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2. Abstract

The level of glycosidically bound phenolic compounds present in grapes can be used as an indication of the overall level of smoke exposure. However, correlations between levels of grape markers at low to moderate levels and the sensory outcomes of resulting wines have hitherto been unknown. This project establishes sensory outcomes of wine made from a range of smoke exposure levels at low to moderate smoke exposure for Shiraz and Pinot Noir dry table and Rosé wine styles. Varying levels of extraction of grapes for Chardonnay wines were also investigated. Excellent correlations between grape markers and sensory ratings for smoke aroma and flavour are presented.

3. Executive summary

Bushfire smoke contains volatile phenol compounds from the thermal decomposition of plant lignin. Grape berries exposed to smoke can absorb these compounds and store them as glycoconjugate forms that are subsequently released during fermentation and wine storage. High levels of phenolic compounds in wines can result in potent aromas such as 'smoky', 'dirty', 'burnt', 'medicinal' and 'ashy'.

The scale and extent of the catastrophic bushfires in vintage 2020 resulted in an extraordinary number of samples submitted at the same time to the few analytical services equipped to provide commercial analysis of grape smoke exposure marker compounds. Consequently, significant delays were encountered in the delivery of analytical results to grape growers in New South Wales.

This project was undertaken to:

1. Establish the analytical capability to analyse grape smoke exposure markers at Charles Sturt University using methods similarly available through the Australian Wine Research Institute (AWRI).
2. Correlate the levels of grape smoke markers to markers in finished wines made from grapes with low to moderate levels of smoke marker compounds (i.e. in the grey zone).
3. Correlate the levels of grape smoke exposure markers to sensory ratings of wines made at varying levels of smoke exposure.
4. Identify the potential for 2D correlative spectroscopy as a tool to measure grape and wine smoke maker compounds.

The work reported in this project could not have been undertaken without the close collaboration and generous support from the Australian Wine Research Institute. Identification of smoke-exposed grapes in vineyards prior to harvest was made known to the research team via Dr Mango Parker and the Commercial Services team at AWRI, along with the transfer of intellectual property for the analysis of grape glycoside compounds. Additional support is acknowledged from the NSW growers who generously allowed harvest from their vineyards and the NSW Wine Industry Association for assistance in the coordination and communication of outcomes arising from this work. The kind provision of 2Dshige software by Shigeaki Morita, Kwanse-Gakuin University is also gratefully acknowledged.

Successful transfer, modification and integration of methods for the analysis of six grape glycosides by LCMS using an expanded set of deuterated internal standards was undertaken at Charles Sturt University using the AWRI method. Inter-laboratory proficiency testing has confirmed the validity of the methods at Charles Sturt University. Approximately 3 mg of each deuterated phenol glycoside standard has been secured, which is sufficient for approximately 3000 analyses.

In all wines produced in this project, a reduction in phenolic glycosides and a concurrent increase in volatile phenols from grape to wine were observed, reflecting the transformation from the former to the latter through the vinification process. Concentrations of glycosides and volatile phenols in wine followed the trends observed in grapes i.e. concentrations increased from low to high levels concomitant with the level of grape smoke exposure.

Good correlations were observed between grape and wine smoke marker compounds, indicating that as the levels of smoke marker compounds in grapes increase, the corresponding counterparts will increase in wine.

Rosé style winemaking substantially reduced the concentration of volatile phenols, decreasing from 55% to 70% in Pinot Noir and 44% to 74% in Shiraz wines, compared to their dry wine counterparts. However, this does not directly translate into wines with improved sensory outcomes. Rosé style wines for both cultivars were also perceived to have higher levels of smoke aroma and flavour, with diminished fruitfulness indicating that this wine style is less suited for smoke exposed grapes. There was a greater array of more apparent fruit characters in the red table wines compared to the rosé wines. This appeared to be the reason why the sensory panel reported that Shiraz wines made from grapes with low to moderate smoke exposure were not significantly different to a control wine. The

fruit attributes in the Pinot Noir wines were rated as having a lower intensity, and only one Pinot Noir wine, that with the lowest glycoside level, was rated as not being significantly different to the control for smoke aroma or taste. The higher fruitfulness in finished dry tables wines (in particular Shiraz) suggests that with careful winemaking, production of wines with commercial potential from grapes with modest levels of smoke exposure is possible.

Reducing pressing yields from 500 to 400 L/tonne resulted in small but consistent reductions in total volatile phenol and phenol glycosides concentration in Chardonnay wines. Overall, the sensory differences between Chardonnay wines with different extraction levels were inconsistent, likely reflecting varying fruit qualities evident from parcels of fruit with similar total phenol glycoside analysis. Encouragingly, the wines produced from grapes with low smoke exposure and the majority of the high exposure grapes were not rated as significantly higher in smoke aroma and flavour than a control wine. While the economic viability of decreased pressing yields would need to be assessed, the investigation suggests it might be possible to make commercially suitable Chardonnay wines from low smoke exposure.

Increased smoke exposure of grapes was clearly associated with increasing levels of smoke flavour and aroma when wines were compared to clean control wines. Sensory descriptive analysis undertaken in the absence of control wines indicated practical winemaking outcomes could be achieved with Shiraz and Chardonnay at modest levels of smoke exposure, provided that sufficient fruit characters were also present. This observation was not apparent for wines from grapes with extreme levels of smoke exposure, regardless of wine style and cultivar.

Targeting predefined bands of total phenol glycosides for grape harvest based on analytical values provided up to two weeks in advance proved to be difficult when final grape analysis was undertaken. Consequently, the goal to select fruit within a range of total glycoside values from low to high could not be achieved for all cultivars. Meaningful comparisons are still evident from the results, and it is of particular interest to compare wines from grapes with similar levels of total glycosides but from differing vineyards and growing conditions. This comparison indicated that grape potential fruitfulness is an important consideration in winemaking outcomes.

These observations suggest that grapes exposed to low, and low-moderate levels of bushfire smoke have the potential to be vinified into acceptable wines, provided that judicious winemaking decisions are made that exemplify the fruitfulness and structure of the wines and limit overall skin contact, macerating and extraction. A balance between fruitfulness and skin contact is thus required for winemaking from smoke-exposed grapes. At high levels of smoke exposure, the resulting smoke-derived compounds may attribute a masking effect to the fruitfulness of wines, thereby making such wines unacceptable from a consumer perspective. Wines with high potential for complex fruitful styles may therefore be more readily amenable to commercial production if they have only been exposed to low and low-moderate levels of bushfire smoke.

Finally, smoke taint markers targeted in grapes using contemporaneous analytical methods have good correlations with smoke aroma and flavour in Pinot Noir and Shiraz. Total grape phenolic glycoside appeared to be a good indicator for levels of smoke taint compounds as well as smoky aroma and flavour in finished wines.

4. Background

The 2019/20, unprecedented bushfires across Australia resulted in an estimated loss of $40 million in the wine sector (ABC news 2020). Bushfire smoke contains volatile phenol compounds from thermal decomposition of lignin in plant materials. These volatile phenol compounds afford potent aromas such as 'smoky', 'dirty', 'burnt', 'medicinal' and 'ashy'. Grape berries that were exposed to smoke can absorb these compounds, convert them enzymatically to glycoconjugated forms, and store them until harvest. Although these glycoconjugates (glycosides) bear no apparent aroma or flavour, they are susceptible to enzymatic and acidic hydrolysis during winemaking, which releases free volatile phenol compounds that give rise to smoke taint characters in the resultant wines. Six of these phenolic glycosides were selected by the Commercial Services group of the Australian Wine Research Institute (AWRI) as the best markers to indicate smoke exposure in grapes. These glycosides are only found in elevated concentrations in grapes that have been exposed to smoke. Thus, grapegrowers and winemakers often rely on the results of analytical testing of these markers in grape samples after a fire event, to make critical harvest decisions. In the 2019/20 season, bushfires of catastrophic scale and extensive geographical range resulted in an extraordinary number of samples being submitted at the same time to the few laboratories in Australia that provided commercial analyses of smoke taint markers, causing delays in test results being delivered to grapegrowers and winemakers. The current project was funded by Wine Australia and the New South Wales Wine Industry Association to establish the analytical capability at Charles Sturt University to analyse smoke taint markers, given its geographic proximity to several key grape growing regions in New South Wales (NSW), in readiness for an emergency response in the event of large-scale bushfires in future years. These markers include six glycoconjugates and seven volatile phenols. Quantitative methods for these compounds in grape and wine samples are detailed in this report.

In addition to establishing analytical methods, the project also sought to address knowledge gaps for the wine industry. The first is to correlate the levels of smoke taint markers in grapes to those in finished wines. Specifically, identified smoke taint levels in grapes were targeted at the 'grey zone' i.e. borderline concentrations of smoke taint compounds where commercial decisions to harvest are currently difficult to make. It was recognised that grape varieties are susceptible to smoke taint to different extents, with one study showing that after a standardised smoking event, Merlot grapes had almost a 10-fold total concentration of glycosides than that of Pinot Noir (Ristic et al. 2011). Therefore, three important varieties in NSW, Pinot Noir, Shiraz and Chardonnay, were included in the current investigation. Furthermore, we aimed to reduce the levels of smoke taint markers through the application of winemaking techniques. As phenol glycosides primarily exist in grape skins, and to a lesser extent, the pulp and seeds, we explored winemaking techniques that limited skin contact in wine, specifically, making Rosé wines out of red varieties and reducing pressing yield by 10% in white varieties. All wines made in this project were subjected to comprehensive sensory evaluation for both smoke taint attributes and overall wine characters. The aim was to explore whether simple winemaking techniques could ameliorate the finished wines made from grapes in the 'grey zone' by reducing the concentrations of smoke taint compounds to levels that make the fault in wine acceptable or undetectable.

5. Project Aims

Overall, this project aimed to establish correlations between the levels of targeted compounds in smoke-exposed grapes to wine sensory outcomes, to assist harvest decisions at low to moderate levels of smoke taint for Shiraz, Chardonnay and Pinot Noir.

The objectives were to:

1. Transfer analytical methods from the AWRI to NWGIC, to establish additional analytical capability for the Australian wine industry, in readiness for an emergency response in the event of large-scale bushfires in future years.
2. Correlate the levels of glycosylated compounds measured in smoke-exposed grapes with the final levels in vinified wines.
3. Correlate the levels of targeted compounds in smoke-exposed grapes with wine sensory outcomes, to assist harvest decisions at low to moderate levels of smoke exposure for Shiraz, Chardonnay and Pinot Noir.
4. Develop rapid analytical methods to classify the degree of smoke taint in grapes and wine using 2D correlative UV-VIS Spectroscopy.
5. Provide sufficient wines for panel training and longitudinal assessment of wines made from varying levels of grape exposure to bushfire smoke.

6. Methods

Grape selection and vineyard location

Ranges of total glycoside concentration indicative of very low to moderate levels of smoke taint for Shiraz, Pinot Noir and Chardonnay were obtained through personal communication with collaborators at AWRI (Table 1). Four hundred kg Shiraz and Pinot Noir, and 500 kg Chardonnay were harvested at low, low-moderate and moderate levels based on the total glycoside measurements performed by AWRI commercial service, two weeks prior to harvest. One vineyard affected by a very high level of smoke taint for all three varieties was also chosen for comparison. Figure 1 shows the vineyard locations where grapes were harvested. A 30-bunch sample was taken from each batch of grapes and frozen at – 20°C for further chemical analyses.

Table 1. Targeted levels, analytical values and corresponding indicative smoke exposure levels based on grape total phenol glycosides.

Sample ID.	Vineyard location	Targeted total glycoside range (µg/kg fruit)	Measured total glycoside (µg/kg fruit)	Nominal smoke exposure level
Pinot Noir				
PN.1	Orange	40 – 70 (Low)	55	Low
PN.2	Orange	70 – 100 (Low – Moderate)	83	Low – moderate
PN.3	Orange	100 – 120 (Moderate)	157	High
PN.4	Canberra	>120 (High)	1037	Extreme
Shiraz				
SH.1	Griffith	70 – 130 (Low)	109	Low
SH.2	Orange	130 – 160 (Low – Moderate)	207	Moderate
SH.3	Orange	160 – 260 (Moderate)	225	Moderate
SH.4	Canberra	>260 (High)	1215	Extreme
Chardonnay				
CH.1	Griffith	10 – 25 (Low)	19	Low
CH.2	Orange	25 – 40 (Low – Moderate)	99	High
CH.3	Orange	40 – 70 (Moderate)	137	High
CH.4	Canberra	>70 (High)	1407	Extreme

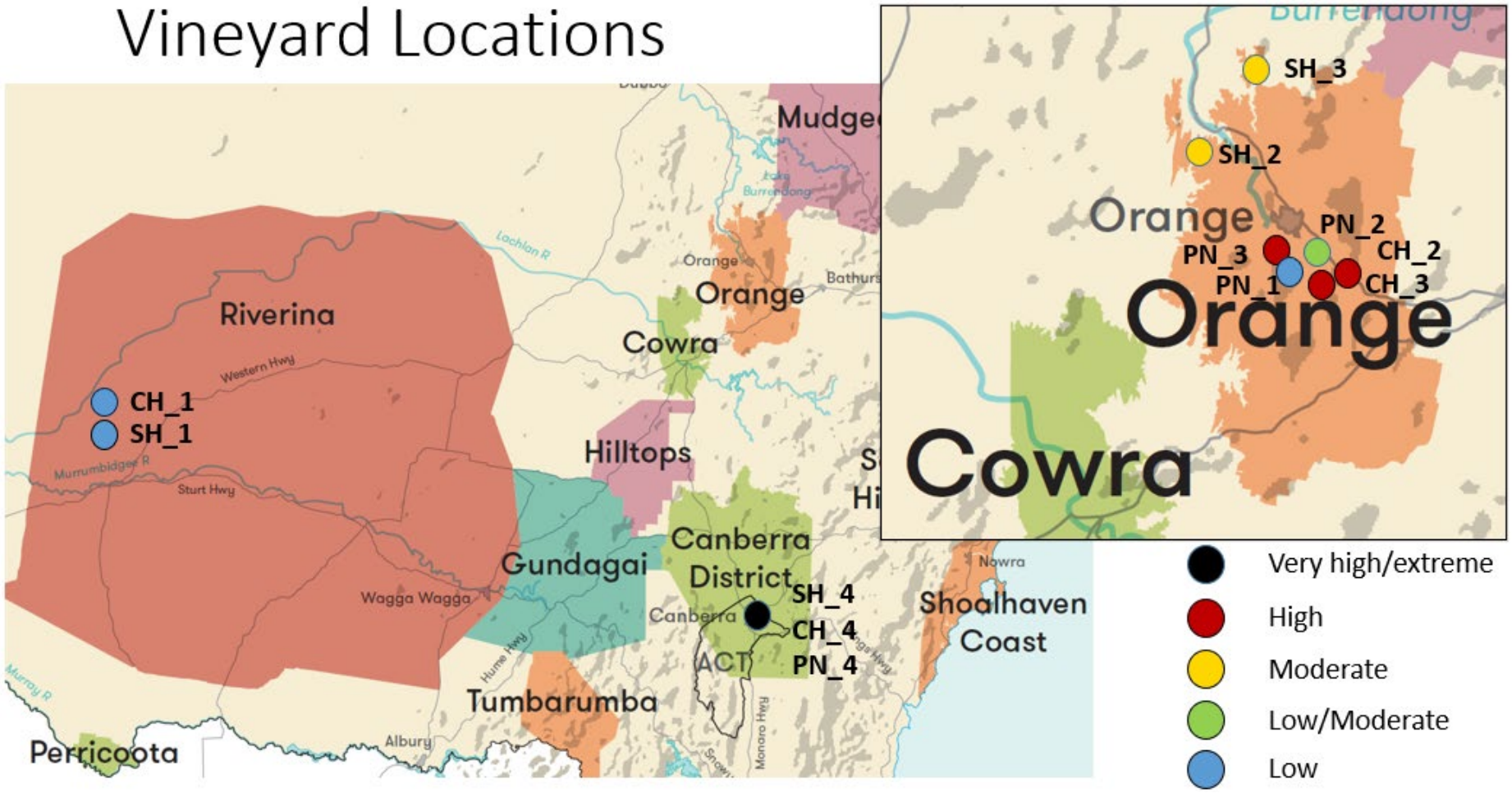


Figure 1. Vineyard locations where grape samples were sourced for winemaking. The dot colours correspond to nominal smoke exposure levels.

Small-lot winemaking

Red and Rosé style winemaking

Grapes were stored at 4°C overnight after being delivered to the winery. For Shiraz and Pinot Noir, each batch of grapes was divided into six of 70 kg parcels, which were made into red and Rosé style wines, in triplicate. Replicates were kept separate during the subsequent vinification processes. For red wine production, grapes were destemmed, crushed and transferred to 100 L stainless steel tanks for fermentation. Acidity was adjusted with tartaric acid to pH 3.6. Grape must was inoculated with 300 mg/L *Saccharomyces cerevisiae* yeast EC1118 (Lalvin) and fermentations were carried out at 25–26°C. After the onset of fermentation, the yeast assimilable nitrogen (YAN) was adjusted to 220 mg/L for treatments that had an initial total soluble solids (TSS) level below 23.4 °Brix using a combination of Fermaid K (Lallemand) and diammonium phosphate. Ferments that had a TSS in excess of 23.4 °Brix were adjusted to 250 mg/L YAN. Malolactic fermentation was carried out by co-inoculation of Enoferm Alpha (*Oenococcus oeni*) (Lallemand) at a rate of 10 mg/L, two days after the start of alcoholic fermentation. Wines were pressed off skins with a small hydraulic basket press up to a pressure of 1 Bar when the residual sugar level had dropped below 0.5 g/L. Pressed wines were maintained at 22°C until the completion of malolactic fermentation. Wines were then dosed with 80 mg/L of potassium metabisulfite and pH was adjusted to 3.6. Wines were cold stabilised for 21 days at 0–2°C and free SO_2 was re-adjusted to 30 mg/L prior to bottling in 0.75 L screw cap bottles. For Rosé style wines, the crushed grapes macerated at 25–26°C for two hours before the juice was pressed off skin with a small hydraulic basket press up to a pressure of 1 Bar, and thereafter vinified in the same way as the red wines.

White winemaking

Chardonnay grapes were harvested and stored at 4°C overnight at the winery before being destemmed/crushed and pressed using a hydraulic basket press. Pressing yields of approximately 400 L/tonne and 500 L/tonne were obtained. Only one replicate was made for each pressing level. Juice was dosed with 30 mg/L potassium metabisulfite, and subsequently held for 24 h at 4 ± 1°C for clarification by cold settling. Afterwards, juice was racked and inoculated with 300 mg/L *Saccharomyces cerevisiae* DV10 yeast (Lallemand), together with 300 mg/L GoFerm Protect (Lallemand). Fermentations were maintained at 15 ± 1 °C until residual sugar of less than 0.5 g/L was achieved. Eighty mg/L potassium metabisulfite was then added. Wines were held at 4 ± 1°C for ~ 2-3 weeks to allow clarification by sedimentation and subsequently racked. Then, wines were cold stabilised at −2°C for seven days after addition of 0.6-1.0 g/L sodium bentonite, racked for clarification and tested for heat stability. Potassium metabisulfite was added to give a molecular SO_2 of 0.5–0.8 mg/L. Acid adjustments were only performed on juice/ wine if a pH > 3.3 was measured. Wines were filtered (0.45 µm) and bottled in 0.750 L glass bottles that were flushed with CO_2 gas immediately prior to filling. Bottles were screw capped and stored at 15 ± 1 °C.

Smoke exposure marker analyses in grape and wine

The following methods were established at the NWGIC laboratories following transfers of detailed methods from the Australian Wine Research Institute. Modifications to the AWRI method have been made to accommodate an expansion of deuterated standards for glycosylated grape constituents from one to six analogues, and to adapt mass spectrometry detection methods to different equipment.

Chemicals

All chromatographic solvents were LCMS-grade, while all other solvents involved in sample preparation were analytical grade. Water was obtained from a Milli-Q purification system. Guaiacol, 4-methylguaiacol, *o*-cresol, *m*-cresol, *p*-cresol, syringol and 4-methylsyringol were obtained from Sigma Aldrich (Macquarie Park, Australia), while their deuterated analogues, d_3-Guaiacol, d_3-4-methylguaiacol, d_7-*p*-cresol and d_3-syringol, were purchased from CDN Isotopes (Pointe-Claire, Canada). Seven volatile phenol glycosides, Guaiacol rutinoside (GuRG), 4-methylguaiacol rutinoside (MGuRG), phenol rutinoside (PhRG), *p*-cresol rutinoside (CrRG), syringol gentiobioside (SyGG) and 4-methylsyringol gentiobioside (MSyGG), as well as their deuterated analogues d_3-GuRG, d_6-

MGuRG, d_5-PhRG, d_7-CrRG, d_6-SyGG and d_6-MSyGG were purchased from Toronto Research Chemicals (North York, Canada).

Phenol glycoside analysis

Grape and wine sample preparation

The 30-bunch grape samples taken at harvest were destemmed by hand while frozen. The berries were mixed thoroughly, and a 500 g aliquot was homogenised. Five grams of homogenate was spiked with a mixture of deuterated internal standards to a concentration of 200 µg/kg for d_6-SyGG and 100 µg/kg for all other glycosides before being centrifuged at 3750 rpm for five minutes. The supernatant was then poured onto a preconditioned Extract Clean C18-HF SPEC 500 mg/4 mL cartridge (Adelab, Thebarton, Australia). The loaded cartridge was washed with 10 mL of MilliQ water, followed by elution with 2 mL methanol, which was dried under a gentle stream of nitrogen at 50°C and reconstituted in 0.3 mL MilliQ water. The extract was then filtered through a 0.2 µm regenerated cellulose syringe filter directly into an amber glass vial with 100 µL insert, ready for analysis.

A 1 mL aliquot of wine was spiked with a mixture of deuterated internal standards to a concentration of 200 µg/L for d_6-SyGG and 100 µg/L for all other glycosides. It was filtered through a 0.2 µm regenerated cellulose syringe filter directly into an amber glass vial, ready for analysis.

HPLC-MS/MS analysis

An Agilent 6470B triple quadrupole LC/MS (Agilent Technologies, Mulgrave, Australia) was used for glycoside analysis. The chromatographic conditions were kept as similar as possible to those used in the smoke panel analysis offered by the AWRI Commercial Services group (Hayasaka et al. 2013). However, changes were made to the MS parameters as the instruments in the two labs were equipped with different types of ionisation source.

Ten µL of either a grape extract or a wine sample was injected onto a 150 mm × 2 mm Synergi 4u Hydro-RP 80 Å (Phenomenex, Lane Cove West, Australia), protected by a guard column of the same material. The column temperature was maintained at 25°C during the HPLC run. A binary gradient with mobile phases consisting of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in acetonitrile (solvent B) was used. A flow rate of 0.3 mL/min was used, with a linear gradient of Solvent B from 10% to 30% in 10 min, and rapidly increased to 95% at 10.1 min, which was kept for a further 5 min before the system was reverted to the starting condition of 10% solvent B for a 10 min re-equilibration time.

The effluent from the column was passed directly into an Agilent Dual ESI Jet Stream ionisation source, operated in the negative ionisation mode. Sheath gas was nitrogen heated to 250°C with a flow rate of 11 L/min and drying gas was nitrogen heated to 200°C with a flow rate of 10 L/min. Nebuliser pressure was set at 45 psi, nozzle voltage was set at 0 V, and capillary voltage was set at -3500 V. Fragmentor voltage and collision energy were optimised for each compound. For multiple reaction monitoring, the mass transition for each compound (Table 2) was monitored with a dwell time of 50 ms.

Table 2. Monitored mass transitions for phenol glycosides and their deuterated analogues.

Name	Retention time (min)	Targeted transition	Other monitored transitions
Syringol gentiobioside (SyGG)	3.995	537 → 323	537 → 477
Syringol gentiobioside - d6 (SyGG - d6)	3.942	543 → 323	
Phenol rutinoside (PhRG)	4.323	461 → 307	461 → 401
Phenol rutinoside – d5 (PhRG – d5)	4.272	466 → 307	
Guaiacol rutinoside (GuRG)	4.96	491 → 307	491 → 431
Guaiacol rutinoside – d3 (GuRG)	4.934	494 → 307	
4-methylsyringol gentiobioside (MSyGG)	5.472	551 → 323	551 → 491
4-methylsyringol gentiobioside – d6 (MSyGG – d6)	5.422	557 → 323	
Cresol rutinoside (CrRG)	6.173	475→ 307	475 → 415
Cresol rutinoside – d7 (CrRG – d7)	6.073	482 → 307	
4-methylguaiacol rutinoside (MGuRG)	6.551	505 → 307	505 → 445
4-methylguaiacol rutinoside – d3 (MGuRG – d3)	6.575	508 → 307	

Calibration function

Frozen Shiraz grapes from 2015 vintage and Riesling wine from 2018 vintage, both free of smoke markers, were used to prepare calibration samples. The grape homogenate or wine was spiked with a mixture of phenol glycosides to create a series of calibration samples containing SyGG at 0, 5, 10, 25,

50, 100, and 200 µg/kg for homogenate or µg/L for wine, as well as all other glycosides at 0, 2.5, 5, 12.5, 25, 50 and 100 µg/kg for homogenate and µg/L for wine. These calibration samples were then subjected to HPLC-MS/MS analysis. A calibration function was constructed for each glycoside by plotting the peak area ratio of the authentic standard to that of its deuterated analogue.

Reproducibility and accuracy

Shiraz grapes used to prepare the standards for calibration were also used to prepare standards for reproducibility and accuracy testing. The homogenate was spiked with a mixture of phenol glycosides to create a standard with glycoside concentrations at the lower end of the calibration range (25 µg/kg for SyGG and 12.5 µg/kg for all other glycosides) and a standard at the high end of the calibration range (200 µg/kg of SyGG and 100 µg/kg for all other glycosides). Six replicates were prepared for each concentration level and were analysed by HPLC-MS/MS. Accuracy was evaluated by dividing the mean concentration of the replicates by the spiked concentration. Reproducibility was evaluated based on coefficient of variation (cv, %) calculated from the replicates.

Volatile phenol analysis

Grape and wine sample preparation

Twenty-five grams of grape homogenate was spiked with 1 mL of absolute ethanol and a mixture of deuterated internal standard mixture (containing d_3-Guaiacol, d_3-4-methylguaiacol, d_7-*p*-cresol and d_3-syringol) to a final concentration of 100 µg/kg each. The homogenate was centrifuged at 3750 rpm for 5 min. A 5 mL aliquot was then transferred into a 10 mL centrifuge-safe borosilicate glass vial, mixed with 2 mL of pentane/ethyl acetate (1:1) and vortexed. The sample was placed in the dark for an hour for liquid-liquid extraction, centrifuged at 3800 rpm for 3 minutes, and 1 mL of the organic layer was placed in an amber vial ready for analysis. For wine samples, a 5 mL aliquot was spiked with a mixture of deuterated internal standard mixture to a final concentration of 100 µg/L each and was extracted as above.

Calibration function

Riesling grapes free of smoke markers were homogenised and spiked with a mixture of targeted volatile phenols, creating a series of calibration samples containing each volatile phenol at 0, 1, 10, 20, 50, 100 µg/kg for homogenate or µg/L for wine. These calibration samples were then subjected to GC-MS analysis. A calibration function was constructed for each volatile phenol by plotting the peak area ratio of the authentic standard to that of its deuterated analogue.

GC-MS analysis

Analysis was performed on an Agilent 7890A gas chromatograph equipped with a Gerstel MPS autosampler (Gerstel, Mülheim an der Ruhr, Germany), and coupled to an Agilent 5975C mass spectrometer. Two µL of extract was injected into a multimode inlet held at 200°C at 25 psi. The split valve was opened at 0.6 min with a purge flow of 50 mL/min. Helium was used as the carrier gas with a flow rate of 1.2 mL/min through a DB-WAXetr capillary column (30 m × 0.25 mm, 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA), where the volatile phenol compounds were separated. The column oven was maintained at 50°C for 1 min, ramped at a rate of 8°C/min to 250°C, and held for 10 min. the MS was operated in electron ionisation mode using selective ion monitoring (SIM) mode. The ions monitored for each compound are reported in Table 3, with the dwell times set at 20 ms for target ions and 10 ms for qualifier ions.

Table 3. MS Acquisition ions for volatile phenol compounds and their deuterated analogues

Compound	Target ion	Qualifier ions		
***d*$_3$-guaiacol**	127	109	81	
guaiacol	124	109	81	
***d*$_3$-4-methylguaiacol**	141	123	95	
4-methylguaiacol	138	123	95	
***d*$_7$-*p*-cresol**	113	115	114	
***o*-cresol**	108	107	77	79
***p*-cresol**	108	107	77	79
***m*-cresol**	108	107	77	79
***d*$_3$-syringol**	157	142	96	
syringol	154	139	111	
4-methylsyringol	168	153	125	

Sensory evaluation

All wines made in the current project were characterised in a two-part sensory evaluation carried out by AWRI Commercial Services. In the first part, intensities of 'smoke' aroma and flavour were assessed by a panel of experienced judges. In the second part, a descriptive analysis where the wines were profiled in more detail in terms of their aroma, flavour, taste and mouthfeel characters in order to investigate the effects of different treatments. Two out of three fermentation replicates were evaluated for rosé and red wines. Since only one wine was made for each treatment for Chardonnay grapes, duplicates of the same wine were presented at the evaluations.

Smoke intensity evaluation

Three panels of eight, qualified and highly experienced AWRI judges were convened to assess the wines. Judges were chosen for their ability to perceive smoke flavour from phenol glycosides, and on the basis of previous experience and performance in smoke exposure sensory panels.

The Chardonnay set was presented first, followed by the rosé wines, and then red wines. For both rosé and red wines, Pinot Noir wines were evaluated before Shiraz wines. The judges were asked to rate intensities of smoke aroma (defined as any type of smoke aroma, including hickory or artificial smoke, phenolic, burnt aroma associated with ashes, ashtray, fire ash, including also medicinal, band aid) and smoke flavour (defined as including bacon, smoked meat and ashy aftertaste).

For each of the wine sets, a clean commercial control wine was included in the assessment. These control wines were 2020 Yalumba 'Y series' Chardonnay, 2021 Bird in Hand Pinot Noir Rosé, 2019 Little Yering Pinot Noir, and 2019 Jacobs Creek Reserve Shiraz.

Each wine was given a three-digit code to mask its identity. Wines were presented in 30 mL aliquots in covered, ISO XL5 standard wine glasses at 22–24°C, with each judge receiving the same set of wines in a different and randomised order. Judges evaluated wines in isolated booths under colour-

masking LED lighting. Only the Chardonnay set was presented in duplicate, with all other samples being assessed as single presentation replicates of the winemaking duplicates. The judges were forced to have a two minute rest between samples. Water was provided for palate cleansing.

The intensity of each attribute was rated using an unstructured 15 cm line scale (0 to 10), with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data were acquired using Compusense20™ sensory evaluation software.

The results were analysed by analysis of variance (ANOVA), for the fixed effects of wine and presentation replicate, the random effect of judge, and their two-way interactions, followed by a Dunnett's means comparison test to determine whether the samples were rated significantly higher in smoke aroma and flavour than the corresponding marker-free control.

Descriptive analysis

The rosé wines, both Pinot Noir and Shiraz, were evaluated first using the quantitative sensory descriptive analysis method, with each variety assessed separately. The Chardonnay, Pinot Noir red wines and Shiraz red wines were then evaluated.

Rosé wines

A panel of twelve assessors (10 females, two males) with an average age of 51 years (SD=8.3) was convened to evaluate the Pinot Noir and Shiraz rosé wines. All panellists were part of the external AWRI trained descriptive analysis panel pool with experience in wine sensory descriptive analysis and were selected from the larger panel for their ability to perceive smoke flavour from phenol glycosides (at a concentration of 0.5 mg/L). All training and formal assessment of the Pinot Noir rosé wines were completed before the panel started assessing the Shiraz rosé wines.

Training for sensory descriptive analysis – Pinot Noir rosé

Assessors attended two two-hour training sessions to determine appropriate descriptors for rating in the formal evaluation sessions. All the wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus-based approach.

Wines were assessed by appearance, aroma and palate. In the second session, standards for aroma, basic taste and mouthfeel attributes were presented and discussed and these standards were also available during subsequent training sessions, the booth practice session and the formal assessment sessions. As a warm-up exercise, panellists revisited these aroma and palate standards at the beginning of each formal assessment session.

Following the second training session, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal evaluation sessions. After the practice session, any terms that needed adjustment were discussed and the final list of terms and standards were determined. For the Pinot Noir rosé wines, this list was refined to include one appearance term, seven aroma terms (six defined and one 'Other' term) and nine palate terms (eight defined and one 'Other' term), which are presented in Table 4.

Table 4. Pinot Noir rosé wine sensory attributes and definitions.

Attribute	Definition/Synonyms
Appearance	
Rose Gold	The degree of rose gold colour intensity in the sample
Aroma	
Stone Fruit A	Intensity of the aroma of peach and nectarine
Citrus A	Intensity of the aroma of lemon, lime and orange
Bruised Apple A	Intensity of the aroma of bruised crushed apples
Dried Fruit A	Intensity of the aroma of quince paste and raisins
Smoke A	Intensity of the aroma of smoke, burnt ash and Band-Aids.
Pungency	Intensity of the aroma and effect of alcohol
Palate	
Stone Fruit F	Intensity of the flavour of peach and nectarine
Citrus F	Intensity of the flavour of lemon, lime and orange
Smoke F	Intensity of the flavour of smoke, burnt ash and Band-Aids.
Acidity	Intensity of acid taste in the mouth including aftertaste
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel
Sweetness	Intensity of sweet (sucrose) taste in the mouth including aftertaste.

Training for sensory descriptive analysis - Shiraz rosé wines

Following the formal Pinot Noir rosé assessment, panellists attended a one hour training session to determine if the same attributes from the previous wine set would be appropriate for rating in the Shiraz rosé formal sessions. Wines from the study were progressively used during training sessions to generate and refine appropriate descriptive attributes and definitions through a consensus-based approach.

Following the training session, tasters participated in a practice session in the sensory booths under the same conditions as those for the formal sessions. After the practice session, any terms that needed adjustment were discussed and the final list of terms and standards were determined. For the Shiraz rosé wine formal sessions, this list was refined to include two appearance terms, nine aroma terms (eight defined and one 'Other' term) and ten palate terms (nine defined and one 'Other' term). The final list of attributes is shown in Table 5.

Table 5. Shiraz rosé wine sensory attributes and definitions.

Attribute	Definition/Synonyms
Appearance	
Pink	The degree of pink colour intensity in the sample
Orange	The degree of orange colour intensity in the sample
Aroma	
Cherry A	Intensity of the aroma of cherry and cough syrup
Citrus A	Intensity of the aroma of lemon and orange
Boiled Lolly A	Intensity of the aroma of boiled lollies
Stone Fruit A	Intensity of the aroma of peach and apricot
Jammy A	Intensity of the aroma of quince paste and jam
Smoke A	Intensity of the aroma of smoke, burnt ash and Band-Aids.
Dusty A	Intensity of the aroma of dust and dry earth
Pungency	Intensity of the aroma and effect of alcohol
Palate	
Cherry F	Intensity of the flavour of cherry and cough syrup
Citrus F	Intensity of the flavour of lemon and orange
Smoke F	Intensity of the flavour of smoke, burnt ash and Band-Aids.
Acidity	Intensity of acid taste in the mouth including aftertaste
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel
Sweetness	Intensity of sweet (sucrose) taste in the mouth including aftertaste.

Formal Evaluation – Pinot Noir rosé and Shiraz rosé

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24°C, in isolated booths under daylight-type fluorescent lighting. Wines were served to panellists in an incomplete Williams Latin Square random block design provided by Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada). The assessors were forced to wait for one minute before they could finalise the palate ratings for their assessments, to account for any lingering attributes and aftertastes. There was also a forced two minute rest between samples where panellists were encouraged to rinse with water, with a minimum ten minute rest between trays.

The intensity of each attribute listed in Tables 4 and 5 was rated using an unstructured 15 cm line scale (numericised 0 to 10), with indented anchor points of 'low' and 'high' placed at 10% and 90%, respectively. Data were acquired using Compusense Cloud sensory evaluation software.

Chardonnay wines, Pinot Noir red wines and Shiraz red wines

The training and formal evaluation sessions for Chardonnay, Pinot Noir red wines and Shiraz red wines were conducted following the exact protocol of the rosé wines. The three varieties were assessed separately, in the order of Chardonnay, Pinot Noir and Shiraz. The sensory attributes generated and rated by the panel are reported in Table 6, 7 and 8.

Table 6. Chardonnay wine sensory attributes and definitions.

Attribute	Definition/Synonyms
Appearance	
Yellow	The degree of yellow colour intensity in the sample
Aroma	
Stone Fruit A	Intensity of the aroma of peach and nectarine
Citrus A	Intensity of the aroma of lemon, lime and orange
Bruised Apple A	Intensity of the aroma of bruised crushed apples
Dried Fruit A	Intensity of the aroma of quince paste and raisins
Smoke A	Intensity of the aroma of smoke, burnt ash and Band-Aids.
Pungency	Intensity of the aroma and effect of alcohol
Palate	
Stone Fruit F	Intensity of the flavour of peach and nectarine
Citrus F	Intensity of the flavour of lemon, lime and orange
Smoke F	Intensity of the flavour of smoke, burnt ash and Band-Aids.
Acidity	Intensity of acid taste in the mouth including aftertaste
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel

Attribute	Definition/Synonyms
Sweetness	Intensity of sweet (sucrose) taste in the mouth including aftertaste.

Table 7. Pinot Noir wine sensory attributes and definitions.

Attribute	Definition/Synonyms
Appearance	
Red	The degree of red colour intensity in the sample
Orange	The degree of orange colour intensity in the sample
Aroma	
Dark Fruit A	Intensity of the aroma of blackcurrant, blackberries, plums and blueberries
Red Fruit A	Intensity of the aroma of cherry, strawberry and raspberry
Smoke A	Intensity of the aroma of smoke, burnt ash and Band-Aids.
Spices A	Intensity of the aroma of pepper, cinnamon and cloves
Eucalypt A	Intensity of the aroma of eucalyptus, green vegetable stalks and fennel
Red Confection	Intensity of the aroma of red cordial and musk lollies
Jammy A	Intensity of the aroma of jam and honey
Earthy A	Intensity of the aroma of dust and dry earth
Vinegar A	Intensity of the aroma of vinegar
Pungency	Intensity of the aroma and effect of alcohol
Palate	
Dark Fruit F	Intensity of the flavour of blackcurrant, blackberries, plums and blueberries
Red Fruit F	Intensity of the flavour of cherry, strawberry and raspberry
Smoke F	Intensity of the flavour of smoke, burnt ash and Band-Aids.
Acidity	Intensity of acid taste in the mouth including aftertaste
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel

Table 8. Shiraz wine sensory attributes and definitions.

Attribute	Definition/Synonyms
Appearance	
Red	The degree of red colour intensity in the sample
Purple	The degree of purple colour intensity in the sample
Opaque	The degree to which light cannot pass through the sample (colour intensity).
Aroma	
Dark Fruit A	Intensity of the aroma of blackcurrant, blackberries, plums and blueberries
Red Fruit A	Intensity of the aroma of cherry, strawberry and raspberry
Smoke A	Intensity of the aroma of smoke, burnt ash and Band-Aids.
Earthy A	Intensity of the aroma of dust and dry earth
Spices A	Intensity of the aroma of pepper and sweet spices: cinnamon, nutmeg and cloves
Jammy/Confection A	Intensity of the aroma of jam, marzipan and red confection
Pungency	Intensity of the aroma and effect of alcohol
Palate	
Dark Fruit F	Intensity of the flavour of blackcurrant, blackberries, plums and blueberries
Red Fruit F	Intensity of the flavour of cherry, strawberry and raspberry
Smoke F	Intensity of the flavour of smoke, burnt ash and Band-Aids.
Acidity	Intensity of acid taste in the mouth including aftertaste
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration
Viscosity	The perception of the body, weight or thickness of the wine in the mouth. Low = watery, thin mouth feel. High = oily, thick mouth feel

2D Correlation Spectroscopy (2D-COS) to identify smoke exposure markers

2-Dimensional spectroscopy was first proposed in the field of NMR spectroscopy to visualise complex spectra consisting of many overlapping peaks. It was introduced more recently to IR, NIR Raman and UV-Vis spectroscopy in a very different form, perturbation based two-dimensional spectroscopy (Lasch and Noda, 2019). In this case the perturbation was the introduction of smoke into a sample.

Smoke was generated with a Masterpro food services smoke generator (figure 2), using smouldering wood as the source of smoke. A small diaphragm pump in the device introduced smoke directly into the samples. To control any small changes due to aspiration of the samples, smokeless air was pumped into control samples for the same time period (only a few seconds).

Spectra were collected with a Shimadzu 2600i UV-VIS-NIR spectrophotometer, with an integrating sphere to scan in reflectance mode (220 – 1400 nm). This allows scanning of clarified wine (using a reflective backing), cloudy wine or grape juice, and also grape homogenates. Ultimately an assay for smoke indicators may be applied directly to grapes, with homogenisation as the only sample preparation step.

CSV files of the spectra were compiled as matching pairs of control and smoked versions of the samples and imported into 2Dshige version 1.3 software, kindly provided free of charge by Shigeaki Morita from the group that originally developed this concept at Kwanse-Gakuin University.

Initial work was undertaken with model wine (12% v/v ethanol in 2 g/L tartrate buffer, pH 3.4) to provide a matrix that had no UV fingerprint in the wavelength region associated with phenolics, maximising the ability to detect volatile phenols from smoke.



Figure 2. Masterpro smoke generator

7. Results and Discussion

Establishment and validation of analytical methods

The first objective of the current project was to transfer AWRI smoke marker analytical methods and standards to Charles Sturt University laboratories, which would enable testing of grape, juice and wine samples for a range of smoke-related markers including six phenolic glycosides and seven volatile phenols (Hayasaka et al. 2010). This would allow for testing smoke contaminated samples closer to NSW producers at peak risk fire season, to build future resilience of the NSW wine industry.

After volatile phenols enter grape berries, they are rapidly metabolised into glycosidic forms and accumulate in grapes until harvest. It was shown that the glycosides in grapes provide more reliable identification of smoke exposure in grapes than volatile phenols (Hayasaka et al. 2013). The six glycosides targeted as markers are guaiacol rutinoside (GuRG), 4-methylguaiacol rutinoside (MGuRG), phenol rutinoside (PhRG), *p*-cresol rutinoside (CrRG), syringol gentiobioside (SyGG) and 4-methylsyringol gentiobioside (MSyGG), among which SyGG is the most abundant regardless of grape variety or vintage. For the analytical method set up at the NWGIC laboratories, all targeted glycosides showed good linearity ($R^2 > 0.99$, Table 9). Measurement accuracy was within ± 10% of the expected values. Coefficient of variance (cv) is the ratio of standard deviation to the mean and shows the extent of variability in measuring replicated samples. Lower cv values indicate that the analytical method gives reproducible and hence reliable results. Cvs for all targeted glycosides were less 10% except for GuRG, which was slightly higher. These data showed that the method set up at NWGIC provides accurate results for phenol glycosides in grape samples.

Table 9. Calibration range, linearity, accuracy and reproducibility of the six phenol glycosides in grape homogenates.

Compound	Range (µg/kg)	R^2	At lower end of the calibration range		At higher end of the calibration range	
			Accuracy (%)	Reproducibility (%)	Accuracy (%)	Reproducibility (%)
Syringol gentiobioside SyGG	1 – 200	0.9993	104.4	4.0	98.5	5.4
4-Methylsyringol gentiobioside MSyGG	1 – 100	0.9978	104.6	8.8	105.0	6.8
Phenol rutinoside PhRG	1 – 100	0.9988	100.3	6.9	108.1	3.1
Guaiacol rutinoside GuRG	1 – 100	0.9991	106.4	19.0	108.0	10.5
Cresol rutinoside CrRG	1 – 100	0.9995	98.6	7.3	99.5	3.8
4-methylguaiacol rutinoside MGuRG	1 – 100	0.9990	102.0	9.7	105.6	5.5

With funding provided by the NSW Government (Industry Sector Development Grants), an additional 3 mg was purchased for each deuterated phenol glycosides. This is enough to analyse at least 3000

grape samples. For reference, AWRI Commercial Services analysed more than 4600 samples for smoke markers for the 2020 season (AWRI Annual Report 2020). The reserve of analytical standards at the NWGIC labs is sufficient to provide extensive sample analysis in the future in case of another catastrophic fire season.

Interlab proficiency testing program

We participated in a smoke exposure marker proficiency testing program coordinated by AWRI Commercial Services in December 2021. The program consisted of 17 laboratories in Australia, Europe, South America and the United States testing 10 sample wines (five red and five white) from the United States with varying degrees of smoke exposure. Smoke exposure markers, including the six phenol glycosides and seven volatile phenols examined in the current program, were measured by all participating labs. Results were communicated back to us in the form of Z-scores (Table 10). Z-scores calculate the number of standard deviations of a measurement deviating from the group mean. A Z-score smaller than -2 or larger than 2 indicates that our measurement was substantially different to the values measured by other labs. A Z-score close to zero indicates that our measurement was very close to the mean value measured by all 17 participating laboratories.

Overall, no Z-score was outside the -2 to 2 range, indicating that none of the measurements were outliers amongst results from all 17 participating laboratories. The volatile phenol measurements showed good agreement with the group mean, while the results for phenol glycosides deviated more from the group mean (higher Z-scores). As the protocols for measuring volatile phenols were relatively similar across all laboratories, this variation is difficult to explain Currently, the interpretation and recommendation from the data collected through this proficiency testing is still pending from the organiser. Further optimisation of our methods may be needed, based on any feedback received in the future

Table 10. Z-scores for all targeted smoke exposure markers calculated from the inter-laboratory proficiency testing (n = 17 labs)

	Smp. 1	Smp. 2	Smp. 3	Smp. 4	Smp. 5	Smp. 6	Smp. 7	Smp. 8	Smp. 9	Smp. 10	Mean Z-score
Phenolic glycosides											
Syringol gentiobioside	-0.9	-1.5	1.2	-1.4	0.6	-1.5	-1.4	-1.1	1.4	-1.5	-0.61
4-Methylsyringol gentiobioside	0.0	0.0	-1.3	-1.4	-0.9	0.0	0.0	0.0	0.0	-1.4	-0.50
Phenol rutinoside	-0.5	-0.8	-1.0	-0.6	-0.4	-0.8	-0.7	-0.6	-0.6	-1.2	-0.72
Guaiacol rutinoside	0.0	-1.1	-1.3	-1.3	-1.2	-1.7	-0.9	-0.7	-0.5	-0.8	-0.95
Cresol rutinoside	-0.7	-0.9	-1.2	-1.2	-1.5	-0.9	-1.1	-1.0	-1.5	-1.2	-1.12
4-methylguaiacol rutinoside	0.6	1.4	1.0	0.7	0.9	-1.0	1.0	0.8	0.6	0.9	0.69
Volatile phenols											
Guaiacol	-1.0	-0.7	-0.9	-1.0	-1.0	0.0	-0.5	-0.9	-0.8	-1.2	-0.80
4-methylguaiacol	-0.6	0.3	0.1	0.0	0.3	0.8	0.0	-0.6	-0.6	0.2	-0.01
o-cresol	-0.2	-0.7	-1.3	-0.8	-1.0	0.6	0.5	-0.7	-0.8	0.2	-0.42
p-cresol	0.2	-0.5	-0.6	-0.5	-0.6	0.5	0.0	-0.6	-0.8	-0.3	-0.32
m-cresol	-0.2	-0.1	-0.5	-0.3	-0.5	0.4	0.2	-0.4	-0.6	-0.4	-0.24
syringol	-1.0	-0.7	-1.2	-0.9	-1.1	-0.6	-0.6	-1.5	-0.6	-0.8	-0.90
4-methylsyringol	-0.2	-0.1	-0.3	-0.3	-0.4	-0.4	-0.4	-0.4	-0.4	-0.6	-0.35

Winemaking

Basic wine chemistry data for completed wines in this investigation are presented in Table 11.

Table 11. Mean finished basic wine chemistry for wines in this study.

	Glucose (g/L)	Fructose (g/L)	pH	Titratable Acidity (g/L)	Malic acid (g/L)	Acetic acid (g/L)	Turbidity (NTU)	Alcohol (% v/v)	Free SO_2 (mg/L)	Total SO_2 (mg/L)
Dry Wines										
PN.1	0.41	0.38	3.47	6.1	0.07	0.23	6.6	12.3	33	86
PN.2	0.6	0.33	3.47	5.4	0.06	0.19	7.0	13.8	35	128
PN.3	0.39	0.35	3.49	5.3	0.06	0.29	7.2	12.9	30	80
PN.4	0.61	0.29	3.42	5.7	0.06	0.27	6.0	12.0	34	88
SH.1	0.39	0.36	3.63	6.2	0.09	0.23	1.7	12.5	44	90
SH.2	0.48	1.14	3.27	7.6	0.18	0.4	7.3	12.4	32	59
SH.3	0.46	0.43	3.47	5.0	0.07	0.5	3.4	13.8	37	93
SH.4	0.6	0.3	3.21	7.4	0.05	0.17	5.0	12.5	31	65
Rose										
PN.1	0.49	1.26	3.50	5.9	2.25	0.33	1.7	12.0	28	79
PN.2	0.68	1.21	3.35	6.3	1.16	0.14	2.6	12.7	35	146
PN.3	0.43	1.25	3.53	5.2	2.08	0.27	5.7	12.1	32	99
PN.4	0.58	1.10	3.39	4.8	0.41	0.19	1.0	11.6	33	77
SH.1	0.56	0.85	3.25	5.9	1.38	0.24	2.4	14.3	49	150
SH.2	0.51	3.04	3.31	6.3	1.00	0.59	6.2	13.8	34	67
SH.3	0.5	1.99	3.55	5.9	0.89	0.46	3.0	13.3	31	87
SH.4	0.55	1.41	3.60	5.0	2.21	0.18	6.3	12.0	29	81
Chardonnay										
CH.1 (400)	0.56	0.31	3.31	6.9	1.41	0.30	1.5	13.4	37	51
CH.1 (500)	0.56	0.25	3.27	6.9	1.46	0.29	1.7	12.8	30	57
CH.2 (400)	0.60	0.79	3.48	6.1	1.63	0.27	1.1	13.2	30	65
CH.2 (500)	0.65	1.02	3.41	5.7	1.71	0.15	0.8	11.3	29	90
CH.3 (400)	0.65	0.50	3.35	5.1	2.01	0.40	0.9	13.0	36	87
CH.3 (500)	0.59	0.56	3.27	6.8	2.08	0.27	1.3	12.8	31	48
CH.4 (400)	0.62	0.35	3.53	5.3	2.79	0.12	0.7	11.3	38	107
CH.4 (500)	0.87	0.35	3.53	4.9	2.88	0.15	0.7	11.3	26	111

Correlate levels of grape smoke exposure markers to markers in finished wines

Following establishment of analytical methods to quantify smoke exposure markers in grape and wine, this part of the project sought to find correlations between targeted smoke exposure markers in grapes and their concentrations in finished wines. Grapes in the 'grey zone', exposed to low to moderate levels of smoke, were especially targeted since commercial decisions to harvest for these grapes are currently difficult to make and the resultant wine quality is unknown. Three important grape varieties in NSW i.e. Shiraz, Pinot Noir and Chardonnay, were examined in this project. Furthermore, options for reducing concentrations of the smoke marker compounds were also explored. For red varieties, both red and rosé wines were made in order to examine whether reduced skin contact time would result in lower levels of marker compounds in finished wines. For the white variety (Chardonnay), control wines were made with standard pressing yields at 500 L/t, and treatment wines were made with lower pressing yields at 400 L/t, in order to examine whether a small reduction in pressing yields could lead to reduction of marker compounds in finished wines. The results from this part of the project aimed to help winemakers interpret grape testing results in order to make harvest decisions for grapes that are low to moderately exposed to smoke. In addition, the results of this project would also assist winemakers in using winemaking techniques to ameliorate grapes to lower the levels of smoke marker compounds in finished wines.

Correlating levels of smoke exposure markers in grapes to finished wines

Levels of smoke exposure identified in the 'grey zone' i.e. low, low to moderate and moderate (Table 1) were targeted for grape harvest. Pre-harvest glycoside analysis results were derived from samples taken up to two weeks prior to harvest and consequently glycoside values for harvested grapes were not always within the target band. Actual harvest values for grape glycoside markers placed the harvests into the nominal smoke exposure categories presented in Table 12. Levels of phenolic glycosides and volatile phenols in all grape batches are summarised in Table 13 and nominal exposure levels presented in Figure 3.

Table 12. Nominal smoke severity ratings for grape harvest based upon total glycoside analysis with total glycoside values indicated.

Exposure severity	Pinot Noir wine ID	Shiraz wine ID	Chardonnay wine ID
Low	PN.1 (55µg/kg)	SH.1 (109 µg/kg)	CH.1 (19 µg/kg)
Low-Moderate	PN.2 (83 µg/kg)		
Moderate		SH.2 & SH.3 (207 & 225 µg/kg)	
High	PN.3 (157 µg/kg)		CH.2 & CH.3 (99 & 137 µg/kg)
Extreme	PN.4 (1037 µg/kg)	SH.4 (1215µg/kg)	CH.4 (1407 µg/kg)

Both phenolic glycosides and volatile phenols increased in grapes from low to high smoke exposure levels (Tables 12 and 13). Notably, phenolic glycosides were present in grapes at substantially higher levels than volatile phenol compounds, and this observation is supported by other studies (Ristic et al. 2011, Hayasaka et al. 2013). Phenolic glycosides and volatile phenols are naturally present at low

levels in grapes. A recent study showed that the upper background limits of total glycosides were 14.9, 34.7 and 8.9 µg/kg for Pinot Noir, Shiraz and Chardonnay respectively (Coulter et al. 2022). Low smoke exposure level harvests for all three varieties exceeded the background limits, indicating that even the least smoke-exposed samples in this project were affected by smoke. Furthermore, AWRI Commercial Services reported to have analysed 1305 grape samples from NSW in 2019/20 season (Smoke Taint New Knowledge Seminars 2021), and the range of total glycoside concentration ranged from 12.5 to 1508 µg/kg with an average of 304 µg/kg for Pinot Noir, from 16.5 to 1835 µg/kg with an average of 297 µg/kg for Shiraz, and from 3.5 to 2677 µg/kg with an average of 232 µg/kg for Chardonnay. These ranges are also presented in Figure 3.

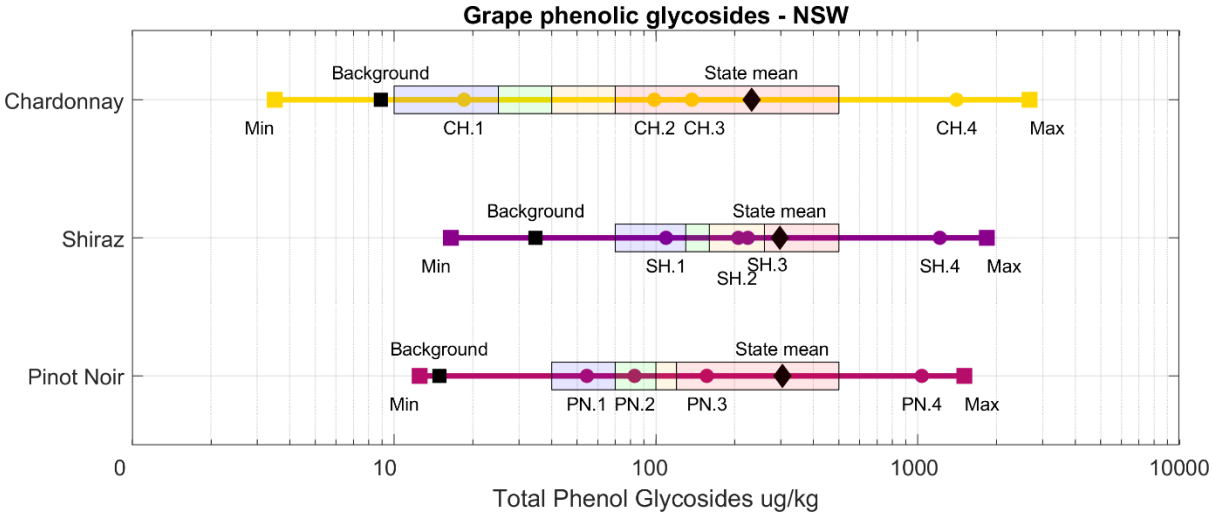


Figure 3. Grape phenol glycoside concentrations relative to state min, max and mean levels for each grape sample for Chardonnay, Shiraz and Pinot Noir grapes. Shaded area represented target grape glycosides levels of low (blue), low-moderate (green); moderate (yellow) and high (red).

All grape harvests with nominal 'moderate' or 'high' level smoke exposure for this project were below the state average, while the heavily exposed grape samples were close to the upper range seen in NSW and are therefore nominally categorised as 'extreme'.

In all wines produced in this project, a reduction in phenolic glycosides and a concurrent increase in volatile phenols from grape to wine were observed (Tables 14 - 16), which reflected the transformation from the former to the latter through the vinification process. Concentrations of glycosides and volatile phenols in wine followed the trends observed in grapes i.e. concentrations generally increased from low to high levels of smoke exposure detected in the grapes.

Good correlations were observed i.e. above 0.7 (Table 17) between the concentrations of smoke exposure markers in red grapes (Pinot Noir and Shiraz) and subsequent red wines (n = 8). Both grape volatile phenols and glycosides had good correlations with the total wine volatile phenol contents, as well as the individual volatile phenols. Individual grape glycosides have better correlations with wine glycosides than grape volatile phenols. Furthermore, total grape glycosides levels had good correlations with all wine volatile phenols, individual wine glycosides and almost perfect correlation with the total wine glycosides. This further corroborated the reliability of grape glycosides as markers to indicate final concentrations of smoke associated compounds in finished wine. Overall, good correlations were observed between grape and wine smoke marker compounds, indicating that the levels of smoke exposure compounds in grapes had a direct correlation with the level of the corresponding compounds in the finished wines, although their correlation to the perceived smoky aroma and flavour in wine need to be further examined (reported in section 3.3).

For Chardonnay, correlations between compounds in grapes and wine were all above 0.7. However, since there were only four pairs of grape and wine for Chardonnay, the small sample size creates

larger uncertainties for calculated correlation values. The correlation matrix for Chardonnay is therefore not presented in this report.

Investigation of winemaking techniques to reduce levels of smoke marker compounds in wine

Limiting on-skin fermentation for red varieties and lowering pressing yield for white varieties are two techniques both aimed to reduce extraction of glycosides from the grape skin, since it has a higher proportion of total glycosides than pulp (Dungey et al. 2011). In Shiraz and Pinot Noir, rosé wine styles possessed substantially reduced concentrations of all volatile phenols (Table 14 - 16) with levels decreasing between 44 to 74% in Shiraz and 55-70% in Pinot Noir compared to their red wine counterparts.

The trend for wine glycosides in red wines is less clear. Limiting skin contact for rosé wine production did achieve a reduction in wine glycosides in Pinot Noir wines compared to dry wine counterparts, but this trend was reversed for wines made from Shiraz.

This result contrasts with previous work (Ristic et al. 2011), and is deserving of further study and may reflect specific cultivar differences, the level and origin of smoke exposure. The Ristic study was undertaken using Grenache deliberately exposed to smoke derived from controlled burns of straw with vines enclosed in purposely constructed smoke tents. Bottle aging has also been shown to change wine glycoside composition, increasing concentrations in some cases, due to breakdown of more complex precursors to yield the disaccharide glycosides targeted in this study (Ristic et al. 2017), thus differences in bottle age at wine analysis may also account for some of the observed differences.

In Chardonnay wines, a modest reduction in pressing yields resulted in small but consistent reductions in total volatile phenol concentration, ranging between 8% and 39%. It is worth noting that the Chardonnay wines had considerably lower volatile phenol concentrations and substantially lower grape glycoside marker compounds than the red wines. Most volatile phenols were present at below the quantitation limit (1 µg/L) in Chardonnay wines made from 'grey zone' grapes. Thus, the reduction of volatile phenol concentrations for Chardonnay wines was small in terms of absolute values.

Overall, it has been demonstrated that there is a direct link between the levels of smoke exposure markers, especially the total glycoside concentration, and their counterparts in finished wines. Moreover, winemaking techniques that sought to reduce skin contact and the level of extraction can reduce the concentration of smoke marker compounds in wine.

Table 13. Concentration of phenolic glycosides and volatile phenols in grape samples.

	Pinot Noir				**Shiraz**				**Chardonnay**			
	PN.1	PN.2	PN.3	PN.4	SH.1	SH.2	SH.3	SH.4	CH.1	CH.2	CH.3	CH.4
Region	Orange	Orange	Orange	Canberra	Griffith	Orange	Orange	Canberra	Griffith	Orange	Orange	Canberra
Phenolic glycosides µg/kg												
Syringol gentiobioside	4.8	18	45	494	67	126	137	928	8.0	50	47	1037
Methyl syringol gentiobioside	14	9.1	16	63	10	23	28	120	<3	8.3	9.8	96
Phenol rutinoside	6.2	5.7	6.2	42	9.4	8.4	5.2	16	<3	3.4	4.2	20
Guaiacol rutinoside	17	29	56	196	10	24	24	45	<3	13	47	71
Cresol rutinosdie	7.1	9.6	15	122	4.5	8.8	6.7	22	<3	11	12	60
4-methyl guaiacol rutinoside	5.7	11	18	120	8.3	17	23	84	3.3	14	17	124
Total glycosides	55	83	157	1037	109	207	225	1215	19	99	137	1407
Volatile phenols µg/L												
guaiacol	2.6	3.3	6.0	17	4.5	3.0	<1	9.0	<1	1.1	<1	6.3
4-methylguaiacol	<1	<1	<1	2.7	n.d.	n.d.	n.d.	<1	n.d.	<1	<1	1.9
o-cresol	3.4	2.2	4.4	14	n.d.	n.d.	n.d.	3.5	n.d.	2.1	<1	11
p-cresol	3.8	4.4	6.4	29	n.d.	n.d.	n.d.	4.3	n.d.	6.0	4.1	22
m-cresol	1.1	<1	1.8	8.3	n.d.	n.d.	n.d.	1.8	n.d.	1.3	<1	9.5
syringol	n.d.	n.d.	n.d.	<1	n.d.	n.d.	n.d.	<1	n.d.	n.d.	n.d.	n.d.
4-methylsyringol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total Volatile phenols	11	11	19	70	4.5	3.0	<1	20	<1	11	6.3	51

The lowest level in the calibration curve was considered the quantitation limit of each compound. Therefore, measurements lower than the limit was reported as '< quantitation limit' instead of specific values.

Table 14. Mean concentration of phenolic glycosides and volatile phenols in red and rosé wines made from Pinot Noir grapes (n = 3 replicated ferments)

	PN.1		**PN.2**		**PN.3**		**PN.4**	
Wine style	red	rosé	red	rosé	red	rosé	red	rosé
Phenolic glycosides µg/kg								
Syringol gentiobioside	128	14	8.7	17	33	38	313	328
4-Methylsyringol gentiobioside	15	11	12	5.0	26	17	68	49
Phenol gentiobioside	<3	<3	<3	<3	5.2	6.0	14	14
Guaiacol rutinoside	<3	<3	<3	<3	<3	<3	25	17
Cresol rutinoside	5.9	4.1	4.4	3.0	8.4	5.8	24	18
4-methylguaiacol rutinoside	<3	<3	<3	<3	7.6	5.2	24	22
Total glycosides	27	33	27	27	82	73	469	447
Volatile phenols µg/L								
guaiacol	11	3.3	15	3.5	20	6.4	67	21
4-methylguaiacol	1.4	<1	3.4	<1	3.4	<1	22	5.9
o-cresol	8.0	2.4	7.7	2.1	12	3.5	32	10
p-cresol	4.0	1.0	7.9	2.2	9.6	3.1	33	13
m-cresol	6.6	1.7	8.5	2.1	12.6	3.5	41	13
syringol	5.8	2.1	11	2.3	29	15	35	12
4-methylsyringol	<1	<1	2.4	<1	5.5	3.0	12	3.5
Total Volatile Phenols	37	11	56	13	91	36	242	79

The lowest level in the calibration curve was considered the quantitation limit of each compound. Therefore, measurements lower than the limit was reported as '< quantitation limit' instead of specific values.

Table 15. Mean concentration of phenolic glycosides and volatile phenols in red and rosé wines made from Shiraz grapes (n = 3 replicated ferments)

	SH.1		**SH.2**		**SH.3**		**SH.4**	
Wine style	red	rosé	red	rosé	red	rosé	red	rosé
Phenolic glycosides µg/kg								
Syringol gentiobioside	26	65	65	92	81	123	417	372
4-Methylsyringol gentiobioside	15	8.5	19	12	16	7.9	35	20
Phenol gentiobioside	6.0	6.4	13	9.1	8.5	5.9	30	20
Guaiacol rutinoside	0.6	<3	3.6	3.9	11	9.4	43	25
Cresol rutinoside	3.9	4.9	8.1	7.2	6.3	6.1	16	12
4-methylguaiacol rutinoside	4.3	2.8	9.4	5.6	11	7.8	37	31
Total glycosides	55	88	119	129	133	161	579	480
Volatile phenols µg/L								
guaiacol	47	14	24	6.6	16	4.0	77	50
4-methylguaiacol	1.1	<1	<1	<1	1.4	<1	15	9.3
o-cresol	1.5	<1	2.8	6.4	1.7	4.5	15	4.1
p-cresol	<1	<1	1.1	<1	1.1	n.d.	15	4.4
m-cresol	1.5	<1	1.8	<1	1.6	<1	16	5.9
syringol	25	5.5	23	4.5	20	3.4	38	22
4-methylsyringol	4.4	1.2	1.4	<1	1.0	<1	10	6.9
Total Volatile Phenols	81	21	55	18	42	13	184	102

The lowest level in the calibration curve was considered the quantitation limit of each compound. Therefore, measurements lower than the limit was reported as '< quantitation limit' instead of specific values.

Table 16. Concentration of phenolic glycosides and volatile phenols in Chardonnay wines made with two pressing levels

	CH.1		**CH.2**		**CH.3**		**CH.4**	
Wine style	400 L/T	500 L/T	400 L/T	500 L/T	400 L/T	500 L/T	400 L/T	500 L/T
Phenolic glycosides µG/KG								
Syringol gentiobioside	10	9.4	16	28	8.5	17	226	212
4-Methylsyringol gentiobioside	<3	<3	<3	<3	<3	<3	25	26
Phenol gentiobioside	<3	<3	<3	<3	<3	<3	11	11
Guaiacol rutinoside	<3	<3	<3	<3	<3	<3	26	20
Cresol rutinoside	<3	<3	<3	<3	<3	<3	14	14
4-methylguaiacol rutinoside	<3	<3	<3	<3	<3	<3	20	21
Total glycosides	14	13	24	37	13	23	321	305
Volatile phenols µG/l								
guaiacol	<1	<1	<1	<1	<1	1.1	5.2	5.8
4-methylguaiacol	n.d.	n.d.	<1	<1	<1	<1	1.9	2.4
o-cresol	n.d.	<1	<1	1.0	<1	<1	1.4	2.0
p-cresol	n.d.	n.d.	<1	<1	<1	<1	2.3	2.9
m-cresol	n.d.	n.d.	<1	<1	<1	<1	2.2	3.1
syringol	<1	<1	<1	<1	<1	1.1	8.5	9.9
4-methylsyringol	n.d.	<1	<1	<1	<1	<1	2.4	2.7
Total Volatile Phenols	1.1	1.2	2.3	3.8	3.8	5.1	24	29

The lowest level in the calibration curve was considered the quantitation limit of each compound. Therefore, measurements lower than the limit was reported as '< quantitation limit' instead of specific values.

Table 17. Pearson correlation matrix between grape (G) smoke taint markers and wine (W) smoke taint markers in red varieties (Pinot Noir and Shiraz), SyG: syringol gentiobioside; PhR: phenol rutinoside; GuR: guaiacol rutinoside; MsyG: 4-methyl syringol gentiobioside; CrR: Cresol rutinoside; MGuR: 4-methyl guaiacol rutinoside.

	(W) guaiacol	(W) 4-methyl guaiacol	(W) *o*-cresol	(W) *p*-cresol	(W) *m*-cresol	(W) syringol	(W) 4-methyl syringol	(W) total volatiles	(W) SyG	(W) PhR	(W) GuR	(W) MsyG	(W) CrR	(W) MGuR	(W) Total glycosides
(G) guaiacol	0.79	0.95	0.95	0.96	0.96	0.72	0.94	0.97	0.75	0.97	0.49	0.63	0.94	0.67	0.77
(G) 4-methyl guaiacol	0.64	0.94	0.98	0.98	0.99	0.57	0.84	0.90	0.67	0.98	0.35	0.55	0.94	0.58	0.70
(G) *o*-cresol	0.51	0.87	0.98	0.96	0.98	0.43	0.76	0.82	0.55	0.92	0.19	0.43	0.87	0.45	0.58
(G) *p*-cresol	0.50	0.86	0.96	0.95	0.97	0.43	0.74	0.81	0.52	0.93	0.17	0.39	0.86	0.41	0.55
(G) *m*-cresol	0.58	0.90	0.98	0.97	0.99	0.50	0.79	0.86	0.60	0.96	0.26	0.47	0.90	0.50	0.63
(G) total volatiles	0.59	0.91	0.98	0.98	0.99	0.52	0.82	0.87	0.60	0.96	0.27	0.48	0.90	0.51	0.63
(G) SyG	0.88	0.78	0.55	0.59	0.54	0.76	0.77	0.80	0.98	0.62	0.94	0.99	0.76	0.98	0.97
(G) PhR	0.73	0.92	0.91	0.92	0.93	0.61	0.83	0.91	0.70	0.97	0.43	0.58	0.93	0.61	0.73
(G) GuR	0.55	0.88	0.95	0.95	0.97	0.54	0.77	0.84	0.58	0.96	0.27	0.45	0.90	0.49	0.61
(G) MSyG	0.83	0.75	0.53	0.56	0.51	0.73	0.73	0.76	0.97	0.60	0.94	0.99	0.74	0.98	0.96
(G) CrR	0.59	0.89	0.93	0.94	0.96	0.51	0.76	0.85	0.60	0.96	0.28	0.46	0.90	0.50	0.63
(G) MGuR	0.83	0.98	0.88	0.91	0.90	0.74	0.90	0.96	0.91	0.94	0.69	0.84	0.98	0.86	0.93
(G) Total glycosides	0.90	0.91	0.74	0.77	0.73	0.79	0.87	0.91	1.00	0.81	0.87	0.96	0.90	0.97	1.00

Correlate levels of grape smoke exposure markers to sensory ratings of wines

In section 3.1 and 3.2, we demonstrated that there is a direct link between the levels of smoke exposure precursors in grapes and those in finished wines. Current testing methods of smoke precursors can effectively identify smoke exposure in grapes. However, interpreting the levels of these compounds to estimate the sensory outcomes in finished wines remains a key knowledge gap for the wine industry. In this section, we present the sensory properties of wines made from grapes with the various smoke exposure marker levels shown in the last section. The links between the levels of smoke markers in grapes and perceived smoke aroma and flavour in wine were also explored.

Sensory evaluation – Smoke Intensity evaluation

Pinot Noir and Shiraz

Sensory comparison of wines for smoke flavour and aroma with control wines that have been made from grapes without smoke exposure need to be contextualised within the frame of potential false positive ratings. Sensory analysis is a highly subjective activity and even experienced panels for rating smoke attributes may assess the control wines with low levels of smoke aroma and or flavour, typically in the range of 0.5 to 1.5 using the methods employed in this project.

Wines made from this project were compared to clean commercial controls in terms of smoke aroma and flavour. All Pinot Noir rosé wines were rated to have significantly higher smoky aroma and flavour, compared to a control wine (Table 18). The intensity of both aroma and flavour increased as the concentrations of volatile phenol and glycosides elevated in wine.

In the Pinot Noir red wines, PN.1 (low), was not rated significantly higher in smoke aroma and flavour than a control wine, while the rest of the wines were (Table 19). Moreover, unlike their rosé wine counterparts, PN.3 red wine was not perceived to be smokier than PN.2, despite having higher amounts of volatile phenols and glycosides (Table 14).

For the Shiraz rosé wines made from grapes of low level of smoke exposure, (SH.1) was perceived to have higher smoke aroma than a control wine, but this was not evident for smoke flavour (Table 20). SH.2 and SH.3 rosé (both moderate levels of smoke exposure) were not significantly different from each other, in agreement with the chemical analysis of the grapes with both wines having similar levels of volatile phenols and glycosides (Table 15) but were rated higher for smoke aroma and flavour than the control (Table 20).

The Shiraz red wines, with low (SH.1) and moderate (SH.2 & SH.3) levels of smoke exposure, were not rated to have significantly different smoke aroma and flavour profiles than the control (Table 21). Only SH.4 (extreme exposure) wineswere perceived to be noticeably smoky.

The overall perceptions of smoke aroma and smoke flavour in dry red wines and rosé style are in good agreement with increasing levels of grape glycosides and wine glycosides for the grapes and resulting wines. Consideration on the level of smokiness for wines should be tempered with an understanding of the level of glycosides for wine 4 in each data set. Wine 4 has extremely high levels of glycosides and overt smokiness. These wines are not specifically in the grey zone and sensory results are likely to have been skewed through their inclusion.

Table 18. Smoke mean scores, rated on a 0 to 10 scale for Pinot Noir rosé wines (n=8 assessors x 2 replicates).

Wine	Smoke aroma	Smoke Flavour	Significantly higher than control?
PN.1	1.23*	1.58**	Yes, aroma and flavour
PN.2	1.46**	2.00***	Yes, aroma and flavour
PN.3	2.36***	3.43***	Yes, aroma and flavour
PN.4	6.71***	6.69***	Yes, aroma and flavour
Clean Pinot Noir rosé control	0.00	0.12	-

Table 19. Smoke mean scores, rated on a 0 to 10 scale for Pinot Noir red wines (n=8 assessors x 2 replicates).

Wine	Smoke aroma	Smoke Flavour	Significantly higher than control?
PN.1	0.59	1.39	No
PN.2	2.80***	3.14***	Yes, aroma and flavour
PN.3	2.74***	3.83***	Yes, aroma and flavour
PN.4	5.61***	5.76***	Yes, aroma and flavour
Clean Pinot Noir control	0.30	0.69	-

Table 20. Smoke mean scores, rated on a 0 to 10 scale for Shiraz rosé wines (n=8 assessors x 2 replicates).

Wine	Smoke aroma	Smoke Flavour	Significantly higher than control?
SH.1	0.60*	0.70	Yes, aroma only
SH.2	1.22***	1.61***	Yes, aroma and flavour
SH.3	0.82***	0.84*	Yes, aroma and flavour
SH.4	6.84***	6.58***	Yes, aroma and flavour
Clean Shiraz rosé control	0.1	0.21	-

Table 21. Smoke mean scores, rated on a 0 to 10 scale for Shiraz red wines (n=8 assessors x 2 replicates).

Wine	Smoke aroma	Smoke Flavour	Significantly higher than control?
SH.1	0.97	1.36	No
SH.2	0.81	0.94	No
SH.3	1.15	1.46	No
SH.4	4.75***	5.88***	Yes, aroma and flavour
Clean Shiraz control	0.52	0.77	-

Sensory Descriptive Analysis of Wines

To further understand the sensory characters of red and rosé wines made from smoke-exposed grapes, sensory descriptive analyses were performed. The wines were assessed not only in terms of smoke aroma and flavour, but were profiled comprehensively, including colour, various fruit aromas and flavours, tastes and mouthfeel characters. Sensory descriptive analysis was undertaken in the absence of 'control wines' made from grapes without smoke exposure, however, the inclusion of wines made from extremely high levels of smoke-exposed grapes may influence overall levels of smoke perceptions at low to moderate levels of exposure. Presentation of wines in random order along with repeat panellist appraisal helps to avoid this confounding influence on results.

Pinot Noir Rosé

Figure 4 illustrates a biplot of sensory ratings and spider plot for Pinot Noir rosé wines using the mean sensory scores. Pinot Noir rosé wines made from grapes with low (PN1), low-to-moderate (PN2) and high (PN3) levels of smoke exposure were not rated as having obvious smoke aroma and flavour.

From the ANOVA, nine attributes rated by the panel differed significantly ($P<0.05$) among the treatments including rose-gold (colour intensity), stonefruit aroma, citrus aroma, bruised apple aroma, dried fruit aroma, smoke aroma, stonefruit flavour, citrus flavour and smoke flavour. There was a significant judge effect for many of the attributes indicating that the assessors were utilising different sections of the line scale for their evaluations. There were significant ($P<0.05$) Judge by Wine interaction effects for several attributes, indicating some concept alignment issues between some judges. Some Judge by Wine variation is expected within sensory panels. There was no Judge by Wine interaction for the smoke related terms.

Wines on the right of the biplot in Figure 4 were highest in attributes positively loaded on PC1, notably stonefruit aroma, stonefruit flavour, acidity and citrus flavour. These wines were also rated as low in the attributes smoke aroma and smoke flavour. The wines to the upper part of the biplot were highest in attributes such as rose-gold (colour intensity), and lower in attributes loaded negatively on PC2, such as pungency.

Interpretation of the positioning of wines in the biplot must be considered in light of the significant judge effect indicating varying use of scale, concept alignment and that mean scores have been used in the Principal Components Analysis. Moreover, the influence of growing conditions for grapes and winemaking influences may manifest in differences in wine colour and fruit profile. This is evident in the position of PN1 (low exposure) wines close to the Bruised Apple and Dried Fruit attributes which may indicate some oxidative mishandling of these wines. Clearly evident is the strong association of smoke aroma and flavour with wines PN4 (extreme exposure) whereas wines made from with low (PN1), moderate (PN2) and high (PN3) levels of smoke exposure are positioned in the opposing section of the biplot.

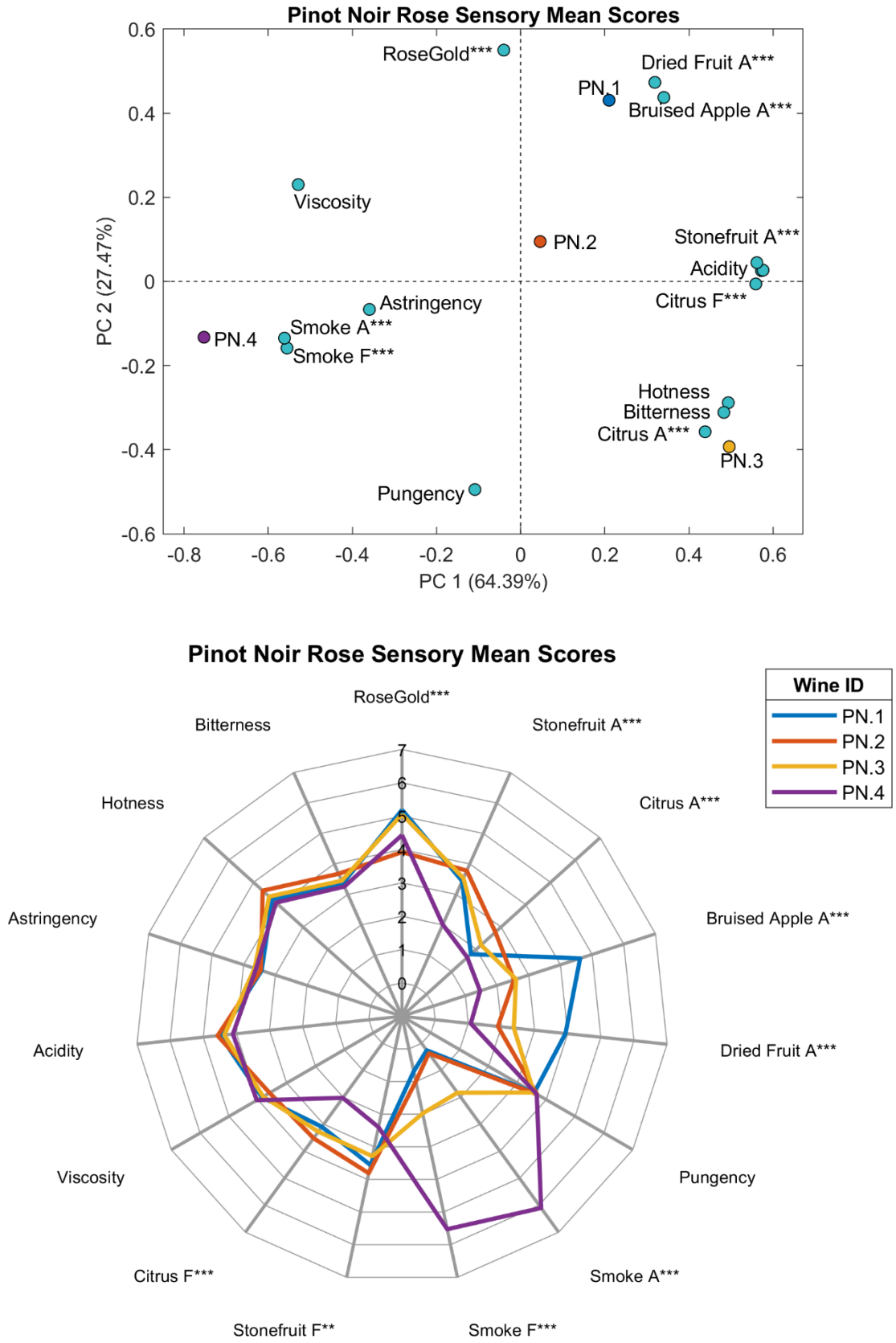


Figure 4. Principal Components Analysis biplot and spider-plot of the mean values for the sensory attributes for the Pinot Noir rosé wines. Line colours represent different samples. A: aroma, F: Flavour. Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Shiraz Rosé

From the ANOVA for the Shiraz rosé, fifteen attributes rated by the panel differed significantly ($P<0.05$) including pink (colour intensity), orange (colour intensity), cherry aroma, citrus aroma, confection aroma, stonefruit aroma, jammy aroma, smoke aroma, pungency, hotness (alcohol burn), bitterness, sweetness, cherry flavour, citrus flavour and smoke flavour. There was a significant judge effect for many of the attributes indicating that the assessors were utilising different scale sections for rating attributes. Some minor variations ($P<0.05$) between wine treatment replicates are evident for pink (colour intensity), orange (colour intensity) and smoke aroma. There were also significant ($P<0.05$) Judge by Wine interaction effects for several attributes.

Figure 5 illustrates a Principal Components Analysis biplot and spider plot of the mean sensory ratings for Shiraz rosé wines. Wines on the far right of the biplot (SH.1 - low, SH.2 - moderate and SH.3 - moderate) were highest in attributes positively loaded on PC1, notably pink (colour intensity), sweetness, hotness (alcohol burn), jammy aroma, confection aroma, dusty aroma and pungency. These wines were also rated as low in the attributes smoke aroma, smoke flavour, bitterness and orange (colour intensity). The wines high in PC2 (SH.4 - extreme) were highest in attributes such as viscosity, and lower in attributes loaded negatively on PC2, such as stonefruit aroma. The level of smokiness in wines SH.4 is clearly driving the separation of wines along PC1.

Shiraz rosé wines with all three wines made from grapes with low or moderate levels (SH.1, SH.2 and SH.3) of smoke exposure rated very similarly to each other. This was possibly due to their contrast with the very high intensity of smoke aroma and flavour in the wines made from grapes with extreme levels of smoke exposure (SH.4). There were some differences between the wines for taste attributes (hotness, bitterness and sweetness) and the fruit characters were also different between the wines and this best illustrated in the spider plot for the wines. The sensory differences for hotness, sweetness and bitterness are likely influenced by the growing conditions of the grapes with SH.1 being harvested from a warm to hot region with subsequent higher alcohol levels compared to grapes from cooler origins (SH.2-4) (14.3% v/v, 12.0-13.8% v/v (Table 11)).

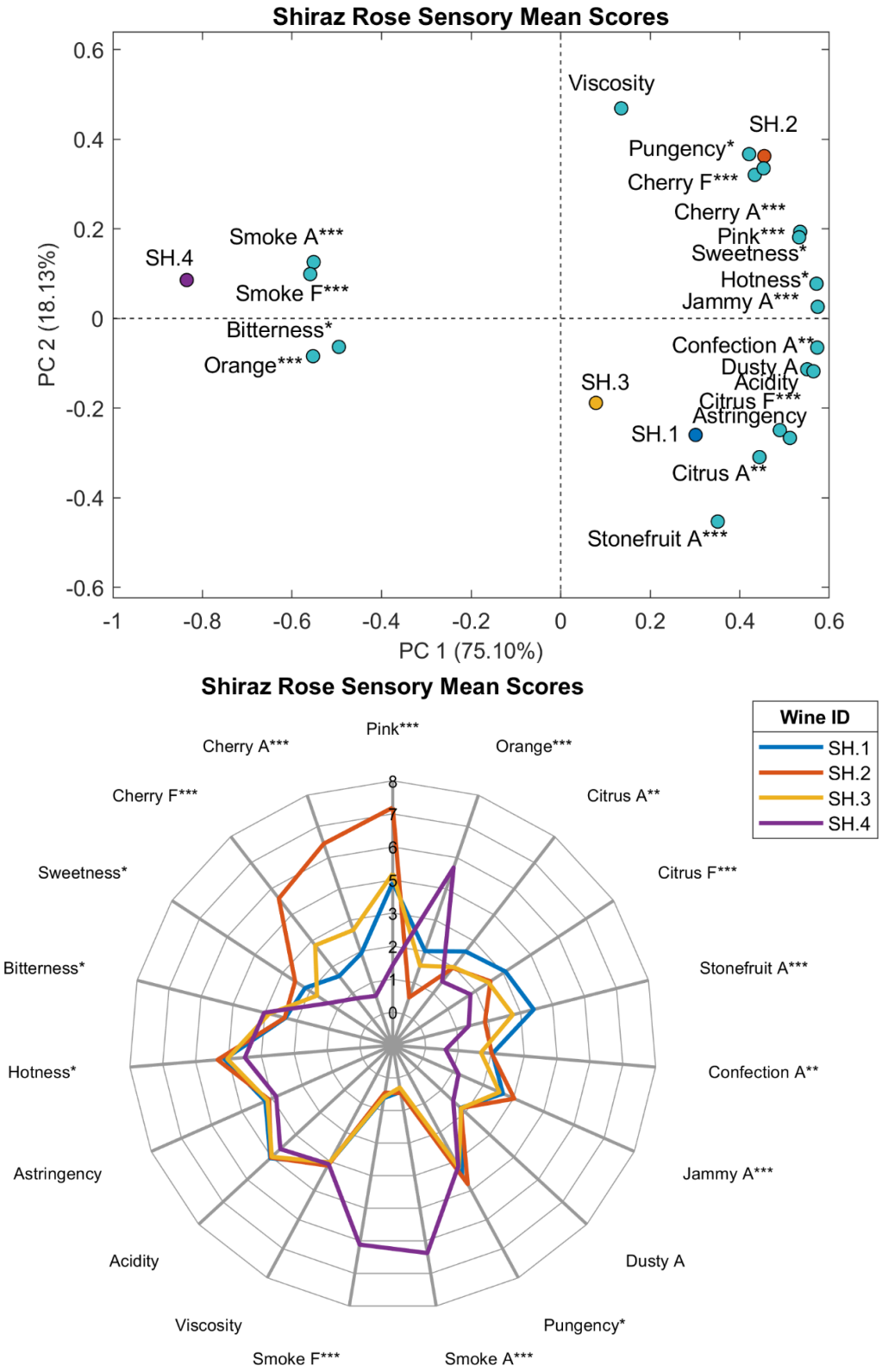


Figure 5. Principal Components Analysis biplot and spider-plot of the mean values of sensory attributes for the Shiraz rosé wines. Line colours represent different samples. A: aroma, F: Flavour. Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Pinot Noir Dry Red Wines

From the ANOVA for the Pinot Noir dry red wines, ten attributes rated by the panel differed significantly ($P<0.05$) including red (colour intensity), orange (colour intensity), dark fruit aroma, red fruit aroma, smoke aroma, spices aroma, red confection aroma, dark fruit flavour, red fruit flavour and smoke flavour. There was a significant judge effect for many of the attributes indicating different use of scale, and significant ($P<0.05$) Judge by Wine interaction effects for several attributes.

Figure 6 illustrates the Principal Component Analysis biplot and spider plot derived from the mean sensory ratings. PCs 1 and 2 explained 57% and 28% of the variation in the data. Wines (PN.1 – low and PN.2 low-moderate) on the far right were highest in attributes positively loaded on PC1, notably eucalypt aroma, red fruit aroma, red fruit flavour, red confection aroma, vinegar aroma and orange (colour intensity). These wines were also rated low in the attributes smoke aroma, smoke flavour, viscosity, earthy aroma red (colour intensity) and pungency. The wines high on PC2 (PN.3 – High) were highest in attributes such as dark fruit aroma, dark fruit flavour, hotness and astringency, and lower in attributes loaded negatively on PC2, such as bitterness.

Wine PN.2 (low-moderate) was rated highest in orange (colour intensity), whilst the other three wines were higher for red (colour intensity). It is also notable that wine PN.4 (extreme), was rated highest in smoke flavour, followed by PN.3 (high), and wines PN.1 (low) and PN.2 (low-moderate) had very low ratings for the smoke attributes, which is consistent with the Pinot Noir rosé wine set.

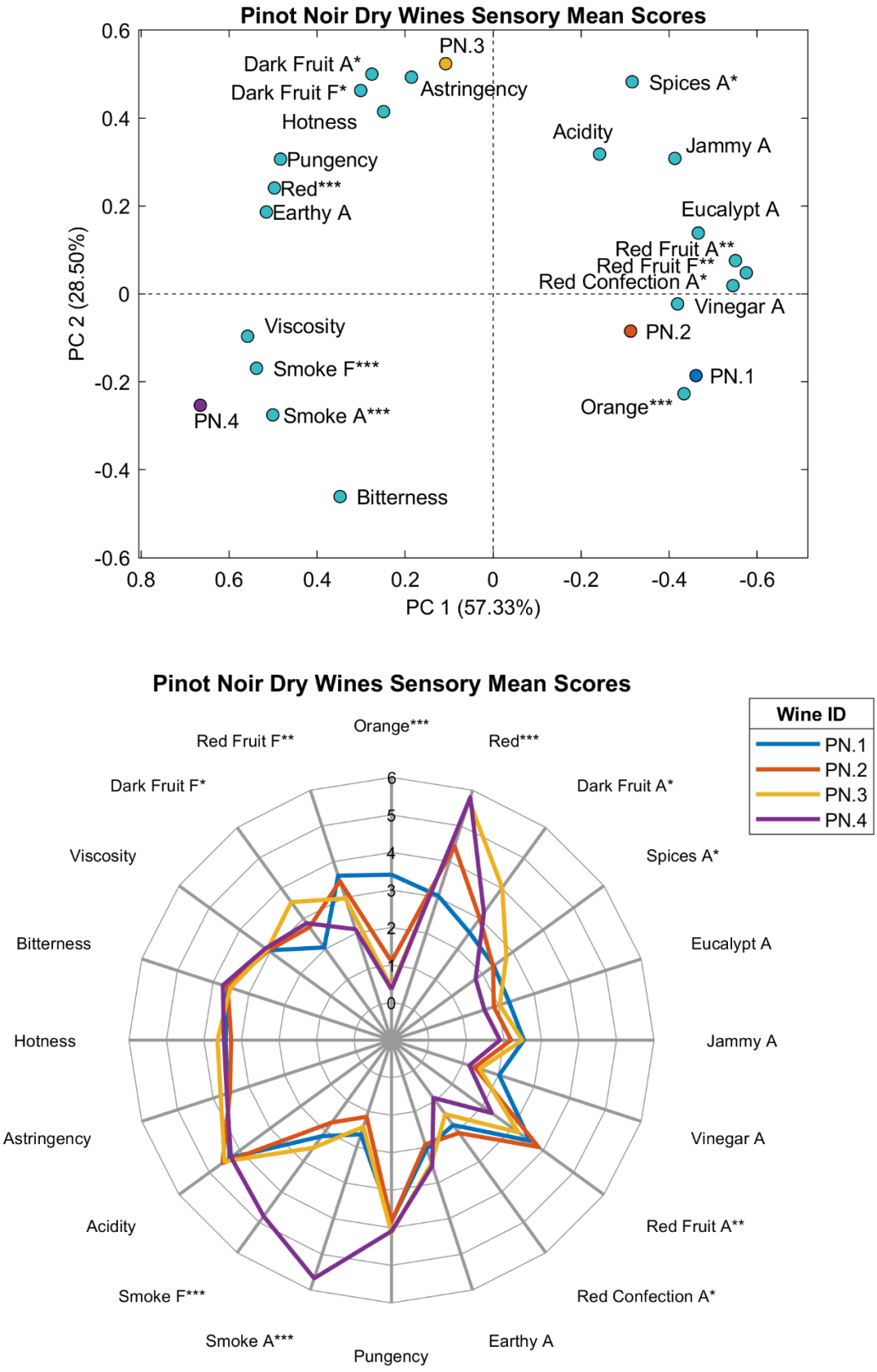


Figure 6. Principal Components Analysis biplot and spider-plot of the mean values of sensory attributes for the Pinot Noir red wines. Line colours represent different samples. A: aroma, F: Flavour. Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Shiraz Dry Wines

From the ANOVA for the Shiraz dry wines, fourteen attributes were rated significantly different ($P<0.05$). These included red (colour intensity), purple (colour intensity), opacity, dark fruit aroma, red fruit aroma, smoke aroma, spices aroma, jammy/confection aroma, pungency, astringency, hotness (alcohol burn), viscosity, dark fruit flavour, and smoke flavour. There was a significant judge effect for many of the attributes indicating a lack of consistency for use of scale. It is notable that inconsistent use of scale will contribute to a masking of differences in figures that are constructed with mean ratings. There were significant replicate effects for purple (colour intensity), opacity and earthy aroma indicating variation between replicates for some wines. There was significant ($P<0.05$) Judge by Wine interaction effects for several attributes.

Figure 7 shows the Principal Components Analysis biplot and spider plot of the mean sensory ratings PCs 1 and 2 explained 55.7% and 32.8% of the variation in the data. Wines on the far right of the biplot (SH.2 and SH.3 – moderate) were highest in attributes positively loaded on PC1, notably earthy aroma, dark fruit aroma, opacity, dark fruit flavour, purple (colour intensity), viscosity and hotness. These two wines were also rated as low in the attributes smoke aroma, smoke flavour, red (colour intensity) and red fruit flavour. The wines positioned higher on PC2 (SH.1 – low) were highest in attributes such as red fruit aroma, spices aroma and jammy/confection aroma, and lower in attributes loaded negatively on PC2, such as acidity and bitterness.

SH.4 (extreme) was rated highest in smoke aroma and smoke flavour and is an important leverage component for PC1, whilst the other three treatments did not have significant differences in smoke attributes. Observations for the Shiraz dry wines are consistent with the Shiraz rosé wine set.

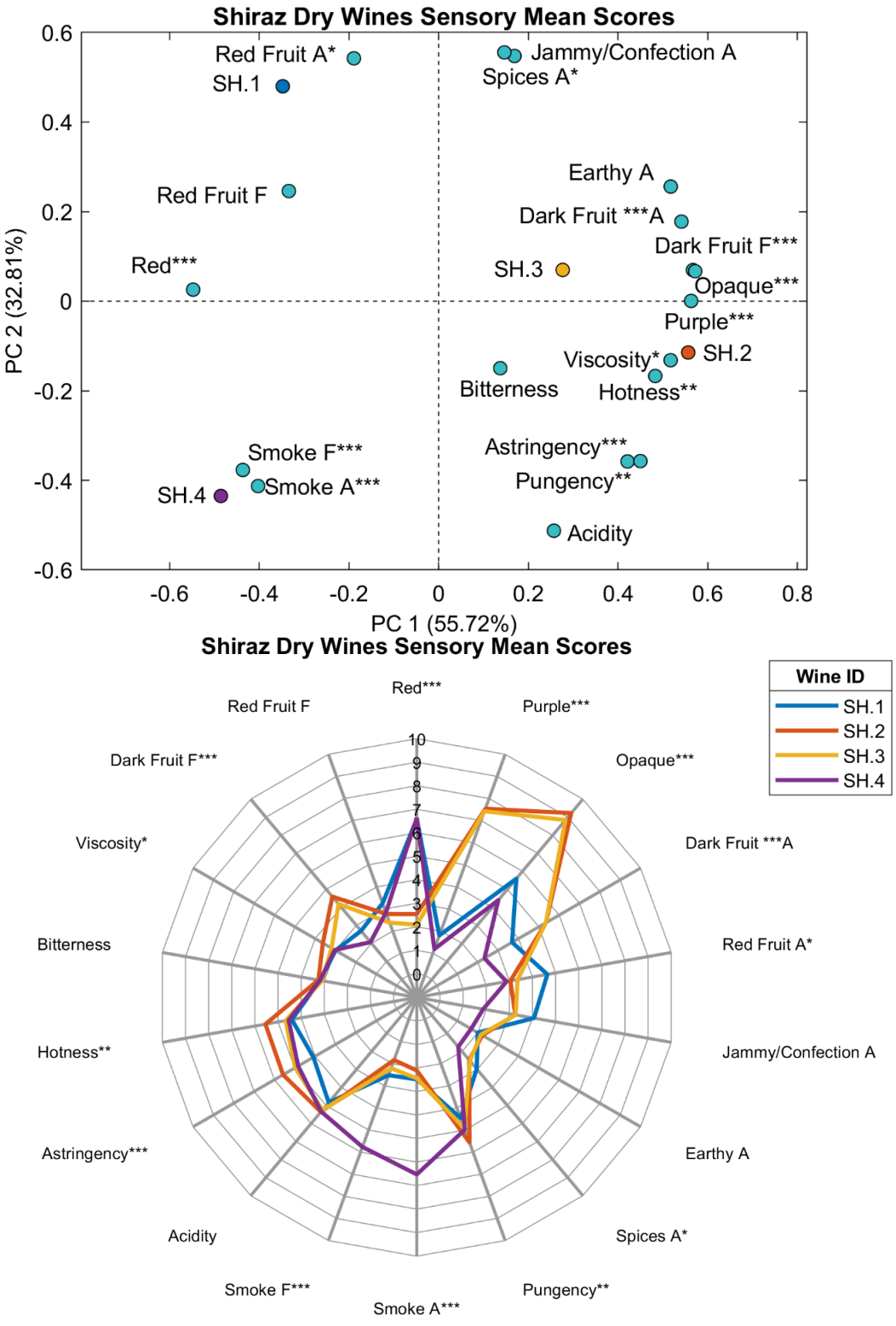


Figure 7. Principal Components Analysis biplot and spider-plot of the mean values of sensory attributes for the Shiraz red wines. Line colours represent different samples. A: aroma, F: Flavour. Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Wines made from grapes with extreme smoke exposure grapes, PN.4 and SH.4 were perceived to be overtly smoky. More non smoke-related characters could be perceived in red wines than in rosé wines; in particular, spice aroma was rated to be significantly higher in wines of low to moderate levels of smoke taint than those of high smoke taint, irrespective of variety. Similarly to the rosé wines, red wines from grapes with higher levels of smoke exposure were perceived to have reduced fruit characters compared to their less tainted counterparts, though some differences were not obvious. In the absence of grape samples of varying levels of smoke exposure obtained from the same location one should be cautious in interpreting the significance of colour and fruit flavour attribute differences between the wines. It is well known that cooler climates will increase the concentration of anthocyanins in grapes, and those grapes will have a different fruit sensory domain to those grown in warm to hot climates. Shiraz grapes were obtained from warm to hot (SH.1) to temperate (SH.2-4) climates. It is possible that smoke attributes do mask fruitfulness in some of the wines and such masking is more evident in the Pinot Noir wines (PN1-3) obtained from the Orange region (temperate).

High smoke exposure rosé wines were rated the lowest in fruit related aroma and flavours, although there were a few exceptions for the dry red wines. For example, Pinot Noir wine from high level of smoke taint (PN.3) was perceived to have significantly higher dark fruit aroma than wines from other smoke exposure levels. In Shiraz wines, red fruit flavour was not rated differently among wines of all exposure levels, while red fruit aroma was rated significantly higher in wines of low smoke exposure (SH.1) compared to all other wines.

Previous studies have established that the volatile phenol compounds are highly correlated to 'smoke' and 'medicinal' aromas. The best-estimate odour detection threshold in red wine has been reported for five of them, including guaiacol (23 µg/L), 4-methylguaiacol (65 µg/L), *o*-cresol (62 µg/L), *m*-cresol (20 µg/L) and *p*-cresol (64 µg/L) (Boidron et al. 1988, Parker et al. 2012). By comparison, syringol and 4-methylsyringol have very high sensory thresholds that are unlikely to be found in wine (Parker et al. 2012), and therefore they are generally considered to contribute to the smoky flavour through synergistic effects with other volatile phenols. Most red and rosé wines made in this project contained levels of volatile phenols below reported sensory thresholds. The only exception was the guaiacol concentration in SH.1 red wine, which exceeded the sensory threshold (Table 15). Even in high smoke-exposed wines, only guaiacol and *m*-cresol exceeded the reported threshold. However, it was observed in this project that wines contained sub-threshold of volatile phenols can still exhibit pronounced smoke aroma and flavour, and this was particularly evident when the low to moderately affected wines were compared to clean controls unaffected by smoke. This result is supported by previous studies and indicated that synergistic and/or additive effects of volatile phenols compounds in imparting smoke aroma and flavour may contribute to the overall smoky aromas in wine (Parker et al. 2012). Smoke taint can still be found in wines containing all volatile phenols below their detection thresholds. The potential for compounds other than those targeted for analysis in this investigation, to contribute to smoky aromas and flavours, cannot be understated and is deserving of further investigation to better inform winemakers on the likely outcomes of grapevine smoke exposure and winemaking outcomes.

In addition to volatile phenol compounds, non-volatile glycosides can also contribute to the perceived smoke taint in wine. Guaiacol monoglucoside has been found to be hydrolysable by saliva which leads to guaiacol release. Furthermore, Mayr and colleagues (2013) conducted a study where glycosides from smoke tainted grapes were spiked into clean control wines and reported that they directly contributed to 'smoky' and 'medicinal' flavours. It was also found that the wines spiked with high levels of both volatile phenols and glycosides exhibited the highest smoke flavour and ashy aftertaste, in comparison with wines spiked with only volatile phenols or glycosides, or spiked at lower concentrations. This further pointed to the additive effect that may exist among various volatile or non-volatile smoke taint compounds. Therefore, measuring a range of volatile phenol compounds, rather than measuring just one or two compounds (e.g. guaiacol) is more informative in providing a comprehensive picture of the potential impact of grape smoke exposure in resulting wine.

One further observation is that Shiraz wines, especially Shiraz dry red wines seem to be less affected by smoke taint than Pinot Noir wines. In every smoke exposure level targeted in this project, both

Shiraz rosé and red wines contained higher concentrations of glycosides than their Pinot Noir counterparts. SH.1 (low) contained a total volatile phenol concentration between PN.2 (low – moderate) and PN.3 (high), while SH.2 and SH.3 (both moderate) contained total volatile phenol concentrations close to PN.3. However, while PN.2 and PN.3 were both rated to be significantly higher in smoke aroma and flavour (Table 19), SH.2 and SH.3 were not (Table 21).

While the absolute values between wine varietal and style ratings cannot be directly compared, the protocol for rating was the same across all panels i.e., the intensity of each sensory attribute was rated on a 15 cm line scale. Lower absolute ratings may indicate a less perceptible smoke aroma and flavour in Shiraz red wines compared to the Pinot Noir wines, which may indicate the Shiraz wines possessing more readily apparent and obvious desirable fruit and spice attributes. This was indicated by the greater number of fruit-related attributes the panellists perceived in Shiraz red wines compared to the Pinot red wines and all rosé wines. It has been shown in sensory studies that fruit characters could have a masking effect on the smoke characters (Ristic et al. 2017, Culbert et al. 2021). Thus, Shiraz red wines might have benefited from having higher intensity and complexity of fruit characters that moderated the perceived smoky characters.

Chardonnay

The ANOVA of sensory attributes for the Chardonnay wines indicated fifteen attributes rated by the panel differed significantly ($P<0.05$) including *yellow* (colour intensity), *stonefruit aroma, tropical fruit aroma, floral aroma, apple aroma, smoke aroma, pungency, acidity, hotness* (alcohol burn)*, astringency, viscosity, sweetness, stonefruit flavour, tropical fruit flavour* and *smoke flavour.* There was a significant replicate effect for *tropical fruit aroma,* indicating variation between some replicates within the treatments and a significant ($P<0.05$) judge by wine interaction effect.

Figure 8 shows the PCA biplot and spider plot of mean sensory ratings for the Chardonnay wines. Notable is the relatively close positioning of juice extraction levels (400 and 500) for each grape harvest. Wines positioned in the positive half of PC1 (CH.1 400; CH.1 500 - low; CH.3 400 and CH.3 500 - high) are highest in stonefruit aroma, tropical fruit flavour, hotness (alcohol burn), apple aroma, stonefruit flavour and sweetness. These wines were also rated as low in the attributes smoke aroma, smoke flavour and acidity. The wines high in PC2 (CH.3 400 and CH.3 500 - high) were highest in attributes such as astringency, and lower in attributes loaded negatively on PC2, such as yellow (colour intensity). It is notable that wines made from grapes with extreme levels of smoke exposure (CH.4 400 and CH.4 500) were rated highest in smoke aroma and smoke flavour, while the other wines had lower smoke ratings. There were some differences between the wines for in-mouth attributes (acidity, hotness, astringency, viscosity, and sweetness) and may reflect the final ethanol concentration in the wines, a factor of climate and ripeness of grapes when harvested.

Chardonnay wines had lower volatile phenol and glycoside concentrations compared to red and rosé wines of the same smoke exposure level (Table 16). The Chardonnay wines made from grapes of low (CH.1), and high (CH.2 and CH.3) levels of smoke exposure were mostly not perceived to have significantly higher smoke aroma and flavour than the control (Table 22). Only one wine (CH.2 500 – high) was rated moderately high for these attributes. This appears to be encouraging as it indicates that wine can made from Chardonnay 'grey zone' smoke exposure levels when decreased pressing yields are employed. However, the lack of winemaking replicates and the significant judge and wine effect from the ANOVA means it is prudent to not over-interpret this observation. Further investigations utilising decreased pressing yields of Chardonnay are warranted.

Overall, the sensory descriptive analysis of the Chardonnay wines made from grapes with high smoke exposure level were rated higher in smoke aroma and flavour, and lower in all desirable fruit characters such as stonefruit, tropical fruit and apple.

Results of CH.2 (High) wines were rated to be slightly, yet significantly, higher in smoke aromas, as well as lower in stonefruit aroma and floral aroma. CH.2 wines did not contain higher concentrations volatile phenol compounds than those made from grapes with similar levels of smoked exposure (CH.3), but had slightly higher glycoside concentrations. Unlike the red varieties, a smaller

difference in the concentrations of volatile phenols and glycosides could be important in determining the sensory characters in white wines, possibly due to the less complex fruit characters in white wines.

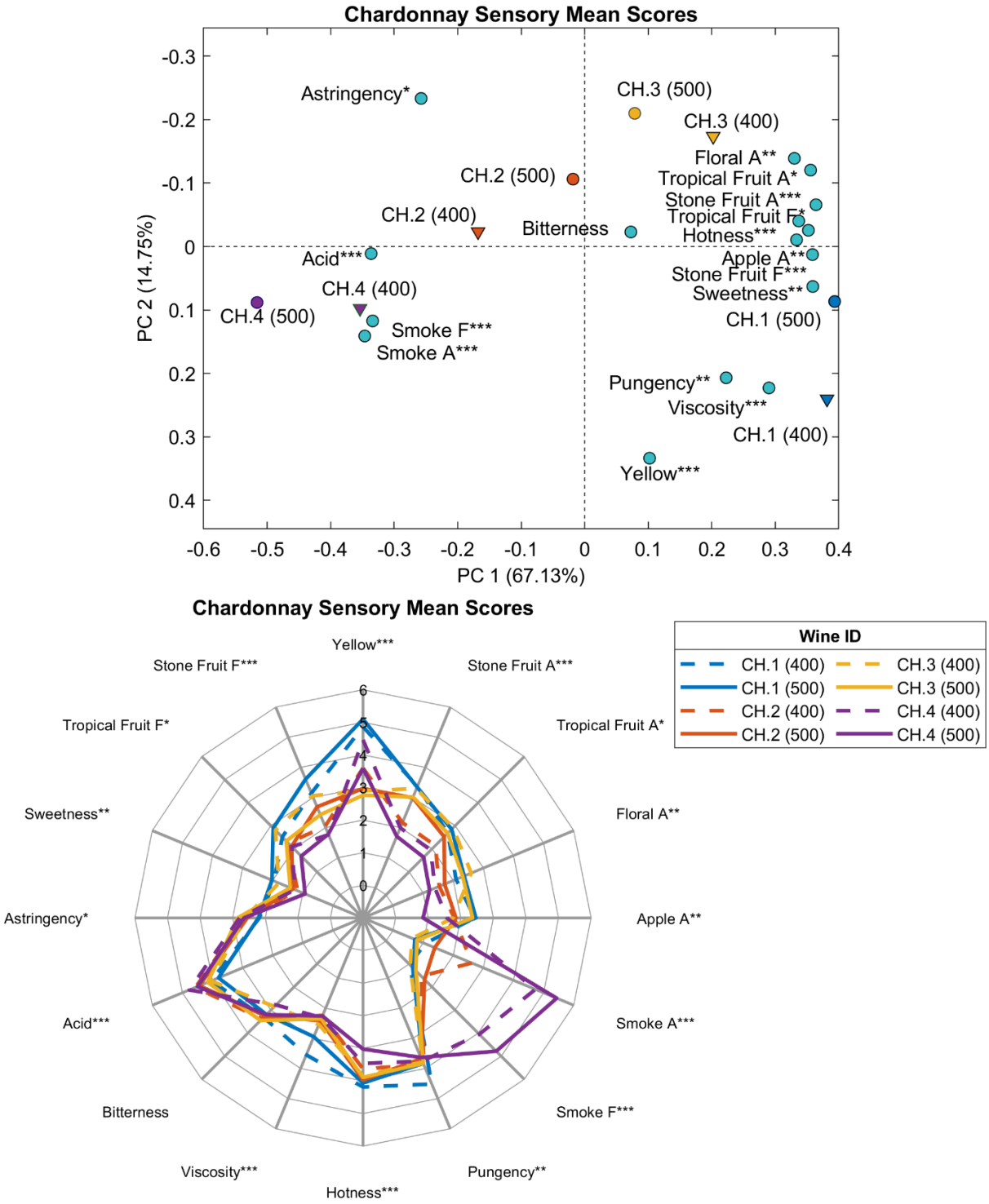


Figure 8. Principal Components Analysis biplot and spider-plot of the mean values of sensory attributes for the Chardonnay wines. Line colours represent different samples. A: aroma, F: Flavour. Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$;

Table 22. Smoke mean scores, rated on a 0 to 10 scale for the Chardonnay wines (n=8 assessors x 2 replicates).

Wine	Smoke aroma	Smoke flavour	Significantly higher than control
CH.1 (400)	0.67	1.11	No
CH.1 (500)	0.40	1.75	No
CH.2 (400)	1.16	1.84	No
CH.2 (500)	1.44*	2.51*	Yes, aroma and flavour
CH.3 (400)	0.54	1.96	No
CH.3 (500)	0.53	2.03	No
CH.4 (400)	3.56***	4.59***	Yes, aroma and flavour
CH.4 (500)	4.68***	5.39***	Yes, aroma and flavour
Chardonnay Control	0.35	0.97	

Correlations between grape smoke exposure marker compounds and smoke aroma and flavour ratings in wine

Correlations between grape smoke exposure markers (seven volatile phenol compounds and six glycosides) in Shiraz and Pinot Noir (Table 11) and the intensities of smoke aroma and smoke flavour in corresponding red wines (Table 19 and 21) are presented in Figure 9. The results are visualised in the two right hand side columns in Figure 7 (outlined in red). Blank spaces denote insignificant correlations (P>0.05), and thus were not shown. All significant correlations were positive, indicating that all targeted smoke exposure markers contributed to smoke aroma and flavour in the final wines. All markers highly correlated with smoke aroma, with methylguaiacol rutinoside and guaiacol having the highest correlation at 0.9 and 0.89 respectively. Fewer compounds were correlated with smoke flavour, among which methylguaiacol rutinoside and syringol have the highest correlation. However, the correlation between individual grape markers and smoke aroma/flavour in wine requires further investigation with larger sample sizes. Total grape glycosides correlated well with both smoke aroma and flavour, with coefficients of 0.86 and 0.85 respectively. Total grape volatile phenols also correlate well with both smoke aroma and flavour in wine, although to a lesser extent at 0.82 and 0.76 respectively.

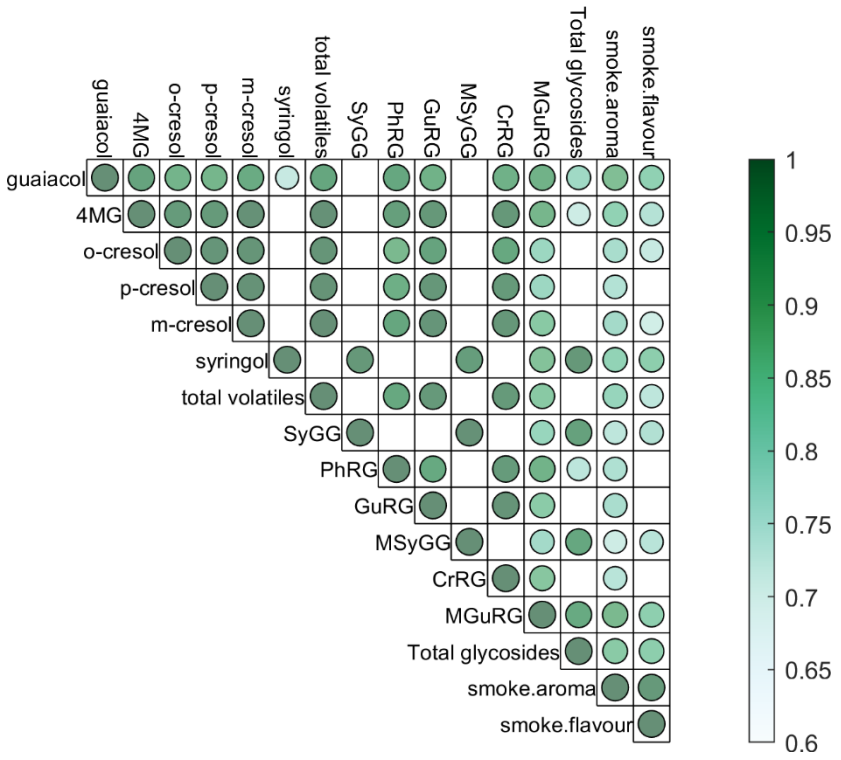


Figure 9. Correlation matrix between grape smoke exposure markers and intensities of smoke aroma and flavour in wine.

2D Correlation Spectroscopy (2D-COS) to identify smoke exposure markers

Initial work was performed with model wine (12% ethanol in 2 g/L tartrate buffer, pH 3.4) to provide a matrix that had no UV fingerprint in the wavelength region associated with phenolics, maximising the ability to detect volatile phenols from smoke. With samples that had barely perceptible smoke taint by sensory analysis, direct examination of the spectra revealed very minor changes related to the introduction of smoke, with no distinct peaks. Contour plots from 2D-COS analysis revealed four peaks in the 220- 235 nm range and eight peaks in the 260-300 nm range (Figure 10). These regions are associated with UV absorbance spectra of phenolic compounds, including smoke taint compounds (Liu et al, 2021).

Spectra were collected over a 220-1400 nm range (UV-Vis-NIR) but absorbance in the Vis-NIR range was too low, illustrating the sensitivity of the UV range in detecting phenolic compounds.

High absorbance in the UV region allows detection of low concentrations of phenolic compounds, including smoke taint, but detecting a small smoke-related signal in wines is difficult, as wines have a strong UV absorbing matrix. Red wine had to be diluted 1/100 in model wine and white wines 1/30 to bring them on scale, diluting out the small signals due to the introduction of smoke. This could in part be overcome by using a spectrophotometer with a higher absorbance range, but such devices are expensive. A simple solution may be to reduce the background UV absorbing matrix with fining that selectively removes high MW phenolics e.g. methyl cellulose, without removing smoke taint compounds.

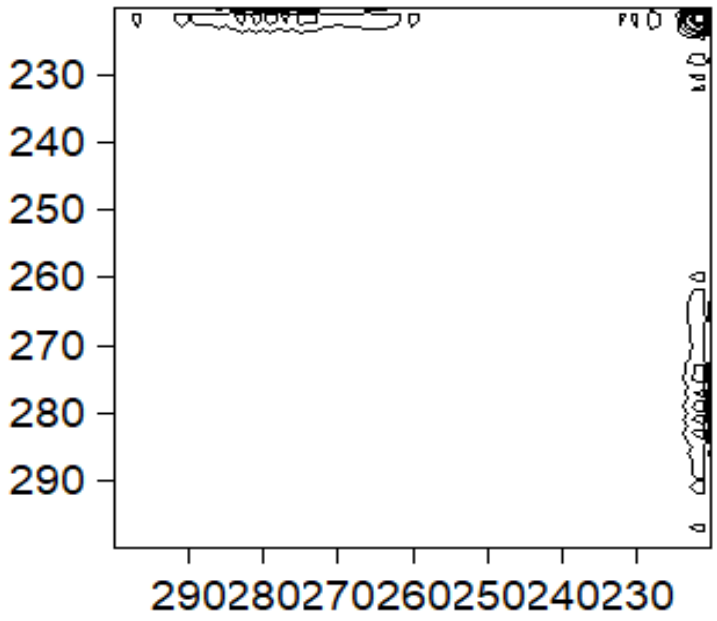


Figure 10. 2-D COS contour map of smoked model wine (220-300 nm)

Further investigation of the use of UV-spectroscopy and multivariate data analysis may provide a rapid screening method for smoke taint in grapes and wine, with positives confirmed by more complex quantitative analysis methods.

8. Outcome/Conclusions

Successful calibration ranges and reproducibility targeting six grape glycoside markers of smoke exposure have been established using an expanded set of deuterated internal standards. Approximately 3 mg of each deuterated phenol glycoside standard has been secured, which is sufficient for approximately 3000 future analyses.

Overall results for grape glycoside calibration and reproducibility are shown in Table 23 and demonstrate a successful implementation of a modified AWRI protocol.

Table 23. Calibration range, linearity, accuracy and reproducibility of the six phenol glycosides in grape homogenates.

Target Compound	Calibration range (µg/kg)	R^2	Accuracy (%)	Reproducibility (%)
Syringol gentiobioside	1 – 200	0.9993	101.5	4.7
4-Methylsyringol gentiobioside	1 – 100	0.9978	104.8	7.8
Phenol rutinoside	1 – 100	0.9988	104.2	5.0
Guaiacol rutinoside	1 – 100	0.9991	107.2	14.8
***p*-Cresol rutinoside**	1 – 100	0.9995	99.1	5.6
4-Methylguaiacol rutinoside	1 – 100	0.9990	103.8	7.6

This investigation demonstrated a direct positive correlation between the levels of smoke taint markers, especially the total glycoside concentration, and their counterparts in finished wines. In all wines produced in this project, a reduction in phenolic glycosides and a concurrent increase in volatile phenols from grape to wine were observed reflecting the transformation from the former to the latter through the vinification process. Concentrations of glycosides and volatile phenols in wine both followed the trends observed in grapes i.e. concentrations generally increased from low to high levels of smoke taint detected in the grapes.

The trend for wine glycosides is less clear than the observations for volatile phenols. A reduction in wine glycosides was noted but the effect was indistinct in Shiraz, where the rosé wine had a slightly higher total glycoside concentration than the dry red table wine. This observation contradicts a previous report for rosé wine styles made with smoke exposed grapes (Ristic et al. 2011). Several factors account for differences in previously published work including differences in cultivars (Coulter et al. 2022; Crews et al. 2022), smoke generation methods, smoke exposure intensity, and wine bottle age at analysis.

Production of a rosé style substantially reduced concentrations for all volatile phenols, by 55% to 70% in Pinot Noir and 44% to 74% in Shiraz wines, in comparison with their table wine counterparts. Reduction of pressing yields in Chardonnay from 500 to 400 L/tonne resulted in small but consistent reductions in total volatile phenol concentration, ranging between 8% and 39%. Both these winemaking techniques attempted to reduce skin contact and extraction, and the analyses obtained indicated a reduction in the concentration of smoke exposure marker compounds in the final wine. However, this did not always directly translate into wines with an improved sensory outcome.

The Shiraz and Pinot Noir rosé wines made from grapes with low to moderate levels of smoke exposure possessed more intense fruit characters than those made from grapes with high exposure

levels. As smoke exposure level increased, the intensity of fruit flavour and aroma diminished in all wines. All Pinot Noir rosé wines were also rated as having significantly higher smoky aroma and flavour compared to a control wine made from grapes without smoke exposure. The intensity of smoke aroma and flavour increased with rising concentrations of volatile phenol and glycosides in the wine. The Shiraz rosé wines made from grapes with low level of smoke exposure were also perceived to have significantly higher smoke aroma than a control wine. Intriguingly, this did not translate into an increased smoke flavour and this observation may be reflective the level of fruit intensity in Shiraz compared to Pinot Noir. However, for the most part, as the levels of grape glycoside markers increased in Pinot Noir and Shiraz, the level of smoke aroma and flavour increased in both dry table and rosé wine styles.

In the Pinot Noir red wines, only the PN.1 wine, made from grapes of a low smoke exposure level (37 µg/kg) was not perceived as having significantly higher smoke aroma and flavour than the control. The other Pinot Noir wines (PN.2, 3 and 4), produced from grapes with low-to-moderate, moderate and high levels of smoke exposure respectively, were all rated with a higher smoke aroma and smoke flavour than the control. Conversely, none of the Shiraz red wines made from low to moderate levels of smoke taint (SH.1, SH.2 and SH.3) were rated to have significantly higher smoke aroma and flavour.

Importantly, fruit-derived characters (e.g. Red and Dark Fruit flavours) were attributes of the table wines but not the rosé wines. These fruit characters were also rated as being considerably higher in the Shiraz table wines in comparison to the Pinot Noir wines. The results show that obvious fruit characters appear to have a masking effect on the smoke aroma and flavour in wines made from grapes produced from low-moderate levels of smoke taint. These observations suggest that it is possible to produce wine styles or varieties (such as Shiraz) without perceptible smoke characters from grapes that fall within the 'grey zone' smoke exposure levels, provided that readily apparent, strong fruit flavours are also present.

Almost all of the Chardonnay wines made from grapes of low and high levels of smoke exposure were not perceived to have significantly higher smoke aroma and flavour. The one exception was the Chardonnay wine (CH.2 (500)) produced using a 500 L/tonne pressing yield. It is also interesting to note that the mean rating for 'Smoke Flavour' was higher in all wines produced made using a 50% extraction rate, although only the CH.2 (500) wine was statistically significantly higher. It is important to acknowledge that all these wines were made by pressing grapes to achieve a lower juice extraction percentage than is typically performed in commercial wineries. However, the current data limit interpretation and extrapolation due to the lack of winemaking replicates.

Finally, smoke exposure markers measured in grapes using contemporaneous analytical methods have good correlations with smoke aroma and flavour in Pinot Noir and Shiraz. Total grape phenolic glycosides appeared to be a good indicator for levels of smoke taint compounds as well as smoky aroma and flavour in finished wines.

9. Recommendations

This investigation has helped further confirm that smoke exposure markers, including six phenolic glycosides and seven volatile phenols, in grapes were well correlated to their levels in finished wines. In particular, total grape glycoside concentration had a good correlation with every smoke taint compound in subsequent wines. Winemakers should have increased confidence that the concentration of these grape smoke exposure markers will be an accurate indication of levels found in wines produced from these grapes.

Limiting skin contact in red wine production i.e. making rosé wine from Shiraz and Pinot Noir was able to reduce the levels of volatile phenols in finished wines. However, sensory evaluations indicated that all rosé wines made from Pinot Noir, even those made from grapes at low smoke exposure levels, were perceived to have higher smoke aroma and flavour than clean control wines. Only the Shiraz rosé produced from grapes with the lowest concentration of grape glycoside was not perceived to be

significantly higher than a control wine. This investigation therefore indicates that rosé wine production from grapes in the 'grey zone' is not recommended, irrespective of the variety.

Reduction of pressing yields resulted in small but consistent reductions in total volatile phenol and phenol glycosides concentration in Chardonnay wines. A promising outcome was that wines produced from both the low smoke exposure grapes and even the majority of the high exposure grapes were not rated as significantly higher in smoke aroma and flavour than a control wine. However this is tempered by the fact that lower pressing yields (400 and 500 L/tonne) were used than are normal for commercial production. The small number of samples in this investigation also needs to be considered and further investigations are recommended.

Shiraz red wines made from grapes of low and moderate level smoke taint were not perceived to have significantly higher smoke taint than a clean control wine, probably a result of having full flavour and obvious fruit characters that help mask the taint. This indicates that Shiraz grapes that fall in the 'grey zone' for smoke exposure might still have the potential to produce wines without perceptible smoke characters. However, only one Pinot Noir wine from grapes harvested from grey zone smoke-exposed grapes i.e. the sample with the lowest grape glycoside level, was not rated as significantly different than the control wine for smoke aroma and taste. This indicates that any level of smoke exposure will likely make commercial Pinot Noir production extremely difficult.

Finally, smoke exposure marker compounds measured in grapes by the current analytical methods have good correlations with smoke aroma and flavour in Pinot Noir and Shiraz. Total grape phenolic glycoside appeared to be a good indicator for levels of smoke marker compounds as well as smoke aroma and flavour in finished wines.

10. Appendix 1: Communication

Publications

Industry presentations and extension activities

2021 National Wine Sector Bushfire Conference

Dr John Blackman, Dr Sijing Li and Prof. Leigh Schmidtke Assessment of smoke exposure on grapes – Blind Tasting and Discussion

Dr Sijing Li & Professor Leigh Schmidtke, Australian Wine Industry Technical Conference Workshop Smoke Taint The Latest Research

Prof. Leigh Schmidtke, Assessment and impact of bushfire smoke exposure on grapes and wine, KPMG Traction Tour, August 2022

Webinars

Television and radio news broadcasts.
N/A

11. Appendix 2: Intellectual property

N/A

12.Appendix 3: References

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13. Appendix 4: Project Staff

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Winemaking

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Technical advice

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