–53.

Sheppard, S.I., Dhesi, M.K. and Eggers, N.J. (2009) Effect of pre- and postveraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. American Journal of Enology and Viticulture 60, 98–103.

Sims, C.A., Eastridge, J.S. and Bates, R.P. (1995) Changes in phenols, color, and sensory characteristics of Muscadine wines by pre- and post-fermentation additions of PVPP, casein and gelatine. American Journal of Enology and Viticulture 46, 155–158.

Singh, D.P., Chong, H.H., Pitt, K.M., Cleary, M., Dokoozlian, N.K. and Downey, M.O. (2011) Guaiacol and 4-methylguaiacol accumulate in wines made from smoke-affected fruit because of hydrolysis of their conjugates. Australian Journal of Grape and Wine Research 17, S13–S21.

Stone, H. and Sidel, J.L. (2004) Descriptive analysis. In: Sensory evaluation practices, Third edn. Ed. S.L. Taylor (Elsevier Academic Press: San Diego, CA) pp. 201–245.

Wilkinson, K.L., Ristic, R., Pinchbeck, K.A., Fudge, A.L., Singh, D.P., Pitt, K.M., Downey, M.O., Baldock, G.A., Hayasaka, Y., Parker, M. and Herderich, M.J. (2011) Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. Australian Journal of Grape and Wine Research 17, S22–S28.

Supporting information

Additional Supporting Information may be found in the online version of this article: http://onlinelibrary.wiley.com/doi/10.1111/j.1755-0238.2012.00200.x/abstract

Table S1. Mean ratings for sensory attributes of smoke-affected Cabernet Sauvignon and Merlot wines before and after treatment with selected fining agents.

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Chapter 3
Spectroscopic Analysis of Smoke Taint

Classification of Smoke Tainted Wines Using Mid-Infrared Spectroscopy and Chemometrics

**Anthea L. Fudge[1], Kerry L. Wilkinson[1],
Renata Ristic[1], and Daniel Cozzolino[2]**

[1]*School of Agriculture, Food and Wine, The University of Adelaide, PMB 1,
Glen Osmond, SA 5064, Australia*
[2]*The Australian Wine Research Institute, P.O. Box 197,
Glen Osmond, SA 5064, Australia*

Statement of Authorship

Classification of Smoke Tainted Wines Using Mid-Infrared Spectroscopy and Chemometrics

Anthea Fudge (Candidate)

Performed spectroscopic and sensory analysis of wines, analysed and interpreted data, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate.

Signed.. ....*Date.*25/9/15

Kerry Wilkinson

Supervised experimental work, analysed and interpreted data, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed..... ....*Date.*25./9/15

Renata Ristic

Assisted with chemical and sensory analysis of wines, and revised the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed... ....*Date.*25-9-15

Daniel Cozzolino

Assisted with spectroscopic analysis of wines, performed chemometric analysis of data, drafted and revised the manuscript, and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed..   ....*Date....* 25/9/2015

Synchronous Two-Dimensional Correlation Spectroscopy (2D-COS) as a Novel Method for Screening Smoke Tainted Wine

Anthea L. Fudge[1], Kerry L. Wilkinson[1], Renata Ristic[1] and Daniel Cozzolino[2,3]

[1]*School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia*
[2]*The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia*
Current Address: [3]The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia

Statement of Authorship

Synchronous Two-Dimensional Correlation Spectroscopy (2D-COS) as a Novel Method for Screening Smoke Tainted Wine

Food Chemistry - 2013, 139 (1-4), pp 115-119

Anthea Fudge (Candidate)

Performed analysis of wines, analysed and interpreted data, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate.

Signed. ....*Date*.25|9|15

Kerry Wilkinson

Supervised experimental work, analysed and interpreted data, and drafted and revised the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed....... ....*Date*.25/9/15

Renata Ristic

Assisted with analysis of wines and revised the manuscript.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed..... ....*Date*.25/9/15

Daniel Cozzolino

Assisted with spectroscopic analysis of wines, performed chemometric analysis of data, drafted and revised the manuscript, and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for inclusion of the paper in this thesis.

Signed....*Date*.......25./ 9 /20 15

Chapter 3

Spectroscopic Analysis of Smoke Taint

The content of this chapter is based on the following two publications:

i)   *Classification of Smoke Tainted Wines Using Mid-Infrared Spectroscopy and Chemometrics, Journal of Agricultural and Food Chemistry, 2012, 60 (1), pp 52–59*

ii) *Synchronous Two-Dimensional Correlation Spectroscopy (2D-COS) as a Novel Method for Screening Smoke Tainted Wine, Food Chemistry, 2013, 139 (1-4), pp 115-119*

This chapter describes the use of spectroscopy as a rapid analytical method for screening wines for the presence of smoke taint. The capability of spectroscopic techniques, in combination with chemometrics, for classification and discrimination of wine is also introduced. The need for rapid detection of smoke taint in grapes and wines was identified as a priority for industry; especially following a fire event when demand for analysis of grapes and wine is high. Winemakers have considerable time and resource constraints during vintage, thus rapid determinations of fruit quality, including the assessment of smoke exposure by fruit, are important. A predictive method employing mid-infrared (MIR) spectroscopy was developed by scanning control and smoke-affected wines, and applying multivariate data analysis techniques (i.e. chemometrics) to the resulting data to generate a calibration model for the classification of smoke tainted wines. Two-dimensional correlation spectroscopy (2D-COS) was also investigated as a novel method for characterising smoke taint in wines.

A general overview of infrared (IR) spectroscopy is provided below; however, while both near-infrared (NIR) and MIR spectroscopic techniques were evaluated, the main compositional differences were observed within the MIR fingerprint region. As such, the papers presented in this chapter describe only the MIR related results.

Introduction

IR spectroscopy is a technique used to determine the unique structure and bonding of a molecule. Infrared radiation is passed through a sample and absorption can cause bonded atoms within molecules to vibrate. Bonds have different bending and stretching motions and give rise to particular frequencies (Silberberg 2000), such that the resulting absorption peaks correspond to particular wavelengths. The total spectrum for a given molecule therefore comprises vibrations at different wavelengths indicative of the atoms/bonds of that molecule (Suslick 2000). The electromagnetic spectrum is divided into the far (400 to 10 cm^{-1}), mid (4,000 to 400 cm^{-1}) and near (14,000 to 4,000 cm^{-1}) infrared regions (Luykx & Van Ruth 2008).

In the NIR region, spectra typically comprise broad overlapping bands, so quantitative NIR spectroscopy measurements require calibration against established reference methods (Guillén & Cabo 1997; Garde-Cerdán et al. 2010). In contrast, spectra in the MIR region comprise well defined bands that can be used for qualitative analysis to identify organic constituents and/or to differentiate samples (Guillén & Cabo 1997; Rodriguez-Saona & Allendorf 2011). NIR spectra typically exhibit vibrational bands due to C-H, O-H and N-H functional groups and bands in the spectra are due to the fundamental vibrational transitions (Rodriguez-Saona & Allendorf 2011). Spectra from the MIR region are used for structural identification as the 'fingerprint' region can help to identify organic compounds as the absorbance bands are due to fundamental vibrations of specific functional groups (Guillén & Cabo 1997).

Gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) are both powerful analytical techniques that have been used for the compositional characterisation of various foods and beverages. Volatile and non-volatile components can be separated on the basis of their interactions with a solid phase (i.e. by chromatographic separation); subsequent detection, by mass spectrometry for example, can enable their identification (Karasek & Clement 1988). Sample preparation is usually required, for example isolation and/or pre-concentration steps prior to analysis, particularly for trace components, and these processes can significantly increase the time and cost associated with analysis. In contrast, IR spectroscopy techniques require little or

no sample preparation, are usually non-destructive and in most cases require considerably less sample than traditional analytical methods (i.e. GC-MS and HPLC). IR spectroscopy is therefore a rapid and convenient method of analysis

IR spectroscopy generates an overall fingerprint of a given sample, from which analytical information can be gained following the application of mathematical multivariate analysis techniques, such as principal component analysis (PCA).

Spectroscopic techniques have been widely used in the food, beverage and agricultural industries for various broad applications. Recent studies have demonstrated the use of IR spectroscopy to identify egg freshness (Zhao et al. 2010), the cultivar of table olives (Casale et al. 2010), quality assessment of plums during storage (Pérez-Marín et al. 2010), discrimination of different beers (Di Egidio et al. 2011), determination of tomato quality (Ścibisz et al. 2011) and detection of Chinese honey adulterated by the addition of high fructose corn syrup (Chen et al. 2011). A review on the use of Fourier Transform Infrared Spectroscopy (FTIR) for rapid authentication and detection of food adulteration demonstrates the increasing interest in this work (Rodriguez & Allendorf, 2011), as food authenticity is important for quality control and food safety. These studies demonstrate the utility of IR spectroscopy for compositional analysis.

In recent years, spectroscopic techniques have also been adopted by the wine industry for rapid wine analysis. NIR and MIR has been used to predict oenological parameters of red wine fermentation (Di Egidio et al. 2010), to discriminate wines after malolactic fermentation (Cozzolino, McCarthy, & Bartowsky 2012) and to discriminate rice wines on the basis of quality (Yu et al. 2008). Other studies have described the discrimination of organic and non-organic wines (Cozzolino et al. 2009), and the geographical classification of Tempranillo (Liu et al. 2006; Cynkar et al. 2010) and Sauvignon Blanc wines (Cozzolino et al. 2011). This highlights the potential of spectroscopy for authentication of wine.

Ultraviolet-visible spectroscopy (UV-Vis), together with chemometrics and data analysis techniques such as PCA or linear discriminant analysis (LDA) have similarly been used to classify Chilean Pinot Noir wines (Saavedra et al. 2011); while NIR spectroscopy has been

used to assess grape quality parameters (Kemps et al. 2010) and to determine the concentration of wine phenolic compounds (Cozzolino et al 2004; Tarantilis 2008), fermentation-derived volatile compounds (Smyth et al. 2008) and oak-derived volatile compounds (Garde-Cerdán et al. 2010). These studies described spectroscopic methods as rapid cost-effective analytical techniques, particularly compared with traditional methods such as GC-MS and HPLC.

In this chapter, the application of MIR spectroscopy as a technique for rapid detection of smoke taint in wine are presented.

Classification of Smoke Tainted Wines Using Mid-Infrared Spectroscopy and Chemometrics

Anthea L. Fudge,[†] Kerry L. Wilkinson,[†] Renata Ristic,[†] and Daniel Cozzolino*[,‡]

[†]School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[‡]The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia

ABSTRACT: In this study, the suitability of mid-infrared (MIR) spectroscopy, combined with principal component analysis (PCA) and linear discriminant analysis (LDA), was evaluated as a rapid analytical technique to identify smoke tainted wines. Control (i.e., unsmoked) and smoke-affected wines (260 in total) from experimental and commercial sources were analyzed by MIR spectroscopy and chemometrics. The concentrations of guaiacol and 4-methylguaiacol were also determined using gas chromatography—mass spectrometry (GC-MS), as markers of smoke taint. LDA models correctly classified 61% of control wines and 70% of smoke-affected wines. Classification rates were found to be influenced by the extent of smoke taint (based on GC-MS and informal sensory assessment), as well as qualitative differences in wine composition due to grape variety and oak maturation. Overall, the potential application of MIR spectroscopy combined with chemometrics as a rapid analytical technique for screening smoke-affected wines was demonstrated.

KEYWORDS: *classification, guaiacol, linear discriminant analysis, mid-infrared spectroscopy, principal component analysis, smoke taint, wine*

■ INTRODUCTION

Spectroscopic techniques in the mid-infrared (MIR) region have been applied to the analyses of a diverse range of agricultural products, for example, olive oils,[1,2] honey,[3,4] meat products,[5] cheese,[6] and fruits and vegetables,[7−9] for purposes including authentication, classification, quality control, and compositional determinations. Infrared (IR) spectroscopy measures changes in the absorption of IR radiation by organic compounds due to the vibration of fundamental frequencies (in particular C−H, N−H, and O−H bonds) within different functional groups.[1,10] Spectra in the MIR region (4,000 to 400 cm⁻¹) comprise well-defined bands that can be used for qualitative analysis to identify organic constituents or to differentiate samples.[1,10]

Wine is a complex medium, and the diverse nature of its chemical constituents, many of which are present at only trace concentrations, can complicate its analysis. Traditional analytical techniques, such as those using gas (GC) or liquid chromatography (LC), are often time-consuming and costly due to sample preparation requirements. By comparison, spectroscopic techniques are rapid and nondestructive, thereby offering significant time and cost savings.[10] Not surprisingly, numerous studies have therefore applied MIR spectroscopy to te analysis of wine. Some recent examples describe the classification of wines on the basis of geographical origin;[11,12] red wine fermentation monitoring;[13] analysis of red wine tannins;[14] the use of red wine phenolic extracts to discriminate between different cultivars;[15,16] and the authentication of wine[17] and organic grape production systems.[18]

The objective of this study was to evaluate the suitability of MIR spectroscopy as a rapid analytical technique for the detection of smoke taint in wine. Vineyard exposure to smoke from bushfires occurring in close proximity to wine regions can result in the uptake of smoke-derived volatile compounds by grapes, and in some cases, unpalatable smoke-related sensory attributes in the resulting wines.[19,20] A number of volatile phenols have been identified in smoke-affected grapes and wine.[19−23] Since these compounds can be readily quantified by existing gas chromatography—mass spectrometry (GC-MS) based methods,[24] they have been used as marker compounds for assessing the smoke exposure of grapes and wine, but they are not considered to be solely responsible for smoke taint.[19] Furthermore, recent studies have shown that smoke-derived phenols can accumulate in glycoconjugate precursor forms.[25,26] A comparison of methods for the determination of glyco-conjugate derivatives of smoke-derived volatile phenols has recently been reported.[27] Wineries typically rely on commercial laboratories for smoke taint related analyses, and during periods of high demand, winemakers can experience delays in results being returned. As such, there is clearly a need for a rapid analytical method for differentiating smoke-affected grapes and wine, to give winemakers the opportunity to make informed processing decisions, within the time constraints of vintage.

■ MATERIALS AND METHODS

Wine Samples. Samples (260 in total) included experimental wines sourced from several previous studies,[20,28,29] smoke tainted wines provided by industry, and commercial wines (i.e., without smoke taint). Experimental wine samples (Table 1) comprised (i) 27 smoke-affected Shiraz wines from a winemaking trial involving the addition of oak or tannin to fermentations of grapes harvested from a Victorian vineyard exposed to smoke from bushfires that occurred between 7 February and 14 March, 2009 (9 treatments each conducted in

Table 1. Concentration of Guaiacol and 4-Methylguaiacol in Control and Smoke-Affected Experimental Wines

wine samples		guaiacol (μg/L)	4-methylguaiacol (μg/L)
Shiraz	smoke-affected[a] (n = 27)	19–27	1–5
Viognier	control (n = 12)	na[c]	na
	smoke-affected[b] (n = 12)	na	na
Grenache	control (n = 30)	nd[c]	nd
	smoke-affected[b] (n = 30)	2–9	nd – 2
Chardonnay	control (n = 6)	nd	nd
	smoke-affected[b] (n = 9)	1–5	nd
Shiraz	control (n = 3)	9	nd
	smoke-affected[b] (n = 3)	26	2
Cabernet Sauvignon	control (n = 3)	2	nd
	smoke-affected[b] (n = 3)	20	3
Merlot	control (n = 3)	2	nd
	smoke-affected[b] (n = 3)	18	3
Pinot Noir	control (n = 3)	nd	nd
	smoke-affected[b] (n = 3)	6	1
Pinot Gris	control (n = 3)	nd	nd
	smoke-affected[b] (n = 3)	10	2
Chardonnay	control (n = 3)	nd	nd
	smoke-affected[b] (n = 3)	1	nd
Sauvignon Blanc	control (n = 3)	nd	nd
	smoke-affected[b] (n = 3)	1	nd

[a]Bushfire smoke. [b]Experimental smoke. [c]na, not available; nd, not detected; n, number of samples.

Table 2. Concentration of Guaiacol and 4-Methylguaiacol in Smoke-Affected Industry Wines

wine samples	guaiacol (μg/L)	4-methyl-guaiacol (μg/L)	sensory description	sensory score[a]
Red Blend 1	51	29	smoked meat aroma; ashy AT[b]	4.3
Red Blend 2	34	17	fruit and oak aroma; drying palate	6.8
Red Blend 3	48	33	barnyard and wood smoke aromas; ashy and bitter AT	4.5
Red Blend 4	6	nd[b]	toasty, smoke aroma; sour and drying palate	6.7
Shiraz 1	55	23	wood smoke and gamey palate	4.8
Shiraz 2	28	8	plum, spice and oak aromas; metallic AT	9.0
Shiraz 3	6	nd	smoky, gamey aroma; ashy AT	5.0
Cabernet Sauvignon 1	2	nd	fruit aromas; astringent palate	7.8
Merlot 1	3	nd	fruit aromas; metallic palate	6.7
Pinot Noir 1	15	4	unpleasant AT; bandaid palate	5.7
Pinot Noir 2	7	3	subdued fruit; smoky aroma; dusty, ashy palate	6.8
Pinot Noir 3	21	7	fruit, burnt sugar and bandaid aromas; ashy palate	6.0
Graciano	4	nd	barnyard aroma	6.5
White Blend 1	11	3	yeast and rancid aromas; smoky AT	5.3
Sauvignon Blanc 1	2	nd	fruit aromas; lacks fruit on palate; AT	8.3
Sauvignon Blanc 2	nd	nd	fruit and perfume aromas; sour finish	7.3
Semillon 1	2	nd	fruit aromas; flat finish	8.7
Semillon 2	2	nd	fruit aromas; slightly sour; 9.2	9.2
Pinot Grigio 1	5	nd	fruit and oak aromas; sour palate; 7.0	7.0
Pinot Grigio 2	1	nd	fruit and yeast aromas; 8.7	8.7

[a]Mean scores from three judges using a 10 point scale where 1 = "unacceptable, tainted" and 10 = "acceptable". [b]nd, not detected; AT, aftertaste.

triplicate); (ii) 12 control and 12 smoke-affected Viognier wines from a winemaking trial involving the fermentation of control and smoke-affected grapes by indigenous yeast (2 treatments each with 12 replicates), where smoke-affected grapes were harvested from grapevines exposed to smoke for 30 min under experimental conditions and control grapes were harvested from unsmoked grapevines; (iii) 30 control and 30 smoke-affected Grenache wines from winemaking trials involving either different skin contact times (4 treatments each conducted in triplicate) or selection of different yeast strains for fermentation (16 treatments each conducted in triplicate) where smoke-affected grapes were harvested from grapevines exposed to smoke for 20 min under experimental conditions, and control grapes were harvested from unsmoked grapevines; (iv) 6 control and 9 smoke-affected Chardonnay wines from a viticultural trial involving defoliation and/or smoke exposure of grapevines (5 treatments each conducted in triplicate) where smoke-affected grapes were harvested from grapevines exposed to smoke for 60 min under experimental conditions, and control grapes were harvested from unsmoked grapevines; and (v) 21 control and 21 smoke-affected wines from a varietal trial involving Shiraz, Cabernet Sauvignon, Merlot, Pinot Noir, Pinot Gris, Chardonnay, and Sauvignon Blanc grapes (2 treatments per variety each conducted in triplicate) where smoked-affected grapes were harvested from grapevines exposed to smoke for 60 min under experimental conditions, and control grapes were harvested from unsmoked grapevines. Experimental smoke treatments involved grapevines being enclosed in purpose-built smoke tents and exposed to straw-derived smoke using methodology described previously.[21] Twenty wines were provided by industry (Table 2); these wines were made from grapes harvested from vineyards thought to be exposed to smoke during bushfires that occurred between 7 February and 14 March, 2009 and/or were considered by their respective winemakers to exhibit the smoke-related sensory attributes typically associated with smoke taint; i.e., smoky, ashy, medicinal, and smoked meat aroma and flavor attributes.[19,20] Sixty-eight commercial wines were also included as examples of control (i.e., unsmoked) wines. Finally, two cask wines, one white and one red, were also included, with and without the addition of guaiacol (at approximately 30 mg/L). The presence/absence of smoke taint in industry and commercial wines was determined by informal sensory assessment by a panel of three experienced tasters using a 10 point scale where 1 = unacceptable, tainted, and 10 = acceptable. Wines were stored at constant temperature (15 °C) prior to analysis.

Determination of Smoke Taint Marker Compounds. The concentrations of guaiacol and 4-methylguaiacol were quantified in experimental and industry wines, as markers of smoke taint, according to stable isotope dilution assay methods reported previously.[24] This publication describes the preparation of internal standards and instrument operating conditions used herein. Analyses were performed by the Australian Wine Research Institute's Commercial Services Laboratory (Adelaide, Australia) using an Agilent 6890N gas chromatograph coupled to a 5975 inert source mass spectrometer (Agilent, Palo Alto, CA, USA).

MIR Spectroscopy Measurements. Wine samples (ca. 20 mL) taken from freshly opened bottles were centrifuged (4300g for 5 min) and scanned in transmission mode using a UV–vis flow cell (1 and 0.2 mm path length) in the MIR region (400–4000 cm^{-1}; 25 μm path length) in a Multispec system Bacchus/Multispec System equipped with a Thermo Nicolet, Avatar 380 FT-MIR spectrometer (Microdom, Taverny, France). The fingerprint range was 1000–1500 cm^{-1}. Spectral data and instrument diagnostics were collected using

Bacchus acquisition software (Quant, version 4, 2001) as described previously.[18]

Multivariate Data Analysis and Wine Classification. Multivariate data analysis was performed using The Unscrambler software (version 9.5, Camo ASA, Oslo, Norway). Spectral data were examined for unusual outlier samples before classification methods were used by applying principal component analysis (PCA). Discrimination models were developed using linear discriminant analysis (LDA), a supervised classification technique by which the number of categories and the samples that belong to each category are previously defined.[30] The criterion of LDA for the selection of latent variables is the maximum differentiation between the categories and minimizes the variance within categories. This method produces a number of orthogonal linear discriminant functions, equal to the number of categories minus one, that allow samples to be classified in one or another category. LDA was carried out using the score values of the first three principal components which gave the highest level of separation (high variance) in the PCA models developed (JMP, v. 5.01, SAS Institute, Inc., Cary, NC). In this technique, each sample is assigned with a dummy variable as a reference value, which is an arbitrary number designating whether the sample belongs to a particular group. In the current study, smoke-affected wines were assigned a numeric value of 1 and control wines with the value 2; the cutoff was set at 0.5. Both LDA and PCA models were developed using full cross-validation (leave-one-out). The MIR spectra were preprocessed using standard normal and variate transformation (SNV).[30]

■ RESULTS AND DISCUSSION

The intensity of smoke-related sensory attributes and concentrations of smoke-derived volatile phenols in wine have been shown to be influenced by the duration of grapevine exposure to smoke.[22,27] Therefore, a diverse sample set was sourced for this study, comprising wines derived from (i) unsmoked grapes, i.e., commercial and experimental control wines; (ii) grapes exposed to smoke for short durations (20 to 60 min) under experimental conditions, i.e., experimental smoke-affected wines; and (iii) grapes exposed to bushfire smoke for longer (but unknown) durations, i.e., industry wines. The guaiacol and 4-methylguaiacol concentrations of experimental and industry wines were determined by GC-MS to evaluate the extent of smoke taint (Tables 1 and 2).

For experimental wines (Table 1), guaiacol and 4-methylguaiacol were not detected in the majority of control wines; the exceptions were Shiraz, Cabernet Sauvignon, and Merlot control wines, which contained 2 or 9 μg/L of guaiacol as a natural grape component, in agreement with previous studies.[31] The highest volatile phenol levels were observed in Shiraz wines made from bushfire smoke-affected grapes; lower levels were observed in wines made from grapes exposed to smoke under experimental conditions. This was as expected, given the different durations of smoke exposure. The variation in guaiacol and 4-methylguaiacol concentrations for experimental smoke-affected wines was attributed to different winemaking practices; specifically, the different durations of skin contact for red and white winemaking, which has previously been shown to influence the intensity of smoke taint.[20] However, it is acknowledged that these results may also reflect varietal differences.

For industry wines (Table 2), the volatile phenol content varied considerably. Guaiacol was detected in all but one wine, at concentrations between 1 and 55 μg/L; while 4-methylguaiacol was detected in only half the wines, at concentrations ranging from 3 to 33 μg/L. Again, this is likely to be attributable to differences in the duration of grapevine exposure to smoke, but it was not possible to ascertain the exact

timing, duration, and density of smoke exposure by commercial vineyards. As such, the occurrence of smoke taint in industry wines was further evaluated by informal sensory analysis. Seven wines received scores of 6.0 or less: Red Blend 1, Red Blend 3, Shiraz 1, Shiraz 3, Pinot Noir 1, Pinot Noir 3, and White Blend 1. These wines generally contained the highest volatile phenol levels and were also considered to exhibit apparent smoke-related sensory attributes (Table 2), i.e., they were considered to be heavily smoke tainted. Red Blend 2 and Shiraz 2 also contained high concentrations of guaiacol and 4-methylguaiacol, but from oak maturation, since these wines were described by fruit and oak-related sensory attributes, rather than smoky characters, albeit drying and metallic characters were reported. Smoke-related aromas were also identified in Red Blend 4 and Pinot Noir 2. These wines contained moderate volatile phenol levels and received sensory scores of 6.7 and 6.8, respectively, indicative of moderate levels of smoke taint. The remaining industry wines contained low levels of guaiacol and 4-methylguaiacol, and were not described by specific sensory attributes associated with smoke taint. That said, these wines were considered to lack fruit intensity and/or to exhibit drying, sour or metallic characters, which might be indicative of low levels of smoke taint.

Commercial wines were similarly subjected to informal sensory assessment, but there was no evidence of smoke taint in these wines (data not shown). No visual differences were apparent in the MIR spectra obtained for the wine samples (data not shown); therefore, PCA was performed on the spectral data to investigate qualitative differences within the sample set. Of the 260 wine samples analyzed, three outliers were identified (data not shown): two control Viognier wines (from the winemaking trial) and one commercial Riesling wine. Spectral data collected for these samples were therefore removed prior to further chemometric analyses.

Separate PCA models were performed as follows on five sets of experimental wines, with the inclusion of commercial wines of the same variety as controls and industry wines, with the inclusion of red and white cask wines with and without the addition of guaiacol. PCA was also developed using a larger data set ($n = 245$) comprising all wines, with the exception of the previously identified outliers and the experimental control Viognier wines. Figures 1 and 2 show the score plots of the MIR spectra from the Shiraz and varietal wine sets, respectively.

For the Shiraz wine set (Figure 1), clear separation between control and smoke-affected wines was observed, i.e., samples clustered on opposite sides of the score plot. PC1 and PC2 accounted for 73% and 10% of the variation, respectively. Loadings were investigated to identify the wavenumber regions that corresponded to the PCA separation. For PC1, wavenumbers between 1000 and 1100 cm^{-1} can be assigned to C—O vibrations of residual carbohydrates, i.e., fructose and glucose[15] (Figure 3). For PC2, the highest loadings were observed around 1045 cm^{-1}, due to C—O stretching, for example, from the oxygen atom of hydroxyl groups (Figure 3). These wavenumbers could potentially be associated with the presence of smoke-derived volatile phenols, such as guaiacol and 4-methylguaiacol.

For Viognier wines, separation was observed between the smoke-affected wines, which were located in the top left quadrant (together with the two control outliers) and the remaining control wines, which clustered within PC1 in the other three quadrants (data not shown). The loading plot for Viognier (data not shown) showed a large positive peak at
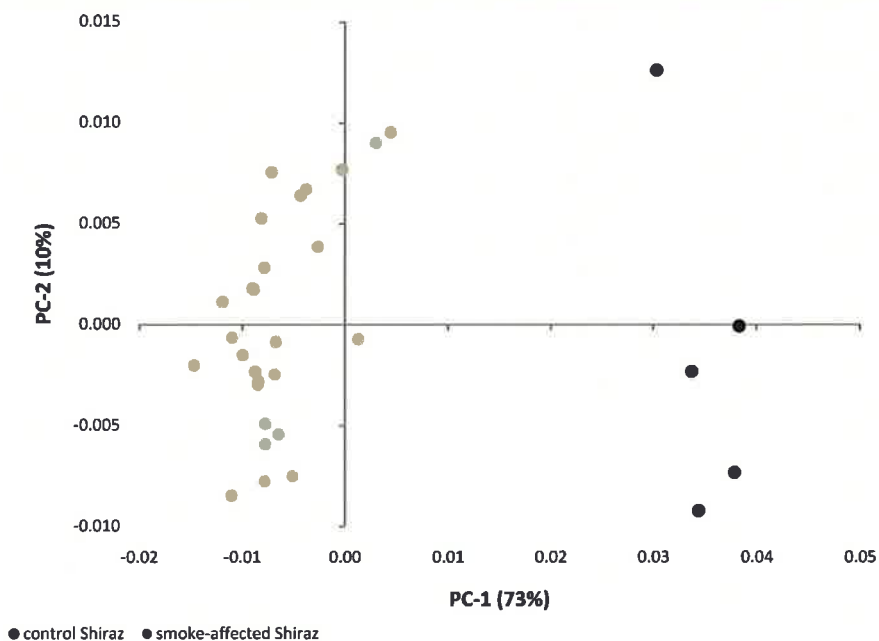
Figure 1. Score plot of the first two PCs derived from the MIR spectra of control and smoke-affected Shiraz wines.
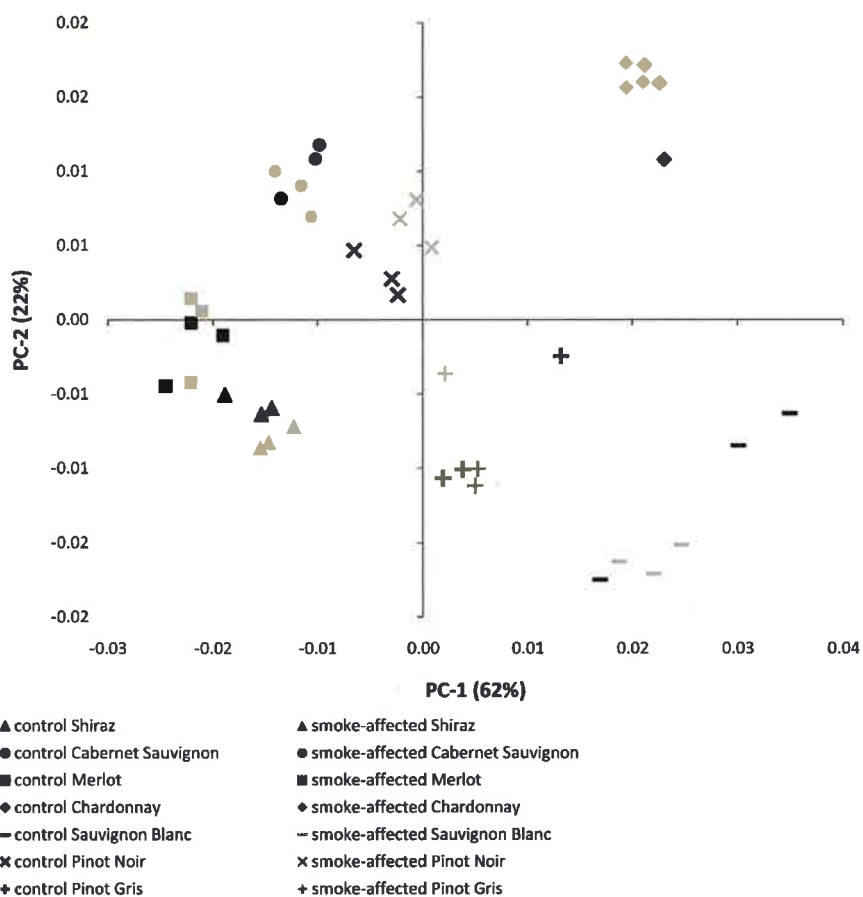

Figure 2. Score plot of the first two PCs derived from the MIR spectra of control and smoke-affected Shiraz, Cabernet Sauvignon, Merlot, Chardonnay, Sauvignon Blanc, Pinot Noir, and Pinot Gris wines.
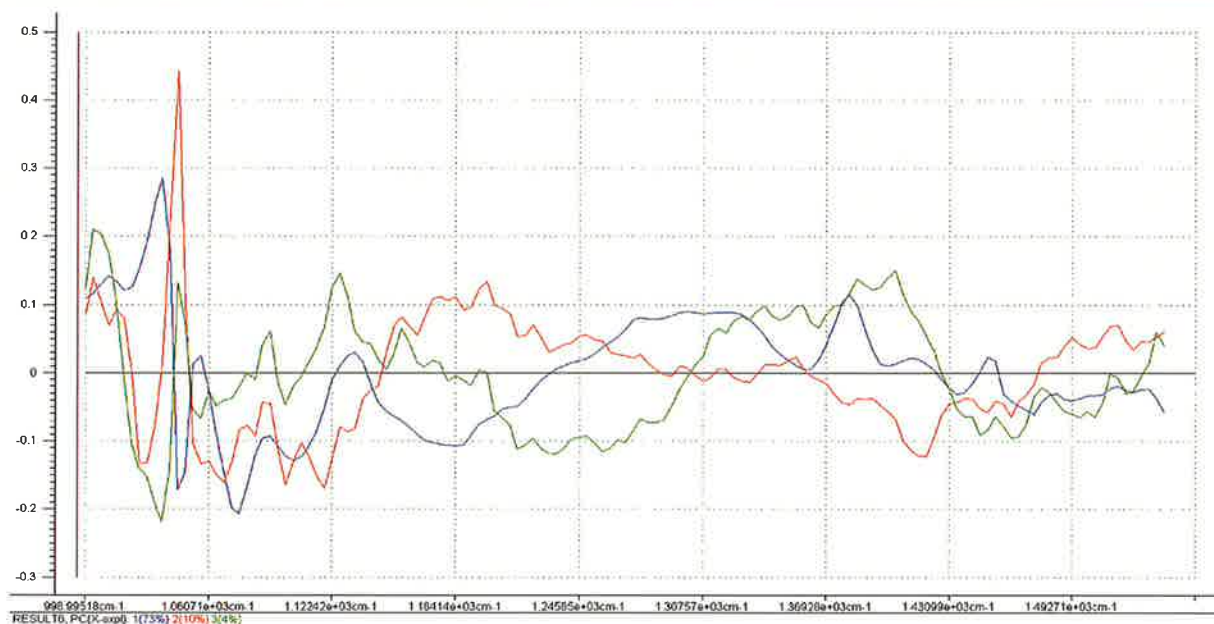
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Figure 3. Loadings for the first three PCs of the fingerprint region derived from Shiraz MIR spectra.

1045 cm^{-1} wavenumbers for PC1, which accounted for 90% of variation. Negatively correlated peaks due to PC3 (which explained only 2% of variation) were observed at approximately 1130 cm^{-1} and 1415 cm^{-1} wavenumbers, respectively. The latter peak occurs in the 1500 to 1400 cm^{-1} region typically associated with aromatic C−C stretching, and so these wavenumbers could also be associated with the presence of volatile phenols.

Control and smoke-affected Grenache wines showed reasonable separation (data not shown). Control wines were generally positively correlated with PC2, while smoke-affected wines were negatively correlated with PC2. The Grenache loadings (Figure 4) also showed the influence of the 1500 to 1400 cm^{-1} region, again due to aromatic C−C stretching. The wavenumbers observed around 1080 cm^{-1} and 1045 cm^{-1} could be attributed to the C−O and C−C bonds of ethanol and other alcohols.[32] Wavenumbers at 1470 and 1380 cm^{-1} could be associated with vibrations of phenolic hydroxyl groups,[33] i.e., signals which volatile phenols would influence.

The PCA models developed for Chardonnay wines (data not shown) did not show separation between control and smoke-affected experimental wines. Smoke-affected wines clustered in the top right quadrant, whereas experimental and control wines were distributed throughout the other quadrants. Significant variation was observed between control Chardonnay wines, which likely reflects differences in geographical origin, vintage and/or production methods, as reported in previous studies.[15−18] The loadings plot also showed broader peaks and thus less defined information. The C−O stretch for primary alcohols[34] at 1050 cm^{-1} was prominent for both PC1 and PC2 (which explained 79% total variation). Characteristic bands due to C−O−C bonds were observed at 1218 and 1108 cm^{-1},[35] suggesting the presence of substituted phenols such as syringol and its derivatives, compounds which have also been identified as components of smoke-affected wines.[26] Bands in the fingerprint region between 1500 and 1200 cm^{-1} were attributed to deformations of −CH$_2$ and also C−C−H and H−C−O,

whereas bands between 1200 and 950 cm^{-1} were attributed to stretching modes of C−C and C−O.[36] These regions were observed in all of the data sets analyzed.

Table 3 shows the classification of wines as control or smoke-affected, according to the LDA of MIR spectra. Classification was performed on both individual wine sets and the larger data set (i.e., all wines, as described above).

For the Shiraz wine set, 100% correct classification rates were obtained for both control and smoke-affected wines. This indicates qualitative differences in the composition of smoke-affected wines that enabled the classification model to differentiate these samples from control wines, i.e., differences presumably attributable to smoke taint. One hundred percent correct classification rates were also obtained for experimental Viognier wines; however, two commercial Viognier wines were incorrectly classified as being smoke-affected. These wines were fermented and matured in oak barrels, so misclassification might be due to the presence of oak derived volatile compounds, which include guaiacol and 4-methylguaiacol.[24]

Classification rates of 68% and 87% were obtained for control and smoke-affected Grenache wines, respectively. The wines incorrectly classified as controls comprised three replicate wines from the winemaking trial that involved different durations of skin contact and one wine from the yeast selection trial. The wines incorrectly classified as being smoke-affected were all control wines from the yeast selection trial. For these wines, PCA showed separation according to both wine production methods, (e.g., duration of skin contact and winemaking yeast) and smoke taint (data not shown). This further indicates factors besides grapevine exposure to smoke influence classification.

Smoke-affected Chardonnay wines were 100% correctly classified, but only 75% of control Chardonnay wines were correctly classified. However, the misclassified wines were experimental control wines rather than commercial wines. As indicated above, PCA showed significant separation of commercial
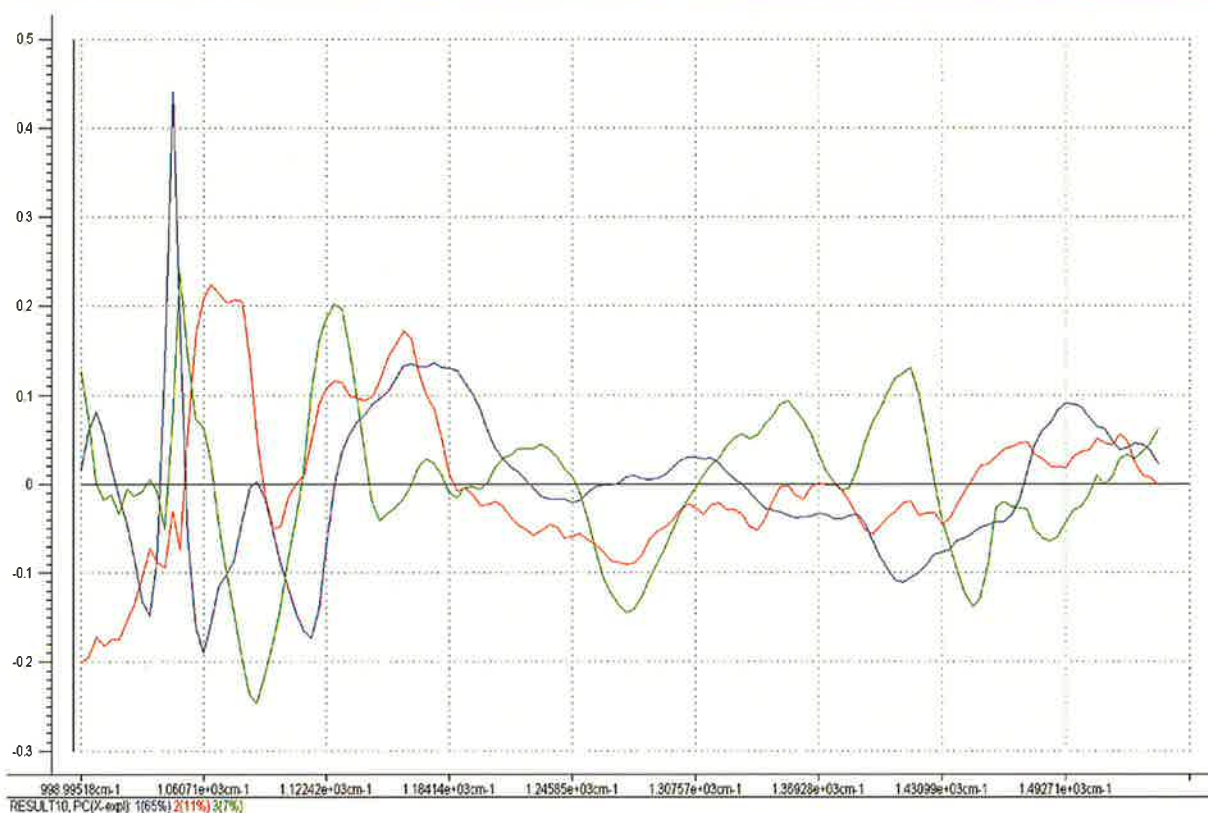
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Figure 4. Loadings for the first three PCs of the fingerprint region derived from Grenache MIR spectra.

Table 3. Classification of Control and Smoke-Affected Wines Using LDA

	wine samples	no. of wines classified as the control	no. of wines classified as smoke-affected	correct classification
Shiraz	control (n^b = 5)	5	0	100%
	smoke-affected (n = 27)	0	27	100%
Viognier	control (n = 14)	12	2	86%
	smoke-affected (n = 10)	0	10	100%
Grenache	control (n = 31)	21	10	68%
	smoke-affected (n = 30)	4	26	87%
Chardonnay	control (n = 16)	12	4	75%
	smoke-affected (n = 9)	0	9	100%
varietal	control (n = 21)	8	13	38%
	smoke-affected (n = 21)	8	13	62%
industry wines[a]	control (n = 2)	1	1	50%
	smoke-affected (n = 22)	7	15	68%
all wines	control (n = 126)	77	49	61%
	smoke-affected (n = 119)	36	83	70%

[a]Includes red and white cask wines with and without the addition of 30 mg/L guaiacol. [b]n, number of samples.

Chardonnay wines, likely due to a combination of vintage, regionality, and production effects.

The poorest classification rates were obtained for experimental wines from the varietal trial, being 38% and 62% for control and smoke-affected wines, respectively. For this sample set, it was observed that the PCA results were influenced more by grape variety than grapevine exposure to smoke (Figure 2). Previous studies have demonstrated the use of MIR spectroscopy to discriminate wines on the basis of grape variety.[16,17] Classification rates are also likely to be influenced by the intensity of smoke taint, with limits of detection for analysis by

MIR spectroscopy, as for analysis by GC-MS. As such, wines with low levels of smoke taint might be classified as controls.

Six industry wines were classified as controls, being Red Blend 3, Shiraz 2, Cabernet Sauvignon 1, and Pinot Noir 1, 2, and 3. The classification of Shiraz 2 and Cabernet Sauvignon 1 as control wines is not unreasonable, given that neither wine exhibited any apparent smoke related sensory attributes; Cabernet Sauvignon 1 contained only 2 μg/L of guaiacol, while the guaiacol and 4-methylguaiacol present in Shiraz 2 derived from oak maturation, rather than grapevine exposure to smoke. As such, the presence of smoke taint in these wines is

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questionable, and so they might be more appropriately classified as control wines. In contrast, Red Blend 3 and Pinot Noir 1, 2, and 3 did exhibit smoke related sensory attributes and contained guaiacol and 4-methylguaiacol, so the reason for their misclassification is unclear. In the case of Red Blend 3, the presence of high levels of 4-ethylguaiacol and 4-ethylphenol (data not shown), which are indicative of *Brettanomyces/Dekkera* spoilage,[37] may have confounded classification. The industry wine set also included red and white cask wines with and without the addition of guaiacol. Both white cask wines were classified as controls, indicating that the presence of guaiacol alone does not determine classification. Both red cask wines were classified as smoke-affected; again misclassification was attributed to oak maturation of this wine.

When all wines were analyzed, correct classification rates of 61% and 70% were obtained for control and smoke-affected wines, respectively. These results demonstrate qualitative differences exist between wines made from unsmoked grapes and wines made from smoke-affected grapes; differences that can be observed by MIR spectroscopy and, when combined with chemometrics, used to screen wines for the presence of smoke taint. Some limitations were identified with the classification model developed in the current study, in particular that compositional differences due to grape variety and oak maturation may override differences resulting from grapevine exposure to smoke (especially at low levels of smoke exposure). However, further refinement of the model using a sample set that represents a broader range of grape varieties, production methods, vintages, and levels of smoke taint would enable improved classification rates.

■ AUTHOR INFORMATION

Corresponding Author
*Tel: + 61 8 8303 6600. Fax: + 61 8 8303 6601. E-mail: Daniel. Cozzolino@gmail.com.

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■ REFERENCES

(1) Guillén, M. D.; Cabo, N. Infrared spectroscopy in the study of edible oils and fats. *J. Sci. Food Agric.* **1997**, *75*, 1−11.

(2) Sinelli, N.; Cerretani, L.; Di Egidio, V.; Bendini, A.; Casiraghi, E. Application of near (NIR) infrared and mid (MIR) infrared spectroscopy as a rapid tool to classify extra virgin olive oil on the basis of fruity attribute intensity. *Food Res. Int.* **2010**, *43*, 369−375.

(3) Kelly, J. F. D.; Downey, G.; Fouratier, V. Initial study of honey adulteration by sugar solutions using midinfrared (MIR) spectroscopy and chemometrics. *J. Agric. Food Chem.* **2004**, *52*, 33−39.

(4) Ruoff, K.; Iglesias, M. T.; Luginbühl, W.; Bosset, J. O.; Bogdanov, S.; Amadò, R. Quantitative analysis of physical and chemical measurands in honey by mid-infrared spectrometry. *Eur. Food Res. Technol.* **2006**, *223*, 22−29.

(5) Al-Jowder, O.; Defernez, M.; Kemsley, E. K.; Wilson, R. H. Mid-infrared spectroscopy and chemometrics for the authentication of meat products. *J. Agric. Food Chem.* **1999**, *47*, 3210−3218.

(6) Pillonel, L.; Luginbühl, W.; Picque, D.; Schaller, E.; Tabacchi, R.; Bosset, J. O. Analytical methods for the determination of the geographic origin of Emmental cheese. Mid and near-infrared spectroscopy. *Eur. Food Res. Technol.* **2003**, *216*, 174−178.

(7) Quilitzsch, R.; Baranska, M.; Schulz, H.; Hoberg, E. Fast determination of carrot quality by spectroscopy methods in the UV-VIS, NIR and IR range. *J. Appl. Bot. Food Qual.* **2005**, *79*, 163−167.

(8) Di Egidio, V.; Sinelli, N.; Limbo, S.; Torri, L.; Franzetti, L.; Casiraghi, E. Evaluation of shelf-life of fresh-cut pineapple using FT-NIR and FT-IR spectroscopy. *Postharvest Biol. Technol.* **2009**, *54*, 87−92.

(9) Ścibisz, I.; Reich, M.; Bureau, S.; Gouble, B.; Causse, M.; Bertrand, D.; Renard, C. Mid-infrared spectroscopy as a tool for rapid determination of internal quality parameters in tomato. *Food Chem.* **2011**, *125*, 1390−1397.

(10) Rodriguez-Saona, L. E.; Allendorf, M. E. Use of FTIR for rapid authentication and detection of adulteration of food. *Annu. Rev. Food Sci. Technol.* **2011**, *2*, 467−483.

(11) Cozzolino, D.; Cynkar, W. U.; Shah, N.; Smith, P. A. Can spectroscopy geographically classify Sauvignon Blanc wines from Australia and New Zealand? *Food Chem.* **2011**, *126*, 673−678.

(12) Riovanto, R.; Cynkar, W. U.; Berzaghi, P.; Cozzolino, D. Discrimination between Shiraz wines from different Australian regions: The role of spectroscopy and chemometrics. *J. Agric. Food Chem.* **2011**, *59*, 10356−10360.

(13) Di Egidio, V.; Sinelli, N.; Giovanelli, G.; Moles, A.; Casiraghi, E. NIR and MIR spectroscopy as rapid methods to monitor red wine fermentation. *Eur. Food Res. Technol.* **2010**, *230*, 947−955.

(14) Fernandez, K.; Agosin, E. Quantitative analysis of red wine tannins using Fourier-transform mid-infrared spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 7294−7300.

(15) Edelmann, A.; Diewok, J.; Schuster, K. C.; Lendl, B. Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. *J. Agric. Food Chem.* **2001**, *49*, 1139−1145.

(16) Tarantilis, P. A.; Troianou, V. E.; Pappas, C. S.; Kotseridis, Y. S.; Polissiou, M. G. Differentiation of Greek red wines on the basis of grape variety using attenuated total reflectance Fourier transform infrared spectroscopy. *Food Chem.* **2008**, *111*, 192−196.

(17) Bevin, C. J.; Dambergs, R. G.; Fergusson, A. J.; Cozzolino, D. Varietal discrimination of Australian wines by means of mid-infrared spectroscopy and multivariate analysis. *Anal. Chim. Acta* **2008**, *621*, 19−23.

(18) Cozzolino, D.; Holdstock, M.; Dambergs, R. G.; Cynkar, W. U.; Smith, P. A. Mid infrared spectroscopy and multivariate analysis: A tool to discriminate between organic and non-organic wines grown in Australia. *Food Chem.* **2009**, *116*, 761−765.

(19) Kennison, K. R.; Wilkinson, K. L.; Williams, H. G.; Smith, J. H.; Gibberd, M. R. Smoke-derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. *J. Agric. Food Chem.* **2007**, *55*, 10897−10901.

(20) Ristic, R.; Osidacz, P.; Pinchbeck, K. A.; Hayasaka, Y.; Fudge, A. L.; Wilkinson, K. L. The effect of winemaking techniques on the intensity of smoke taint in wine. *Aust. J. Grape Wine Res.* **2011**, *17*, S29−S40.

(21) Kennison, K. R.; Gibberd, M. R.; Pollnitz, A. P.; Wilkinson, K. L. Smoke-derived taint in wine: The release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. *J. Agric. Food Chem.* **2008**, *56*, 7379−7383.

(22) Kennison, K. R.; Wilkinson, K. L.; Pollnitz, A. P.; Williams, H. G; Gibberd, M. R. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. *Aust. J. Grape Wine Res.* **2009**, *15*, 228−237.

54

(23) Sheppard, S. I.; Dhesi, M. K.; Eggers, N. J. Effect of pre- and post-veraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. *Am. J. Enol. Vitic.* **2009**, *60*, 98–103.

(24) Pollnitz, A. P.; Pardon, K. H.; Sykes, M.; Sefton, M. A. The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. *J. Agric. Food Chem.* **2004**, *52*, 3244–3252.

(25) Hayasaka, Y.; Dungey, K. A.; Baldock, G. A.; Kennison, K. R.; Wilkinson, K. L. Identification of a *β*-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. *Anal. Chim. Acta* **2010**, *660*, 143–148.

(26) Hayasaka, Y.; Baldock, G. A.; Parker, M.; Pardon, K. H.; Black, C. A.; Herderich, M. J.; Jeffrey, D. W. Glycosylation of smoke-derived volatile phenols in grapes as a consequence of grapevine exposure to bushfire smoke. *J. Agric. Food Chem.* **2010**, *58*, 10989–10998.

(27) Wilkinson, K. L.; Ristic, R.; Pinchbeck, K. A.; Fudge, A. L.; Singh, D. P.; Pitt, K. M.; Downey, M. O.; Baldock, G. A.; Hayasaka, Y.; Parker, M.; Herderich, M. J. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. *Aust. J. Grape Wine Res.* **2011**, *17*, S22–S28.

(28) Dungey, K. A.; Grbin, P. R.; Wilkinson, K. L. Impact of Smoke on Grape Berry Microflora and Yeast Fermentation. In: *State-of-the-Art in Flavour Chemistry and Biology*; Hofmann, T., Meyerhof, W., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 2011; pp 249–253.

(29) Wilkinson, K. L.; Pinchbeck, K. A.; Fudge, A. L.; Hayasaka, Y.; Ristic, R. Effect of leaf removal and smoke exposure on the chemical composition and sensory properties of Chardonnay wine, unpublished work.

(30) Naes, T.; Isaksson, T.; Fearn, T.; Davies, T. *A User-Friendly Guide to Multivariate Calibration and Classification*, NIR Publications: Chichester, United Kingdom, 2002.

(31) Wirth, J.; Guo, W.; Baumes, R.; Günata, Z. Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. *J. Agric. Food Chem.* **2001**, *49*, 2917–2923.

(32) Colthup, N. B.; Daly, L. H.; Wiberly, S. E. *Introduction to Infrared and Raman Spectroscopy*, 3rd ed.; Academic Press Inc.: New York, 1990.

(33) Kislenko, V. N.; Oliinyk, L. P. Kinetics of hydrogen peroxide decomposition in guaiacol solution, catalyzed by hexacyanoferrate(II). *Russ. J. Gen. Chem.* **2003**, *73*, 114–118.

(34) Bevin, C. J.; Fergusson, A. J.; Perry, W. B.; Janik, L. J.; Cozzolino, D. Development of a rapid "fingerprinting" system for wine authenticity by mid-infrared Spectroscopy. *J. Agric. Food Chem.* **2006**, *54*, 9713–9718.

(35) Guillén, M. D.; Manzanos, M. J. Characterization of the components of a salty smoke flavouring preparation. *Food Chem.* **1997**, *58*, 97–102.

(36) Stuart, B. *Modern Infrared Spectroscopy*, 2nd ed.; Ando, D. J., Ed.; John Wiley and Sons: New York, 1996.

(37) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Pons, M. The origin of ethylphenols in wine. *J. Sci. Food Agric.* **1992**, *60*, 165–178.
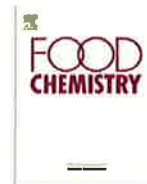
Analytical Methods

Synchronous two-dimensional MIR correlation spectroscopy (2D-COS) as a novel method for screening smoke tainted wine

Anthea L. Fudge [a], Kerry L. Wilkinson [a], Renata Ristic [a], Daniel Cozzolino [b,a,*]

[a] The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia
[b] The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia

ABSTRACT

In this study, two-dimensional correlation spectroscopy (2D-COS) combined with mid-infrared (MIR) spectroscopy was evaluated as a novel technique for the identification of spectral regions associated with smoke-affected wine, for the purpose of screening taint arising from grapevine exposure to smoke. Smoke-affected wines obtained from experimental and industry sources were analysed using MIR spectroscopy and chemometrics, and calibration models developed. 2D-COS analysis was used to generate synchronous data maps for red and white cask wines spiked with guaiacol, a marker of smoke taint. Correlations were observed at wavelengths that could be attributable to aromatic C–C stretching, i.e., between 1400 and 1500 cm^{-1}, indicative of volatile phenols. These results demonstrate the potential of 2D-COS as a rapid, high-throughput technique for the preliminary screening of smoke tainted wine.

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1. Introduction

Spectroscopic techniques are emerging as rapid, non-destructive tools for compositional analysis. Whereas chromatography-based techniques such as high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) are widely recognised as benchmarks for qualitative and quantitative chemical analysis, they typically involve time-consuming sample preparation and can be expensive in terms of reagent and instrumentation costs. By comparison, spectroscopic techniques require little or no sample preparation and consequently offer significant time and cost savings (Fragoso, Aceña, Guasch, Busto, & Mestres, 2011; Zhang et al., 2010).

Two-dimensional correlation spectroscopy (2D-COS) was originally developed by Noda (1989, 1990) and involved the study of spectral changes associated with mechanical deformations (or perturbations) of samples as a function of time (Noda & Ozaki, 2004). Noda et al. subsequently introduced a generalised 2D-COS theory (Noda, Dowrey, Marcott, Story, & Ozaki, 2000), which involved a series of spectra being recorded from one or more samples during or following perturbation, with the resulting spectral data matrix enabling identification of compositional differences within and between samples (Kirwan, Clark, Barnett, Nierre, & Adams, 2008; Noda et al., 2000); where perturbations have included variables such as temperature, pressure, spatial orientation and concentration (Noda, 1993; Noda et al., 2000). In this way, 2D-COS has greatly aided structural analysis, where spectral peaks were not readily distinguishable in one-dimension. 2D correlation analysis not only enhances spectral resolution, but also provides insight into variation in composition and structure (Noda, 1989; Wynne, Clark, Adams, & Barnett, 2007; Zhang et al., 2010). Changes in data are visualised according to 2D spectral maps: the synchronous matrix represents correlations between measured variables and the asynchronous matrix represents sequential variations occurring in the data (Kirwan et al., 2008; Wynne et al., 2007). Synchronous and asynchronous matrices are displayed as contour maps; peaks located on the diagonal (autopeaks) show regions of the spectrum which are changing with respect to the average spectrum of the series, while peaks off the diagonal (cross peaks) show the correlation intensity (positive or negative) of different bands (Noda, 1989).

Generalised 2D-COS has been applied to a wide range of spectroscopic studies, based on nuclear magnetic resonance (NMR), fluorescence, X-ray absorption, UV–visible (Vis), Fourier transform (FT) infrared (IR), near infrared (NIR) and mid infrared (MIR) techniques (Noda, 2004). Applications include structural analysis of ligand binding sites (Roselli, Burie, Mattioli, & Boussac, 1995), minerals (Gadaleta, Gericke, Boskey, & Mendelsohn, 1996), polymers (Ozaki, Liu, & Noda, 1997), proteins (Sefara, Magtoto, &

* Corresponding author at: The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia. Tel.: +61 8 8313 6738; fax: +61 8 8313 7109.

E-mail address: d.cozzolino@adelaide.edu.au (D. Cozzolino).

Richardson, 1997), carbohydrates (Liu, Himmelsbach, & Barton, 2004) and even water (Segtnan, Šašić, Isaksson, & Ozaki, 2001). Despite the increased use of spectroscopic techniques for wine analysis in recent years, there are few wine-related studies involving 2D-COS reported in the literature. Two-dimensional correlation techniques have enabled red wine fermentation to be monitored by FTIR (Wynne et al., 2007) and NMR (Kirwan et al., 2008) spectroscopy; demonstrating the application of 2D-COS for process analysis. More recently, Zhang et al. (2010) employed 2D IR correlation spectroscopy to discriminate red wines on the basis of sugar content. 2D correlation analysis has also been used to investigate the effect of temperature on the volatile compounds present in the headspace of red and white wines analysed by mass spectrometry electronic nose (Cozzolino, Cynkar, Dambergs, & Smith, 2010). These studies highlight the potential of 2D-COS, as a multivariate data processing technique, for wine analysis. Most importantly, 2D-COS is a rapid chemometric tool, thereby facilitating analysis within the time constraints of commercial wine production.

In recent years, a number of wine regions throughout the world have experienced an unusual taint, characterised by objectionable 'smoke' and 'ash' characters, attributed to vineyard exposure to smoke from forest fires or prescribed burns (Kennison, Wilkinson, Williams, Smith, & Gibberd, 2007; Ristic et al., 2011). Several volatile phenols have been identified, in free and glycoconjugate forms, in smoke-affected grapes and wine using GC–MS and HPLC based analytical techniques (Dungey, Hayasaka, & Wilkinson, 2011; Hayasaka, Dungey, Baldock, Kennison, & Wilkinson, 2010; Kennison, Gibberd, Pollnitz, & Wilkinson, 2008; Kennison, Wilkinson, Pollnitz, Williams, & Gibberd, 2011; Kennison et al., 2007; Ristic et al., 2011; Sheppard, Dhesi, & Eggers, 2009). Whilst these methods provide grapegrowers and winemakers with a quantitative means of assessing the extent of smoke taint in grapes and wine, during periods of high demand, i.e., following a major fire event, commercial laboratories are unlikely to be able to provide rapid results due to sample preparation requirements. As such, the main objective of this study was to evaluate 2D-COS as a rapid analytical tool for the preliminary screening of smoke tainted wines. A novel application of two-dimensional correlation analysis, i.e., to investigate the impact of grapevine exposure to smoke on the composition of wine, is demonstrated.

2. Materials and methods

2.1. Wine samples

A sub-sample of wines (59) was randomly selected from an earlier study concerning the classification of smoke tainted wines using MIR spectroscopy and chemometrics (Fudge, Wilkinson, Ristic, & Cozzolino, 2012). Samples included: experimental wines made from grapes exposed to smoke during field trials; industry wines made from grapes exposed to bushfire smoke; and red and white cask wines, with and without the addition of guaiacol (at approximately 30 mg/L). The extent of smoke taint present in each wine was determined by informal sensory assessment and/or quantification of guaiacol and 4-methylguaiacol as smoke taint marker compounds, as previously described (Fudge et al., 2012). Wines were stored at constant temperature (15 °C) prior to analysis.

2.2. Spectral analysis

MIR spectra of wine samples (ca. 20 mL) were recorded in transmission mode using a Multispec™ system Bacchus/Multispec System equipped with a Thermo Nicolet, Avatar 380 FT-MIR spectrometer (Microdom, Taverny, France), with a UV–Vis flow cell

(1 and 0.2 mm path length) and in the MIR region (400–4000 cm^{-1}) at 25 μm path length. The fingerprint range between 1000 and 1500 cm^{-1} was selected and used in further chemometric analysis. Spectral data and instrument diagnostics were collected using Bacchus acquisition software (Quant, version 4, 2001), as described previously (Cozzolino, Holdstock, Dambergs, Cynkar, & Smith, 2009).

2.3. Data processing

Partial least squares (PLS) calibration models were developed between data collected from MIR spectra and GC–MS reference data (guaiacol and 4-methylguaiacol concentrations) using The Unscrambler® software (version 9.5, Camo ASA, Oslo, Norway) with cross-validation. Spectral data were examined for unusual outlier samples before classification methods were used by applying principal component analysis (PCA). Three PLS models were developed using 'red' ($n = 12$), 'white' ($n = 17$) and 'red and white' ($n = 30$) smoke-affected wines, i.e., to account for compositional differences in wines attributable to variety and/or the extent of smoke taint, and full cross validation (CV) to validate the classification models. The number of latent variables used was automatically selected by the software using the predicted residual error sum-of-squares (PRESS) function in order to avoid over-fitting of the models. The calibrations were evaluated using the root mean square of the standard error in cross validation (RMSEP) and the coefficient of correlation (R) as criteria (Naes, Isaksson, Fearn, & Davies, 2002). The MIR spectra were pre-processed using standard normal and variate transformation (SNV) (Naes et al., 2002). The synchronous 2D correlation spectrum processing was subsequently performed using 2Dshige© 1.3 software (Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005), according to the theory of generalised 2D correlation analysis developed by Noda and Ozaki (2004).

3. Results and discussion

3.1. PLS calibration models

The extent of smoke taint in wine, which is currently assessed on the basis of volatile phenol concentrations (in free and glycoconjugate forms) and the intensity of smoke-related sensory attributes, has been shown to be influenced by the timing and duration of grapevine exposure to smoke (Kennison, Wilkinson, Pollnitz, Williams, & Gibberd, 2009; Kennison et al., 2011), as well as different winemaking techniques (Ristic et al., 2011). Grape variety has also been suggested as a factor which might influence the uptake of smoke (Fudge et al., 2012). Therefore, three PLS calibration models were developed using MIR spectral data and guaiacol and 4-methylguaiacol concentrations to account for compositional differences in wines attributable to variety and/or the extent of smoke taint.

The correlations plots from each PLS calibration model (Figs. 1–3), developed using the fingerprint region (1400–1500 cm^{-1}), were presented. The fingerprint region selected was the most important for the separation of wines within each sample set, with respect to 'smokiness'. The PLS loadings (not shown) between 1450 and 1510 cm^{-1} could be associated with aromatic ring stretching (Stuart, 1996) and could therefore be related to volatile phenols such as guaiacol. For the red and white wine set (Fig. 1), PLS loadings were positively correlated in principal component one (PC1) and negatively correlated in PC2, and accounted for 77 % of variation. The PLS calibration gave a coefficient of correlation (R) of 0.90 and RMSEP of 3.84 (Table 1). For the white wine set (Fig. 2), poorer PLS calibrations were obtained, being an R of 0.75 and RMSEP of 1.86. These results suggest that there were other matrix differences
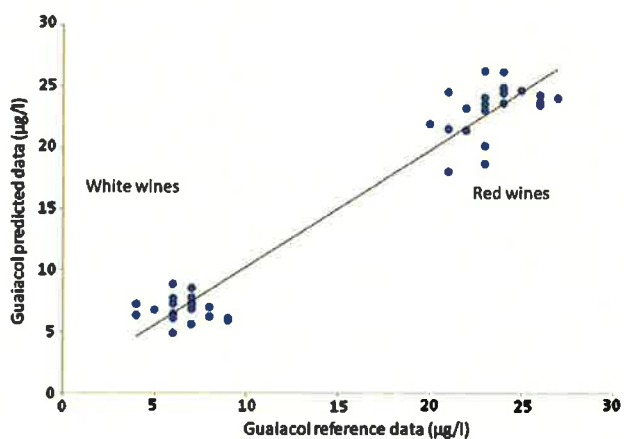
Fig. 1. Correlations plot between guaiacol reference data and predicted values using mid infrared spectroscopy in the set of red and white samples comprising Chardonnay, Sauvignon Blanc, Pinot Gris, Shiraz, Merlot, Cabernet Sauvignon and Pinot Noir wines.
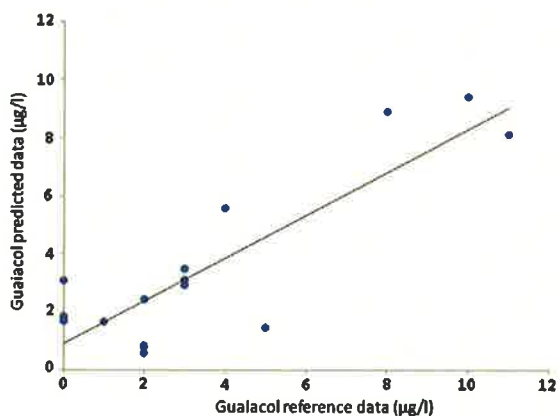

Fig. 2. Correlations plot between guaiacol reference data and predicted values using mid infrared spectroscopy for white wine samples comprising Chardonnay, Sauvignon Blanc (SAB) and Pinot Gris (Pinot) wines.
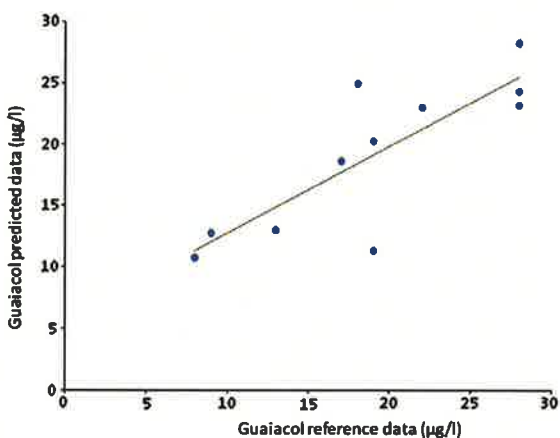

Fig. 3. Correlations plot between guaiacol reference data and predicted values using mid infrared spectroscopy for red wine samples comprising Shiraz, Merlot, Cabernet Sauvignon (Cabernet) and Pinot Noir (Pinot) wines.

between red and white wines, besides guaiacol content, that more strongly influenced the calibrations obtained. This is not surprising, given that the white wines from this set contained the lowest

Table 1
Performance parameters of the calibration models.

Samples	Correlation	RMSEP	Bias	Slope	LV
Smoke-affected red and white wines ($n = 30$)	0.90	3.84	0.007	0.84	3
Smoke-affected red wines ($n = 12$)	0.80	4.85	0.313	0.70	2
Smoke-affected white wines ($n = 17$)	0.75	1.86	0.003	0.71	3

n, number of samples; RMSEP, root mean square error of prediction; LV, latent variables.

levels of guaiacol, i.e., exhibited less taint (Table 1). The PLS calibration obtained for the red wine set (Fig. 3) yielded an R of 0.80 and RMSEP of 4.85. Similar loadings to those observed for the smoke-affected wine set were obtained in the fingerprint region between 1400 and 1500 cm^{-1}. It is important to note that a bimodal distribution was observed for this wine set (Fig. 1). Two distinct clusters, comprising white wines with lower guaiacol levels and red wines with higher guaiacol levels were observed. Again this indicated a clear matrix effect, but irrespective of this, the performance parameters obtained for all three models were good (Table 1). The objective of this study was not the accurate prediction of the levels of guaiacol or other smoke taint marker compounds in smoke-affected wines, but rather the capacity of MIR coupled with 2D-COS to reproduce and replicate the screening results obtained using traditional analytical techniques like GC–MS and HPLC. The results showed that wines can be easily and rapidly screened using spectroscopic techniques, so that only tainted wines need be further processed to examine the exact levels of taint.

3.2. Two-dimensional correlation analysis

Once the PLS calibration models had been developed using the volatile phenol concentrations obtained by GC–MS, 2D-COS analysis was applied in order to confirm that the PLS loading models were the result of changes in the MIR fingerprint region associated with the level of guaiacol (and thus level of smoke taint) and not just matrix effects. The application of 2D spectroscopy allowed measurement of changes in the wine due to interactions with the molecular structure of guaiacol and other smoke-derived volatile phenols, in particular. Figs. 4 and 5 show the synchronous 2D autopeaks derived from the correlation spectrum obtained from the dynamic MIR spectra of the cask wine samples spiked with
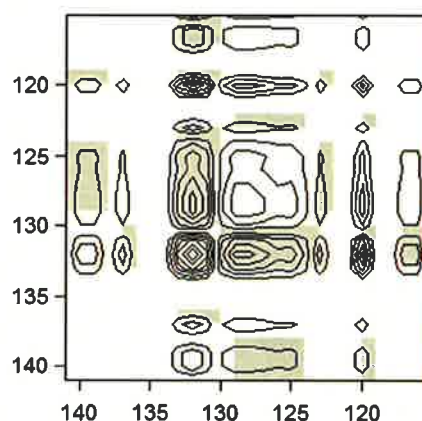

Fig. 4. Two-dimensional correlation: synchronous plot of red cask wine spiked with guaiacol using MIR spectroscopy (120 = 1454 cm^{-1}, 130 = 1492 cm^{-1}, 132 = 1495 cm^{-1}).
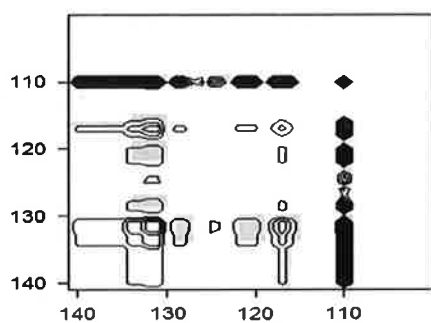
Fig. 5. Two-dimensional correlation: synchronous plot of white cask wine spiked with guaiacol using MIR spectroscopy ($110 = 1415$–$1417\ cm^{-1}$, $130 = 1492\ cm^{-1}$, $131 = 1495\ cm^{-1}$).

guaiacol. The commonality between the response patterns of organic constituents (i.e., guaiacol content due to spiking) was monitored by the MIR spectroscopy probes and detected as correlation intensities. The autopeaks in the synchronous 2D correlation spectrum for the red cask wine sample set showed that corresponding MIR bands sharing the autopeak (i.e., at 1454, 1492 and $1500\ cm^{-1}$) either increased or decreased together and showed the same origin or change in-phase from one sample to the other. In the infrared spectra of the wines analysed, the broad peaks that occurred at about 3310, 2140 and $1640\ cm^{-1}$ due to water, and the peaks typical of alcohol observed at 1044 and $1085\ cm^{-1}$ were not included for analytical purposes. However the C–O stretch asymmetrically coupled to the C=C stretch, at 1385 and $1455\ cm^{-1}$, were included as they were considered to be qualitative fingerprint peaks (Table 2). In the range of 1225–$1500\ cm^{-1}$, strong auto peaks at 1454 and $1492\ cm^{-1}$, were associated with the relative intensities of the peak around $1454\ cm^{-1}$. The interpretation of the autopeaks indicated these originated from volatile compounds present in the wine matrix. In Fig. 4, the autopeaks corresponded to wavenumbers associated with aromatic ring stretching (Stuart, 1996). These could be due to the aromatic ring stretching of volatile phenols; although bands due to deformations of $–CH_2$, and C–C–H and H–C–O bending modes can also occur between 1200 and $1500\ cm^{-1}$ (Bevin, Fergusson, Perry, Janik, & Cozzolino, 2006; Stuart, 1996). Similar correlations were observed for the set of white cask wines spiked with guaiacol (Fig. 5). Correlations were observed at wavelengths corresponding to 1415–1417, 1492 and $1495\ cm^{-1}$. Although quantification of guaiacol and other volatile phenols can be achieved using GC–MS analysis, interpretation of the 2D correlation maps showed that information about the chemical structure of wines is present in the MIR spectra. From the data shown, it can be observed that correlation spectroscopy highlights the overall similarity of the spectrum; i.e., similarity between the red cask wine, which already contained guaiacol as a result of oak maturation (a common red winemaking practice) and the wine

spiked with guaiacol. This made the interpretation of loadings for the set of red wines more complex. In contrast, the white cask wines, which were not oaked, only exhibited these peaks after spiking.

The data obtained from analysing wines by MIR spectroscopy, with further interpretation by 2D correlation analysis, offers a high-throughput tool that could be applied for preliminary screening of smoke tainted wines. IR spectroscopy has already gained wide acceptance as a fast and easy-to-operate technique for routine analysis in the wine industry. Considering the continual improvements in hardware and software design, and the need for real-time, multi-parametric analysis in commercial wine production, it is anticipated that IR spectroscopy will become a routine analytical tool for process control and monitoring of wine production in the near future. MIR spectroscopy combined with 2D-COS has the potential to dramatically improve the efficiency of wine laboratories, considering the range of data that can be collected. However, these technologies will require a new approach to analysis and a significant paradigm shift; in particular, a move away from the classical idea of wine chemical data being isolated numbers reflecting changes to a model, to a more dynamic approach, whereby compositional change provides the information required to trigger process responses. Importantly, this would facilitate rapid analysis, and therefore decision-making, during the time constraints of vintage, with significant cost and time savings to winemakers.

4. Conclusions

This study has shown that 2D IR correlation spectroscopy can be used as a tool to identify compositional differences arising in wines as a result of grapevine exposure to smoke; for example, the presence of smoke derived volatile phenols such as guaiacol. The technique can be used as a means of preliminary screening to rapidly identify smoke-affected wines; thereby enabling time and cost intensive GC–MS and HPLC based techniques to be employed more strategically, i.e., when there is a need for the exact concentrations of specific taint marker compounds to be ascertained. Given the continual improvements in hardware and software design, together with the wine industry's need for real-time and/or multi-parametric analysis, it is anticipated that in the near future rapid analytical techniques may become routine tools for process control and monitoring of grapes and wines; in particular, for determining quality. The 2D-COS approach will allow a more complete interpretation of IR spectra, than is possible from simple models alone.

Table 2
Correlation loading values.

	Smoke-affected red wines	Smoke-affected white wines	Smoke-affected red and white wines	Region
Highest negative (cm^{-1})	1492	1412	1508	~1500
Highest positive (cm^{-1})	1495	1488	1488	~1500
% Correlation	76	31	77	

References

Bevin, C. J., Fergusson, A. J., Perry, W. B., Janik, L. J., & Cozzolino, D. (2006). Development of a rapid "fingerprinting" system for wine authenticity by mid-infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 54*, 9713–9718.

Cozzolino, D., Cynkar, W., Dambergs, R., & Smith, P. (2010). Two-dimensional correlation analysis of the effect of temperature on the fingerprint of wines analysed by mass spectrometry electronic nose. *Sensors and Actuators B, 145*, 628–634.

Cozzolino, D., Holdstock, M., Dambergs, R. G., Cynkar, W. U., & Smith, P. A. (2009). Mid-infrared spectroscopy and multivariate analysis: A tool to discriminate between organic and non-organic wines grown in Australia. *Food Chemistry, 116*, 761–765.

Dungey, K. A., Hayasaka, Y., & Wilkinson, K. L. (2011). Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. *Food Chemistry, 126*, 801–806.

Fragoso, S., Aceña, L., Guasch, J., Busto, O., & Mestres, M. (2011). Application of FT-MIR spectroscopy for fast control of red grape phenolic ripening. *Journal of Agricultural and Food Chemistry, 59*, 2175–2183.

Fudge, A. L., Wilkinson, K. L., Ristic, R., & Cozzolino, D. (2012). Classification of smoke tainted wines using mid-infrared spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry, 60*, 52–59.

Gadaleta, S. J., Gericke, A., Boskey, A. L., & Mendelsohn, R. (1996). Two-dimensional infrared correlation spectroscopy of synthetic and biological apatites. *Biospectroscopy, 2*, 353–364.

Hayasaka, Y., Dungey, K. A., Baldock, G. A., Kennison, K. R., & Wilkinson, K. L. (2010). Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. *Analytica Chimica Acta, 660*, 143–148.

Kennison, K. R., Gibberd, M. R., Pollnitz, A. P., & Wilkinson, K. L. (2008). Smoke-derived taint in wine: The release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. *Journal of Agricultural and Food Chemistry, 56*, 7379–7383.

Kennison, K. R., Wilkinson, K. L., Pollnitz, A. P., Williams, H. G., & Gibberd, M. R. (2009). Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. *Australian Journal of Grape and Wine Research, 15*, 228–237.

Kennison, K. R., Wilkinson, K. L., Pollnitz, A. P., Williams, H. G., & Gibberd, M. R. (2011). Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. *Australian Journal of Grape and Wine Research, 17*, S5–S12.

Kennison, K. R., Wilkinson, K. L., Williams, H. G., Smith, J. H., & Gibberd, M. R. (2007). Smoke-derived taint in wine: Effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. *Journal of Agricultural and Food Chemistry, 55*, 10897–10901.

Kirwan, G. M., Clark, S., Barnett, N. W., Nierre, J. O., & Adams, M. J. (2008). Generalized 2D-correlation NMR analysis of a wine fermentation. *Analytica Chimica Acta, 629*, 128–135.

Liu, Y., Himmelsbach, D. S., & Barton, F. E. (2004). Two-dimensional Fourier transform Raman correlation spectroscopy determination of the glycosidic linkages in amylase and amylopectin. *Applied Spectroscopy, 58*, 745–749.

Naes, T., Isaksson, T., Fearn, T., & Davies, T. (2002). *A user-friendly guide to multivariate calibration and classification*. Chichester, United Kingdom: NIR Publications.

Noda, I. (1989). Two-dimensional infrared spectroscopy. *Journal of the American Chemical Society, 111*, 8116–8118.

Noda, I. (1990). 2-Dimensional infrared (2D IR) spectroscopy – theory and applications. *Applied Spectroscopy, 44*, 550–561.

Noda, I. (1993). Generalized 2-dimensional correlation method applicable to infrared, Raman, and other types of spectroscopy. *Applied Spectroscopy, 47*, 1329–1336.

Noda, I. (2004). Advances in two-dimensional correlation spectroscopy. *Vibrational Spectroscopy, 36*, 143–165.

Noda, I., Dowrey, A. E., Marcott, C., Story, G. M., & Ozaki, Y. (2000). Generalized two-dimensional correlation spectroscopy. *Applied Spectroscopy, 54*, 236A–248A.

Noda, I., & Ozaki, Y. (2004). *Two-dimensional correlation spectroscopy-applications in vibrational and optical spectroscopy*. Chichester, United Kingdom: John Wiley & Sons.

Ozaki, Y., Liu, Y., & Noda, I. (1997). Two-dimensional near-infrared correlation spectroscopy study of premelting behavior of nylon 12. *Macromolecules, 30*, 2391–2399.

Ristic, R., Osidacz, P., Pinchbeck, K. A., Hayasaka, Y., Fudge, A. L., & Wilkinson, K. L. (2011). The effect of winemaking techniques on the intensity of smoke taint in wine. *Australian Journal of Grape and Wine Research, 17*, S29–S40.

Roselli, C., Burie, J.-R., Mattioli, T., & Boussac, A. (1995). Two-dimensional correlation method applied to Yb^{3+} vibronic sideband spectroscopy: Discrimination of fluorescence spectral features arising from different Yb^{3+} binding sites. *Biospectroscopy, 1*, 329–339.

Sefara, N. L., Magtoto, N. P., & Richardson, H. H. (1997). Structural characterization of p-lactoglobulin in solution using two-dimensional FT mid-infrared and FT near-infrared correlation spectroscopy. *Applied Spectroscopy, 51*, 536–540.

Segtnan, V. H., Šašić, Š., Isaksson, T., & Ozaki, Y. (2001). Studies on the structure of water using two-dimensional near-infrared correlation spectroscopy and principal component analysis. *Analytical Chemistry, 73*, 3153–3161.

Sheppard, S. I., Dhesi, M. K., & Eggers, N. J. (2009). Effect of pre- and post-veraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. *American Journal of Enology and Viticulture, 60*, 98–103.

Stuart, B. (1996). *Modern infrared spectroscopy*. New York: John Wiley & Sons.

Wynne, L., Clark, S., Adams, M. J., & Barnett, N. W. (2007). Compositional dynamics of a commercial wine fermentation using two-dimensional FTIR correlation analysis. *Vibrational Spectroscopy, 44*, 394–400.

Zhang, Y., Chen, J., Lei, Y., Zhou, Q., Sun, S., & Noda, I. (2010). Discrimination of different red wine by Fourier-transform infrared and two-dimensional infrared correlation spectroscopy. *Journal of Molecular Structure, 974*, 144–150.

Chapter 4: Conclusions and Future Directions

Conclusions

Smoke taint represents a significant challenge, not only for the Australian wine industry, but for wine producers around the world, in particular, in those regions predicted to be vulnerable to the increased temperature and drought conditions associated with climate change; i.e. conditions conducive to bushfires. The research described in this thesis has helped to address the issue of smoke taint by demonstrating: (i) the capacity of commercial fining agents and RO solid phase adsorption to ameliorate smoke tainted wine; and (ii) the potential application of IR spectroscopy as a rapid analytical method for screening smoke-affected grapes and wine.

The research identified an activated carbon and, to a lesser extent, a synthetic mineral which were able to reduce the level of taint in smoke-affected wines. Further research would enable those particular fining agents to be tested at other addition rates, to optimise the fining required to remove smoke taint, but retain varietal characteristics. It would be most beneficial to the winemaking community that this type of research is completed where additions of fining agents can be properly calculated to manage different levels of smoke taint present in both white and red wines.

One limitation of these treatments was that precursor compounds were not removed, but remained in the wines. Over time these precursors may be hydrolysed, releasing smoke taint volatiles, replacing those removed by the fining or RO treatment. As a more detailed picture of the timing of precursor hydrolysis is established, the cellar potential, or lack thereof, of wines treated by these amelioration methods can be determined.

A rapid method using MIR spectroscopy combined with chemometrics enabled wine samples to be screened quickly for the presence of smoke taint. Control and smoke-affected wines were analysed using the classification method developed, with 61% of control samples and 70% of smoke-affected samples being correctly classified. The level of accuracy was influenced by the degree of smoke taint, and in the case of control samples, any prior oak maturation. Samples were analysed by GC-MS (to quantify guaiacol and 4-methylguaiacol, as markers of smoke taint), as well as informal sensory assessment. Classification of wines with low levels of smoke taint were more strongly influenced by grape variety and/or oak treatment than their concentrations of smoke-derived volatile compounds or intensity of smoke-related sensory attributes. This explained the classification rates obtained for smoke-affected wines; while oak

maturation was the confounding factor for incorrect classification of some control wines. The inclusion of additional samples, i.e. wines from a broader range of grape varieties, wine styles and vintages, would allow the classification method to be refined, to improve the accuracy of diagnostic screening for detection of samples affected by smoke taint.

2D-COS was also evaluated as a method for characterisation of smoke taint and data maps were constructed using the spectroscopic region between 1400 and 1500 cm^{-1}, i.e. the region which might display signals attributable to the C-C aromatic stretching expected for volatile phenols. In this way, the potential for 2D-COS to identify compositional differences between control and smoke tainted wines was also demonstrated.

Ideally, the wine industry needs to be able to screen grape and/or juice samples, rather than wine, so as to reduce costs associated with harvesting, processing and fermenting fruit. However, direct analysis of juice by MIR spectroscopy is problematic due to the high sugar content, which increases the viscosity of samples thereby influencing the efficacy of automatic sampling pumps within the MIR instrument. Nevertheless, MIR analysis could be combined with other sample preparation methods, such as those developed by Schneider et al. (2004), which might overcome the issue of high sugar content of juice, to allow the potential for recurrence of smoke taint in wines treated with RO or fining agents to be determined. A rapid method was developed using FTIR and chemometrics for determining glycosidic precursors in grapes (Schneider et al. 2004). The steps of pre-purification and concentration of grape samples employed allowed for injection into the FTIR transmission cell. Key steps employed included: crushing and centrifugation of the grapes; filtering of the juice through glass wool and treatment with PVPP, further sample clean-up using C_{18} reverse phase solid phase extractor (SPE) cartridges – with glycoconjugates eluted using methanol and the extract concentrated to dryness before being dissolved with 500 µL of methanol for sample injection. Using predictive partial least squares (PLS) a predictive error of 22% was obtained for volatile phenols (Schneider et al. 2004).

Future Directions

The occurrence of vineyard exposure to smoke is almost certain to be reported again in the future, primarily because winegrapes are typically grown in regions with long, hot, dry summers (Sheppard et al. 2009) and often in close proximity to forests or bushland. Since 2003, considerable research has been undertaken to improve our current understanding of the impact of bushfire smoke on grapes and wine. However, whilst many of the early knowledge gaps concerning smoke taint have been addressed, the issue has not yet been fully resolved. The success and sustainability of the Australian wine industry relies, in part, on its ability to address the various adversities which affect grape and wine production, including the occurrence of smoke taint. The frequency of smoke events cannot be readily predicted, but the potential consequences for grape and wine quality have been demonstrated and are sufficient to ensure that smoke taint remains an issue of concern. The papers presented in this thesis represent a significant contribution to both wine science and analytical chemistry, and hopefully enable industry to make more informed viticultural and winemaking decisions so as to minimise smoke taint in finished wine. However, further research is still required to fully address the impact of smoke on grape and wine production. Several potential research avenues are briefly outlined below:

1) *Identification of other smoke-derived volatile compounds responsible for smoke taint:* To date, a series of volatile phenols, including guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, syringol and cresols (in both free and glycoconjugate forms) have been quantified to determine the extent to which grapes and wine are smoke tainted. However, several of these compounds are routinely identified as components of oak-aged wines, without any association with smoke taint, and as such, whilst they may contribute to smoke taint, they cannot be considered solely responsible for the objectionable smoke-related aromas and flavours characteristic of smoke taint. Wine is a complex matrix and it typically comprises several hundred volatile compounds which influence aroma and flavour to varying degrees. Further GC-MS analysis of smoke and/or smoke-affected grapes, juice and wine should be undertaken, to identify other compounds responsible for smoke taint. Techniques such as pyrolysis-GC-MS and GC-olfaction in particular might prove useful. Spectroscopic and

chemometric methods could also be used to identify compositional differences between control and smoke affected wines to facilitate identification of new smoke taint marker compounds. Once additional compounds have been identified, their isotopically-labelled derivatives can be synthesised for use as internal standards, enabling the development of quantitative methods using stable isotope dilution analysis (SIDA). This will enable the occurrence of these compounds to be determined in smoke, as well as their accumulation in smoke-affected grapes and impact on wine sensory properties to be studied.

2) *Investigation into the glycosylation of smoke derived volatile compounds following grapevine exposure to smoke:* The activity of grapevine glucosyltransferase enzymes following smoke exposure could be also studied, together with the stability of different glycosides (i.e. glucosides vs. disaccharides vs. rutinosides etc.) during fermentation and storage – i.e. the ability of different yeasts to metabolise the different precursors; their susceptibility to acid and/or enzyme catalysed hydrolysis; and whether or not it might be possible to selectively remove glycosides from smoke-affected wine post-fermentation.

3) *Methods for detecting and monitoring smoke in the vineyard:* Finally, it would also be beneficial for industry to be able to conduct real-time monitoring of grapevine exposure to smoke in the vineyard. Nephelometers have previously been used to evaluate smoke particulates in real-time – for example, by natural resource management and public health organisations to monitor air quality (Brauer et al. 2000; Williamson & Bowman 2008). It is possible this technology could be modified for use in vineyards to capture data that can be used to assess grapevine smoke exposure, e.g. the density and duration of smoke. Nephelometers are reasonably portable optical instruments that could provide real-time data for monitoring smoke. Particles in the air are measured by means of light scattering and a sensor detects the scattered light to provide an indirect measurement of particulate concentrations (Williamson & Bowman 2008). Williamson and Bowman (2008) also suggest that in addition to nepthelometers, portable air monitoring stations could be used in proximity to a fire, together with mobile spatial monitoring, to track smoke. Brauer et al. (2000) noted that portable nephelometers are

capable of continual monitoring and as such, can differentiate intermittent and variable exposure. In their study, Brauer and colleagues monitored indoor particle levels, but this approach could easily be adapted for use in the field. Real-time analysis of smoke in the vineyard would provide a clearer picture of grapevine smoke exposure.

Smoke taint is better understood than ever before, but with changing climate conditions and a propensity for fires, and therefore smoke, in areas where wine grapes are grown, there is still further work to be done. Until all of the volatile compounds responsible for smoke taint are identified, there is scope for further research. In addition, improved methods for monitoring fire events in real-time and a better understanding of the biochemical pathways involved in glycosylation of smoke-derived volatiles is required. Only then can the issue of smoke taint be fully addressed.

Appendix

Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine

K.L. WILKINSON[1], R. RISTIC[1], K.A. PINCHBECK[1], A.L. FUDGE[1], D.P. SINGH[2], K.M. PITT[2], M.O. DOWNEY[2], G.A. BALDOCK[3], Y. HAYASAKA[3], M. PARKER[3] and M.J. HERDERICH[3]

[1] The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia

[2] The Department of Primary Industries, PO Box 905, Mildura, Vic. 3502, Australia

[3] The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia

Corresponding authores: Dr Kerry Wilkinson, fax +61 8 8303 7116, email kerry.wilkinson@adelaide.edu.au; Dr Davinder Singh, fax +61 3 5051 4523, email davinder.singh@dpi.vic.gov.au; Dr Yoji Hayasaka, fax +61 8 8303 6601, email yoji.hayasaka@awri.com.au

Abstract

Background and Aims: Australian grape growers and winemakers have typically relied on guaiacol and 4-methylguaiacol measurements to determine smoke exposure of grapes following bushfires or prescribed burns. However, the guaiacol and 4-methylguaiacol content of grapes does not always correlate with the extent of taint in resultant wines. This study compared several methods for the analysis of smoke related phenols and their conjugates in grapes and wine, to determine their capacity as diagnostic assays for smoke exposure.

Methods and Results: Smoke-affected grapes were sourced from commercial vineyards exposed to bushfire smoke and from experimental field trials involving the application of smoke to grapevines, and small-scale wines were made from a number of these samples. Several analytical methods were applied to grapes and wine to determine the concentration of smoke related phenols and their conjugates. Strong correlations were observed between the glycoconjugate content of smoke-affected grapes and the concentration of guaiacol and 4-methylguaiacol released following acid hydrolysis of juice.

Conclusions: Where smoke-affected grapes contain low or non-detectable levels of guaiacol and 4-methylguaiacol, analytical methods that quantify their glycoconjugate forms (either directly or indirectly) provide a better indication of the extent of smoke exposure.

Significance of the Study: This is the first study to compare different methods for assessing smoke exposure in grapes and wine, through analysis of free and bound guaiacol and 4-methylguaiacol. These methods will allow grape growers and winemakers to more reliably assess smoke exposure of grapes, enabling better informed decisions to be made with regards to harvesting and processing smoke-affected grapes.

Keywords: 4-methylguaiacol, glycoconjugate, grape, guaiacol, smoke, wine

Introduction

The impact of grapevine exposure to smoke on the composition and sensory attributes of grapes and wine has been the subject of several recent publications (e.g. Kennison et al. 2008, Sheppard et al. 2009, Hayasaka et al. 2010a, Dungey et al. 2011, Singh et al. 2011), in response to the recurrent incidence of bushfires in proximity to wine regions in Australia, as well as overseas. Smoke comprises numerous volatile compounds (Baltes et al. 1981, Maga 1988); however its characteristic aroma is thought to be largely caused by the presence of volatile phenols (Wittkowski et al. 1992). Kennison et al. (2007) reported guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol at elevated concentrations in wine made from grapes exposed to straw-derived smoke post-harvest. Of these, guaiacol and 4-methylguaiacol were most abundant. While guaiacol and 4-methylguaiacol are not considered solely responsible for the objectionable 'smoky', 'burnt' and 'smoked meat' characters associated with smoke-tainted wine (Kennison et al. 2009), they are useful marker compounds for assessing the exposure of grapes to smoke; particularly given

they can be readily quantified by existing gas chromatography-mass spectrometry (GC-MS)-based analytical methods (Pollnitz et al. 2004).

However, the guaiacol and 4-methylguaiacol content of grapes is not always a reliable indicator of the extent of smoke exposure by grapes. Grapes harvested from grapevines exposed to smoke for 1 h at either pre-veraison, post-veraison or maturity were found to contain guaiacol and 4-methylguaiacol at levels that ranged from 2 to 26 µg/kg and from 0 to 4 µg/kg, respectively (Sheppard et al. 2009). In contrast, Kennison et al. (2008) detected only trace levels of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol (i.e. ≤1 µg/L) in free-run juice derived from fruit harvested from grapevines exposed to repeated smoke applications between veraison and maturity; but significant quantities of these phenols were evolved when the juice was subsequently fermented or hydrolysed under strong acid or β-glucosidase enzyme conditions. This disparity is attributed to the glycoconjugation of volatile phenols in grapes, following grapevine smoke exposure (Hayasaka et al. 2010a, Dungey et al. 2011). Hayasaka et al. (2010a) confirmed the

presence of a β-D-glucopyranoside of guaiacol in juice of smoke-affected Chardonnay and Sangiovese grapes. Subsequent stable isotope tracer experiments tentatively identified six additional guaiacol disaccharides (Hayasaka et al. 2010b). Glycoconjugate forms of smoke related volatile phenols are not detected by current GC-MS analytical methods and so the extent of smoke exposure in grapes can be under-estimated by these methods. The assessment of smoke exposure is further complicated given that guaiacol has been identified in both free and conjugate (bound) forms, as a natural component of grapes of several *Vitis vinifera* varieties, including Merlot (Sefton 1998), Shiraz (Wirth et al. 2001), Tempranillo and Grenache (Lopez et al. 2004).

Grape growers and winemakers clearly need reliable diagnostic assays for grape exposure to smoke, in order to make informed decisions with regards to harvesting and processing smoke-affected fruit. This study was therefore undertaken to compare methods for the analysis of smoke related phenols and their conjugates in grapes and wine, in order to investigate their capacity as diagnostic assays for smoke exposure. Analytical methods included: (i) a GC-MS based stable isotope dilution assay (SIDA) method for the quantification of guaiacol and 4-methylguaiacol (Pollnitz et al. 2004); (ii) a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)-based SIDA method for the quantification of guaiacol glycoconjugates (Dungey et al. 2011); and (iii) a direct acid hydrolysis technique based on the glycosyl-glucose (GG) assay (Williams et al. 1995) and an adaption of this assay incorporating solid phase extraction (SPE), for the quantification of glycosylated guaiacol and 4-methylguaiacol (Singh et al. 2011).

Materials and methods

Smoke-affected grapes: grapes exposed to experimental smoke

Control and smoke-affected Grenache, Merlot and Viognier grapes were sourced from field trials involving the application of smoke to grapevines under experimental conditions described by Ristic et al. (2011) and Dungey et al. (2011). Grenache vines (approximately 100) growing in a vineyard located in the Barossa Valley region of South Australia (34°30′S, 138°59′E) and Merlot and Viognier vines (three vines per variety, in triplicate) growing in vineyards located at the University of Adelaide's Waite campus in Adelaide, South Australia (34°58′S, 138°38′E) were exposed to straw-derived smoke at approximately 7 days post-veraison, i.e. at juice total soluble solids (TSS) concentrations of approximately 15°Brix, determined using a digital handheld refractometer (PAL-1, Atago, Tokyo, Japan). The duration of smoke exposure was 20 min for Grenache vines and 30 min for Merlot and Viognier vines. Grapes were harvested from smoked and control vines when juice TSS reached 23 ± 1°Brix.

Smoke-affected grapes: grapes exposed to bushfire smoke

Smoke-affected grapes were sourced from several Victorian vineyards exposed to smoke from bushfires that occurred between February 7 and March 14, 2009. Shiraz grapes were sourced from Yarra Valley (37°42′S, 145°30′E) and Shiraz, Cabernet Sauvignon, Chardonnay and Pinot Noir grapes were sourced from Goulburn Valley (37°42′S, 145°30′E). The Yarra Valley and Goulburn Valley Shiraz are hereafter differentiated as Shiraz 1 and Shiraz 2, respectively. Fruit was harvested at juice TSS levels of 23 ± 1°Brix, except for Cabernet Sauvignon, which was harvested at TSS of 19 ± 1°Brix and Shiraz 1, which was harvested at TSS > 30°Brix. Fruit was stored at −20°C prior to analysis and winemaking.

Winemaking

Fruit was randomly divided into parcels (approximately 5 kg) and processed (in triplicate) according to small-lot winemaking procedures (Holt et al. 2006). Irrespective of variety, all fermentations were conducted with skin contact to eliminate any effect of winemaking practice, particularly given the duration of skin contact during fermentation has been shown to influence the intensity of smoke taint in wine (Ristic et al. 2011). Bunches were de-stemmed and crushed, and tartaric acid added to adjust the pH to 3.5, prior to inoculation with Maurivin PDM yeast (200 ppm). Musts were fermented on skins for 7 days at 15°C, with the cap plunged at least twice per day. The wine was pressed at 1 °Baume, transferred to stainless steel vessels and held at 20°C until the residual sugar approached 0 g/L. Wines were then racked from gross lees and cold stabilised (at 4°C for 3 weeks). Wine pH and free SO_2 were adjusted to 3.4 and between 20 and 40 ppm, respectively, before filtration (Z6 grade pad for non-sterile, 0.45 μm for sterile) and bottling (under screw cap closures). Because of the extremely high TSS content of Shiraz 1 (i.e. >30°Brix), water (700 mL) was added to each fermentation replicate, but all other winemaking protocols were as described earlier. Acid adjustments were not made to Chardonnay juice or wine, because of high natural acid content (i.e. pH 3.0), but all other winemaking protocols were as described above. Malolactic fermentation was not carried out for any of these wines.

Determination of guaiacol and 4-methylguaiacol by GC-MS

Guaiacol and 4-methylguaiacol were quantified according to SIDA methods developed by Pollnitz et al. (2004). For fruit analysis, grapes (200 berries, in triplicate) were randomly collected from each fruit parcel and average berry weight determined. Samples were then homogenised (T18 Ultra Turrax, IKA, Staufen, Germany) and an aliquot (25 g) of the resulting whole berry homogenate accurately weighed into centrifuge vials. Following addition of d_3-guaiacol and d_3-4-methylguaiacol (10 μg/mL in ethanol, 100 μL) as internal standards, samples were centrifuged (4300× g for 5 min). Organic solvent (n-pentane c. 2 mL) was added to an aliquot (5 mL) of the resulting juice and the mixture shaken briefly. A portion of the organic layer (c. 1.5 mL) was then taken for instrumental analysis. For wine analysis, internal standards (10 μg/mL in ethanol, 100 μL) were added to the sample (10 mL) in a screw cap vial and extracted with organic solvent as mentioned earlier, prior to analysis. The preparation of internal standards, method development and validation, and instrument operating conditions have been published previously (Pollnitz et al. 2004). Analyses were performed by the Australian Wine Research Institute's Commercial Services Laboratory (Adelaide, Australia) using an Agilent 6890 gas chromatograph (Agilent, Palo Alto, CA, USA), coupled to a 5973 mass selective detector. For these methods: linearity was confirmed for a concentration range from 1 to 1000 μg/L, with correlation coefficients of 0.999 and 1.000 for guaiacol and 4-methylguaiacol, respectively; the limit of detection (3 × standard deviation) was 1 μg/L; and the precision was better than 5% relative standard deviation.

Determination of guaiacol glycoconjugates by HPLC-MS/MS

Guaiacol glycoconjugates were quantified according to the SIDA methods previously described (Dungey et al. 2011, Ristic et al. 2011), using an Agilent 1200 HPLC system, coupled to a 4000 Q TRAP hybrid tandem mass spectrometer. For fruit analysis, d_4-guaiacol β-D-glucopyranoside (100 μg/mL in water, 50 μL) was added as an internal standard to a subsample (10 g) of

whole berry homogenate (as mentioned earlier) and the mixture centrifuged (4300× g for 5 min). The glycoconjugate fraction of the resulting juice was purified using C18-HF SPE cartridges (Grace, Rowville, Australia) and extracts filtered through a 0.45-µm GHP membrane (Acrodisc®, PALL Life Sciences, Cheltenham, Australia) prior to instrumental analysis. For wine analysis, d_4-guaiacol β-D-glucopyranoside (100 µg/mL in water, 10 µL) was added to the sample (1 mL), the mixture shaken briefly and filtered as mentioned earlier, prior to analysis. The preparation of internal standards, method development and validation, and instrument operating conditions have been previously published (Dungey et al. 2011, Ristic et al. 2011). For these methods: linearity was confirmed for a concentration range from 1 to 10 000 µg/kg (for homogenate) and 10 to 50 000 µg/L (for wine), with correlation coefficients of 0.993 and 0.995 for homogenate and wine, respectively; the limit of detection (3 × standard deviation) was 25 µg/kg (for homogenate) and 7 µg/L (for wine); and the precision was better than 5% relative standard deviation.

Determination of glycosylated guaiacol and 4-methylguaiacol by GC-MS after acid hydrolysis

Direct acid hydrolysis. Acid hydrolysates were prepared from each whole berry homogenate (as above). Homogenate samples (20 g) were centrifuged (4300× g for 5 min) and an aliquot (10 mL) of the resulting juice acidified to pH 1.0 with concentrated sulphuric acid, then heated at 100°C for 1 h, i.e. conditions used in the glycosyl-glucose assay (Williams et al. 1995). Samples were subsequently analysed by GC-MS and HPLC-MS/MS as mentioned earlier to determine guaiacol, 4-methylguaiacol and guaiacol glycoconjugate concentrations.

SPE/acid hydrolysis. Bound forms of guaiacol and 4-methylguaiacol were quantified according to the modified glycosyl-glucose assay (Williams et al. 1995) as described by Singh et al. (2011). Whole berry homogenate samples (20 g) were centrifuged (2500× g for 10 min) and to an aliquot (10 mL) of the resulting juice was added aqueous sodium hydroxide (1.5 mL, 10 M), followed by vortex mixing and filtration through a 0.45 µm syringe filter. Wine (20 mL) was freeze-dried and the residue reconstituted in distilled water (10 mL), followed by the addition of aqueous sodium hydroxide (1.5 mL, 10 M) and filtration, as mentioned earlier. Homogenate and wine-derived filtrates (1 mL) were extracted for bound forms of guaiacol and 4-methylguaiacol using a 2 mL 96-well plate system (Oasis® HLB Plate, Waters Corporation, Milford, MA, USA) and vacuum manifold (96 Well Plate manifold, Waters Corporation). Extracts were acidified to pH 1.0 with sulphuric acid (2.5 M), and then heated at 100°C for 1 h. Samples were subsequently analysed by GC-MS to determine guaiacol and 4-methylguaiacol concentrations, using similar methodology as mentioned earlier, except: acid hydrolysates (5 mL) were extracted with n-hexane (2 mL); d_5-4-ethylguaiacol (10 µL) was added as the internal standard; and analyses were performed with an Agilent 7890A gas chromatograph coupled with a 5975 mass selective detector. For these methods: linearity was confirmed for a concentration range from 1 to 500 µg/L, with correlation coefficients of 0.999; the limit of detection (3 × standard deviation) was 0.4 µg/L; and the precision was better than 10% relative standard deviation (Singh et al. 2011).

Statistical analysis
Chemical data were analysed using JMP (Version 5.0.1, SAS Institute, Cary, NC, USA) to generate a correlation matrix.

Results and discussion
The timing and duration of grapevine smoke exposure have been shown to influence the extent of smoke taint in wine, with prolonged or repeated exposure resulting in wines with higher volatile phenol content and more intense smoke related sensory attributes (Kennison et al. 2009). Grapevine smoke exposure can range from several hours to several weeks, depending on the burn time of a bushfire or prescribed burn and the prevailing wind conditions. In the current study, Grenache, Merlot and Viognier grapevines were exposed to smoke (for 20 or 30 min) under experimental conditions, whereas Shiraz, Cabernet Sauvignon, Chardonnay and Pinot Noir grapevines were exposed to bushfire smoke over a 5-week period, but specific details regarding the timing, duration and density of smoke exposure were not known. While the impact of grapevine exposure to smoke can be readily determined in wine via sensory analysis, it would be beneficial for the wine industry to objectively ascertain the extent of smoke exposure prior to the expense and effort associated with harvesting and processing grapes. The diagnostic potential of a range of analytical methods for the quantification of free and bound guaiacol and 4-methylguaiacol were therefore evaluated. These methods were applied to control and smoke-affected grapes, and where sufficient fruit was available for winemaking, their corresponding small-lot wines. Chemical data from grape and wine analysis are reported in Table 1.

Analysis of free guaiacol and 4-methylguaiacol in grapes by GC-MS
Guaiacol was detected in all of the bushfire smoke-affected grapes, at concentrations ranging from 4 µg/L for Chardonnay to 34 µg/L for Pinot Noir. For fruit derived from experimental field trials, control and smoke-affected Viognier grapes contained 1 µg/L and 5 µg/L guaiacol, respectively, but guaiacol was not detected in control or smoked Grenache and Merlot grapes. 4-Methylguaiacol was detected in Cabernet Sauvignon and Pinot Noir only, i.e. the two varieties containing the highest concentrations of guaiacol. The higher abundance of guaiacol relative to 4-methylguaiacol is consistent with literature concerning the composition of both wood smoke (Kornreich and Issenberg 1972) and smoke-tainted wine (Kennison et al. 2007, 2008).

Analysis of guaiacol glycoconjugates in grapes by HPLC-MS/MS
Guaiacol glycoconjugates (measured by HPLC-MS/MS) were present in all grape samples: at low concentrations for control grapes (approximately 30 µg/kg), as natural grape components; at concentrations between 253 and 358 µg/kg for grapes exposed to smoke under experimental conditions; and at considerably higher levels for grapes exposed to bushfire smoke (875 to 3474 µg/kg). Differences in grape glycoconjugate concentrations are likely to reflect the duration of smoke exposure. No smoke exposure for control grapevines, short (20 or 30 min) smoke exposure for Grenache, Merlot and Viognier grapevines under experimental conditions and prolonged smoke exposure for Shiraz, Cabernet Sauvignon, Chardonnay and Pinot Noir grapevines located in bushfire-affected regions. Pinot Noir and Cabernet Sauvignon grapes contained substantial quantities of guaiacol and 4-methylguaiacol and relatively high glycoconjugate concentrations, 1079 and 1526 µg/kg, respectively. However, guaiacol glycoconjugates were most abundant in

Table 1. Concentrations of free guaiacol and 4-methylguaiacol (by gas chromatography-mass spectrometry (GC-MS)), guaiacol glycoconjugates (by HPLC-MS/MS), and bound guaiacol and 4-methylguaiacol (by GC-MS after direct acid hydrolysis or solid phase extraction (SPE)/acid hydrolysis) in smoke-affected grapes and wine derived from grapevines exposed to experimental or bushfire smoke. Control grapes and wine were not exposed to smoke.

	Grenache Control	Grenache Smoked§	Merlot Control	Merlot Smoked§	Viognier Control	Viognier Smoked§	Shiraz 1 Smoked¶	Shiraz 2 Smoked¶	Cabernet Sauvignon Smoked¶	Chardonnay Smoked¶	Pinot Noir Smoked¶
Grapes											
Guaiacol† (µg/kg)	nd	nd	nd	nd	1	5	10	8	12	4	34
4-methylguaiacol† (µg/kg)	nd	nd	nd	nd	nd	nd	nd	nd	2	nd	5
Guaiacol glycoconjugates† (µg/kg)	30	294	34	358	34	253	875	3474	1526	1012	1079
Acid hydrolysate guaiacol† (µg/L)	4	26	3	37	6	30	79	283	124	69	83
Acid hydrolysate 4-methylguaiacol† (µg/L)	2	7	2	10	2	7	8	55	30	25	20
Acid hydrolysate guaiacol glycoconjugates† (µg/L)	nd	tr	nd	nd	nd	nd	nd	31	tr	tr	54
SPE/acid hydrolysate guaiacol‡ (µg/kg)	8	24	8	35	10	30	72	283	113	63	79
SPE/acid hydrolysate 4-methylguaiacol‡ (µg/kg)	nd	7	3	11	2	6	17	81	36	30	27
Wine											
Guaiacol† (µg/L)	tr	5	—	—	—	—	19	—	28	7	60
4-methylguaiacol† (µg/L)	nd	1	—	—	—	—	tr	—	7	4	13
Total guaiacol glycoconjugates† (µg/L)	38	290	—	—	—	—	825	—	1660	980	880
SPE/acid hydrolysate guaiacol‡ (µg/L)	12	41	—	—	—	—	121	—	182	98	87
SPE/acid hydrolysate 4-methylguaiacol‡ (µg/L)	nd	16	—	—	—	—	16	—	56	47	28

†Values are means from three sample replicates (n = 3) and were in agreement to c. 10%. ‡Values are means from three sample replicates, each measured in triplicate (n = 9) and were in agreement to c. 10%. §Smoke-affected grapes sourced from grapevines exposed to experimental smoke. ¶Smoke-affected grapes sourced from grapevines exposed to bushfire smoke. nd, not detected; tr, trace (i.e. positive identification but <1 µg/L for guaiacol and 4-methylguaiacol or <25 µg/L for guaiacol glycoconjugates). HPLC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry.

Shiraz 2 (3474 µg/kg). While a proportion of these glycoconjugates might represent natural grape constituents, as observed in control grapes in the current study, as well as in previous studies (Wirth et al. 2001, Singh et al. 2011), the majority of the glycoconjugate pool is likely because of the glycosylation of smoke-derived guaiacol as demonstrated by Hayasaka et al. (2010b) and Dungey et al. (2011).

Analysis of bound guaiacol and 4-methylguaiacol in grapes by GC-MS after acid hydrolysis
Good agreement was seen between the two hydrolysis methods for bound guaiacol (i.e. direct acid hydrolysis and SPE/acid hydrolysis) and across the different types of grapevine smoke exposure (control, experimental or bushfire). As expected, hydrolysates of control grapes contained the least guaiacol being between 3 and 6 µg/L following direct acid hydrolysis and between 8 and 10 µg/L following adapted acid hydrolysis. Guaiacol concentrations between 24 and 37 µg/L were measured in hydrolysates prepared from smoke-affected Grenache, Merlot and Viognier grapes, following either direct or adapted acid hydrolysis. Hydrolysis of bushfire smoke-affected grapes yielded the highest guaiacol concentrations, ranging from 63 and 69 µg/L for Chardonnay, 72 and 79 µg/L for Shiraz 1, 79 and 83 µg/L for Pinot Noir, 113 and 124 µg/L for Cabernet Sauvignon, to 283 µg/L for Shiraz 2, depending on which acid hydrolysis based analytical method was employed. A similar trend was observed for 4-methylguaiacol. The lowest 4-methylguaiacol concentrations (2 to 4 µg/L) were observed in hydrolysates of control grapes; whereas hydrolysates from smoke-affected Grenache, Merlot and Viognier grapes contained between 6 and 11 µg/L. For bushfire smoke-affected grapes, greater amounts of 4-methylguaiacol were measured following adapted acid hydrolysis (from 17 µg/L for Shiraz 1 to 81 µg/L for Shiraz 2) than following direct acid hydrolysis (from 8 µg/L for Shiraz 1 to 55 µg/L for Shiraz 2). However, the relative order of concentrations within varieties was consistent regardless of the analytical method applied, i.e. Shiraz 2 > Cabernet Sauvignon > Chardonnay > Pinot Noir > Shiraz 1. Interestingly, despite containing the least amount of guaiacol, Chardonnay hydrolysates contained more 4-methylguaiacol than Pinot Noir and Shiraz 1 hydrolysates. At this stage it is unclear if this reflects differences in grape metabolism, smoke composition or perhaps the physiological responses of different grapevine varieties to smoke.

HPLC-MS/MS analysis after acid hydrolysis indicated small amounts of guaiacol glycoconjugates remained intact in hydrolysates of Shiraz 2 and Pinot Noir, i.e. 31 and 54 µg/L, respectively. However, in most cases, glycoconjugates were either not detected after hydrolysis, or detected at trace levels only (i.e. <25 µg/L), indicating near complete degradation under the acid hydrolysis conditions employed. On the contrary, a quantitative recovery of guaiacol was not observed. Given the molecular mass of guaiacol and its conjugate forms, i.e. 124 (free guaiacol), 286 (glucoside) and 448 (glucose-glucose disaccharide) atomic mass units, the guaiacol concentrations observed represent a recovery rate from acid hydrolysis between 15 and 30%. While enzyme hydrolysis occurs via cleavage of the glycosidic C–O linkage to release the aglycones, i.e. guaiacol or 4-methylguaiacol in the current study, Sefton (1998) suggested acid-catalysed hydrolysis of phenolic glycosides to involve cleavage of the phenolic ether linkage, to generate a reactive anisol carbocation intermediate. The low recovery of guaiacol after hydrolysis may therefore be attributed to the instability or reactivity of this intermediate, which is expected to rapidly undergo either further degradation and/or chemical reaction.

Analysis of smoke related phenols and their conjugates in wine
Compared with the aggressive conditions employed during the direct and adapted acid hydrolysis methods, i.e. high temperature and low pH, fermentation conditions are quite mild and in the current study resulted in hydrolysis of a considerably lower proportion of conjugated guaiacol and 4-methylguaiacol. Evolution of free guaiacol and 4-methylguaiacol during fermentation is most likely attributable to yeast enzyme activity, because smoke-derived phenols were shown not to be liberated under weak acid hydrolysis conditions (Kennison et al. 2008). Trace levels of guaiacol were detected in the control Grenache wine; with low levels (5 µg/L guaiacol, 1 µg/L 4-methylguaiacol) observed in the corresponding smoked Grenache wine. For wines made from bushfire smoke-affected grapes, Pinot Noir contained the highest guaiacol concentration (60 µg/L), followed by Cabernet Sauvignon (28 µg/L), Shiraz 1 (19 µg/L) and Chardonnay (7 µg/L). The free guaiacol concentrations of these wines were approximately twice that determined for their corresponding grapes. As such, for these particular wines, the grape guaiacol content was, to some extent, indicative of the level of smoke-derived volatile phenols in the resulting wine. However, this was not evident for the smoked Grenache wine, because neither guaiacol nor 4-methylguaiacol could be detected in smoke-affected Grenache grapes. The Grenache is therefore an example of a potential 'false-negative' classification, i.e. smoke-affected fruit for which determination of free guaiacol and 4-methylguaiacol would not accurately reflect the extent of smoke exposure by grapes. Application of a diagnostic assay involving quantification of glycoconjugates, either directly by HPLC-MS/MS or indirectly by acid hydrolysis would address this limitation.

As in previous studies (Hayasaka et al. 2010c, Ristic et al. 2011), significant concentrations of guaiacol glycoconjugates remained in the finished wine, having endured the fermentation process. Control Grenache wine contained the lowest glycoconjugate concentration (38 µg/L), followed by the smoked Grenache wine (290 µg/L) and then the bushfire smoke-affected Shiraz 1, Pinot Noir, Chardonnay and Cabernet Sauvignon wines, which contained 825, 880, 980 and 1660 µg/L, respectively. Significant quantities of bound guaiacol and 4-methylguaiacol were similarly observed following analysis of wine using the adapted acid hydrolysis method. The guaiacol and 4-methylguaiacol content of smoke-tainted wines has been shown to increase with bottle age (Kennison et al. 2008, Singh et al. 2011), which the authors attributed to the hydrolysis of glycoconjugate precursors remaining in wine after fermentation. Given the glycoconjugate content of wines in the current study, a similar increase in guaiacol and 4-methylguaiacol concentrations would be expected with time. Measurement of wine glycoconjugate concentrations, again either directly by HPLC-MS/MS or indirectly by acid hydrolysis, might therefore indicate the potential for smoke taint to intensify during storage.

Comparison of methods for the analysis of smoke-related phenols and their conjugates
Statistical analysis of compositional data obtained from the different analytical methods was used to generate a correlation matrix (Table 2). Grape guaiacol and 4-methylguaiacol concentrations were found to be poorly correlated with measurements of conjugate forms, whether by HPLC-MS/MS or either of the acid hydrolysis methods. Correlation coefficients ranged from

Table 2. Correlation matrix for grape compositional data obtained from the different analytical methods.

	Guaiacol	4-methylguaiacol	Guaiacol glycoconjugates	Acid hydrolysate guaiacol	Acid hydrolysate 4-Methylguaiacol	SPE/acid hydrolysate guaiacol	SPE/acid hydrolysate 4-Methylguaiacol
Guaiacol	1.000	0.937	0.354	0.339	0.344	0.316	0.350
4-methylguaiacol	0.937	1.000	0.172	0.149	0.208	0.128	0.181
Guaiacol glycoconjugates	0.354	0.172	1.000	0.997	0.974	0.995	0.995
Acid hydrolysate guaiacol	0.339	0.149	0.997	1.000	0.961	0.998	0.986
Acid hydrolysate 4-methylguaiacol	0.344	0.208	0.974	0.961	1.000	0.955	0.989
SPE/acid hydrolysate guaiacol	0.316	0.128	0.995	0.998	0.955	1.000	0.983
SPE/acid hydrolysate 4-methylguaiacol	0.350	0.181	0.995	0.986	0.989	0.983	1.000

Correlation coefficients above 0.900 are significant at $P < 0.001$.

0.316 to 0.354 for guaiacol and from 0.128 to 0.208 for 4-methylguaiacol. This is not surprising, given the disparity in free and bound guaiacol and 4-methylguaiacol content observed for the different grape varieties. Namely, guaiacol and 4-methylguaiacol were not detectable in smoke-affected Grenache or Merlot grapes. Low levels of free guaiacol in Shiraz 2 accompanied high levels of guaiacol glycoconjugates and bound guaiacol and 4-methylguaiacol. High levels of free guaiacol in Pinot Noir corresponded to only moderate levels of guaiacol glycoconjugates and bound guaiacol and 4-methylguaiacol. These findings again highlight the potential for grapevine smoke exposure to be considerably underestimated by GC-MS measurement of free grape guaiacol and 4-methylguaiacol alone, and the need for glycoconjugate forms to be taken into consideration also.

In contrast, all direct and indirect measurements of guaiacol glycoconjugates and bound guaiacol and 4-methylguaiacol were strongly correlated, with correlation coefficients ranging from 0.955 to 0.998 (Table 2). In the current study, the guaiacol glycoconjugate content of grapes was indicative of the amount of guaiacol and 4-methylguaiacol released during either direct or adapted acid hydrolysis. Glycoconjugate and hydrolysate guaiacol concentrations gave correlation coefficients greater than 0.995, whereas glycoconjugate and hydrolysate 4-methylguaiacol gave correlation coefficients greater than 0.974. Strong correlations were also observed between the direct and adapted acid hydrolysis methods, being 0.998 for bound guaiacol and 0.989 for bound 4-methylguaiacol. The strong correlations established for these different analytical methods indicate consistency in selectivity and relative robustness of the quantitative data generated across a range of samples, such that any of the methods could be implemented as diagnostic assays for the quantification of conjugated guaiacols.

Because the different techniques are characterised by similar and sufficient analytical method performance data, method selection may be determined by the analytical and instrumental capabilities of individual wine, research or commercial laboratories. Relying exclusively on the quantification of free guaiacol and 4-methylguaiacol in grapes has obvious limitations as discussed above. Quantification of bound guaiacol (after acid hydrolysis of grapes or wine) or guaiacol released from glycoconjugates isolated by SPE (Singh et al. 2011) would provide a suitable first pass screening tool and an alternative to the quantification of free guaiacol, offering improved diagnostic value. However, the concentrations and profiles of smoke related phenolic glycoconjugates have been shown to vary significantly between varieties and smoke events (Hayasaka et al. 2010c, Dungey et al. 2011) and the recovery of guaiacol from acid hydrolysis is relatively low (Hayasaka et al. 2010c) and may be affected by matrix variation. Hence, even quantification of guaiacol and 4-methylguaiacol following acid hydrolysis of glycoconjugates does not entirely resolve the risk of 'false negative' results and should be augmented through HPLC-MS/MS analysis of the full glycoconjugate profile where possible.

Conclusion

The methods for determination of bound guaiacol and 4-methylguaiacol evaluated in the current study were found to be highly correlated and yielded more informative data than quantification of free guaiacol and 4-methylguaiacol alone. Guaiacol and 4-methylguaiacol were again shown to occur naturally in some grape samples. As such, their presence at trace concentrations in grapes does not always provide definitive evidence of grapevine exposure to smoke. Furthermore,

glycoconjugation of smoke-derived volatile phenols can result in trace or undetectable levels of free guaiacol and 4-methylguaiacol in smoke-affected grapes. Hence, analytical methods that either directly or indirectly account for their glycoconjugate forms are better suited to detect grapevine smoke exposure and may allow development of predictive models that assess the risk for smoke taint developing in wine.

Acknowledgements

References

Baltes, W., Wittkowski, R., Söchtig, I., Block, H. and Toth, L. (1981) Ingredients of smoke and smoke flavour preparations. In: The quality of food and beverages. Eds. G. Charalambous and G. Inglett (Academic Press: New York) pp. 1–19.

Dungey, K.A., Hayasaka, Y. and Wilkinson, K.L. (2011) Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. Food Chemistry **126**, 801–806.

Hayasaka, Y., Dungey, K.A., Baldock, G.A., Kennison, K.R. and Wilkinson, K.L. (2010a) Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. Analytica Chimica Acta **660**, 143–148.

Hayasaka, Y., Baldock, G.A., Pardon, K.H., Jeffrey, D.W. and Herderich, M.J. (2010b) Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine *Vitis vinifera* L. cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. Journal of Agricultural and Food Chemistry **58**, 2076–2081.

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

Holt, H.E., Iland, P.G. and Ristic, R. (2006) A method for mini-lot fermentation for use in research and commercial viticultural and winemaking trials. Australian and New Zealand Grapegrower and Winemaker **509**, 74–81.

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith, J.H. and Gibberd, M.R. (2007) Smoke-derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry **55**, 10897–10901.

Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry **56**, 7379–7383.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research **15**, 228–237.

Kornreich, M.R. and Issenberg, P. (1972) Determination of phenolic wood smoke components as trimethylsilyl ethers. Journal of Agricultural and Food Chemistry **20**, 1109–1113.

López, R., Ezpeleta, E., Sánchez, I., Cacho, J. and Ferreira, V. (2004) Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. Food Chemistry **88**, 95–103.

Maga, J.A. (1988) Flavour chemistry of wood smoke. In: Smoke in food processing. Ed. J.A. Maga (CRC Press: Boca Raton) pp. 49–88.

Pollnitz, A.P., Pardon, K.H., Sykes, M. and Sefton, M.A. (2004) The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. Journal of Agricultural and Food Chemistry **52**, 3244–3252.

Ristic, R., Osidacz, P., Pinchbeck, K.A., Hayasaka, Y., Fudge, A.L. and Wilkinson, K.L. (2011) The effect of winemaking techniques on the intensity of smoke taint in wine. Australian Journal of Grape and Wine Research. **17**, S29–S40.

Sefton, M.A. (1998) Hydrolytically-released volatile secondary metabolites from a juice sample of *Vitis vinifera* grape cvs. Merlot and Cabernet Sauvignon. Australian Journal of Grape and Wine Research **4**, 30–38.

Sheppard, S.I., Dhesi, M.K. and Eggers, N.J. (2009) Effect of pre- and postveraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. American Journal of Enology and Viticulture **60**, 98–103.

Singh, D.P., Chong, H.H., Pitt, K.M., Cleary, M., Dokoozlian, N.K. and Downey, M.O. (2011) Guaiacol and 4-methylguaiacol accumulate in wines made from smoke-affected fruit because of hydrolysis of their conjugates. Australian Journal of Grape and Wine Research. **17**, S13–S21.

Williams, P.J., Cynkar, W., Francis, I.L., Gray, J.D., Iland, P.G. and Coombe, B.G. (1995) Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. Journal of Agricultural and Food Chemistry **43**, 121–128.

Wirth, J., Guo, W., Baumes, R. and Günata, Z. (2001) Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. Journal of Agricultural and Food Chemistry **49**, 2917–2923.

Wittkowski, R., Ruther, J., Drinda, H. and Rafiei-Taghanaki, F. (1992) Formation of smoke flavor compounds by thermal lignin degradation. In: Flavour precursors. Eds. R. Teranashi, G.R. Takeoka and M. Günert ACS Symposium Series 490 (American Chemical Society: Washington) pp. 232–243.

The effect of winemaking techniques on the intensity of smoke taint in wine

R. RISTIC[1], P. OSIDACZ[2], K.A. PINCHBECK[1], Y. HAYASAKA[2], A.L. FUDGE[1] and K.L. WILKINSON[1]

[1] The University of Adelaide, School of Agriculture, Food and Wine, PMB 1, Glen Osmond, SA 5064, Australia
[2] The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia
Corresponding author: Dr Renata Ristic, fax +61 8 8303 7116, email renata.ristic@adelaide.edu.au

Abstract

Background and Aims: The chemical composition and sensory properties of smoke-affected grapes and wine has been the subject of several recent studies. However, while this research has addressed early knowledge gaps surrounding the effect of smoke on grapes and wine, to date, no practical solutions that mitigate the incidence or severity of smoke taint have been reported. The current project therefore aimed to identify winery processing methods that minimise the sensory impact of smoke taint in wine.

Methods and Results: Different winemaking techniques were used to process smoke-affected grapes, i.e. cold maceration, the use of different yeast strains for primary fermentation and the use of oak and tannin additives. The extent of smoke taint in the resulting wines was determined by quantification of volatile phenols using GC-MS, followed by descriptive sensory analysis.

Conclusions: Applied winemaking practices showed significant impacts on reducing the negative implications of smoke exposure on the chemical composition and sensory properties of wines. Winery processing methods that reduced skin contact time enhanced fruit character, produced wines with reduced 'smoke' aromas and flavours and less apparent taint. Selected yeast strains can be used as a tool for altering smoke-related aromas, flavours, colour and chemical composition of wines. The addition of oak chips and tannin enhanced the complexity of wines thereby reducing the perception of smoke-related attributes.

Significance of the Study: These findings will enable winemakers to make more informed decisions when processing smoke-affected grapes, in order to minimise the severity of smoke taint perception in resulting wine.

Keywords: cold maceration, guaiacol, oak, smoke taint, tannin, wine, winemaking, yeast

Introduction

Recent research has established that grapevine exposure to smoke from bushfires or prescribed burning can result in smoke-tainted grapes and wine (Kennison et al. 2007, 2008, 2009, Sheppard et al. 2009). Kennison et al. (2007) reported the presence of several volatile phenols (including guaiacol and 4-methylguaiacol) and objectionable 'smoke' characters in wines made from grapes exposed to smoke post-harvest, but not in control wines made from unsmoked grapes. In subsequent field-based experiments, guaiacol and 4-methylguaiacol were identified in grapes (Sheppard et al. 2009) and wines (Kennison et al. 2009) following the application of smoke to grapevines at several different phenological growth stages.

The first evidence to support the accumulation of smoke-derived volatile phenols in glycoconjugate form was reported by Kennison et al. (2008), in a study concerning the evolution of smoke taint during primary and malolactic fermentation. Trace levels (i.e. ≤ 1 μg/L) of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol were detected in free run juice derived from fruit harvested from grapevines exposed to repeated smoke applications; but significant quantities of these phenols were released when the juice was subsequently fermented, or hydrolysed with strong acid or β-glucosidase enzymes. Glycoconjugation of volatile phenols following

grapevine smoke exposure was confirmed using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). The β-D-glucopyranoside of guaiacol was identified in juice of Sangiovese grapes affected by bushfire smoke and Chardonnay grapes affected by smoke under experimental conditions (Hayasaka et al. 2010b). Several disaccharide precursors were subsequently identified through stable isotope feeding experiments with potted vines, the most abundant of which were four glucose-pentose glycoconjugates (Hayasaka et al. 2010a). Using an HPLC-MS/MS based stable isotope dilution analysis (SIDA) method, Dungey et al. (2011) investigated the accumulation of guaiacol glycoconjugates in grapes following grapevine smoke exposure. Glycoconjugates were detected within days of smoke exposure, with a higher proportion of total glycoconjugates (by mass) found to be present in skins, rather than pulp (Dungey et al. 2011).

While the studies described earlier have addressed many of the early knowledge gaps surrounding the effect of smoke on grape and wine composition, they do not offer industry any practical solutions to mitigate the incidence or severity of smoke taint. This paper therefore concerns an investigation into the amelioration of smoke taint during winemaking and reports the effect of skin contact time, yeast selection and the use of oak and tannin additives on the concentration and sensory impact of smoke-derived volatiles in wine.

Materials and methods

Smoke-affected grapes

Smoke-affected Grenache grapes were sourced from experimental field trials conducted in a vineyard located in Nuriootpa, in the Barossa Valley district of South Australia (34°30′S, 138°59′E, altitude 274 m). Grapevines (approximately 100 vines) were exposed to smoke in a series of treatments, in which vines (four per treatment) were enclosed in a purpose-built smoke tent and exposed to straw-derived smoke (for 20 min) using experimental conditions described previously (Kennison et al. 2008). Smoke was applied approximately 7 days post-veraison. Shiraz grapes (200 kg) were sourced from a commercial vineyard located in Coldstream, the Yarra Valley district of Victoria (37°42′S, 145°30′E, altitude 130 m), which were exposed to smoke from bushfires that occurred between 7 February and 14 March 2009.

Red and rosé winemaking styles. Grenache fruit (approximately 400 kg) was harvested from smoked and control (unsmoked) grapevines when juice total soluble solids (TSS levels) reached 23 ± 1°Brix. The fruit was randomly sorted into parcels, i.e. six parcels of smoked fruit and six parcels of control fruit (approximately 50 kg each). The fruit parcels were then processed to produce four experimental wines (in triplicate): (i) a control red table wine; (ii) a smoked red table wine; (iii) a control rosé style table wine; and (iv) a smoked rosé style table wine. For red table wines, bunches were de-stemmed and crushed, with the addition of 50 mg/L sulphur dioxide (added as an 8% solution of potassium meta-bisulphite (PMS)) to each replicate. Tartaric acid was added to adjust the pH to 3.5, prior to inoculation with Maurivin PDM yeast (200 ppm). Musts were fermented on skins for 7 days at 15°C, with the cap plunged twice per day. After pressing, the wine was transferred to 20 L stainless steel vessels and held at 20°C until the residual sugar approached 0 g/L. Wines were then racked from gross lees and inoculated with Lallemand VP41 LAB (1 g/hL) to initiate malolactic fermentation. When malic acid reached 0.1 g/L (via enzymatic determination), wines were again racked and 60 ppm SO_2 added, prior to cold stabilisation (at 0°C for 4 months). Wine pH and free SO_2 were adjusted to 3.6 and 30 ppm, respectively, before filtration and bottling (under screw cap closures). For rosé style table wines, bunches were de-stemmed and crushed, with the addition of 50 mg/L sulphur dioxide (SO_2) to each replicate. Tartaric acid was added to adjust the pH to 3.5 and the must was cold soaked (3 days at 0°C). Musts were then pressed, cold settled (3 days at 0°C) and racked, prior to inoculation with Maurivin PDM yeast (200 ppm). Juice was fermented at 15°C until the residual sugar approached 0 g/L. Wines were then racked from gross lees, prior to cold stabilisation (at 0°C for 4 months). Wine pH and free SO_2 were adjusted to 3.5 and 30 ppm, respectively, before filtration and bottling (under screw cap closures). Bottles were stored at 15°C until chemical and sensory analysis.

Yeast selection trial. Grenache fruit harvested from smoked and control vines was frozen at −20°C prior to winemaking. The fruit was divided into 24 parcels of smoked fruit and 24 parcels of control fruit (4.5 kg each) and processed according to small-lot winemaking procedures at the University of Adelaide (Holt et al. 2006). Eight experimental wines from smoked and control fruit were made in triplicate, each fermented with a different yeast as follows: *Saccharomyces cerevisiae* Lalvin ICV D254, Lalvin ICV GRE, Enoferm BDX (Lallemand Australia), SIHA Aktiv hefe 3 (Begerow, Germany); *S. uvarum* Lalvin S6U (Lallemand,

Australia); *S. bayanus* AWRI 1176 (Lallemand, Australia); *S. cerevisiae × S. kudriavzevii* AWRI 1503 (Maurivin, Australia); or *S. cerevisiae × S. cariocanus* AWRI Fusion (Maurivin, Australia). Bunches were de-stemmed and crushed, with the addition of 50 mg/L sulphur dioxide (SO_2) to each replicate. Tartaric acid was added to adjust the pH to 3.5, prior to inoculation with the corresponding yeast (300 ppm) and the addition of diammonium phosphate (150 mg/L). Musts were fermented on skins for 6 days at 20°C, with the cap plunged four times per day. The wine was pressed and transferred to 5 L glass vessels and held at 20°C until the residual sugar approached 0 g/L. Wines were then racked from gross lees and cold stabilised (at 4°C for 4 weeks). Wine pH and free SO_2 were adjusted to 3.4 and 40 ppm, respectively, before filtration and bottling (under screw cap closures). Malolactic fermentation was not carried out for any of those wines. Bottles were stored at 15°C until chemical and sensory analysis.

Oak and tannin additives trial. Smoke-affected Shiraz fruit was harvested at a TSS level of >30° Brix and kept frozen at −20°C until winemaking. The fruit was divided into approximately 5 kg parcels and processed according to small-lot winemaking procedures (Holt et al. 2006) to produce nine experimental wines (in triplicate). Three wines were fermented with untoasted, medium toasted or heavy toasted American oak chips (5 g/kg); five wines were fermented with blended tannins (0.5 g/kg) containing predominantly ellagic chestnut tannins (Tannin 1), catechins (Tannin 2), gallic tannins (Tannin 3), ellagic oak tannins (Tannin 4) and proanthocyanins (Tannin 5) (Laffort, Australia); and one was the control wine with neither oak or tannin additions. Bunches were de-stemmed and crushed, with the addition of 50 mg/L sulphur dioxide (SO_2) to each replicate. Because of the extremely high TSS content (i.e. >30°Brix), water (700 mL) was added to each replicate and pH adjusted to 3.5. Prior to inoculation with Lalvin EC1118 yeast (300 ppm), diammonium phosphate (150 mg/L) was added. Musts were then fermented on skins at 24°C, with the cap plunged three times per day. Where stuck fermentations occurred, a starter culture comprising grape juice, active dry EC1118 yeast (400 mg/L), a yeast nutrient (Go Ferm Protect, Lallemand, Australia, 500 mg/L) and Fermaid A (3 × 100 mg/L) (Lallemand, Australia, technical information) was used to resume fermentation. Wines were pressed at a TSS level of <10°Brix and transferred to glass vessels and held at 20°C until the residual sugar approached 0 g/L. Wines were then racked from gross lees and cold stabilised (at 4°C for 4 weeks). Wine pH and free SO_2 were adjusted to 3.4 and 40 ppm, respectively, before filtration and bottling (under screw cap closures). Malolactic fermentation was not carried out for any of these wines. Bottles were stored at 15°C until chemical and sensory analysis.

Wine analysis

Titratable acidity (TA), volatile acidity (VA), and glucose and fructose content were determined by near infrared spectroscopy (NIRS) using a FOSS6500 Vis-NIR spectrophotometer. Alcohol content was measured by an alcolyser (Anton Paar, Graz, Austria). Determination of wine colour and phenolics was carried out using a Cary Varian UV-visible spectrophotometer according to the methods of Somers and Evans (1977) and Iland et al. (2004). Modified wine colour density was calculated as the sum of the absorbance at 520 and 420 nm after adjusting wine pH to 3.40 and the addition of acetaldehyde to the wine samples to allow for any effect of pH and sulphur dioxide concentrations (Iland et al. 2004). Spectral radiance measurements (CIELAB) were made with μQuant spectroradiometer in the range 380 to 780 nm at 4-nm intervals.

Guaiacol glycoconjugates were quantified in grapes and wine according to the SIDA method described by Dungey et al. (2011), using an Agilent 1200 HPLC system coupled to a 4000 Q TRAP hybrid tandem mass spectrometer (Agilent Technologies, Forest Hill, Vic, Australia). For grape analysis, a solution of $[^2H_4]$-guaiacol β-D-glucopyranoside (5.00 μg) in water (50 μL) was added to a subsample (10 g) of whole berry homogenate and the mixture centrifuged (4300 g for 5 min). A 2 mL aliquot of the resulting juice was loaded onto a C18-HF SPE cartridge (Grace, Australia) and the cartridge washed with water (10 mL). The retained compounds were then eluted with methanol (2 mL). The methanol fraction was then concentrated to dryness and the resulting residue reconstituted in water (300 μL). Extracts were filtered through a 0.45-μm GHP membrane (Acrodisc®, PALL Life Sciences, East Hills, NY, USA) followed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. For wine analysis, a solution of $[^2H_4]$-guaiacol β-D-glucopyranoside (1.00 μg) in water (10 μL) was added to a subsample (1 mL) of wine. The mixture was shaken briefly and filtered (as mentioned earlier) prior to HPLC-MS/MS analysis. The internal standards were prepared as previously described (Dungey et al. 2011). Guaiacol and 4-methylguaiacol concentrations were determined using an Agilent 6890N gas chromatograph coupled to a 5975 inert source mass spectrometer and stable isotope dilution assay methods reported previously (Pollnitz et al. 2000, 2004). The analysis was performed by the Australian Wine Research Institute's Commercial Services Laboratory (Adelaide, Australia).

Sensory assessment of wines
Descriptive sensory analysis (Stone and Sidel 2004) of wines, undertaken four months after bottling, consisted of a series of training and formal evaluation sessions was. Prior to sensory sessions, each wine replicate was assessed informally by a panel of eight tasters with extensive experience in order to assess any artefacts or off-flavours in the replicates and to determine the scope of differences among the treatments. During the training period judges generated appropriate descriptive terms and gained familiarity in recognising and scoring the intensity of selected and defined specific attributes, following the procedure outlined in Lawless and Heymann (1999). A tasting panel for Grenache wines was convened of nine female and four male and for Shiraz wines of seven female and five male staff and students from the University of Adelaide and the Australian Wine Research Institute (AWRI). All panellists except one had several years of extensive wine sensory descriptive analysis experience. Formal sessions were conducted daily in isolated booths at 22–23°C under sodium lights (to mask potential colour differences). All wines were presented as 30 mL samples in three-digit coded, covered, ISO standard glasses. Only one fermentation replicate of each treatment was presented in any one of three sensory session replicates. Panellists rated five aroma attributes and seven palate attributes (Table 1). Palate attributes included assessment of the characteristics of the wine in the mouth and after expectorating (aftertaste). The intensity of sensory attributes was rated on a 15-cm unstructured line scale from 0 to 10 marked with anchor points 'low' (10% of the line) and 'high' (90% of the line). Panellists rinsed thoroughly with pectin solution (1 g/L) and rested for at least 45 s between samples. Data acquisition was carried out using the software Fizz (Version 2.2, Biosystemes, Couternon, France).

Statistical methods
Chemical data were analysed by two-way analysis of variance (ANOVA) using JMP (Version 7, SAS Institute, Cary, NC, USA).

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Table 1. Aroma and palate sensory attributes used for Grenache and Shiraz wines.

Attribute	Description
Aroma	
Fruit aroma intensity	The intensity of the overall fruit aroma, includes red fruit, red berry, dark berry, strawberry, raspberry, cherry, apple
Cold ash	Burnt aroma associated with ashes, includes ashtray, earthy, muddy, tarry
Smoke	Perception of any type of smoke aroma, whether it be hickory or artificial, maybe phenolic, includes charry, smoked meat, bacon
Medicinal	Aromatic characteristic of band-aids, disinfectant-like or phenols, includes cleaning product, disinfectant, phenols, band-aid, doctor's waiting room
Solvent	The volatile aroma associated with solvents, includes varnish, shoe polish
Palate	
Fruit flavour intensity	The intensity of the overall fruit flavour; includes red fruit, red berry, dark berry, strawberry, raspberry, cherry, apple
Smoky	Perception of smoke flavour, includes bacon and smoked meat
Sour	Intensity of sour/ acid taste
Metallic	The 'tinny' flavour associated with metals
Bitter	Intensity of bitter taste, bitter aftertaste
Ashy aftertaste	Length of taste associated with residue of ashtray perceived in the mouth after expectorating, includes coal ash, ashtray, tarry, acrid
Drying	Drying, puckering mouth-feel after expectorating the wine

For sensory assessment each attribute rated by judges was subjected to ANOVA to ascertain the effects of treatment, fermentation replicate nested within treatment, judge and presentation replicate, using a mixed-model ANOVA and treating judges in a random effect. The method used to discriminate among the means was Fisher's least significant difference procedures, $P < 0.05$ using SenPaq (Version 4.82 (c) Qi Statistics 2009, UK). Principal component analysis (PCA) was performed, using JMP (Version 7, SAS Institute), on the correlation matrix of mean ratings across the wines for all attributes that showed significant differences according to the ANOVA, focusing on the principal components (PCs) that showed eigenvalues higher than one. Chemical parameters were projected onto sensory data to show possible correlations between sensory and chemical data.

Results

Chemical composition of wine
The alcohol content, pH, TA, VA, colour and phenolic content of all wines are reported in Table 2. Red style Grenache table wines had higher pH and lower TA than rosé style Grenache wines, while VA concentrations differed only slightly between winemaking styles. In the yeast selection trial, the alcohol content ranged from 12.4 to 13.1% in smoked wines and from 12.7 to 13.3% in control wines, while TA was measured between 6.2 and 7.5 g/L, and 6.7 and 8.0 g/L for smoked and control,

Table 2. Modified wine colour density, wine hue, concentration of anthocyanins, phenolics, and polymeric pigments, alcohol content, pH, titratable acidity (TA) and volatile acidity (VA) as acetic acid, in Grenache wines made from control (C) and smoke-affected (S) grapes, and Shiraz wines made from bushfire smoke-affected grapes.

Treatment	Wine colour density	Wine hue	Anthocyanins (mg/L)	Phenolics (au)	Polymeric pigments (mg/L)	Alcohol % v/v	pH	TA (g/L)	VA (g/L)
Grenache									
Rosé and red									
C Rosé	0.7 ± 0.0^b	0.59 ± 0.02	49 ± 4^b	8.1 ± 0.5^c	0.1 ± 0.0^b	13.6 ± 0.1	3.48 ± 0.01^b	6.0 ± 0.1^a	0.24 ± 0.01^a
S Rosé	0.8 ± 0.0^b	0.56 ± 0.00	59 ± 4^b	9.9 ± 0.7^c	0.1 ± 0.0^b	13.6 ± 0.1	3.44 ± 0.01^b	6.0 ± 0.1^a	0.23 ± 0.01^{ab}
C Red	3.3 ± 0.2^a	0.60 ± 0.03	231 ± 15^a	25.0 ± 1.1^a	0.5 ± 0.0^a	13.3 ± 0.3	3.64 ± 0.02^a	5.7 ± 0.0^b	0.19 ± 0.01^c
S Red	3.0 ± 0.1^a	0.58 ± 0.01	199 ± 12^a	21.6 ± 1.3^b	0.5 ± 0.0^a	13.4 ± 0.0	3.64 ± 0.01^a	5.7 ± 0.0^b	0.21 ± 0.01^{bc}
P	*<0.0001*	*ns*	*<0.0001*	*<0.0001*	*<0.0001*	*ns*	*<0.0001*	*0.0075*	*0.0175*
Yeast trial									
C ICV D254	7.4 ± 0.3^{abcd}	0.49 ± 0.02^{cdef}	300 ± 5^{abc}	33.7 ± 0.7^{abc}	0.9 ± 0.1^{cdef}	13.0 ± 0.1^{ab}	3.35 ± 0.05^{cd}	7.5 ± 0.2^{abcd}	0.16 ± 0.00^f
C BDX	6.9 ± 0.3^{cdef}	0.49 ± 0.02^{cdef}	283 ± 14^{bcd}	31.7 ± 1.9^{abcd}	0.8 ± 0.1^{def}	12.7 ± 0.2^b	3.34 ± 0.11^{cd}	7.8 ± 0.4^{ab}	0.22 ± 0.03^{bcde}
C ICV GRE	8.2 ± 0.5^a	0.55 ± 0.03^{abcd}	325 ± 21^a	36.4 ± 2.6^a	1.0 ± 0.1^{abcd}	13.0 ± 0.2^{ab}	3.46 ± 0.04^{abc}	6.7 ± 0.1^{cdef}	0.22 ± 0.02^{bcde}
C S6U	6.6 ± 0.2^{defg}	0.52 ± 0.01^{bcdef}	254 ± 8^{defg}	29.9 ± 1.2^{cde}	0.9 ± 0.1^{bcdef}	13.0 ± 0.2^{ab}	3.49 ± 0.01^{ab}	7.3 ± 0.3^{bcd}	0.20 ± 0.02^{def}
C AWRI 1176	7.6 ± 0.2^{abc}	0.51 ± 0.01^{bcdef}	271 ± 9^{bcde}	30.5 ± 0.9^{cde}	1.1 ± 0.1^{abcd}	12.9 ± 0.1^{ab}	3.53 ± 0.03^a	7.5 ± 0.2^{abc}	0.34 ± 0.01^a
C AWRI 1503	8.0 ± 0.3^{ab}	0.48 ± 0.02^{ef}	303 ± 12^{cdefg}	33.1 ± 1.3^{abc}	1.0 ± 0.1^{abcd}	13.3 ± 0.1^a	3.46 ± 0.01^{abc}	7.1 ± 0.1^{bcde}	0.21 ± 0.02^{bcde}
C Fusion	6.6 ± 0.2^{defg}	0.46 ± 0.01^f	255 ± 6^{abcd}	27.5 ± 0.4^{de}	0.7 ± 0.0^f	12.9 ± 0.2^{ab}	3.33 ± 0.06^d	8.0 ± 0.2^a	0.21 ± 0.01^{bcde}
C SIHA 3	7.2 ± 0.4^{bcde}	0.48 ± 0.02^{cf}	266 ± 8^{bcdef}	29.3 ± 0.7^{de}	0.8 ± 0.1^{def}	13.1 ± 0.1^{ab}	3.32 ± 0.02^d	7.8 ± 0.6^{ah}	0.20 ± 0.01^{def}
S ICV D254	7.7 ± 0.2^{abc}	0.61 ± 0.01^a	233 ± 5^{efgh}	31.1 ± 1.0^{bcd}	1.2 ± 0.1^a	12.8 ± 0.1^b	3.41 ± 0.06^{bcd}	6.2 ± 0.2^f	0.19 ± 0.00^{def}
S BDX	7.5 ± 0.3^{abc}	0.61 ± 0.01^a	227 ± 2^{jgh}	29.4 ± 0.5^{cde}	1.2 ± 0.1^{ab}	12.8 ± 0.2^b	3.43 ± 0.02^{abcd}	6.7 ± 0.2^{def}	0.25 ± 0.01^{bc}
S ICV GRE	8.0 ± 0.5^{ab}	0.56 ± 0.01^{abcd}	255 ± 20^{defg}	31.7 ± 3.2^{abcd}	1.1 ± 0.1^{bcdef}	12.7 ± 0.1^b	3.41 ± 0.01^{bcd}	6.4 ± 0.1^{cf}	0.23 ± 0.01^{bcd}
S S6U	6.5 ± 0.4^{cfg}	0.57 ± 0.04^{abc}	199 ± 15^{hg}	26.2 ± 1.8^{de}	1.0 ± 0.1^{bcdef}	12.9 ± 0.1^{ab}	3.42 ± 0.01^{ab}	6.9 ± 0.1^{cdef}	0.32 ± 0.01^a
S AWRI 1176	6.4 ± 0.4^{dcfg}	0.56 ± 0.03^{ab}	226 ± 2^h	27.8 ± 1.0^c	0.9 ± 0.1^{abcdc}	12.4 ± 0.1^c	3.48 ± 0.02^{abcd}	6.9 ± 0.0^{cdef}	0.31 ± 0.01^a
S AWRI 1503	6.3 ± 0.1^{cfg}	0.53 ± 0.01^{bcde}	261 ± 35^{cdefg}	30.9 ± 3.8^{cde}	0.9 ± 0.0^{cdef}	13.1 ± 0.1^{ab}	3.43 ± 0.00^{abcd}	6.8 ± 0.1^{cdef}	0.25 ± 0.01^{bc}
S Fusion	5.8 ± 0.4^g	0.52 ± 0.01^{bcdef}	290 ± 15^{dcfg}	36.2 ± 1.3^a	0.7 ± 0.1^{cf}	12.7 ± 0.2^{bc}	3.35 ± 0.05^{cd}	7.5 ± 0.3^{abcd}	0.24 ± 0.01^{bcd}
S SIHA 3	6.2 ± 0.4^{fg}	0.52 ± 0.04^{bcdef}	300 ± 10^{abc}	35.8 ± 1.4^{ab}	0.8 ± 0.1^{cf}	12.9 ± 0.1^{ab}	3.32 ± 0.02^d	7.2 ± 0.2^{bcde}	0.25 ± 0.01^b
P	*<0.0001*	*0.0011*	*<0.0001*	*0.0014*	*0.0131*	*0.0187*	*0.0173*	*0.0019*	*<0.0001*
Shiraz									
Control	20.7 ± 0.9	0.49 ± 0.01^d	363 ± 13^b	57.1 ± 1.2^d	11.4 ± 0.5^b	13.0 ± 0.1^b	3.41 ± 0.06^c	7.9 ± 0.0^a	0.54 ± 0.01^b
UT oak	19.4 ± 0.4	0.51 ± 0.01^{bc}	355 ± 6^{abc}	57.7 ± 0.2^d	10.6 ± 0.9^{cd}	13.7 ± 0.2^a	3.54 ± 0.01^{ab}	7.2 ± 0.1^c	0.50 ± 0.01^{bc}
MT oak	18.9 ± 0.2	0.54 ± 0.00^a	298 ± 7^d	58.9 ± 0.9^{cd}	11.5 ± 0.1^{ab}	13.7 ± 0.1^a	3.56 ± 0.01^{ab}	7.1 ± 0.0^c	0.50 ± 0.01^{bc}
HT oak	19.5 ± 0.2	0.51 ± 0.00^c	380 ± 6^a	58.7 ± 0.5^{cd}	10.2 ± 0.2^d	13.6 ± 0.1^a	3.57 ± 0.01^{ab}	7.1 ± 0.1^c	0.48 ± 0.01^c
Tannin 1	20.3 ± 0.5	0.51 ± 0.00^c	359 ± 11^{abc}	62.0 ± 0.8^b	10.8 ± 0.3^{bcd}	13.6 ± 0.1^a	3.56 ± 0.01^{ab}	7.2 ± 0.1^c	0.49 ± 0.01^{bc}
Tannin 2	19.1 ± 0.1	0.52 ± 0.00^{bc}	333 ± 10^c	58.7 ± 0.8^{cd}	10.5 ± 0.2^{cd}	13.5 ± 0.2^a	3.53 ± 0.01^b	7.1 ± 0.0	0.52 ± 0.02^{bc}
Tannin 3	20.1 ± 0.4	0.51 ± 0.00^c	351 ± 2^{bc}	70.1 ± 0.7^a	11.0 ± 0.2^{abc}	13.5 ± 0.1^a	3.54 ± 0.03^{ab}	7.5 ± 0.2^b	0.61 ± 0.02^a
Tannin 4	18.9 ± 0.1	0.53 ± 0.00^{ab}	257 ± 13^c	57.1 ± 0.5^d	11.7 ± 0.3^a	13.5 ± 0.1^a	3.58 ± 0.03^a	7.2 ± 0.2^c	0.49 ± 0.01^{bc}
Tannin 5	20.0 ± 0.3	0.51 ± 0.01^c	368 ± 3^b	60.7 ± 0.5^{bc}	10.5 ± 0.1^{cd}	13.7 ± 0.0^a	3.58 ± 0.03^a	7.2 ± 0.1^c	0.49 ± 0.02^{bc}
P	*ns*	*0.0004*	*<0.0001*	*<0.0001*	*0.0092*	*0.0046*	*0.0001*	*0.0002*	*0.0005*

Each value represents the mean of three replicates (±standard error of the mean). Means in columns followed by different letters are significantly different. HT, heavy toasted oak; MT, medium toasted oak; ns, not significant; UT, untoasted oak.

respectively. Increased VA production was observed for smoked Grenache wines fermented with either S6U (0.32 g/L) or AWRI 1176 (0.31 g/L). Interestingly, AWRI 1176 produced high VA in the corresponding control wine also (0.34 g/L), while the lowest VA (0.16 g/L) in the control group was in the wine fermented with ICV D254. VA production was much higher for the Shiraz wines, ranging from 0.48 (HT oak) to 0.61 g/L (Tannin 3). However, the VA of all wines was well below the 1.5 g/L legal limit. Wines fermented with oak or tannins additions had increased pH and alcohol content, but reduced TA, compared with the corresponding control (Table 2).

Wine colour density was significantly higher in red style Grenache wines, compared with rosé style wines, but little difference in colour was observed between smoked and control treatments (Table 2). In the yeast selection trials, wines fermented with AWRI Fusion had the lowest wine colour density (6.6 for control wines and 5.8 for smoked wines), while ICV GRE produced wines with the highest colour density (8.2 for control wines and 6.6 for smoked wines). The extraction of anthocyanins from smoke-affected grapes was highest with SIHA active 3 (300 mg/L) and AWRI Fusion (290 mg/L) and lowest with S6U (199 mg/L) (Table 2). Similarly, AWRI Fusion and SIHA active 3 yeast extracted the most phenolics (35–36 au/L) and S6U the lowest (26 au/L). Given the high extraction of monomeric anthocyanins and phenolics, the formation of polymeric pigments appeared to be low in wines fermented with AWRI Fusion and SIHA active 3 (0.75 g/L), resulting in lower wine colour density (5.8) and wine hue (0.5). In contrast, ICV GRE, ICV D254 and BDX strains showed good extraction of phenolic compounds and formation of polymeric pigments, resulting in high wine colour density (5.8–8.0) (Table 2). The performance of these yeast strains differed when fermenting control fruit; statistical analysis highlighted the diverse yeast/ smoke treatment interactions (Table 2). ICV GRE gave the highest phenolic content and wine colour density, followed by AWRI 1503, ICV D254 and AWRI 1176; while AWRI Fusions and SIHA active 3 gave the lowest (Table 2). For Shiraz wines, no significant difference in wine colour density was observed between treatments. For oak-treated wines, enhanced polymeric pigment formation was observed in wines made with medium toasted oak chip additions; the other oak treatments (untoasted and heavily toasted oak chip additions) gave wines with similar phenolic composition (Table 2). Addition of Tannin 3 increased phenolic concentration (70 au/L), while Tannin 4 enhanced the formation of polymeric pigments (11.7 mg/L), but reduced the concentration of free anthocyanins (257 mg/L). Tannins 1, 2 and 5 had similar effects on wine colour density and wine phenolic composition (Table 2).

Grapes and wines from each of the three winemaking experiments were analysed by GC-MS and HPLC-MS/MS to determine phenol (guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol) and guaiacol glycoconjugate (glucoside, glucosylglucoside, glucose-pentose disaccharide and rutinoside) concentrations, respectively (Table 3). In each case, the glucose-pentose disaccharides represented the most abundant fraction of the guaiacol glycoconjugate pool (i.e. 65–75%); of the remaining glycoconjugate pool, 15–30% was comprised of the rutinoside, 5–14% the glucoside and less than 2% the glucosylglucoside. Of the volatile phenols measured, only guaiacol and 4-methylguaiacol were detected above trace (i.e. > 1 µg/L) levels (Table 3).

Guaiacol and 4-methylguaiacol were not detected in either control or smoked Grenache fruit, but elevated levels of guaiacol glycoconjugates were observed in smoked fruit, compared with control fruit (Table 3). In both the wine style and yeast selection

trials, low or undetectable levels of glycoconjugates and smoke-derived phenols were present in control wines only. The smoked red style wines contained 290 µg/L total glycoconjugates, 4.7 µg/L guaiacol and 1.3 µg/L 4-methylguaiacol, whereas the smoked rosé style wine contained only 204 µg/L total glycoconjugates and 2.0 µg/L guaiacol (4-methylguaiacol was not detected). In the yeast selection trial, smoke-affected Grenache grapes fermented with AWRI 1176 had the highest total concentration of guaiacol glycoconjugates (374 µg/L), followed by ICV GRE (356 µg/L). The lowest total glycoconjugate concentration was observed in wines made with AWRI 1503 (264 µg/L) and ICV BDX (271 µg/L). Differences in total glycoconjugate content appear largely to be a function of glucose-pentose disaccharide and rutinoside precursor levels. A significant difference in guaiacol concentration was also observed between smoked wines fermented with S6U (12.9 µg/L) and ICV GRE (5.3 µg/L) (Table 3). In the oak and tannin addition trial, Shiraz fruit contained 874 µg/kg guaiacol glycoconjugates and 10 µg/L guaiacol (Table 3). The control wines contained 825 µg/L total guaiacol glycoconjugates, whereas concentrations in the oak- or tannin-treated wines ranged from 672 µg/L (Tannin 3) to 771 µg/L (heavy toasted oak chips). The glucose-pentose disaccharides were found to be highest in the control wines (617 µg/L) and wines made with the addition of oak chips (542–558 µg/L); wines made with Tannin 4 (oak derived) were high in the rutinoside precursor (165 µg/L). The addition of oak chips to fermentations resulted in higher wine guaiacol and 4-methylguaiacol concentrations; 10 and 60% higher, respectively.

Sensory assessment
Descriptive sensory analysis was used to quantitatively characterise differences in the perceived aroma and flavour attributes (Table 1) of wines made from fruit exposed to experimental or bushfire smoke, and control (unsmoked) fruit. In Grenache smoked red style wines, the intensity of smoke-related attributes, such as 'smoke' and 'cold ash' aromas, 'smoky' flavour and 'ashy aftertaste' were rated significantly higher than in control red style wines. Rosé style wines, both control and smoked, were given similar ratings, but they both received higher 'fruit' aroma and flavour ratings compared with red style wines (Figure 1).

In the Grenache yeast trial, smoked wines fermented with SIHA activ 3, AWRI Fusion, AWRI 1503 and GRE were given lower ratings for 'smoky' and 'ashy' aromas and flavours, than those smoked wines fermented with ICV D254, AWRI 1176 and BDX (Figure 2). There was little difference in perceived intensity of 'medicinal', 'solvent' and 'metallic' sensory characters in all smoked wines. The intensity of 'fruit' aroma and flavour was also significantly higher in ICV GRE, AWRI Fusion, AWRI 1503 and SIHA active 3 wines, than wines made with AWRI 1176, ICV D254, BDX and S6U (Figure 2). For control wines, the 'smoky' aroma was perceived to be the most intense in wines made with BDX, while the 'drying' character was enhanced in wines made with ICV GRE. These attributes were rated lowest in control wines made with AWRI Fusion and AWRI 1176. No significant difference was noted for any other sensory attributes.

In the oak and tannin addition trial, the use of additives reduced the intensity of smoke-related sensory attributes, compared with the corresponding control; particularly the impact of 'smoky', 'cold ash' and 'medicinal' aromas (Figure 3). Medium toasted oak chip or Tannin 4 (oak based) additions best ameliorated the effect of smoke, reducing the perception of 'smoky' aroma and flavour much more than with Tannin 1 (ellagic chestnut), Tannin 2 (catechins) or Tannin 5 (proanthocyanins).

Table 3. Relative concentration of guaiacol glycoconjugates, guaiacol and 4-methyl guaiacol present in Grenache wines made from control (C) and smoke-affected (S) grapes, and Shiraz wines made from bushfire smoke-affected grapes.

	Glucoside (µg/L)	Glucose-pentose disaccharides (µg/L)	Glucosyl glucoside (µg/L)	Rutinoside (µg/L)	Total glycoconjugates (µg/L)	Guaiacol (µg/L)	4-Methyl guaiacol (µg/L)
Grenache							
Fruit control†	2.4 ± 0.1	19 ± 1.6	4.0 ± 0.5	5 ± 0.5	30 ± 2.5	nd	nd
Fruit smoked†	18.7 ± 10.8	171 ± 14.5	37.2 ± 7.0	67 ± 0.1	294 ± 35.7	nd	nd
Rosé and red							
C Rosé	3.1 ± 0.3^b	14 ± 2.0^c	0.3 ± 0.1	5 ± 0.4^c	22 ± 2.2^c	nd	nd
S Rosé	19.2 ± 1.4^a	122 ± 30.3^b	0.3 ± 0.1	62 ± 2.4^b	204 ± 29.2^b	2.0 ± 0.0^b	nd
C Red	4.9 ± 0.4^b	26 ± 1.2^c	0.5 ± 0.1	7 ± 0.6^c	38 ± 2.2^c	tr	nd
S Red	18.2 ± 3.0^a	177 ± 30.3^a	0.5 ± 0.1	95 ± 14.2^a	290 ± 37.0^a	4.7 ± 0.3^a	1.3 ± 0.3
P	0.0007	0.0005	ns	0.0003	0.0001	<0.0001	0.0029
Yeast trial							
C ICV D254	3.5 ± 0.6^d	29 ± 1.2^c	0.0 ± 0.0^c	6 ± 0.6^c	39 ± 2.0^c	nd	nd
C BDX	3.5 ± 0.6^d	24 ± 4.1^c	0.2 ± 0.1^{bc}	6 ± 0.9^c	33 ± 5.2^c	nd	nd
C ICV GRE	3.2 ± 0.2^d	31 ± 0.5^c	0.1 ± 0.0^{bc}	9 ± 0.3^c	43 ± 0.3^c	nd	nd
C S6U	4.1 ± 1.1^d	27 ± 1.2^c	0.1 ± 0.0^{bc}	8 ± 0.8^c	39 ± 2.1^c	tr	nd
C AWRI 1176	3.6 ± 0.2^d	24 ± 1.1^c	0.1 ± 0.1^{bc}	7 ± 0.4^c	35 ± 1.0^c	nd	nd
C AWRI 1503	3.8 ± 0.8^d	30 ± 1.0^c	0.2 ± 0.2^{bc}	7 ± 0.2^c	41 ± 1.9^c	nd	nd
C Fusion	3.7 ± 1.0^d	29 ± 2.3^c	0.1 ± 0.1^c	6 ± 0.4^c	39 ± 0.4^c	tr	nd
C SIHA 3	3.5 ± 0.5^d	29 ± 0.8^c	0.2 ± 0.0^{bc}	5 ± 0.2^c	38 ± 1.2^c	nd	nd
S ICV D254	14.4 ± 1.4^c	211 ± 21.0^{ab}	0.4 ± 0.0^{ab}	79 ± 3.2^b	305 ± 25.4^{ab}	7.6 ± 0.9^{ab}	tr
S BDX	15.1 ± 1.7^{bc}	183 ± 20.7^{ab}	0.2 ± 0.2^{bc}	73 ± 13.1^b	271 ± 35.4^b	6.3 ± 0.3^{ab}	nd
S ICV GRE	24.0 ± 6.9^{ab}	230 ± 47.1^a	0.7 ± 0.3^a	101 ± 25.3^{ab}	356 ± 79.3^{ab}	5.3 ± 1.3^b	tr
S S6U	17.3 ± 4.4^{abc}	196 ± 32.3^{ab}	0.1 ± 0.0^c	91 ± 22.2^{ab}	304 ± 59.7^{ab}	12.9 ± 0.1^a	tr
S AWRI 1176	26.3 ± 8.2^a	218 ± 25.8^{ab}	0.1 ± 0.0^{bc}	129 ± 41.5^a	374 ± 74.9^a	8.7 ± 2.7^{ab}	1.3 ± 0.3
S AWRI 1503	14.4 ± 0.6^c	175 ± 11.6^b	0.3 ± 0.2^{bc}	75 ± 5.5^b	264 ± 17.3^b	6.3 ± 0.7^{ab}	nd
S Fusion	15.7 ± 3.6^{bc}	201 ± 23.7^{ab}	0.2 ± 0.1^{bc}	75 ± 10.3^b	288 ± 37.0^{ab}	7.3 ± 0.9^{ab}	tr
S SIHA 3	18.3 ± 0.5^{abc}	205 ± 6.6^{ab}	0.1 ± 0.0^c	71 ± 3.6^b	294 ± 10.4^{ab}	6.3 ± 0.3^{ab}	tr
P	<0.0001	<0.0001	0.0359	<0.0001	<0.0001	<0.0001	0.0435
Shiraz							
Fruit†	53.2 ± 6.1	450 ± 64.5	265 ± 30.1	106 ± 10.8	874 ± 80.7	10.0 ± 0.6	nd
Control	84.9 ± 4.9	617 ± 25.7^a	6.3 ± 0.3^a	117 ± 2.2^{bc}	825 ± 29.2^a	18.7 ± 1.2^d	tr.
UT oak	87.1 ± 3.0	556 ± 7.1^b	2.3 ± 0.9^d	120 ± 2.3^{bc}	765 ± 8.3^b	24.0 ± 1.0^{abc}	2.3 ± 0.3^b
MT oak	87.6 ± 2.0	542 ± 11.3^{bc}	3.0 ± 0.2^{bcd}	110 ± 2.1^{cd}	743 ± 12.4^{bc}	25.3 ± 0.7^{ab}	5.0 ± 0.6^a
HT oak	88.0 ± 5.7	558 ± 4.8^b	3.2 ± 0.2^{bcd}	122 ± 5.6^b	771 ± 5.1^b	26.7 ± 1.5^a	4.7 ± 0.3^a
Tannin 1	85.4 ± 1.4	527 ± 4.1^{bcd}	3.4 ± 0.2^{bc}	100 ± 2.7^d	715 ± 7.6^{cd}	22.0 ± 0.6^c	1.3 ± 0.3^{bc}
Tannin 2	88.3 ± 4.3	503 ± 7.3^{de}	3.5 ± 0.6^b	110 ± 4.7^{cd}	705 ± 14.4^{cde}	22.7 ± 0.9^{bc}	1.7 ± 0.3^{bc}
Tannin 3	83.4 ± 3.1	485 ± 2.4^e	2.5 ± 0.3^{cd}	100 ± 1.7^d	672 ± 5.6^d	22.0 ± 1.0^c	1.3 ± 0.3^{bc}
Tannin 4	96.7 ± 3.4	496 ± 8.1^{de}	2.4 ± 0.1^d	165 ± 4.9^a	761 ± 8.2^b	25.0 ± 1.0^{abc}	1.7 ± 0.7^{bc}
Tannin 5	86.5 ± 2.9	510 ± 10.7^{cde}	2.5 ± 0.5^{cd}	103 ± 2.8^d	702 ± 10.4^{de}	22.7 ± 1.2^{bc}	1.7 ± 0.3^{bc}
P	ns	<0.0001	<0.0001	<0.0001	<0.0001	0.004	<0.0001

†expressed as µg/kg. Each value represents the mean of three replicates (±standard error of the mean). Means in columns followed by different letters are significantly different. HT, heavy toasted oak; MT, medium toasted oak; ns, not significant; tr, trace (i.e. positive identification, but <1 µg/L); UT, untoasted oak.
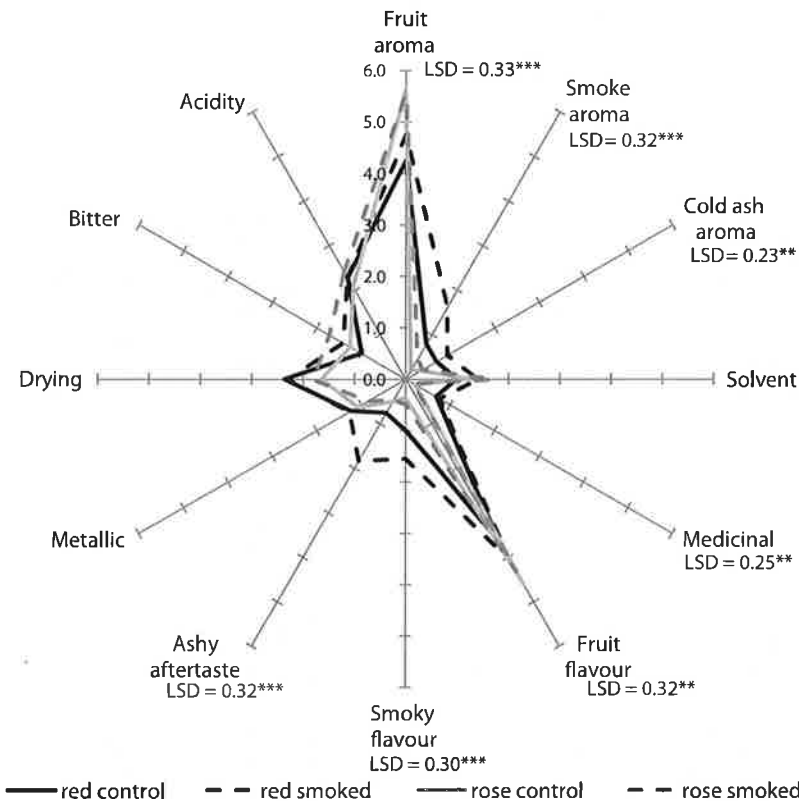
Figure 1. Mean ratings for sensory attributes in red and rosé style Grenache wines. Each value is the mean score from one wine replicate presented to 13 judges in three replicate sessions. LSD is least significant difference and *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

'Ashy' aroma and aftertaste were less intense in wines treated with oak chips, rather than tannin additions, with the exception of Tannin 4 (ellagic oak). The addition of toasted (medium or heavy) oak chips to ferments also intensified 'fruit' aroma of wine; but wines made with untoasted oak chips were given similar 'fruit' flavour ratings as the control wines (Figure 3).

Correlations between chemical and sensory data

In the Grenache yeast study, correlations were established between guaiacol $(_g)$ and total guaiacol glycoconjugates $(_p)$, and the intensity of 'smoke' aroma (correlation coefficient $r_g = 0.63$, $r_p = 0.65$, $P < 0.01$), 'cold ash' aroma $(r_g = 0.62$, $r_p = 0.60$, $P < 0.01$), 'smoky' flavour $(r_g = 0.59$, $r_p = 0.63$, $P < 0.01$) and 'ashy' aftertaste $(r_g = 0.52$, $P < 0.05$, $r_p = 0.52$, $P < 0.01$). Although smoke-related attributes such as 'medicinal', 'solvent' and 'metallic' were rated similarly in all smoked Grenache wines, the correlations were established between guaiacol concentration and the intensity of 'medicinal' $(r = 0.45$, $P < 0.05$) and 'solvent' $(r = 0.46$, $P < 0.05$) aromas.

In Shiraz wines, the concentration of guaiacol and 4-methylguaiacol was enhanced by the addition of oak chips and tannins, thus it was negatively correlated with 'smoke' aroma $(r_g = 0.83$, $P < 0.01$), 'cold ash' aroma $(r_g = 0.95$, $P < 0.001$) and 'smoky' flavour $(r_g = 0.71$, $P < 0.05$). The only correlation that could be established with the guaiacol glycoconjugates was between glucosylglucoside content and 'smoky' aroma $(r = 0.80$, $P < 0.01$).

The intensities of smoke-like aromas and flavours were associated with one another, irrespective of the winemaking techniques employed. Strong correlations $(P < 0.001)$ were established between 'smoke' aroma and: 'cold ash' aroma $(r = 0.88)$; 'smoky' flavour $(r = 0.92)$; and 'ashy' aftertaste' $(r = 0.89)$. 'Smoky' flavour and 'ashy aftertaste' were also highly correlated $(r = 0.92)$, while 'medicinal' aroma was correlated $(P < 0.001)$ with: 'smoke' aroma $(r = 0.61)$, 'cold ash' aroma

(0.62), 'metallic' $(r = 0.79)$ and 'smoky' flavour $(r = 0.49$, $P < 0.01)$.

The correlation matrix generated from the chemical and sensory data for each wine replicate was analysed by the PCA. The first PC accounted for 55% of the total variance and the second for 25% (Figure 4). Red and rosé style Grenache wines were separated on the basis of 'fruit' aroma and flavour, indicating the style of winemaking dominated the effect of smoke exposure. Smoked red style wines were readily differentiated from control wines according to the intensity of smoke-related sensory attributes. In the yeast selection trial, a cluster of smoked wines was associated with 'smoky' and 'ashy' aroma and flavours, and increased wine hue, with wines fermented with AWRI 1176 and ICV D254 positioned at the top of the cluster. The yeast selection had less influence on the corresponding control wines and it was interesting to observe that both smoked and control wines made with AWRI 1503 were positioned very close to each other. The position of the control (untreated) Shiraz wines on the PCA graph was largely determined by the increased anthocyanin concentration and higher intensity of 'smoky' and 'ashy' aroma and flavour. The Shiraz wines treated with oak chips and oak-based Tannin 4 were grouped together on the basis of high intensity of 'fruit' aroma and flavour, 'solvent' aroma and elevated 4-methylguaiacol content, whereas wine colour density, phenolic content and guaiacol concentration separated the Shiraz wines made with different tannin additions.

Discussion

While grapevine exposure to smoke has been shown to affect wine composition and can enhance the perception of smoke-related sensory attributes (Kennison et al. 2007, 2008, 2009), the potential amelioration of smoke taint has not been investigated. This study was therefore undertaken to investigate the efficacy of various winemaking techniques in reducing the intensity of smoke-related sensory attributes, while maintaining
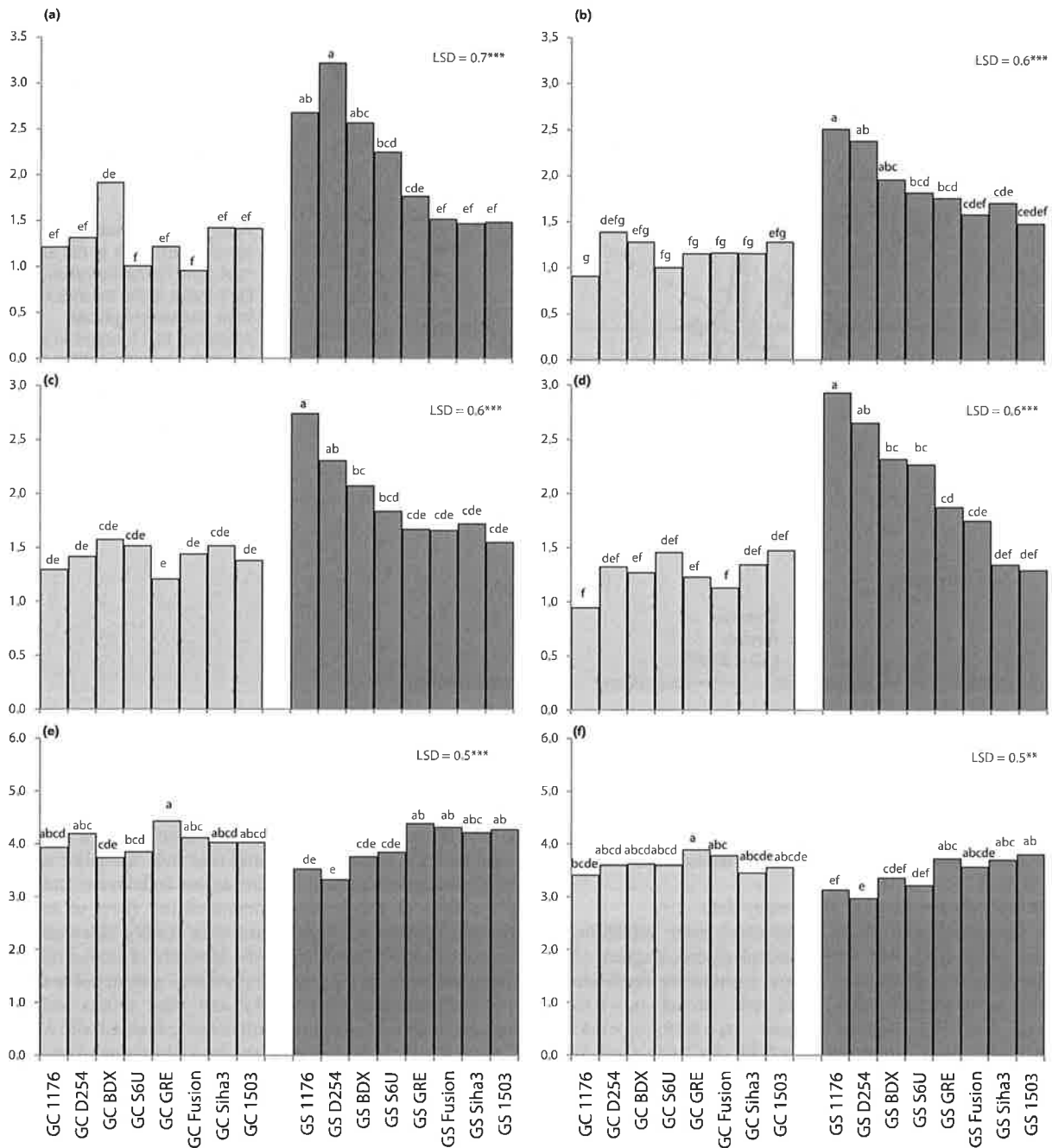
Figure 2. Mean ratings for the intensity of 'smoke' aroma (a), 'smoky' flavour (b), 'cold ash' aroma (c), 'ashy aftertaste' (d), overall 'fruit' aroma (e) and 'fruit' flavour (d) in Grenache wines made from control (▧ GC) and smoke-affected (■ GS) grapes, fermented with different yeast strains. Each value is the mean score from one wine replicate presented to 13 judges in three replicate sessions. LSD is least significant difference and *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

acceptable wine quality. The study evaluated winemaking techniques such as cold maceration (a cold soak to produce a rosé style table wine), fermentation on skins (to produce a red style table wine), fermentation with different yeast strains using Grenache grapes and fermentation with the addition of oak chips or tannins using Shiraz grapes.

Red and rosé style winemaking

Cold maceration has been used widely in white and rosé wine production to improve colour stability, to increase the aromatic profile of wine and to reduce bitterness and astringency

(Salinas et al. 2005, Radeka et al. 2008, De Santis and Frangipane 2010). Because this winemaking practice typically reduces the extraction of aromatic and phenolic compounds, it was anticipated that fermentation of smoke-affected grapes might lead to a reduction in the intensity of smoke-related characteristics.

Red and rosé style winemaking yielded different amounts of anthocyanins, phenolics and polymeric pigments and subsequently differed in wine colour density. The effect of smoke exposure was evident in reduced phenolic concentration in smoked red wines, but there was little difference in wine colour
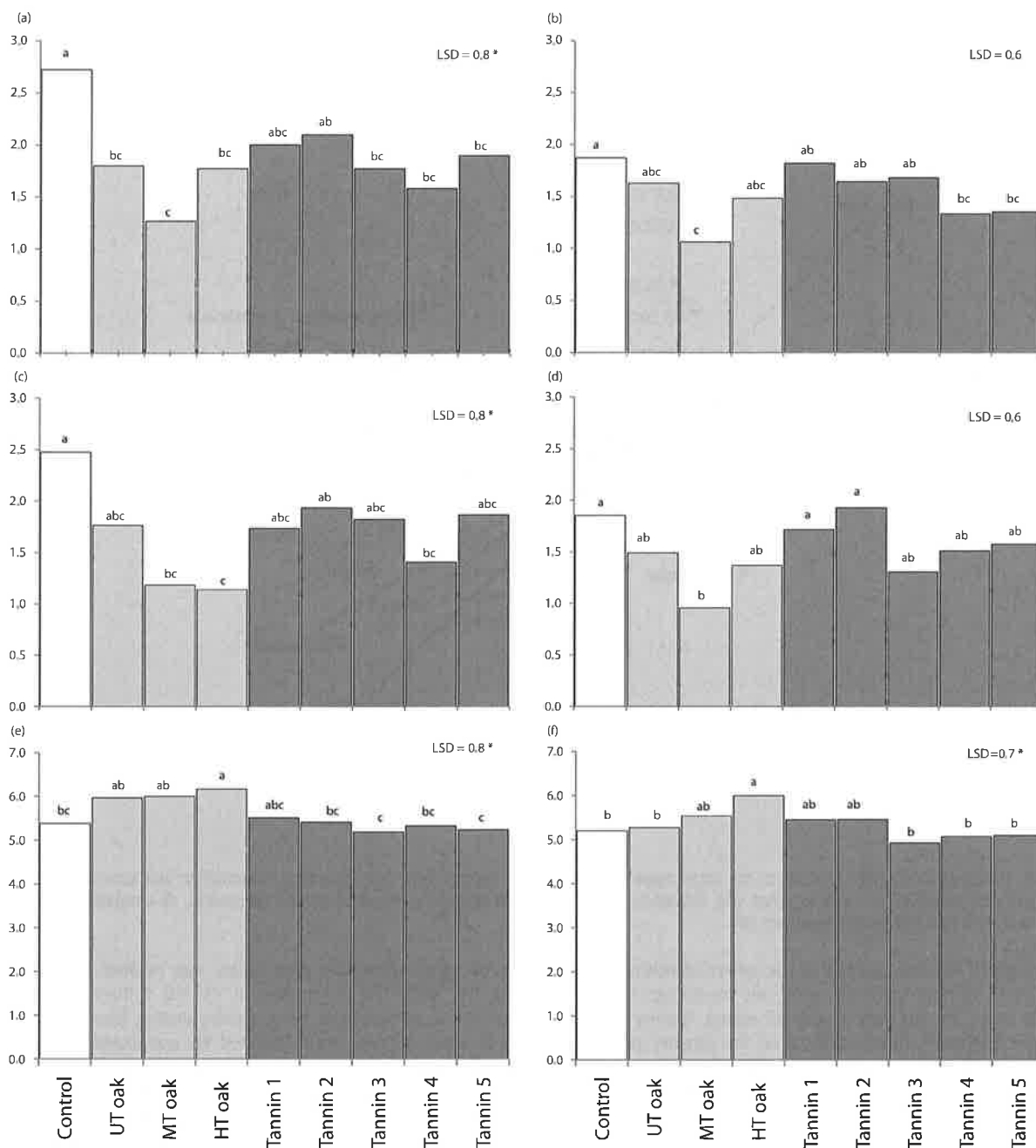
Figure 3. Mean ratings for the intensity of 'smoke' aroma (a), 'smoky' flavour (b), 'cold ash' aroma (c), 'ashy aftertaste' (d), overall 'fruit' aroma (e) and 'fruit' flavour (d) in Shiraz wines made from bushfire smoke-affected grapes, fermented with untoasted (UT) oak, medium toasted (MT) oak and heavy toasted (HT) oak, and five tannin additions. Each value is the mean score from one wine replicate presented to 12 judges in three replicate sessions. LSD is least significant difference and *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

density. CIELAB colour coordinates provided more complex colour measurements and, when subjected to PCA, facilitated separation of smoked rosé style wines (but not red style wines) by the value of $a*$ and hue_{ab} (data not shown). This is consistent with the increased brown hue observed in smoked Verdelho wines from Kennison's (2007) previous work. Guaiacol and 4-methylguaiacol were not detected in either control or smoked Grenache fruit, but elevated levels of guaiacol glycoconjugates were observed in smoked fruit, compared with control fruit. This was attributed to phenol glycoconjugation following grapevine smoke exposure, as demonstrated by Dungey et al. (2011).

Similarly, low or undetectable levels of glycoconjugates and smoke-derived phenols were present in control wines only. The presence of low levels of guaiacol glycoconjugates in control wines indicates these compounds can occur naturally in some grape varieties; several previous studies have reported bound forms of guaiacol as components of grapes and/or juice from Tempranillo, Grenache, Shiraz, Merlot and Viognier (Wirth et al. 2001, Lopez et al. 2004, Dungey et al. 2011). For smoked wines, the duration of skin contact significantly influenced the extraction of glycoconjugates and release of guaiacol and 4-methylguaiacol during fermentation.
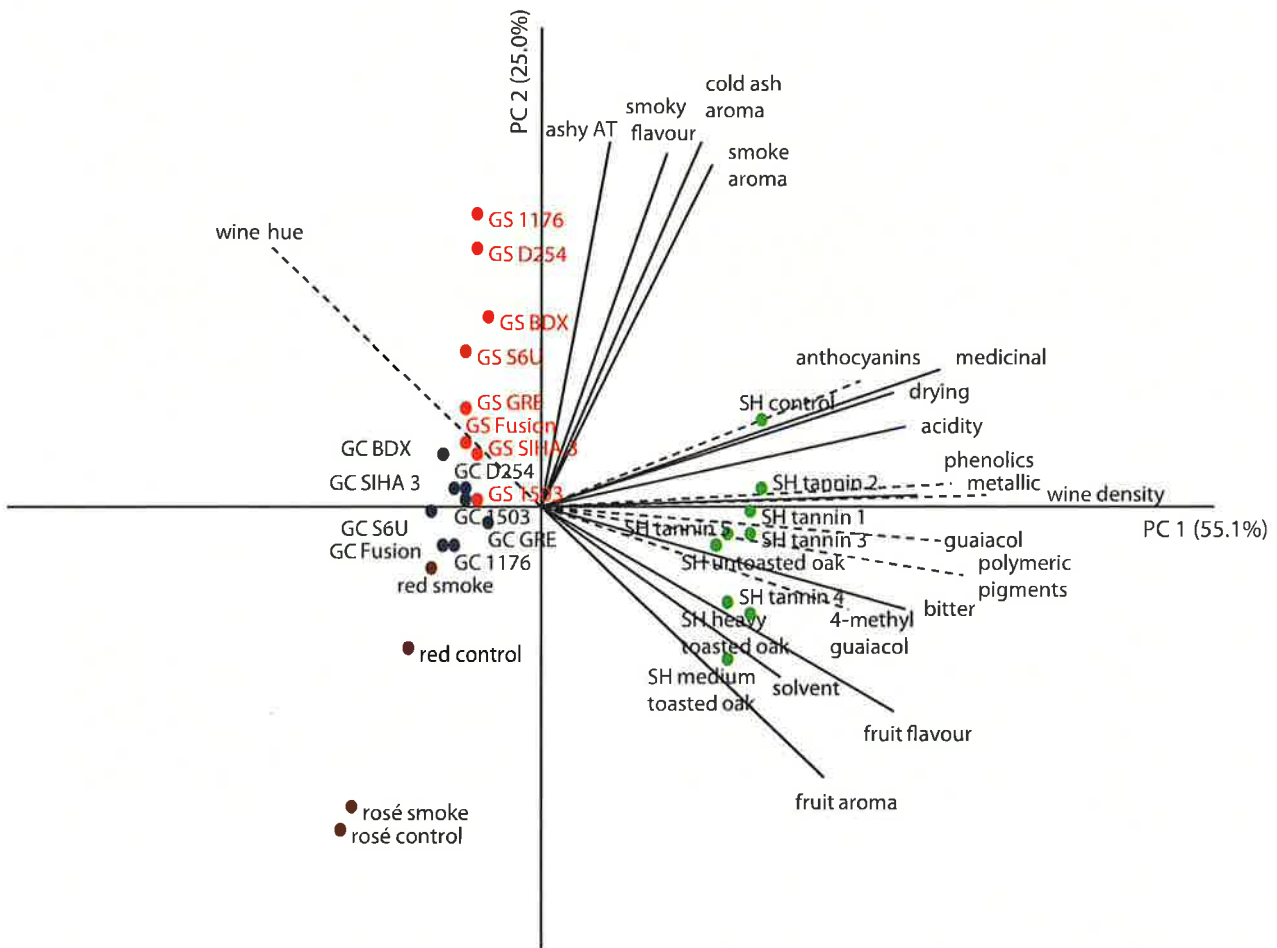
Figure 4. Principal component analysis of the mean value for wine colour density, wine hue, chemical composition and sensory attributes for red and rosé style Grenache table wines (●), Grenache wines fermented with different yeast strains (● control, ● smoked) and Shiraz wines made with oak and tannin additions (●).

Descriptive sensory analysis of the wines identified differences directly attributed to both grapevine smoke exposure and the wine style. 'Smoke' and 'medicinal' aroma, 'smoky' flavour and 'ashy aftertaste' clearly dominated the sensory profile of smoked red style wines. The sensory analysis demonstrated that the effect of smoke exposure was more pronounced in the red style wines, than the rosé style wines, which can be attributed to the greater extraction of smoke-derived components during fermentation on skins, compared with cold maceration. In contrast, 'fruit' aroma and flavour was more prominent in smoked rosé style wines, which indicated that the reduction in volatile phenol content caused by cold maceration might have enabled a more apparent sensory contribution of grape components responsible for the typical 'fruit' aromas and flavours of Grenache. Thus cold maceration and the duration of skin contact are important considerations when processing smoke-affected grapes.

Yeast selection trial
Eight different yeast strains were evaluated for their capacity to suppress or mask the 'smoke' related sensory attributes associated with smoke taint, while maintaining wine quality. Because the same fruit (smoked or control Grenache) was used, differences in chemical composition and sensory properties should be directly attributable to the deliberate application of smoke to grapevines and the action of particular yeast strains.

The effect of smoke application was evident in reduced wine TA, while the production of VA was influenced by the yeast strain, in particular yeast activity during fermentation of smoke-affected grape juice. Elevated VA was found in smoked wines fermented with S6U and AWRI 1176, while ICV D254 significantly decreased VA production, irrespective of smoke exposure. The extraction of phenolic compounds was enhanced with SIHA activ 3 and AWRI Fusion in smoked wines, but not in corresponding controls. In contrast, reduced wine phenolics, in particular anthocyanins, were observed in both smoked and control wines fermented with S6U, which might be because of its capacity to adsorb anthocyanins (Tosi et al. 2009). Furthermore, over the range of yeast strains used in this study, only weak correlations were established between formation of polymeric pigments and extraction of anthocyanins and phenolics. This is most likely attributable to decreased production of the yeast secondary metabolites (i.e. pyruvic acid and acetaldehyde), which are usually involved in the formation of anthocyanin-derived pigments (Hayasaka and Asenstorfer 2002, Asenstorfer et al. 2003, Morata et al. 2003). Based on expression of the wine parameters considered above, ICV GRE was regarded as the preferred yeast strain.

Similar to the wine style trial, guaiacol and 4-methylguiacol were detected only in wines made from smoke-affected grapes, with significant differences found in wines fermented with ICV GRE (5.3 µg/L) and S6U (12.9 µg/L). Quantification of guaiacol

glycoconjugates in wine highlighted ICV GRE as the only yeast strain where high precursor levels did not correspond to high guaiacol levels. With the exemption of ICV GRE, a strong correlation ($r = 0.91$) was established between wine guaiacol and precursor levels for all other yeast strains. Higher concentrations of guaiacol in wines fermented with *S. uvarum* S6U may have resulted from increased activity of β-D-glucosidase, the enzyme responsible for the hydrolysis of aroma precursors (Vazquez et al. 2002), or increased yeast population. It has also been suggested non-*Saccharomyces* yeast strains might be less inhibited by must glucose and pH, and thus display higher glucosidase activity (Otero et al. 2003, Villena et al. 2007). Variation in guaiacol and guaiacol glycoconjugate concentrations may also be associated with the activity of yeast pectinases, which catalyse the hydrolysis of grape skin pectins and enable extraction of compounds located in grape skins (Gainvors et al. 1994).

Smoked Grenache wines were generally rated higher in 'smoky' and 'ashy' and lower (or equal) in 'fruity' aroma and flavour compared with their corresponding control wines. The most intense smoke-related attributes were perceived in smoked wines made with AWRI 1176, ICV D254, BDX and S6U; in the current study, this could be correlated with increased guaiacol and guaiacol glycoconjugate levels. This is in agreement with previous studies that attributed the 'smoky', 'phenolish', 'ash', 'burning wood' and 'burnt' aromas of smoke-tainted wines with increased levels of guaiacol and 4-methylguiacol following grapevine exposure to smoke (Kennison et al. 2007, Hayasaka et al. 2010a). Based on the descriptive sensory analysis results, the yeast strains most capable of reducing 'smoke' aroma and flavour, without compromising 'fruit' characters were AWRI 1503, ICV GRE, AWRI Fusion and SIHA active 3.

Oak chips and tannin trial

Oak chips and tannins are commonly used in winemaking to improve wine colour, stability and aroma. In this trial, the capacity of oak and tannin additives to ameliorate the sensory attributes of smoke taint through enhanced wine complexity was investigated, using Shiraz grapes exposed to bushfire smoke.

The addition of oak chips and tannins reduced VA (except for Tannin 3) and altered phenolic composition. Tannins (in particular Tannin 3) contributed more to phenolic content than oak chips, but less than previously reported for Shiraz wines by Bowyer (2009). The formation of polymeric pigments was enhanced in wines treated with medium toasted oak, but varied in wines treated with tannins, which may be associated with the different composition of tannins used in this study. As a result, wine hue was affected more than wine colour density. Oak or tannin additives increased guaiacol and 4-methylguaiacol concentrations, with greater contributions from oak treatments, including oak-derived Tannin 4, compared with tannin treatments. However, this is not surprising given that guaiacol and 4-methylguaiacol are known oak volatiles (Pollnitz et al. 2004, Garde-Cerdán et al. 2008, Rodriguez-Bencomo et al. 2009). Guaiacol glycoconjugate concentrations were approximately 10% higher in control wines than oak- or tannin-treated wines; possibly indicating some adsorption of glycoconjugates by the oak and tannin. Other smoke constituents might be similarly adsorbed.

The sensory attributes of each wine enabled differentiation of oak and tannin treatments. The intensity of smoke-related attributes was reduced in both oak- and tannin-treated wines compared with the corresponding control wines. Wines produced with medium toasted oak chips were deemed least 'smoky' and 'ashy', followed by wines made with heavily toasted oak, then untoasted oak. Previous studies reported the degree of oak

toasting to be more influential on the release of oak volatiles than oak composition (Guchu et al. 2006, Rodriguez-Bencomo et al. 2008, 2009). Koussissi et al. (2009) found that highly toasted oak chips enhanced 'earthy', 'nutty' and 'vegetative' ('fresh') aromas, medium toasted oak chips 'woody' and 'smoky' aromas, whereas lightly toasted oak chips intensified 'coconut' aroma and 'sweet' taste in Greek Aghiorghitiko wines. In the current study, 'fruit' aroma was enhanced in all oak-treated wines, while 'fruit' flavour was significantly higher only in wines made with heavy toasted oak chips. Furthermore, the wines treated with tannins were perceived as less 'smoky' than control wines, but little effect was noted for 'fruity' attributes. An earlier study reported a considerable effect of oenological tannins on the chemical and sensory properties of Shiraz wines, which received significantly higher preference ranking compared with control or oak-treated wines (Bowyer 2009). We observed an opposite trend instead, consistent with the previously reported low impact of tannins on sensory properties of Monastreli wines (Bautista-Ortin et al. 2005); this is also likely caused by the differences in composition of the tannins.

The sensory results suggest the addition of oak or tannin additives can reduce the intensity of smoke taint, albeit through increased wine complexity, rather than a reduction in the concentration of smoke-derived volatile phenols. Oak is known to favourably influence the sensory properties of wine; volatile compounds, for example *cis*- and *trans*-oak lactone, are extracted during fermentation and/or maturation and can contribute to a wine's overall aroma and flavour, enhancing character and complexity (Maga 1989). It is therefore possible that oak volatiles derived from the oak chips or tannin additions masked the sensory contribution of smoke constituents, such as guaiacol and 4-methylguaiacol. As such, these additives afford an additional winemaking technique for consideration when processing smoke-affected grapes.

Conclusion

The winemaking techniques applied in the current study were shown to be capable of: (i) modifying wine composition; and (ii) reducing the intensity of objectionable smoke-related aroma and flavour attributes. Previous studies have shown that guaiacol glycoconjugates accumulate preferentially in grape skins (Dungey et al. 2011), so winemaking techniques that minimise duration of skin contact (e.g. cold maceration), should reduce the extraction of guaiacol glycoconjugates, and therefore the intensity of smoke taint in finished wine. Different yeast strains modified wine aroma, flavour, colour and complexity to different degrees, so yeast selection is also an important consideration when processing smoke-affected fruit. The addition of oak chips or tannins during winemaking enhanced the complexity of Shiraz wines, again reducing the perception of smoke-related attributes. Guaiacol and 4-methylguaiacol again proved to be useful marker compounds for smoke taint, together with sensory analysis, but the assessment of smoke taint could be extended to include consideration of guaiacol glycoconjugates and other volatile phenols in further studies. Given that significant amounts of the guaiacol glycoconjugate pool remained in the wines after fermentation was completed, it is likely that guaiacol (and thus smoke taint) will continue to evolve slowly in the bottle with age. Quantification of glycoconjugates in grapes and wine might therefore indicate the potential development of smoke taint during fermentation and storage.

Acknowledgements

References

Asenstorfer, R.E., Markides, A.J., Iland, P.G. and Jones, G.P. (2003) Formation of vitisin A during red wine fermentation and maturation. Australian Journal of Grape and Wine Research **9**, 40–46.

Bautista-Ortin, A.B., Martinez-Cutillas, A., Ros-Garcia, J.M., Lopez-Roca, J.M. and Gomez-Plaza, E. (2005) Improving colour extraction and stability in red wines: the use of maceration enzymes and enological tannins. International Journal of Food Science and Technology **40**, 867–878.

Bowyer, K.P. (2009) Tannins vs oak chips: what does each contribute to your wine? The Australian and New Zealand Grapegrower and Winemaker **61**, 61–65.

De Santis, D. and Frangipane, M.T. (2010) Effect of prefermentative cold maceration on the aroma and phenolic profiles of a Merlot red wine Italian. Journal of Food Science **22**, 47–53.

Dungey, K.A., Hayasaka, Y. and Wilkinson, K.L. (2011) Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography-tandem mass spectrometry based stable isotope dilution analysis. Food Chemistry **126**, 801–806.

Gainvors, A., Frezier, V., Lemaresquier, H., Lequart, C., Aigle, M. and Belarbi, A. (1994) Detection of polygalacturonase, pectin-lyase and pectin-esterase activities in a *Saccharomyces cerevisiae* strain. Yeast **10**, 1311–1319.

Garde-Cerdán, T., Lorenzo, C., Carot, J.M., Jabaloyes, J.M., Esteve, M.D. and Salinas, M.R. (2008) Statistical differentiation of wines of different geographic origin and aged in barrel according to some volatile components and ethylphenols. Food Chemistry **111**, 1025–1031.

Guchu, E., Diaz-Maroto, M.C., Perez-Coello, M.S., Gonzalez-Vinas, M.A. and Ibanez, M.D.C. (2006) Volatile composition and sensory characteristics of Chardonnay wines treated with American and Hungarian oak chips. Food Chemistry **99**, 350–359.

Hayasaka, Y. and Asenstorfer, R.E. (2002) Screening for potential pigments derived from anthocyanins in red wine using nonoelectrospray tandem mass spectrometry. Journal of Agricultural and Food Chemistry **47**, 3130–3139.

Hayasaka, Y., Baldock, G.A., Pardon, K.H., Jeffery, D.W. and Herderich, M.J. (2010a) Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine *Vitis vinifera* L. cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. Journal of Agricultural and Food Chemistry **58**, 2076–2081.

Hayasaka, Y., Dungey, K.A., Baldock, G.A., Kennison, K.R. and Wilkinson, K.L. (2010b) Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. Analytica Chimica Acta **660**, 143–148.

Holt, H.E., Iland, P.G. and Ristic, R. (2006) A method for mini-lot fermentation for use in research and commercial viticultural and winemaking trials. The Australian and New Zealand Grapegrower and Winemaker **509a**, 74–81.

Iland, P.G., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004) Chemical analysis of grapes and wine: techniques and concepts (Patrick Iland Wine Promotions Pty Ltd: Adelaide).

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith, J.H. and Gibberd, M.R. (2007) Smoke-derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry **55**, 10897–10901.

Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry **56**, 7379–7383.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research **15**, 228–237.

Koussissi, E., Dourtoglou, V.G., Ageloussis, G., Paraskevopoulos, Y., Dourtoglou, T., Paterson, A. and Chatzilazarou, A. (2009) Influence of toasting of oak chips on red wine maturation from sensory and gas chromatographic headspace analysis. Food Chemistry **114**, 1503–1509.

Lawless, H.T. and Heymann, H. (1999) Acceptance and preferance testing. In: Sensory evaluation of food: principles and practices. Ed. D.R. Heldman (Chapman & Hall, International Thomson Publishing: New York) pp. 430–479.

Lopez, R., Ezpeleta, E., Sanchez, I., Cacho, J. and Ferreira, V. (2004) Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. Food Chemistry **88**, 95–103.

Maga, J.A. (1989) The contribution of wood to the flavour of alcoholic beverages. Food Reviews International **5**, 39–99.

Morata, A., Gomez-Cordoves, A.C., Colomo, B. and Suarez, J.A. (2003) Pyruvic acid and acetaldehyde production by different strains of *Saccharomyces cerevisiae*: relationship with vitisin A and B formation in red wines. Journal of Agricultural and Food Chemistry **51**, 7402–7409.

Otero, R.R.C., Iranzo, J.F.U., Briones-Perez, A.I., Potgieter, N., Villena, M.A., Pretorius, I.S. and van Rensburg, P. (2003) Characterization of the beta-glucosidase activity produced by enological strains of non-*Saccharomyces* yeasts. Journal of Food Science **68**, 2564–2569.

Pollnitz, A.P., Pardon, K.H. and Sefton, M.A. (2000) Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine. Journal of Chromatography A **874**, 101–109.

Pollnitz, A.P., Pardon, K.H., Sykes, M. and Sefton, M.A. (2004) The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. Journal of Agricultural and Food Chemistry **52**, 3244–3252.

Radeka, S., Herjavec, S., Persuric, D., Lukic, I. and Sladonja, B. (2008) Effect of different maceration treatments on free and bound varietal aroma compounds in wine of *Vitis vinifera* L. cv. Malvazlia istarska bijela. Food Technology and Biotechnology **46**, 86–92.

Rodriguez-Bencomo, J.J., Ortega-Heras, M., Perez-Magarino, S., Gonzalez-Huerta, C. and Jose, M. (2008) Importance of chip selection and elaboration process on the aromatic composition of finished wines. Journal of Agricultural and Food Chemistry **56**, 5102–5111.

Rodriguez-Bencomo, J.J., Ortega-Heras, M., Perez-Magarino, S. and Gonzalez-Huerta, C. (2009) Volatile compounds of red wines macerated with Spanish, American, and French oak chips. Journal of Agricultural and Food Chemistry **57**, 6383–6391.

Salinas, M.R., Garijo, J., Pardo, F., Zalacain, A. and Alonso, G.L. (2005) Influence of prefermentative maceration temperature on the colour and the phenolic and volatile composition of rose wines. Journal of the Science of Food and Agriculture **85**, 1527–1536.

Sheppard, S.I., Dhesi, M.K. and Eggers, N.J. (2009) Effect of pre- and post-veraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. American Journal of Enology and Viticulture **60**, 98–103.

Somers, T.C. and Evans, M.E. (1977) Spectral evaluation of young red wines: anthocyanin equilibria, total phenols, free and molecular SO_2, 'chemical age'. Journal of the Science of Food and Agriculture **28**, 279–287.

Stone, H. and Sidel, J.L. (2004) Descriptive analysis. In: Sensory evaluation practices. 3rd edn. Ed. S.L. Taylor (Academic Press: San Diego) pp. 201–245.

Tosi, E., Azzolini, M., Guzzo, F. and Zapparoli, G. (2009) Evidence of different fermentation behaviours of two indigenous strains of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* isolated from Amarone wine. Journal of Applied Microbiology **107**, 210–218.

Vazquez, L.C., Perez-Coello, M.S. and Cabezudo, M.D. (2002) Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airen, and Macabeo grapes. Analytica Chimica Acta **458**, 39–44.

Villena, M.A., Iranzo, J.F.U. and Perez, A.I.B. (2007) β-Glucosidase activity in wine yeasts: application in enology. Enzyme and Microbial Technology **40**, 420–425.

Wirth, J., Guo, W.F., Baumes, R. and Gunata, Z. (2001) Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. Journal of Agricultural and Food Chemistry **49**, 2917–2923.

Effect of leaf removal and grapevine smoke exposure on colour, chemical composition and sensory properties of Chardonnay wines

R. RISTIC[1], K. A. PINCHBECK[1], A.L. FUDGE[1], Y. HAYASAKA[2] and K.L. WILKINSON[1]

[1] School of Agriculture, Food and Wine, The University of Adelaide, Glen Osmond, SA 5064, Australia
[2] The Australian Wine Research Institute, Glen Osmond, SA 5064, Australia
Corresponding author: Dr Kerry L Wilkinson, email kerry.wilkinson@adelaide.edu.au

Abstract

Background and Aims: The effect of leaf removal on the accumulation of smoke-related volatile compounds following exposure of grapevines to smoke under experimental conditions was investigated.

Methods and Results: Chardonnay grapevines were defoliated around the bunches either before or after exposure to smoke. The effect of defoliation and/or smoke treatments on crop yield and vegetative growth was then evaluated. The extent of smoke taint in the resultant wines was determined by quantification of volatile phenols by gas chromatography-mass spectrometry and of guaiacol glycoconjugates by liquid chromatography-tandem mass spectrometry and by descriptive sensory analysis. The concentration of volatile phenols and guaiacol glycoconjugates was highest in wines corresponding to leaf removal pre-smoke exposure. These wines were characterised by intense 'smoky', 'ashy' and 'burnt rubber' attributes, whereas defoliation post-smoke exposure reduced the intensity of 'cold ash' and 'ashy aftertaste' attributes compared with that of other experimental treatments.

Conclusions: Leaf removal had a beneficial effect on wine aroma, enhancing the intensity of 'fruit' attributes. Where leaf removal was employed post-smoke exposure, resulting wines exhibited less intense 'smoke' characters than wines corresponding to grapevines subjected to smoke exposure. Leaf removal prior to smoke exposure, however, did not mitigate the intensity of smoke taint.

Significance of the Study: This study improves the current understanding of the impact on fruit and wine composition of the exposure of grapevines to smoke. It informs grapegrowers and winemakers of viticultural practices that influence the severity of smoke taint in grapes and wine.

Keywords: glycoconjugate, grape, guaiacol, leaf removal, smoke taint, volatile phenol, wine

Introduction

The effect of smoke on grapes and the subsequent development of smoke taint in wine have been reported in several recent studies (Kennison et al. 2007, 2008, 2009, Sheppard et al. 2009). Even short periods of exposure of grapevines to smoke, i.e. 20–30 min, have resulted in an elevated concentration of volatile phenols and their glycoconjugates in grapes and wines (Hayasaka et al. 2010b,c, Dungey et al. 2011) and undesirable 'smoky', 'ashy', 'burnt rubber', 'medicinal' and 'phenolic' wine sensory characteristics (Kennison et al. 2007, Hayasaka et al. 2010c, Ristic et al. 2011). Recent research has investigated methods for the amelioration of smoke taint either during or following winemaking (Fudge et al. 2011, 2012, Ristic et al. 2011), but there is no information on whether viticultural management practices can minimise grapevine uptake of smoke in the vineyard.

Leaf removal (LR) (or defoliation) is a viticultural practice performed during the growing season to regulate canopy density and bunch exposure in order to improve grape quality (Petrie et al. 2000a, Poni et al. 2006). Specific outcomes depend on the timing and severity of LR, as well as grape cultivar, rootstock and climate (Candolfi-Vasconcelos et al. 1994, Bavaresco et al. 2008, Tardaguila et al. 2008). Early defoliation is considered to reduce berry weight and consequently fruit

yield (Poni et al. 2006, Diago et al. 2010), whereas defoliation at or after veraison affects primary and secondary metabolite synthesis (Hunter and Visser 1989, Zoecklein et al. 1992, 1998a,b). In dense canopies, improved bunch microclimate following defoliation has been largely attributed to increased bunch temperature and modification of the ratio of red : far red light (Smart et al. 1988, Dokoozlian and Kliewer 1995a,b). Open canopies, particularly in warmer climates, need to be defoliated more carefully because of the potential risks of sunburn and overheating of the berry, which may result in reduced grape and wine quality (Tarara et al. 2008, Chorti et al. 2010, Ristic et al. 2010).

LR has also been associated with modification of source–sink relationships. LR reduces the surface area available for carbon dioxide (CO_2) assimilation, thereby decreasing photosynthetic rates and altering the grapevine's carbon budget (Candolfi-Vasconcelos et al. 1994); albeit the photosynthetic contribution of leaves is dependent on their age (Candolfi-Vasconcelos and Koblet 1991). Grapevines, however, are capable of growing replacement foliage and/or stimulating existing leaves to function more efficiently (Hunter and Visser 1989, Candolfi-Vasconcelos and Koblet 1991, Petrie et al. 2000c).

Hayasaka and co-workers (2010b) studied the uptake, translocation and conjugation of guaiacol, a smoke marker

compound, in grapevines with stable isotope tracers. Leaves and bunches of potted Cabernet Sauvignon vines were exposed to an aqueous solution containing d_0- and d_3-guaiacol. Leaves and berries in direct contact with the solution contained abundant quantities of d_0- and d_3-guaiacol conjugates, but control leaves and berries from the same vine, which had not been in direct contact with the solution, contained only a trace of these conjugates. The authors therefore concluded that there was limited translocation of either guaiacol or guaiacol conjugates between leaves and berries. The timing of grapevine exposure to smoke, however, has been shown to influence the extent of smoke taint, and the phenological stage at which grapevines are most susceptible to smoke exposure, i.e. approximately 7–10 days post-veraison (Kennison et al. 2009, 2011), coincides with when phloem transport rates are highest (McCarthy and Coombe 1999). This highlights the need for further research to investigate the uptake of smoke-derived volatile compounds by leaves and berries. As such, we established a trial comprising the removal of all main and lateral leaves around bunches, either before or after exposure of grapevines to smoke, and determined the effect of defoliation and/or smoke treatments on wine composition and sensory properties.

Materials and methods

Vineyard site and experimental design
The trial was conducted during 2009/10 in a vineyard located in Nuriootpa in the Barossa Valley district of South Australia (34°30′S, 138°59′E, altitude 274 m). The climate for this region is classified as warm (Dry and Smart 1988), with a mean January temperature of 21.4°C and 1832 biologically effective day degrees (Gladstones 1997). Rainfall is moderate (typically 502 mm) with high summer evaporation and low relative humidity. The soil for this site is classified as a light pass, fine sandy loam (Northcote 1988).

Chardonnay vines planted in 1987 and grown on their own roots were cordon-trained and spur-pruned. Row and vine spacing were 3.7 and 3.1 m, respectively, with north-south row orientation. Vineyard management practices were typical of those employed in the district.

Five different treatments were applied (in triplicate) comprising: (i) 'control', i.e. no defoliation and no smoke exposure; (ii) 'LR', i.e. defoliation but no smoke exposure; (iii) 'smoke', i.e. no defoliation, but smoke exposure; (iv) 'LR pre-smoke', i.e. defoliation before smoke exposure; and (v) 'LR post-smoke', i.e. defoliation after smoke exposure. Treatments were arranged in a completely randomised block design; each replicate consisted of four vines with at least one buffer panel and row between treatments. Defoliation and smoke treatments were imposed at approximately 7 days post-veraison. Defoliation consisted of manual removal of all main and lateral leaves located at nodes opposite a bunch, as well as from nodes directly above and below each bunch, immediately before or after smoke application. Vines were enclosed in a purpose-built smoke tent and exposed to straw-derived smoke for 60 min under the experimental conditions described previously (Kennison et al. 2008, Ristic et al. 2011).

Viticultural parameters
Sunlight intensity at the bunch zone was measured with a ceptometer (Decagon Devices, Cambridge, England) on a cloudless day between 12 noon and 1 pm, 5 days after the experimental treatments were imposed. Readings were taken adjacent to the fruit zone on both sides of each vine, with the ceptometer positioned parallel to the cordon and facing upwards. The pro-

portion of photosynthetically active radiation (PAR) penetrating the canopy at the bunch zone was calculated against ambient measurements taken at bunch zone height outside the canopy.

Empirical models were developed to enable determination of the leaf areas of main and lateral shoots. Approximately one week after the experimental treatments were imposed, representative shoots (104 primary shoots, ranging from 35 to 183 cm in length) were collected from vines in buffer panels (i.e. two to three shoots per vine, from eight buffer vines, in each of five rows). For each shoot, the number and area of main and lateral leaves estimated according to primary vein length (Schultz 1992) were determined. Main leaf area (MLA) per shoot was calculated by summation of the leaf area of all leaves on the main shoot. Lateral leaf area (LLA) per shoot was calculated by summation of the leaf area of all leaves on the lateral shoot. Empirical models were generated by plotting MLA and LLA against shoot length (data not shown). After pruning, the number and length of main and lateral canes for all experimental vines were measured. The main and LLAs of each cane were then calculated from the empirical models and summed to determine MLA and LLA per vine (per replicate per treatment). Total leaf area (TLA) per vine was calculated as the sum of the MLA and LLAs, i.e. MLA + LLA.

Canopy volume was calculated by multiplying canopy height, length and width. Yield per vine represented the total weight of all bunches harvested from one vine, and the mean bunch weight was calculated by dividing yield by the number of bunches per vine. After pruning, the one-year-old wood from each vine was collected and weighed to determine pruning weight. Leaf area/fruit weight (LA/FW) was calculated as a ratio of the TLA (TLA less MLA removed) to total fruit weight (i.e. yield). Fruit weight/pruning weight (FW/PW) was calculated as a ratio of yield to pruning weight.

Berry samples (50 berries per replicate per treatment) were collected weekly from 'pea-size' (E–L stage 31) until 'harvest' (E–L stage 38), and berry weight and sugar accumulation (total soluble solids (TSS), expressed as °Brix) were measured. Additional samples were taken at harvest to enable determination of juice pH, TSS and the concentration of guaiacol glycoconjugates.

Winemaking
Fruit was harvested when juice TSS reached approximately 22°Brix (approximately 3 weeks after experimental treatments were imposed) and stored overnight at 3°C. Bunches (5 kg per field replicate per treatment) were de-stemmed, crushed and pressed with the addition of 50 mg/L sulfur dioxide (added as an 8% solution of potassium metabisulfite) and 0.01 g/L Lallzyme HC (Lallemand, Edwardstown, SA, Australia). Tartaric acid was added to adjust the pH of the must to 3.5, prior to inoculation with 0.3 g/L EC-1118 yeast (Lallemand) and addition of diammonium phosphate (150 mg/L). Musts were fermented at 15°C until the residual sugar approached dryness (i.e. <2 g/L). Wines were then racked from gross lees and cold stabilised (at 0°C for 4 months). No wines underwent malolactic fermentation. Wine pH and free SO_2 were adjusted to 3.5 and 30 mg/L, respectively, before bottling (under screw cap closures). Bottles were stored at 15°C for 6 months prior to chemical and sensory analysis.

Chemical analysis
Wines were analysed for pH, titratable acidity (TA), volatile acidity (VA), glucose, fructose, alcohol content and phenolic composition (Iland et al. 2004). Wine colour was determined using spectral radiance measurements (CIELAB) performed with a μQuant spectroradiometer (Winooski, VT, USA) operating in

the range of 380–780 nm at 4-nm intervals. Guaiacol glycoconjugates were measured by HPLC-MS/MS according to the stable isotope dilution analysis (SIDA) method developed by Dungey et al. (2011). The concentration of the volatile phenols – guaiacol; 4-methylguaiacol; 4-ethylguaiacol; 4-ethylphenol; p-, m- and o-cresols; syringol; and methylsyringol – was determined by The Australian Wine Research Institute's (AWRI) Commercial Services Laboratory (Adelaide, Australia), according to the SIDA methods reported previously (Hayasaka et al. 2010a). These publications describe the preparation of internal standards, method validation and instrumental operating conditions.

Sensory analysis

Descriptive sensory analysis (Stone and Sidel 2004) of wines consisted of a series of training sessions and formal evaluations. A tasting panel comprising seven female and four male staff and students from The University of Adelaide and the AWRI was convened. Prior to sensory analysis, each wine replicate was informally assessed by four experienced tasters in order to assess any artefacts or off-flavours, and to determine the scope of the difference between treatments. During the training period, the tasting panel generated appropriate descriptive terms and gained familiarity in recognising and scoring the intensity of selected and defined attributes following the procedure outlined by Lawless and Heymann (1999). The wines were then formally evaluated in isolated booths at 22–23°C under sodium lights during three consecutive sensory sessions, i.e. with one wine replicate from each experimental treatment presented in each session. All wines were presented as 30-mL samples in three-digit coded, covered ISO standard wine glasses. Panellists rated aroma attributes and several selected mouth-feel attributes (Table 1) while holding the wine in mouth and after expectoration. The intensity of sensory attributes was rated on a 15-cm unstructured line scale marked from 0 to 10, with anchor points of 'low' (at 10% of the line) and 'high' (at 90% of the line). Panellists rinsed thoroughly with pectin solution (1 g/L) and water, and rested for at least 45 s between samples. Data were acquired with Fizz software (Version 2.4, Biosystèmes, Couternon, France).

Statistical methods

Viticultural and chemical data were analysed by analysis of variance (ANOVA) and all pairwise multiple comparisons with the Tukey test using Prism (Version 5.04, GraphPad Software, Inc. La Jolla, CA, USA). Sensory data were analysed using SenPaq (Version 4.82, Qi Statistics, Reading, England) and a mixed model ANOVA to ascertain the effects of treatment, fermentation replicate, presentation replicate and judge (treating judges in a random effect). The method for discrimination among the means was Fisher's least significant difference at $P < 0.1$. The correlation matrix of mean ratings across the wines for all attributes that showed significant differences according to the ANOVA was subjected to principal component analysis (PCA) (JMP, Version 7, SAS Institute, Cary, NC, USA) focusing on the principal components (PCs) that showed Eigenvalues higher than 1. Chemical parameters were projected onto the sensory data to show possible correlations between sensory and chemical data.

Results and discussion

Effect of defoliation and smoke exposure on viticultural parameters

Grapevines were characterised by canopies with a high proportion of lateral foliage growth, approximately 40%, relative to the MLA, approximately 60% (Table 2). There was no statistical

Table 1. Aroma and palate attributes used for sensory analysis of Chardonnay wines.

Attribute	Description
Aroma	
Fruit aroma intensity	The intensity of the overall fruit aroma
Smoke	Perception of any type of smoke aroma, including smoked meat/bacon, toasty, charry, cigar box, estery
Cold ash	Burnt aroma associated with ashes, including ashtray, tarry, campfire
Earthy	Any aroma associated with musty, dusty, wet-wood, barnyard, mushroom-like, dank, mouldy, stagnant, stale
Burnt rubber	Perception of burnt rubber-like aromas
Medicinal	Aromatic characteristic of band-aids, disinfectant-like, including cleaning products, solvents, chemicals
Palate	
Fruit flavour intensity	The intensity of the overall fruit flavour
Smoky	Perception of smoke flavour, including bacon and smoked meat
Ashy aftertaste	Length of taste associated with residue of ashtray perceived in the mouth after expectorating, including coal ash, ashtray, tarry, acrid, camp fire
Woody aftertaste	Length of taste associated with woody residue, including wood, oak, pencil shavings
Acidity	Intensity of sour/acid taste
Metallic	The 'tinny' flavour associated with metals
Bitter	Intensity of bitter taste, bitter aftertaste
Drying	Drying, puckering mouth-feel after expectoration of the wine

difference in the MLA, LLA or TLA of vines allocated to the different experimental treatments. Defoliation, however, resulted in the removal of 0.43–0.45 m² per vine or approximately 9–10% of MLA (Table 2). Previous studies found that partial defoliation applied late in the growing season increased lateral formation in order to achieve a photosynthetically and physiologically efficient leaf area (Reynolds and Wardle 1989, Hunter and Leroux 1992) and/or to enhance the photosynthetic efficiency of remaining leaves (Hunter and Visser 1989, Petrie et al. 2000b). It is not known, however, if similar physiological responses occurred to some extent in the current study. Canopy architecture characterised by measurement of canopy volume, surface area, and the number of main and lateral shoots per vine (data not shown) was similar across the different experimental treatments. This suggests that retention of smoke within the whole canopy would have been similar for all treatments involving smoke application to grapevines.

The application of smoke to Merlot grapevines was found to affect sugar accumulation (Kennison et al. 2008), but in the current study, no significant differences were observed between experimental treatments during ripening (data not shown) or at

Table 2. Viticultural measurements for Chardonnay grapevines.

	Experimental treatments					*P*
	'Control'	'LR'	'Smoke'	'LR pre-smoke'	'LR post-smoke'	
PAR (%)	22.9 ± 1.4[b]	31.4 ± 1.1[a]	24.4 ± 1.6[b]	33.2 ± 0.9[a]	31.8 ± 2.7[a]	0.001
Canopy volume (m³)	3.0 ± 0.1	3.2 ± 0.1	3.4 ± 0.4	2.8 ± 0.2	3.0 ± 0.1	ns
Pruning weight (kg)	1.4 ± 0.2	1.5 ± 0.2	1.3 ± 0.1	1.2 ± 0.0	1.4 ± 0.2	ns
Main leaf area (m²)	4.7 ± 0.2	4.9 ± 0.2	4.9 ± 0.3	4.3 ± 0.2	4.9 ± 0.3	ns
Lateral leaf area (m²)	3.8 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	3.8 ± 0.2	4.0 ± 0.1	ns
Total leaf area (m²)	8.5 ± 0.3	9.1 ± 0.5	8.9 ± 0.4	8.0 ± 0.4	8.9 ± 0.5	ns
Main leaf area removed (m²)	–	0.43 ± 0.0	–	0.44 ± 0.1	0.45 ± 0.1	ns
Berry weight (g)	0.85 ± 0.03	0.86 ± 0.03	0.80 ± 0.08	0.77 ± 0.05	0.79 ± 0.04	ns
Bunch weight (g)	20.9 ± 1.6	19.9 ± 1.2	21.7 ± 2.1	22.0 ± 3.1	25.0 ± 5.5	ns
Yield/vine (kg)	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.4	1.8 ± 0.4	ns
LA†/FW	5.3 ± 0.8	5.3 ± 1.1	5.3 ± 0.4	5.1 ± 2.4	5.2 ± 0.9	ns
FW/PW	1.2 ± 0.3	1.1 ± 0.2	1.3 ± 0.1	1.5 ± 0.4	1.2 ± 0.1	ns

†LA = (TLA–MLA removed). Values represent the mean of three replicates ± standard deviation. Values followed by different letters within rows are significantly different. FW/PW, fruit weight/pruning weight; LA/FW, leaf area/fruit weight; LR, leaf removal; ns, not significant; PAR, photosynthetically active radiation.

harvest (Table 2). Lower average berry weight was observed for fruit harvested from smoke-affected grapevines, but it was not significantly different to that of the control fruit. Furthermore, a significant difference was not observed for berry and bunch weight measurements between defoliated and non-defoliated treatments (Table 2). Berry weight (0.77–0.86 g), bunch weight (20–25 g) and consequently crop yield (1.6–1.8 kg/vine) were extremely low; this was attributed to a combination of dry grown grapevines (i.e. irrigation was not applied in 2009/10 nor in the previous season) and unusually hot weather conditions experienced during flowering and the early stages of berry development (May 1992, Ebadi et al. 1995). LA/FW being 5.1–5.3 and FW/PW being 1.2–1.5 were similar across treatments (Table 2), i.e. despite defoliation. Previous studies found early defoliation contributed to reduced berry and bunch weight because of reduced carbohydrate supply (Petrie et al. 2000a, Poni et al. 2006), although other studies did not observe similar effects (Zoecklein et al. 1992, Intrieri et al. 2008, Tardaguila et al. 2008, Kotseridis et al. 2012). Post-flowering defoliation generally has less effect on berry and bunch weight, but according to Kotseridis et al. (2012), certain cultivars are more sensitive. Defoliation affected the yield and bunch weight of Merlot and Sangiovese but only the berry weight of Cabernet Sauvignon. The phenolic composition of Merlot and Cabernet Sauvignon berries, however, were improved, with little effect on Sangiovese.

Defoliation modified bunch microclimate by increasing PAR and possibly also bunch temperature. Defoliated treatments received 675–710 µmol/m²/s PAR (i.e. 31–34% of ambient radiation) at the bunch zone compared with that of the 'control' and 'smoke' treatments that received 520–540 µmol/m²/s PAR (i.e. 23–24% of ambient radiation) (Table 2). Smoke treatments were applied (in a randomised order) between 8 am and 6 pm on the 13 and 14 January 2010; when the mean daily temperature was 23.8 and 26.5°C, respectively. Canopy temperature measured at the fruit zone fluctuated in a typical diurnal pattern and ranged between 25 and 34°C; berry temperature was not measured. Exposure of bunches to sunlight has previously been found to give a higher level of TSS, anthocyanins and phenolics, and lower acidity, malate and juice pH compared with that of

shaded bunches (Kliewer 1970, Bledsoe et al. 1988, Bergqvist et al. 2001, Ristic et al. 2007), but in a warm climate, the concomitant increase in berry temperature may have a detrimental effect on berry composition (Kliewer 1977, Spayd et al. 2002, Tarara et al. 2008). In this study, the effect of defoliation on the uptake of smoke and therefore fruit and wine composition was likely to have been influenced to some extent by berry temperature, which may have been elevated due to increased sunlight exposure.

The effect of defoliation and smoke exposure on wine composition and sensory properties

Grapevine defoliation and/or exposure to smoke did not significantly affect wine pH, TA, VA or alcohol content (Table 3). While increased sugars, flavours, total and free-glucosides and flavonoids, and decreased acidity were reported in studies where dense canopies were defoliated at veraison (Zoecklein et al. 1992, 1998a,b, Poni et al. 2006), when similar techniques were applied to grapevines with low canopy density, there was little effect on grape juice sugar content, acidity or colour (Reynolds et al. 2007). In the current study, hydroxycinnamates and phenolic compounds were found to be the predominant phenolic group in all wines (Table 3), but statistically significant differences in concentration were not observed between treatments. The 'smoke' treatment showed slightly elevated brown pigments (0.15 au), while 'LR post-smoke' had higher phenolics (2.5 au). There was no sign of browning as either a noticeable change in colour or by spectral measures at A_{420}. CIELAB colour measurements were made to achieve a more accurate evaluation of any colour differences between wines, but similar values for colour coordinates a*, b*, L*, C_{ab}* and h_{ab} were obtained for all treatments (data not shown) and differences between the colour of wines, expressed as ΔE*, were less than 1.0 unit. ΔE* values ≥3 units are thought to be discernable to the human eye for red wines (Martinez et al. 2001). Because white wines are located in a different region of the colour space (i.e. L* ~95 units) than the region occupied by red wines, this estimate is somewhat difficult to ascertain for white wines. In the current study, there was no correlation established between CIELAB

Table 3. Composition of Chardonnay juices and wines.

	Experimental treatments					*P*
	'Control'	'LR'	'Smoke'	'LR pre-smoke'	'LR post-smoke'	
Juice						
TSS (°Brix)	22.3 ± 0.1	22.6 ± 0.2	22.3 ± 0.1	21.9 ± 0.2	21.9 ± 0.1	ns
pH	3.86 ± 0.06	3.79 ± 0.04	3.84 ± 0.04	3.77 ± 0.02	3.91 ± 0.05	ns
Glycoconjugates (μg/kg)	75 ± 2[c]	75 ± 7[c]	792 ± 222[b]	2462 ± 1472[a]	778 ± 97[b]	0.05
Wine						
Glycoconjugates (μg/L)	20 ± 1[c]	47 ± 10[c]	384 ± 53[ab]	496 ± 42[a]	365 ± 39[b]	0.001
Guaiacol (μg/L)	nd	nd	2.3 ± 0.3[b]	3.3 ± 0.9[a]	2.0 ± 0.6[b]	0.001
4-Methylguaiacol (μg/L)	nd	nd	nd	tr	nd	ns
Total cresols (μg/L)	nd	nd	tr	1.7 ± 1.2	tr	ns
Syringol (μg/L)	nd	nd	3.0 ± 0.6[b]	5.7 ± 1.8[a]	2.7 ± 0.3[b]	0.001
Brown pigments (au)	0.08 ± 0.03	0.09 ± 0.00	0.15 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	ns
Hydroxycinnamates (au)	2.2 ± 0.2	2.4 ± 0.2	2.1 ± 0.2	2.0 ± 0.1	2.5 ± 0.2	ns
Flavonoids (au)	0.6 ± 0.2	0.7 ± 0.2	0.9 ± 0.3	0.6 ± 0.1	0.9 ± 0.1	ns
Phenolics (au)	2.1 ± 0.3	2.3 ± 0.3	2.3 ± 0.2	2.0 ± 0.2	2.5 ± 0.2	ns
Alcohol (% v/v)	13.3 ± 0.0	13.2 ± 0.1	13.3 ± 0.1	13.2 ± 0.0	13.0 ± 0.1	ns
pH	3.3 ± 0.1	3.4 ± 0.0	3.4 ± 0.0	3.4 ± 0.0	3.4 ± 0.0	ns
TA (g/L)	6.9 ± 0.5	6.1 ± 0.1	6.1 ± 0.3	6.2 ± 0.4	6.1 ± 0.3	ns
VA (g/L)	0.35 ± 0.0	0.37 ± 0.0	0.37 ± 0.0	0.36 ± 0.0	0.36 ± 0.0	ns

Volatile acidity (VA) expressed as acetic acid. Values represent the mean of three replicates ± standard deviation. Values followed by different letters within rows are significantly different. LR, leaf removal; nd, not detected; ns, not significant; tr, trace (i.e. positive identification, but <1 μg/L); TA, titratable acidity.

colour coordinates and the concentration of volatile phenols or phenolic groups.

The assessment of smoke taint in wine included quantification of both volatile phenols and guaiacol glycoconjugates because it has been established recently that smoke-derived volatile phenols can accumulate in conjugated forms following exposure of grapevines to smoke (Hayasaka et al. 2010c, Dungey et al. 2011). Volatile phenols, including guaiacol, 4-methylguaiacol, cresols and syringol, were detected in wines made only from smoke-affected grapes (Table 3). Similarly, the concentration of total glycoconjugates was considerably higher in wines derived from treatments involving smoke exposure (>365 μg/L) compared with that of unsmoked treatments (<47 μg/L). There was no significant difference between 'smoke' and 'LR post-smoke' treatments; wines from the 'LR pre-smoke' treatment contained the highest concentration of volatile phenols (Table 3). The considerably higher level of glycoconjugates observed in fruit corresponding to 'LR pre-smoke' may reflect increased bunch exposure to smoke and/or enhanced berry metabolism arising from increased bunch temperature. The glycoconjugate pool remaining in 'LR pre-smoke' wine, however, was comparable with that of 'smoke' and 'LR post-smoke' wines. This suggests the glycoconjugate pool accumulated preferentially in grape skins, with only partial extraction during the processing of fruit, in agreement with previous studies (Dungey et al. 2011, Ristic et al. 2011).

Descriptive sensory analysis identified several key differences between experimental treatments that may be directly attributed to defoliation and/or smoke exposure (Table 4):

LR affects the intensity of 'fruit' aroma. Defoliation ('LR') significantly enhanced the intensity of wine 'fruit' aroma compared with that of the 'control' (i.e. 'no LR/no smoke') and of the 'smoke' treatments. 'Earthy' aroma and 'woody aftertaste' were also perceived to be much lower than that in any other treatment. The benefits of defoliation to fruit composition have been previously demonstrated. For example, LR increased the total concentration of glycoconjugates in Riesling and Chardonnay grapes, which was attributed to both assimilate movement (Quinlan and Weaver 1970) and intraplant competition because of the removal of low-efficiency leaves (Reynolds and Wardle 1989). In a later study, Reynolds and co-authors (2007) found that the concentration of glycosidically bound terpenes in Chardonnay was more responsive to defoliation than that of the free monoterpene concentration.

Grapevine smoke exposure affects the intensity of 'smoke'-related sensory attributes. Defoliation prior to the application of smoke ('LR pre-smoke') produced wines with intense 'smoky', 'ashy' and 'burnt rubber' attributes as well as enhanced 'bitterness'. Compared with wines from the 'smoke' treatment, only 'bitterness' was rated significantly higher, but compared with 'control' wines, both 'smoke' aroma and 'ashy aftertaste' were perceived to be more intense. No difference in the perception of 'fruit' attributes was observed when compared with other treatments involving LR.

Timing of LR affects the intensity of 'fruit' and 'smoke' attributes of smoke-affected wines. Defoliation after the application of smoke ('LR post-smoke') reduced the intensity of 'cold ash' and 'ashy aftertaste' attributes compared with that of the 'smoke' treatment, with no apparent reduction in 'fruit' aroma and flavour. This was an interesting observation given that there was no significant difference in the concentration of volatile phenols between these two treatments. It should be

Table 4. Mean ratings for sensory attributes of Chardonnay wines.

	Experimental treatments					LSD	P
	'Control'	'LR'	'Smoke'	'LR pre-smoke'	'LR post-smoke'		
Fruit aroma	3.9[b]	5.2[a]	4.8[ab]	4.1[b]	5.2[a]	1.0	0.05
Smoke	1.9[bc]	1.4[c]	2.5[ab]	3.0[a]	1.8[bc]	0.9	0.05
Cold ash	1.6[ab]	0.8[b]	1.8[a]	2.0[a]	0.9[b]	0.8	0.05
Earthy	2.2[a]	1.3[b]	1.9[ab]	2.2[a]	2.2[a]	0.7	0.1
Burnt rubber	0.5	0.5	0.6	1.0	0.4	0.5	ns
Medicinal	1.0	0.8	1.1	1.0	0.9	0.7	ns
Fruit flavour	4.4[ab]	4.6[ab]	5.1[a]	3.9[b]	5.2[a]	0.9	0.05
Smoky	1.7[ab]	1.0[b]	1.9[a]	2.2[a]	1.8[ab]	0.9	0.1
Ashy aftertaste	1.1[c]	0.9[c]	2.3[ab]	2.6[a]	1.4[bc]	1.0	0.01
Woody aftertaste	1.2[ab]	0.6[b]	1.6[a]	1.5[a]	1.5[a]	0.8	0.1
Metallic	2.3	1.6	1.9	2.3	1.6	0.8	ns
Bitter	2.0	2.2	1.5	2.4	1.9	0.7	ns
Drying	2.9	2.7	2.6	2.8	2.6	0.5	ns
Acidity	7.0[a]	6.1[b]	5.7[b]	6.1[b]	6.0[b]	0.9	0.1

Values represent the mean score from three fermentation replicates presented to 12 judges in three replicated sensory sessions. Values followed by different letters within rows are significantly different. LR, leaf removal; LSD, least significant difference; ns, not significant.
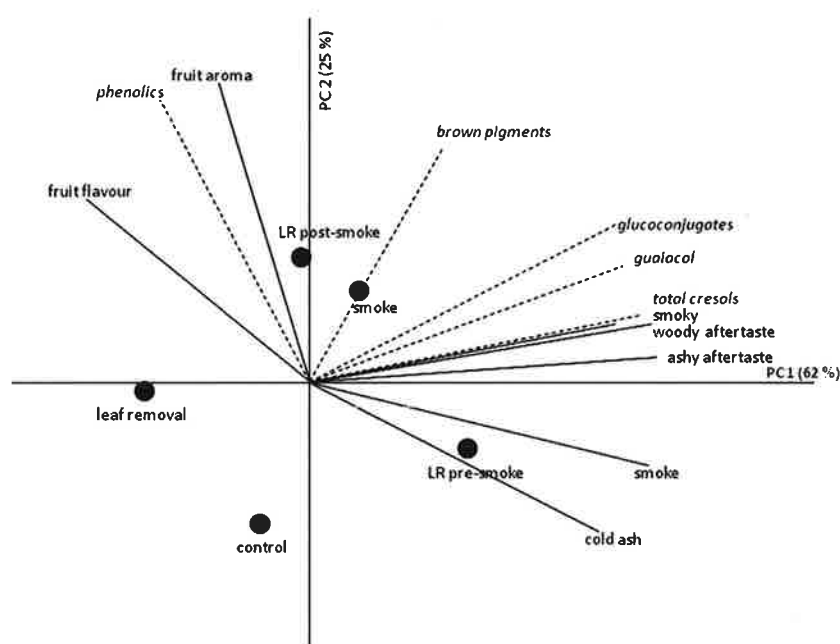

Figure 1. Principal component analysis (PCA) biplot of the mean values of the chemical and sensory data obtained from the five Chardonnay wines. LR, leaf removal.

noted that it remains unclear which of the smoke derived compounds, the volatile phenols, their glycoconjugates or other as yet unidentified compounds, are specifically responsible for the 'ashy' character present in some tainted wines. As expected, smoke-related attributes were given low ratings for wines from the 'control' and 'LR' treatments, with enhanced 'fruit' attributes perceived in the 'LR' wines. These results suggest that LR following grapevine exposure to smoke might result in enhanced 'fruit' attributes, thereby partially masking 'smoke' attributes.

Because it is not clear to what extent defoliation increased the exposure of grapevine leaves and berries to smoke, differences in wine composition and sensory properties observed between experimental treatments might be explained by dif-ferences in grapevine physiological responses. For example, increased sun exposure following defoliation could result in an increased berry temperature and therefore increased metabolic activity. Increased uptake of smoke-derived volatile compounds by berries would increase smoke taint in resulting wines. Nevertheless, the factors responsible for treatment effects are the subject of ongoing research.

Relationship between chemical and sensory data
The correlation matrix generated from the chemical and sensory data obtained for each wine replicate was analysed by PCA. The first PC accounted for 62% of the total variance and the second for 25% (Figure 1). Treatments involving smoke exposure were

readily differentiated from control treatments based on the intensity of smoke-related sensory attributes and on the concentration of guaiacol glycoconjugates and volatile phenols. There was relatively close positioning of the wines from the 'smoke' and 'LR post-smoke' treatments; wine from the 'LR post-smoke' treatment was associated with high phenolics concentration and the 'smoke' treatment related more to brown pigments and 'smoky' and 'ashy' characters. In contrast, the wine from the 'LR pre-smoke' treatment was associated with the highest rating of smoke-related aromas.

Conclusion
The rate of berry growth and sugar accumulation was not affected by either LR or smoke treatments. Defoliation prior to the application of smoke resulted in wines with a higher concentration of smoke-derived volatile phenols and guaiacol glycoconjugates compared with that of other treatments involving smoke exposure. Wines from the 'LR pre-smoke' treatment exhibited the most intense 'smoky', 'ashy' and 'burnt rubber' aromas and flavours. In contrast, defoliation after smoke exposure resulted in wines with less intense 'smoky' and 'ashy' characters possibly because of masking by 'fruit' attributes. Defoliation of grapevines following smoke exposure may therefore enable grapegrowers and winemakers to mitigate the severity of smoke taint in grapes and wine.

Acknowledgements

References

Bavaresco, L., Gatti, M., Pezzutto, S., Fregoni, M. and Mattivi, F. (2008) Effect of leaf removal on grape yield, berry composition, and stilbene concentration. American Journal of Enology and Viticulture **59**, 292–298.

Bergqvist, J., Dokoozlian, N.K. and Ebisuda, N. (2001) Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenanche in the central San Joaquin of California. American Journal of Enology and Viticulture **52**, 1–7.

Bledsoe, A.M., Kliewer, W.M. and Marois, J.J. (1988) Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon Blanc grapevines. American Journal of Enology and Viticulture **39**, 49–54.

Candolfi-Vasconcelos, M.C. and Koblet, W. (1991) Influence of partial defoliation on gas exchange parameters and chlorophyll content of field grown grapevines – Mechanisms and limitations of the compensation capacity. Vitis **30**, 129–141.

Candolfi-Vasconcelos, M.C., Koblet, W., Howell, G.S. and Zweifel, W. (1994) Influence of defoliation, rootstock, training system, and leaf position on gas-exchange of Pinot Noir grapevines. American Journal of Enology and Viticulture **45**, 173–180.

Chorti, E., Guidoni, S., Ferrandino, A. and Novello, V. (2010) Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. American Journal of Enology and Viticulture **61**, 23–30.

Diago, M.P., Vilanova, M. and Tardaguila, J. (2010) Effects of timing of manual and mechanical early defoliation on the aroma of *Vitis vinifera* L. Tempranillo wine. American Journal of Enology and Viticulture **61**, 382–391.

Dokoozlian, N.K. and Kliewer, W.M. (1995a) The light environment within grapevine canopies. I. Description and seasonal changes during fruit development. American Journal of Enology and Viticulture **46**, 209–217.

Dokoozlian, N.K. and Kliewer, W.M. (1995b) The light environment within grapevine canopies. II. Influence of leaf area density on fruit zone light environment and some canopy assessment parameters. American Journal of Enology and Viticulture **46**, 219–226.

Dry, P.R. and Smart, R.E. (1988) The grapegrowing regions in Australia. In: Viticulture. Vol. 1, Resources. Eds. B.G. Coombe and P.R. Dry (Winetitles: Adelaide, SA) pp. 37–60.

Dungey, K.A., Hayasaka, Y. and Wilkinson, K.L. (2011) Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography-tandem mass spectrometry based stable isotope dilution analysis. Food Chemistry **126**, 801–806.

Ebadi, A., Coombe, B.G. and May, P. (1995) Fruit-set on small Chardonnay and Shiraz vines grown under varying temperature regimes between budburst and flowering. Australian Journal of Grape and Wine Research **1**, 3–10.

Fudge, A.L., Ristic, R., Wollan, D. and Wilkinson, K.L. (2011) Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption. Australian Journal of Grape and Wine Research **17**, S41–S48.

Fudge, A.L., Schiettecatte, M., Ristic, R., Hayasaka, Y. and Wilkinson, K.L. (2012) Amelioration of smoke taint in wine by treatment with commercial fining agents. Australian Journal of Grape and Wine Research **18**, 302–307.

Gladstones, J. (1997) Viticulture and environment (Winetitles: Adelaide, SA).

Hayasaka, Y., Baldock, G.A., Pardon, K.H., Jeffery, D.W. and Herderich, M.J. (2010a) Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine *Vitis vinifera* L. cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. Journal of Agricultural and Food Chemistry **58**, 2076–2081.

Hayasaka, Y., Dungey, K.A., Baldock, G.A., Kennison, K.R. and Wilkinson, K.L. (2010b) Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. Analytica Chimica Acta **660**, 143–148.

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

Hunter, J.J. and Leroux, D.J. (1992) The effect of partial defoliation on development and distribution of roots of *Vitis vinifera* L cv Cabernet Sauvignon grafted onto rootstock 99 Richter. American Journal of Enology and Viticulture **43**, 71–78.

Hunter, J.J. and Visser, J.H. (1989) The effect of partial defoliation, leaf position and developmental stage of the vine on leaf chlorophyll concentration in relation to the photosynthetic activity and light intensity in the canopy of *Vitis vinifera* L. cv. Cabernet Sauvignon. South African Journal of Enology and Viticulture **10**, 67–73.

Iland, P.G., Bruer, N., Edwards, G., Weeks, S. and Wilkes, E. (2004) Chemical analysis of grapes and wine: techniques and concepts, ed. (Patrick Iland Wine Promotions Pty Ltd: Adelaide, SA).

Intrieri, C., Filippetti, I., Allegro, G., Centinari, M. and Poni, S. (2008) Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research **14**, 25–32.

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith, J.H. and Gibberd, M.R. (2007) Smoke-derived taint in wine: Effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry **55**, 10897–10901.

Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: The release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry **56**, 7379–7383.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research **15**, 228–237.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2011) Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. Australian Journal of Grape and Wine Research **17**, S5–S12.

Kliewer, M.V. (1970) Effect of day temperature and light intensity on coloration of *Vitis vinifera* L. grapes. Journal of the American Society for Horticultural Science **95**, 693–697.

Kliewer, W.M. (1977) Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. American Journal of Enology and Viticulture **28**, 96–103.

Kotseridis, Y., Georgiadou, A., Tikos, P., Kallithraka, S. and Koundouras, S. (2012) Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L. cultivars grown under semi-arid conditions. Journal of Agricultural and Food Chemistry **60**, 6000–6010.

Lawless, H.T. and Heymann, H. (1999) Acceptance and preference testing. In: Sensory evaluation of food: principles and practices. Ed. D.R. Heldman (Chapman & Hall, International Thomson Publishing: New York) pp. 430–479.

Martinez, J.A., Melgosa, M., Perez, M.M., Hita, E. and Negueruela, A.I. (2001) Note. Visual and instrumental color evaluation in red wines. Food Science and Technology International **7**, 439–444.

May, P. (1992) Studies on fruit set in winegrapes: comparison of Chardonnay vines in the Adelaide Hills and Southern Vales. Australian and New Zealand Wine Industry Journal **7**, 187–188. 193.

McCarthy, M.G. and Coombe, B.G. (1999) Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport?, Australian Journal of Grape and Wine Research **5**, 17–21.

Northcote, K.H. (1988) Soil and Australian viticulture. In: Viticulture. Vol.1. Eds. B.G. Coombe and P.G. Dry (Winetitles: Adelaide, SA) pp. 61–90.

Petrie, P.R., Trought, M.C.T. and Howell, S.G. (2000a) Fruit composition and ripening of Pinot Noir (*Vitis vinifera* L.) in relation to leaf area. Australian Journal of Grape and Wine Research **6**, 46–51.

Petrie, P.R., Trought, M.C.T. and Howell, G.S. (2000b) Growth and dry matter partitioning of Pinot Noir (*Vitis vinifera* L.) in relation to leaf area and crop load. Australian Journal of Grape and Wine Research **6**, 40–45.

Petrie, P.R., Trought, M.C.T. and Howell, G.S. (2000c) Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot Noir) leaves. Vitis **39**, 31–36.

Poni, S., Casalini, L., Bernizzoni, F., Civardi, S. and Interieri, C. (2006) Effects of early defoliation on shoot photosynthesis, yield components and grape composition. American Journal of Enology and Viticulture **57**, 397–407.

Quinlan, J.D. and Weaver, R.J. (1970) Modification of pattern of photosynthate movement within and between shoots of *Vitis vinifera* L. Plant Physiology **46**, 527–530.

Reynolds, A.G. and Wardle, D.A. (1989) Influence of fruit microclimate on monoterpene levels of Gewürztraminer. American Journal of Enology and Viticulture **40**, 149–154.

Reynolds, A.G., Schlosser, J., Power, R., Roberts, R., Willwerth, J. and de Savigny, C. (2007) Magnitude and interaction of viticultural and enological effects. I. Impact of canopy management and yeast strain on sensory and chemical composition of Chardonnay Musque. American Journal of Enology and Viticulture **58**, 12–24.

Ristic, R., Downey, M.O., Iland, P.G., Bindon, K., Francis, I.L., Herderich, M. and Robinson, S.P. (2007) Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. Australian Journal of Grape and Wine Research **13**, 53–65.

Ristic, R., Bindon, K., Francis, L.I., Herderich, M.J. and Iland, P.G. (2010) Flavonoids and C$_{13}$-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. Australian Journal of Grape and Wine Research **16**, 369–388.

Ristic, R., Osidacz, P., Pinchbeck, K.A., Hayasaka, Y., Fudge, A.L. and Wilkinson, K.L. (2011) The effect of winemaking techniques on the intensity of smoke taint in wine. Australian Journal of Grape and Wine Research **17**, S29–S40.

Schultz, H.R. (1992) An empirical model for the simulation of leaf appearance and leaf development of primary shoots of several grapevine (*Vitis vinifera* L. Scientia Horticulturae **52**, 179–200.

Sheppard, S.I., Dhesi, M.K. and Eggers, N.J. (2009) Effect of pre- and post-veraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. American Journal of Enology and Viticulture **60**, 98–103.

Smart, R.E., Smith, S.M. and Winchester, R.V. (1988) Light quality and quantity effects on fruit ripening for Cabernet Sauvignon. American Journal of Enology and Viticulture **39**, 250–258.

Spayd, S.E., Tarara, J.M., Mee, D.L. and Fergunson, J.C. (2002) Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. American Journal of Enology and Viticulture **53**, 171–182.

Stone, H. and Sidel, J.L. (2004) Descriptive analysis. In: Sensory evaluation practices, 3rd edn. Ed. S.L. Taylor (Academic Press: San Diego, CA) pp. 201–245.

Tarara, J.M., Lee, J.M., Spayd, S.E. and Scagel, C.F. (2008) Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in Merlot grapes. American Journal of Enology and Viticulture **59**, 235–247.

Tardaguila, J., Diago, M.P., de Toda, F.M., Poni, S. and Vilanova, M. (2008) Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of cv. Grenache grown under non irrigated conditions. Journal International Des Sciences De La Vigne Et Du Vin **42**, 221–229.

Zoecklein, B.W., Wolf, T.K., Marcy, J.E. and Jasinski, Y. (1998a) Effect of fruit zone leaf thinning on total glycosides and selected aglycone concentrations of Riesling (*Vitis vinifera* L.) grapes. American Journal of Enology and Viticulture **49**, 35–43.

Zoecklein, B.W., Wolf, T.K., Duncan, S.E., Marcy, J.E. and Jasinski, Y. (1998b) Effect of fruit zone leaf removal on total glycoconjugates and conjugate fraction concentration of Riesling and Chardonnay (*Vitis vinifera* L.) grapes. American Journal of Enology and Viticulture **49**, 259–265.

Zoecklein, B.W., Wolf, T.K., Duncan, N.W., Judge, J.M. and Cook, M.K. (1992) Effects of fruit zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and White Riesling (*Vitis vinifera* L.) grapes. American Journal of Enology and Viticulture **43**, 139–148.

References

References (excluding those in papers)

ABC News, Deal will stop wine getting a smoky taste, http://www.abc.net.au/rural/news/content/200805/s2238902.htm, viewed 20 May 2008.

Baigrie, B., *Taints and off-flavours in food*. Woodhead Publishing Limited, Cambridge, UK: 2003.

Brauer, M., Hirtle, R., Lang, B., & Ott, W. (2000). Assessment of indoor fine aerosol contributions from environmental tobacco smoke and cooking with a portable nephelometer. *Journal of Exposure Analysis and Environmental Epidemiology, 10*(2), 136-144.

Boidron, J. N., Chatonnet, P., & Pons, M. (1988). Influence du bois sur certaines substances odorantes des vins. *Connaissance Vigne Vin, 22,* 275–294.

Casale, M., Zunin, P., Cosulich, M. E., Pistarino, E., Perego, P., & Lanteri, S. (2010). Characterisation of table olive cultivar by NIR spectroscopy. *Food Chemistry, 122*(4), 1261-1265.

Chen, L., Xue, X., Ye, Z., Zhou, J., Chen, F., & Zhao, J. (2011). Determination of Chinese honey adulterated with high fructose corn syrup by near infrared spectroscopy. *Food Chemistry, 128*(4), 1110-1114.

Chong, H. H., & Cleary, M. T. (2012). Smoke Taint Aroma Assessment in 2008 California Grape Harvest. *Flavor Chemistry of Wine and Other Alcoholic Beverages,* 67-79.

Cozzolino, D., Cynkar, W. U., Shah, N., & Smith, P. A. (2011). Can spectroscopy geographically classify Sauvignon Blanc wines from Australia and New Zealand?. *Food Chemistry, 126*(2), 673-678.

Cozzolino, D., Holdstock, M., Dambergs, R. G., Cynkar, W. U., & Smith, P. A. (2009). Mid infrared spectroscopy and multivariate analysis: A tool to discriminate between organic and non-organic wines grown in Australia. *Food Chemistry, 116*(3), 761-765.

Cozzolino, D., Kwiatkowski, M. J., Parker, M., Cynkar, W. U., Dambergs, R. G., Gishen, M., & Herderich, M. J. (2004). Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. *Analytica Chimica Acta, 513*(1), 73-80.

Cozzolino, D., McCarthy, J., & Bartowsky, E. (2012). Comparison of near infrared and mid infrared spectroscopy to discriminate between wines produced by different *Oenococcus Oeni* strains after malolactic fermentation: A feasibility study. *Food Control, 26*(1), 81-87.

Cruz, M. G., Sullivan, A. L., Gould, J. S., Sims, N. C., Bannister, A. J., Hollis, J. J., & Hurley, R. J. (2012). Anatomy of a catastrophic wildfire: the Black Saturday Kilmore East fire in Victoria, Australia. *Forest Ecology and Management, 284*, 269-285.

Cynkar, W., Dambergs, R., Smith, P., & Cozzolino, D. (2010). Classification of Tempranillo wines according to geographic origin: Combination of mass spectrometry based electronic nose and chemometrics. *Analytica Chimica Acta, 660*(1), 227-231.

Di Egidio, V., Oliveri, P., Woodcock, T., & Downey, G. (2011). Confirmation of brand identity in foods by near infrared transflectance spectroscopy using classification and class-modelling chemometric techniques—the example of a Belgian beer. *Food Research International, 44*(2), 544-549.

Di Egidio, V., Sinelli, N., Giovanelli, G., Moles, A., & Casiraghi, E. (2010). NIR and MIR spectroscopy as rapid methods to monitor red wine fermentation. *European Food Research and Technology, 230*(6), 947-955.

Dungey, K. A., Hayasaka, Y., & Wilkinson, K. L. (2011). Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. *Food Chemistry, 126*(2), 801-806.

Ferreira, R. B., Piçarra-Pereira, M. A., Monteiro, S., Loureiro, V. B., & Teixeira, A. R. (2001). The wine proteins. *Trends in Food Science and Technology, 12*(7), 230-239.

95

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*(11), 1659-1667.

Fiddler, W., Wasserman, A. E., & Doerr, R. C. (1970). A "smoke" flavor fraction of a liquid smoke solution. *Journal of Agricultural and Food Chemistry, 18*(5), 934-936.

Francis, I. L., & Newton, J. L. (2005). Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research, 11*(2), 114-126.

Garde-Cerdán, T., Lorenzo, C., Alonso, G. L., & Rosario Salinas, M. (2010). Employment of near infrared spectroscopy to determine oak volatile compounds and ethylphenols in aged red wines. *Food Chemistry, 119*(2), 823-828.

Gilbert, J., & Knowles, M. E. (1975). The chemistry of smoked foods: a review. *International Journal of Food Science & Technology, 10*(3), 245–261.

Guillén, M. D., & Cabo, N. (1997). Infrared spectroscopy in the study of edible oils and fats. *Journal of the Science of Food and Agriculture, 75*(1), 1-11.

Guillén, M. D., & Ibargoitia, M. L. (1996). Relationships between the Maximum Temperature Reached in the Smoke Generation Processes from Vitis vinifera L. Shoot Sawdust and Composition of the Aqueous Smoke Flavoring Preparations Obtained. *Journal of Agricultural and Food Chemistry. 44* (5), 1302-1307.

Guillén, M. D., Sopelana, P., & Partearroyo, M. A. (2000). Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. *Journal of Agricultural and Food Chemistry. 48*(10), 5083-5087.

Hayasaka, Y., Dungey, K. A., Baldock, G. A., Kennison, K. R., & Wilkinson, K. L. (2010a). Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. *Analytica Chimica Acta, 660*(1), 143-148.

Hayasaka, Y., Baldock, G. A., Pardon, K. H., Jeffery, D. W., & Herderich, M. J. (2010b). Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine Vitis vinifera L. Cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. *Journal of Agricultural and Food Chemistry, 58*(4), 2076-2081.

Hayasaka, Y., Parker, M., Baldock, G. A., Pardon, K. H., Black, C. A., Jeffery, D. W., & Herderich, M. J. (2012). 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. *Journal of Agricultural and Food Chemistry, 61*(1), 25-33.

Høj, P., Pretorius, I., & Blair, R. (2003). The Australian Wine Research Institute Annual Report 2003. *The Australian Wine Research Institute, Adelaide, Australia*, 37-39.

Iland, P. (2004). *Monitoring the winemaking process from grapes to wine techniques and concepts*. Patrick Iland Wine Promotions Pty, Ltd.

James, T. (2008). Understanding that 'burnt rubber' aroma. http://grape.co.za/News/080601rubber.htm, viewed 4 August 2008.

Jensen, N., Varelis, P., & Whitfield, F. B. (2001). Formation of guaiacol in chocolate milk by the psychrotrophic bacterium *Rahnella aquatilis*. *Letters in Applied Microbiology, 33*, 339–343.

Jones, L. (2008) Smoke taint study aims to understand more about the impacts on grape and wine quality, *Australian Viticulture, 12*(3), 102-103.

Karasek, F. W., & Clement, R. E. (1988). *Basic gas chromatography-mass spectrometry: principles and techniques*. Elsevier.

Kemps, B., Leon, L., Best, S., De Baerdemaeker, J., & De Ketelaere, B. (2010). Assessment of the quality parameters in grapes using VIS/NIR spectroscopy. *Biosystems Engineering, 105*(4), 507-513.

Kennison, K. R., Gibberd, M. R., Pollnitz, A. P., & Wilkinson, K. L. (2008). Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. *Journal of Agricultural and Food Chemistry, 56*(16), 7379-7383.

Kennison, K. R., Wilkinson, K. L., Pollnitz, A. P., Williams, H. G., & Gibberd, M. R. (2009). Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. *Australian Journal of Grape and Wine Research, 15*(3), 228-237.

Kennison, K. R., Wilkinson, K. L., Pollnitz, A. P., Williams, H. G., & Gibberd, M. R. (2011). Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. *Australian Journal of Grape and Wine Research, 17*(2), S5-S12.

Kennison, K. R., Wilkinson, K. L., Williams, H. G., Smith, J. H., & Gibberd, M. R. (2007). Smoke-derived taint in wine: Effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. *Journal of Agricultural and Food Chemistry, 55*(26), 10897-10901.

Kostyra E., & Barylko-Pikielna, N. (2006). Volatiles composition and flavour profile identity of smoke flavourings. *Food Quality and Preference, 17*, 85-95.

Liu, L., Cozzolino, D., Cynkar, W. U., Gishen, M., & Colby, C. B. (2006). Geographic classification of Spanish and Australian Tempranillo red wines by visible and near-infrared spectroscopy combined with multivariate analysis. *Journal of Agricultural and Food Chemistry, 54*(18), 6754-6759.

Luykx, D. M., & Van Ruth, S. M. (2008). An overview of analytical methods for determining the geographical origin of food products. *Food Chemistry, 107*(2), 897-911.

Manessis, N., & Furer, D., (2007). Greek fires threaten wine regions. http://www.decanter.com/news/wine-news/486362/greek-fires-threaten-wine-regions, viewed 25 April 2015.

Maga, J. A., & Chen, Z. (1985). Pyrazine composition of wood smoke as influenced by wood source and smoke generation variables. *Flavour and Fragrance Journal, 1*(1), 37-42.

Martinez, O., Salmeron, J., Guillen, M. D., & Casas, C. (2004). Texture profile analysis of meat products treated with commercial liquid smoke flavourings. *Food Control, 15*(6), 457-461.

Mayer, F., Czerny, M., & Grosch W. (1999). Influence of provenance and roast degree on the composition of potent odorants in Arabica coffees. *European Food Research and Technology, 209*(3-4), 242-250.

Memstar; Wine Membrane Technology, *viewed 2014*, < http://www.memstar.com.au/>.

Oberholster, A., Francis, I. L., Iland, P. G., & Waters, E. J. (2009). Mouthfeel of white wines made with and without pomace contact and added anthocyanins. *Australian Journal of Grape and Wine Research, 15*(1), 59-69.

Parker, M., Osidacz, P., Baldock, G. A., Hayasaka, Y., Black, C. A., Pardon, K. H., Jeffrey, D. W., Geue, J. P., Hederich, 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.

Peddie, C. (2015). Sampson Flat fires have tainted Adelaide Hills wine growers' grapes. News Corp. http://www.adelaidenow.com.au/news/south-australia/sampson-flat-fires-have-tainted-adelaide-hills-wine-growers-grapes/story-fnpp66pk-1227388636162, viewed 6 June 2015.

Pérez-Marín, D., Paz, P., Guerrero, J. E., Garrido-Varo, A., & Sánchez, M. T. (2010). Miniature handheld NIR sensor for the on-site non-destructive assessment of post-harvest quality and refrigerated storage behavior in plums. *Journal of Food Engineering, 99*(3), 294-302.

Pollnitz, A. P., Pardon, K. H., Sykes, M., & Sefton, M. A. (2004). The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring

guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. *Journal of Agricultural and Food Chemistry, 52*(11), 3244-3252.

Port, J. (2007). Wine's bitter end: bushfire smoke ruins state vintage. The Age Company Ltd. http://www.theage.com.au/news/national/wines-bitter-end-bushfire-smoke-ruins-state-vintage/2007/03/09/1173166985987.html, viewed 25 April 2015.

Rankine, B., *Making good wine*. Pan Macmillan, Sydney, Australia: 2004.

Rapp, A., & Mandery, H. (1986). Wine aroma. *Experientia, 42*(8), 873-884.

Ristic, R., Osidacz, P., Pinchbeck, K. A., Hayasaka, Y., Fudge, A. L., & Wilkinson, K. L. (2011). The effect of winemaking techniques on the intensity of smoke taint in wine. *Australian Journal of Grape and Wine Research, 17*(2), S29-S40.

Ristic, R., Pinchbeck, K. A., Fudge, A. L., Hayasaka, Y., & Wilkinson, K. L. (2013). Effect of leaf removal and grapevine smoke exposure on colour, chemical composition and sensory properties of Chardonnay wines. *Australian Journal of Grape and Wine Research, 19*(2), 230-237.

Rodriguez-Saona, L. E., & Allendorf, M. E. (2011). Use of FTIR for rapid authentication and detection of adulteration of food. *Annual Review of Food Science and Technology, 2*, 467-483.

Regodón Mateos, J. A., Pérez-Nevado, F., & Ramírez Fernández, M. (2006). Influence of *Saccharomyces cerevisiae* yeast strain on the major volatile compounds of wine. *Enzyme and Microbial Technology, 40*(1), 151-157.

Saavedra, J., Fuentealba, C., Yáñez, L., Bravo, M., Quiroz, W., Lukacsy, G., & Carot, J. M. (2011). Chemometric approaches for the zoning of Pinot Noir wines from the Casablanca valley, Chile. *Food Chemistry, 127*(4), 1842-1847.

Sauvage, F. X., Bach, B., Moutounet, M., & Vernhet, A. (2010). Proteins in white wines: thermo-sensitivity and differential adsorption by bentonite. *Food Chemistry, 118*(1), 26-34.

Scarlett, N., Needs, S., & Downey, M. O. (2011). Assessing vineyard viability after bushfire. *Australian and New Zealand Grapegrower and Winemaker, 564*, 21-25.

Schneider, R., Charrier, F., Moutounet, M., & Baumes, R. (2004). Rapid analysis of grape aroma glycoconjugates using Fourier-transform infrared spectrometry and chemometric techniques. *Analytica Chimica Acta, 513*(1), 91-96.

Ścibisz, I., Reich, M., Bureau, S., Gouble, B., Causse, M., Bertrand, D., & Renard, C. M. (2011). Mid-infrared spectroscopy as a tool for rapid determination of internal quality parameters in tomato. *Food Chemistry, 125*(4), 1390-1397.

Sheppard, S. I., Dhesi, M. K., & Eggers, N. J. (2009). Effect of pre-and postveraison smoke exposure on guaiacol and 4-methylguaiacol concentration in mature grapes. *American Journal of Enology and Viticulture, 60*(1), 98-103.

Silberberg, M Chemistry: The Molecular Nature of Matter and Change second edition 2000 McGraw-Hill.

Singh, D. P., Chong, H. H., Pitt, K. M., Cleary, M., Dokoozlian, N. K., & Downey, M. O. (2011). Guaiacol and 4-methylguaiacol accumulate in wines made from smoke-affected fruit because of hydrolysis of their conjugates. *Australian Journal of Grape and Wine Research, 17*(2), S13-S21.

Singh, D. P., Zerihun, A., Kelly, D., Cain, N. M., Nankervis, P., & Downey, M. O. (2012). A GC-MS based analytical method for detection of smoke taint associated phenols in smoke affected wines. *Current Bioactive Compounds, 8*(3), 190-199.

Smyth, H. E., Cozzolino, D., Cynkar, W. U., Dambergs, R. G., Sefton, M., & Gishen, M. (2008). Near infrared spectroscopy as a rapid tool to measure volatile aroma compounds in Riesling wine: possibilities and limits. *Analytical and Bioanalytical Chemistry, 390*(7), 1911-1916.

Spagna, G., Barbagallo, R. N., & Pifferi, P. G. (2000). Fining treatments of white wines by means of polymeric adjuvants for their stabilization against browning. *Journal of Agricultural and Food Chemistry, 48*(10), 4619-4627.

Suslick, K. S. 2000. Sonochemistry. Kirk-Othmer Encyclopedia of Chemical Technology.

Suñen, E., Aristimuño, C., & Fernandez-Galian, B. (2003). Activity of smoke wood condensates against *Aeromonas hydrophila* and *Listeria monocytogenes* in vacuum-packaged, cold-smoked rainbow trout stored at 4 °C. *Food Research International, 36*(2), 111-116.

Tarantilis, P. A., Troianou, V. E., Pappas, C. S., Kotseridis, Y. S., & Polissiou, M. G. (2008). Differentiation of Greek red wines on the basis of grape variety using attenuated total reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 111*(1), 192-196.

Vine finders, Smoke taint in winegrapes: VineFinders completes a contract for the VWIA and DSE, February 2008, www.vinefinders.com.au/about.html, viewed 25 April 2015.

Wasserman, A. E. (1966). Organoleptic evaluation of three phenols present in wood smoke. *Journal of Food Science, 31*(6), 1105-1010.

Whiting, J. (2012). Recovery of grapevines from fire damage. *Australian & New Zealand Grapegrower & Winemaker*, (580), 25-31.

Wilkinson, K. L., Ristic, R., Pinchbeck, K. A., Fudge, A. L., Singh, D. P., Pitt, K. M., Downey, M. O., Baldock, G. A., Hayasaka, Y., Parker, M., & Herderich, M. J. (2011). Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. *Australian Journal of Grape and Wine Research, 17*(2), S22-S28.

Williamson, G., & Bowman, D. (2008). Smoke monitoring methods review. University of Tasmania, http://epa.tas.gov.au/documents/db%20smoke%20report%20final.pdf, viewed 14 December 2014.

Wittkowski, R., Baltes, W., & Jennings, W. G. (1990). Analysis of liquid smoke and smoked meat volatiles by headspace gas chromatography. *Food Chemistry, 37*(2), 135-144.

Wittkowski, R., Ruther, J., Drinda, H., & Rafiei-Taghanaki, F. Formation of smoke flavor compounds by thermal lignin degradation. In *Flavor Precursors; ACS Symposium Series*, Teranashi, R., Takeoka, G. R., & Günert, M., Eds. Washington, 1992; vol. 490, pp. 232-243.

Yu, H., Lin, H., Xu, H., Ying, Y., Li, B., & Pan, X. (2008). Prediction of enological parameters and discrimination of rice wine age using least-squares support vector machines and near infrared spectroscopy. *Journal of Agricultural and Food Chemistry, 56*(2), 307-313.

Zhao, J., Lin, H., Chen, Q., Huang, X., Sun, Z., & Zhou, F. (2010). Identification of egg's freshness using NIR and support vector data description. *Journal of Food Engineering, 98*(4), 408-414.