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The Australian Wine Research Institute

Mitigation of climate change impacts on the national wine industry by reduction in losses from controlled burns and wildfires and improvement in public land management



FINAL REPORT TO WINE AUSTRALIA

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Abstract

Beyond the human and community impacts of bushfires, grape and wine businesses are affected in two key ways – through fire damage to vineyards, buildings, plant and equipment, and through smoke impacts on wine-grapes and the resulting wine. This project evaluated a range of possible preventative and remedial treatments for dealing with smoke-affected grapes and wine. A range of commercially available activated carbons was demonstrated to reduce smoke taint in wine, especially when added to grape juice prior to fermentation. Uptake of findings from this project in the context of the 2019/20 bushfires has already been significant.

Executive summary

The objectives of this project were to:

(i) evaluate a range of possible preventative vineyard mitigation options to reduce uptake of smoke taint compounds in the vine and grapes
(ii) evaluate remedial management options for dealing with smoke-affected grapes and wine
(iii) evaluate the impact of treatments on the sensory characteristics of the wines produced
(iv) evaluate consumer preferences for smoke-affected and treated wine
(v) improve our knowledge on background concentrations of volatile phenols and phenolic glycosides in non-smoke-exposed fruit.

Preventative vineyard mitigation options and strategies to reduce uptake of smoke taint compounds

A model system was developed to evaluate under controlled conditions the effectiveness of a range of existing agrochemicals and other materials for reducing/preventing the uptake of volatile phenols from smoke and their subsequent conversion to phenolic glycosides. Grapes were coated with the materials to be tested and then exposed to gaseous phenols in a closed glass container. Treatments typically did not provide any significant protection and many resulted in higher concentrations of volatile phenols and phenolic glycosides in the grapes. The highest concentrations of volatile phenols and their glycosides were observed after application of oily/hydrophobic materials, suggesting that these materials may enhance the adsorption or transfer of volatile phenols into grape berries.

Remedial management options for dealing with smoke-affected grapes and wine

Fifteen commercially-available activated carbon products were evaluated for their ability to remove smoke volatile phenols and phenolic glycosides from smoke-affected juice and wine. The effectiveness of carbon products in removing smoke taint compounds was highly dependent on the type of carbon product used, the matrix (i.e. juice versus wine; red versus white) and the dose applied. Some carbons were better at targeting phenolic glycosides, while others exhibited selectivity for removing volatile phenols. Generally, activated carbon products were more effective at removing phenolic glycosides from juice than wine. The best-performing activated carbons were able at high doses to remove 90% or more of phenolic glycosides from grape juice but also desirable compounds. These results emphasised the importance of selecting the right carbon product for the desired application.

As activated carbons also remove colour from red must or wine, red wine is better suited to treatment with glycosidases. As such, five commercially available glycosidases were evaluated for their ability to degrade smoke taint related glycosides. All were observed to preferentially, and effectively, cleave some phenolic gentiobiosides in wine. However, many other phenolic glycosides were not cleaved.

Sensory impact of treatments

For sensory evaluation of activated carbon treatment, two products were evaluated in small-scale winemaking trials with smoke-affected Chardonnay and Pinot Noir rosé juices treated at three doses (1, 2 or 4 g/L) prior to fermentation. Sensory evaluation of the resulting wines confirmed that smoke-

affected juices can be effectively treated with activated carbon products to reduce the perception of smoke taint in wine. However, the carbon dose must be carefully optimised as the highest dose treatments with 4 g/L activated carbon also showed significant decreases in many of the positive 'fruit/floral' attributes. Notably, the Pinot Noir wines where the juices had been treated with 1 g/L of activated carbon had enhanced 'strawberry' aroma and flavour attributes compared to the control, while also having a significant reduction in smoke aroma and flavour.

A glycosidase was chosen for treatment of a full-bodied Pinot Noir wine, in conjunction with two activated carbon products to reduce the volatile phenols released in the wine due to cleavage of glycosides. However, combining enzyme treatment of wine with carbon fining did not improve the sensory characteristics compared to fining the wine with activated carbon alone. In this particular study, enzyme treatment of the wine led to increases in the rating of 'smoky' characters.
Sensory studies performed on two smoke-affected Pinot Noir wines confirmed that blending can be an effective option for remediation of smoke-affected wine. For both experiments, a blend of 25% or less of the smoke-affected wine with unaffected wine of a similar style was sufficient to lower the smoke taint intensity to be not significantly different from the unaffected wine.

Consumer preferences for smoke-affected and treated wine

In a follow-up study to the blending experiments described above, 82 consumers were asked to describe their liking for a smoke-affected Pinot Noir rosé-style wine in comparison to the tainted wine blended with varying proportions of an unaffected wine. Overall, the least liked wine was the smoke-affected wine and liking scores decreased with greater proportion of smoke-affected wine in the blend. Cluster analysis identified three groups of consumers with significantly different liking scores within each of the clusters. The results from the total sample set, and for the largest cluster group (46% of consumers), demonstrated that even a small proportion of smoke-affected wine in a blend can significantly affect consumer liking. Notably, one cluster consisting of 21% of consumers liked all the wines, suggesting that they may not be sensitive to smoke flavours or might even enjoy such characters.

Baseline concentrations of volatile phenols and phenolic glycosides in non-smoke-exposed fruit

Since volatile phenols and phenolic glycosides occur naturally in grapes at low concentrations, it is important to know background concentrations when assessing if samples have been exposed to smoke. A small database of background concentrations already exists and through this project, an additional 325 non-smoke-exposed grape samples were collected from 23 regions across Western Australia, South Australia, Victoria, New South Wales and Tasmania. This allowed baseline concentration data of smoke taint compounds in both grapes and wine to be expanded to 12 of the main winegrape varieties grown in Australia.

Vineyard variability among grapes exposed to smoke was assessed across two blocks, one Chardonnay, one Pinot Noir, after a bushfire in 2018. Field variability of volatile phenols and smoke glycosides was typically within 20%. Importantly all samples, independent of their location within the vineyard, had post-exposure concentrations of smoke taint markers above concentrations for 'clean' grapes as indicated from comparison to baseline data.

This project was funded by the Commonwealth Department of Agriculture, Water and the Environment Rural R&D for Profit program through Wine Australia with co-investment from The Australian Wine Research Institute.

Abbreviations and glossary

CHA – Chardonnay

CrGG - Cresol gentiobioside

CrMG - Cresol monoglucoside

CrPG - Cresol pentosylglucosides

CrRG - Cresol rutinoside

GCMS - Gas Chromatography Mass Spectrometry

GuGG - Guaiacol gentiobioside

GuMG - Guaiacol monoglucoside

GuPG - Guaiacol pentosylglucosides

GuRG - Guaiacol rutinoside

LCMS - Liquid Chromatography - Mass Spectrometry

MGuGG - Methylguaiacol gentiobioside

MGuMG - Methylguaiacol monoglucoside

MGuPG - Methylguaiacol pentosylglucosides

MGuRG - Methylguaiacol rutinoside

MSyGG - Methylsyringol gentiobioside

MSyPG - Methylsyringol pentosylglucosides

MSyMG - Methylsyringol monoglucoside

PhGG - Phenol gentiobioside

PhMG - Phenol monoglucoside

PhPG - Phenol pentosylglucosides

PhRG - Phenol rutinoside

PN – Pinot Noir

SyGG - Syringol gentiobioside

SyMG - Syringol monoglucoside

SyPG - Syringol pentosylglucosides

VPs – volatile phenols

1 Project rationale and objectives

Since 2003, major fire events in 2003, 2004, 2007, 2009, 2013 and 2015 have resulted in more than $400M worth of grapes and wine being rendered worthless, or products being downgraded, as a result of smoke taint in Australia. The frequency of days with very high and extreme Forest Fire Danger Index (FFDI) are predicted to increase by 4-25% by 2020 and 15-70% by 2050 under various climate change scenarios. In addition to natural bushfires, controlled burn activities by public land management agencies will continue. Thus it is important that the Australian wine industry manages this major financial and reputational risk and develops cost-effective remediation tools for smoke-affected grapes and wine, to minimise the expression of smoke taint characters and retain the quality and integrity of wines in the marketplace.

Smoke taint is known to develop during bottle ageing through continued conversion of non-volatile glycosides into their free volatile forms. Currently the Australian wine sector has a very limited number of effective remediation technologies available such as reverse osmosis, high rates of carbon fining (2-3 g/L) and increased use of oak and exogenous wine tannins to mask the expression of low-level smoke taint problems. Additionally, at-risk wines can be targeted for quick consumption. Hence the Australian wine sector needs more effective remediation options for processing smoke-affected grapes and wine. In this context, the project aims to evaluate a range of, preventative and remedial management options for dealing with smoke-affected grapes and wine.

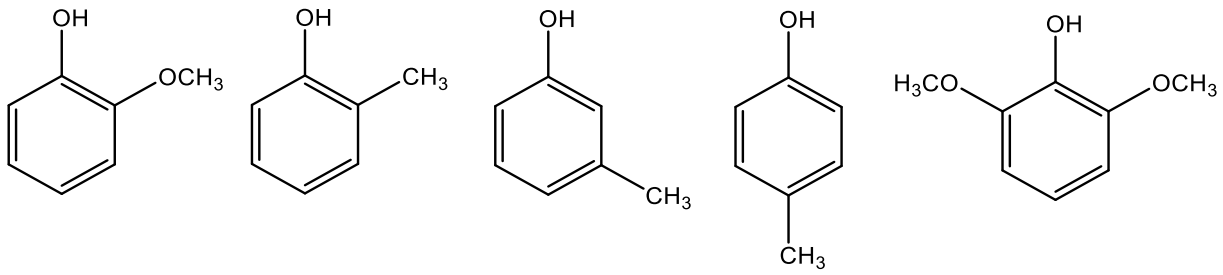
The specific project objectives were to:

- Investigate vineyard mitigation options and strategies to reduce the uptake of smoke taint compounds in the vine and grapes, and/or explore options to limit the conversion of free volatile phenols into their more difficult to remove glycosidic/bound forms.
- Benchmark a range of remediation strategies for managing smoke-affected grapes and wines. This objective aims to examine processing options and materials to remediate wines and minimise the loss of final wine quality.
- Conduct sensory analysis of treated wines, demonstrating the efficacy of a reduction in concentrations of key volatile phenols and their associated glycosides.
- Establish sensory and compositional profiles and consumer preferences for smoke-affected and treated wine.
- Extend the AWRI's current background study to ensure 12 popular varieties are completed with appropriate statistical techniques developed for comparing 'affected' and 'non-affected' samples.
- Conduct an economic evaluation of remediation activities.

2 Method and project locations

2.1 Background – smoke taint in grapes and wine

Exposure of grapes to smoke results in the uptake of volatile phenols from smoke such as guaiacol, isomeric cresols and syringol (Figure 1).



Guaiacol o-Cresol m-Cresol p-Cresol Syringol

Figure 1. Volatile phenols from smoke

Once absorbed into the berry, the volatile phenols are enzymatically converted into a range of non-volatile glycosides (also called glycoconjugates). In grapes, glycosides are typically formed by reaction with glucose followed by addition of a second sugar, leading to the disaccharide structure depicted in Figure 2 and the different compounds listed in Table 1. During winemaking, sugar unit(s) from the glycosides can be cleaved, releasing the volatile phenols back into the wine. In wine, the volatile phenols contribute 'smoky' aromas and their corresponding glycosides can cause unpleasant 'ashy' and 'smoky' sensations and a lingering negative aftertaste. Overall, these sensory characteristics are commonly described as 'smoke taint'.

In tackling smoke taint there are two possible prongs of attack, either by preventing or inhibiting the uptake of volatile phenols from smoke into the grape berry or by remediation/mitigation of smoke-affected grape juice and wine. The former focuses on mitigation in the vineyard and might use materials (or sprays) applied to grapes to prevent uptake of volatile phenols. If the uptake of volatile phenols could be minimised, then minimal glycosides would be formed. Alternatively, if the uptake of volatile phenols by grapes cannot be inhibited, then a 'curative' approach must be undertaken on the resulting grape juice, must or wine.

In smoke-exposed grape berries, much higher concentrations of smoke glycosides than volatile phenols are typically observed; therefore, mitigation approaches should mainly focus on removing the glycosides. In wine, there is typically a mixture of both glycosides and volatile phenols and therefore the removal of both classes should be considered.

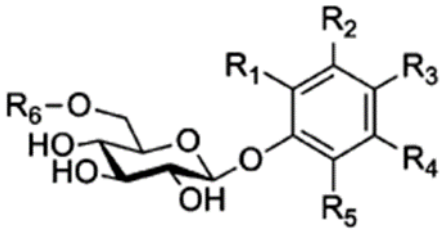


Figure 2. Structure of phenolic glycosides formed in smoke-affected grapes. The R-groups commonly found are listed in Table 1.

Table 1. List of phenolic glycosides found in smoke-affected grapes and the functional groups

Compound	R_1	R_2	R_3	R_4	R_5	R_6
Phenol gentiobioside	H	H	H	H	H	glucose
Phenol pentosylglucosides	H	H	H	H	H	pentose
Phenol rutinoside	H	H	H	H	H	rhamnose
Cresol gentiobioside		R_1, R_2 or R_3 = CH_3				glucose
Cresol pentosylglucosides		R_1, R_2 or R_3 = CH_3				pentose
Cresol rutinoside		R_1, R_2 or R_3 = CH_3				rhamnose
Guaiacol gentiobioside	OCH_3	H	H	H	H	glucose
Guaiacol pentosylglucosides	OCH_3	H	H	H	H	pentose
Guaiacol rutinoside	OCH_3	H	H	H	H	rhamnose
Methylguaiacol gentiobioside	OCH_3	H	CH_3	H	H	glucose
Methylguaiacol pentosylglucosides	OCH_3	H	CH_3	H	H	pentose
Methylguaicol rutinoside	OCH_3	H	CH_3	H	H	rhamnose
Syringol gentiobioside	OCH_3	H	H	H	OCH_3	glucose
Syringol pentosylglucosides	OCH_3	H	H	H	OCH_3	pentose
Methylsyringol gentiobioside	OCH_3	H	CH_3	H	OCH_3	glucose
Methylsyringol pentosylglucosides	OCH_3	H	CH_3	H	OCH_3	pentose

2.2 Methods

2.2.1 Sample preparation

Grape, juice and wine samples for phenolic glycoside and volatile phenol analysis by HPLC-MS/MS and GCMS, respectively, were prepared according to the method described by Hayasaka et al. (2010a) with the exception of the use of d_3-SyGG as internal standard instead of d_3-guaiacol monoglucoside. For volatile phenol analysis, grape homogenate (25 g) was weighed into a 50 mL centrifuge tube and submitted to AWRI Commercial Services for analysis.

For phenolic glycoside analysis in juice, juice (2 mL) was spiked with d_3-SyGG (50 µL of 20 µg/mL solution) to give a final concentration in the juice of 500 µg/L. The dosed juice was then mixed by

vortex for 30 seconds and extracted by SPE as described for grape homogenate. For juice samples containing particulate matter, juice was centrifuged beforehand. For volatile phenol analysis, a minimum of 25 mL of juice was submitted to AWRI Commercial Services.

For phenolic glycoside analysis in wine, wine (1 mL) was spiked with d_3-SyGG (50 µL of 20 µg/mL solution) to give a concentration in the wine of 1000 µg/L, mixed by vortex and filtered (0.45 µm) directly into a HPLC sample vial (1.5 mL) ready for analysis. For volatile phenol analysis in wine, a minimum of 25 mL of wine was submitted to AWRI Commercial Services.

2.2.2 Analytical methods

Volatile phenol analysis

For quantification of volatile phenols in grape, juice and wine, samples were submitted to AWRI Commercial Services for extraction and analysis by gas chromatography mass spectrometry (GCMS) using stable isotope dilution analysis (Pollnitz et al. 2004). AWRI Commercial Services' volatile phenols screen includes the analysis of guaiacol, 4-methylguaiacol, *m*-, *o*- and *p*-cresols, syringol and methylsyringol.

Smoke glycoside analysis

Smoke glycosides were detected and quantified using a previously published method using stable isotope dilution analysis and liquid chromatography mass spectrometry (LCMS) (Hayasaka et al. 2013). 'Total glycosides' are calculated as the sum of the six phenolic glycosides in Table 3.

Basic chemistry of juice and wine samples

Basic chemical analysis of juice and wine samples was performed by AWRI Commercial Services. Analyses included: pH; VA as acetic acid (g/L); free and total SO_2 (mg/L); alpha amino nitrogen (mg/L); ammonia (mg/L); baume (°Baumé); malic acid (g/L); titratable acid pH 7.0 (g/L); titratable acid pH 8.2 (g/L); yeast assimilable nitrogen (mg/L); alcohol (% v/v); glucose + fructose (g/L); specific gravity.

Colour measurements

Colour measurements were performed on a Cary 60 UV-Vis spectrophotometer, model G6860A (Agilent Technologies). CIELab was performed in double beam and transmission modes, with the wavelength range of 380-780 nm, baseline correction (with water) and illuminants of CIE D65 with 10° observers using a 10 mm quartz cuvette. Single wavelength measurements were performed at 280, 320 and 420 nm for Chardonnay and 280, 420 and 520 nm for Pinot Noir using 1, 2 or 10 mm quartz cuvettes. Measurement of absorbance at 280 nm was used to estimate total phenolics (absorbance - 4); Measurement of absorbance at 320 nm was used to estimate the total hydroxycinnamates (absorbance – 1.4); Measurement of absorbance at 420 nm was used as an estimate of brown pigments. A measure of total red pigment colour in wine was obtained by diluting a portion of the wine with 1M HCl and reading the absorbance at 520 nm. CIElab measurements and single wavelength measurements were only performed on juice and wine samples from winery-scale trials (i.e. those involving carbon and glycosidase trials).

2.2.3 Absorption of gaseous phenols by excised grape bunches – proof of concept experiments

Experiments were undertaken to investigate whether excised grape bunches would take up gaseous volatile phenols, generated by their evaporation from an aqueous mixture, and subsequently convert them to phenolic glycosides. Initial experiments were performed with table grapes (sweet white seedless) due to their availability outside of vintage. Grape bunches (n=2) were placed into a desiccator (volume of approximately 6 L) which contained an aqueous volatile phenols mix (300 mg/L, 20 mL) in a glass pyrex dish. A second desiccator contained control grape bunches (n=2) which were not exposed to volatile phenols (contained 20 mL of deionised water) (Figure 3). Desiccators were left outside for approximately 60 hours. Grapes were washed, dried, homogenised and extracted for volatile phenols and phenolic glycoside analysis. Experiments were repeated on white wine grapes (Chardonnay, Sauvignon Blanc and Semillon).

Preparation of volatile phenol mixture (300 mg/L)

Guaiacol (0.12 g), m-cresol (0.12 g), o-cresol (0.12g), 2,6-dimethoxyphenol (0.12 g) and phenol (0.12 g) were weighed into a volumetric flask (200 mL). The flask was made up to the mark with deionised water making a solution with a concentration of 600 mg/L of each of the phenols (or collectively 3 g/L). This solution was further diluted 1 in 10 to give a concentration of 60 mg/L of each of the phenols (or collectively 300 mg/L).



Figure 3. Two desiccators containing sweet white seedless table grapes, with one containing water (left, the control) and the other with aqueous phenol mixture (right).

2.2.4 Glass tank model experiments to evaluate the ability of agrochemicals to prevent uptake of gaseous phenols by wine-grapes

Wine-grapes (Muscat Gordo and Shiraz) were hand-harvested from SARDI's Nuriootpa Viticulture Experiment Station and stored refrigerated until needed. Three separate experiments were performed, two on Muscat Gordo grapes and one on Shiraz grapes, testing twelve different materials (Table 2). Of these, nine were commercial products currently used in viticulture for disease prevention, sunscreen protection and anti-transpiration. The remaining three included activated carbon, titanium dioxide and silicone oil. The ability of activated carbon to adsorb organic compounds is quite well established. As such, carbon was used as a positive control as it was

expected to bind volatile phenols and potentially prevent uptake. Titanium dioxide was used as in the presence of light it can break down phenols. Both carbon and titanium dioxide are solid materials and needed to be applied to grapes with a wetting agent.

To evaluate multiple potentially protective materials in parallel, the model system described above was upscaled to a larger glass vessel (fish tank, external dimensions 910 x 380 x 450 mm, 156-litre capacity). Within the base of the tank, 6 x pyrex dishes (11.5 cm in diameter, 470 mL capacity) were evenly placed. Aqueous phenol solution (total phenols 1.3 g/L, 20 mL) was placed in each of the pyrex dishes and two dishes were under each of three custom made stainless steel platforms (29.5 x 36.5 x 11.5 cm), each containing 17 x 21 holes (5 mm in diameter).

The horticultural products were applied to the surface of grapes by dipping the grape bunches into solutions of the horticultural products/materials in concentrations recommended by the manufacturer and left to dry on paper towel after dipping (Figure 4). Grapes were then placed in the glass vessel and exposed to aqueous volatile phenols (guaiacol, syringol, o-cresol, m-cresol and phenol) for approx. 60 hours. All treatments and controls were performed in triplicate (i.e. three bunches of grapes, with the aim of having grape bunches weigh typically 150-250 g) and the experiment was replicated three times with Shiraz grapes (1x) and Muscat Gordo grapes (2x). The effectiveness of the treatments in preventing the uptake of free phenols by the grapes and their subsequent conversion to phenolic glycosides was evaluated by comparison to ‘non-treated’ control bunches (i.e. not coated with any agrochemicals or materials) which were exposed to the volatile phenols at the same time.

Table 2. List of agrochemicals/coatings investigated for their ability to prevent uptake of gaseous phenols by wine-grapes, their typical application and application rate

Product	**Typical application**	**Other information**	**Preparation**
Biopest	Pest management	Active ingredient is paraffinic oil; highest recommended dose: 3 L per 100 L	Add 15 mL of Biopest to 485 mL of water
Activated carbon (in Raynox)	Salvage chemical compounds	FPS carbon; needed to be used with a wetting agent so Raynox was chosen	Used in a concentration of 30 mg/mL
Deccoshield	Sunburn protectant	Recommended dose: 1 in 50 dilution	10 mL deccoshield in 490 mL water
Ecoprotector	Pest management (including Botrytis)	Fungicidal potassium soap (potassium salts of fatty acids); Highest recommended dose: 1 in 50 dilution	Add 10 mL ecoprotector to 490 mL water
Envy	Anti-transpirant	A water emulsifiable polymer concentrate; forms a semi-permeable biodegradable film, which reduces transpiration; recommended dose: 5 L per 100 L water	Add 25 mL of Envy to 475 mL water
Fruit drying oil	Vine fruit drying	An emusifiable concentrate; ethyl and methyl esters of fatty acids produced from food grade canola oil; modifies surface waxes; highest recommended dose: 1.5 litres per 100 L (with 2 kg of potassium carbonate per 100 L)	Add 7.5 mL fruit drying oil to 492.5 mL water containing 10 g potassium carbonate
Parka Plus	Cuticle supplement	Composition of food-grade phospholipids supplements the plant's cuticle and forms a protective shield necessary to maintain the integrity of fruit and foliage; 1 in 100 dilution	Add 5 mL Parka Plus to 495 mL water
Peratec	Pest management	Highest recommended dose: 1 in 100 dilution	Add 5 mL of peratec to 495 mL of water
Raynox	Sunburn protectant	Recommended dose: 1 in 40 dilution	Add 10mL Raynox to 390 mL water
Silicone oil			Use 'as is' – no dilution required
Surround	Sunburn protectant	A processed and refined kaolin in the form of a wettable powder; recommended dose: 5 kg per 100 L water	Add 25 g of Surround to 500 mL water; 5-10 mL of wetter per 100 L (0.5 mL per 10 L; 0.05 mL per 1 L)
Titanium dioxide (in Raynox)		Titanium dioxide in the presence of light activation can break down phenols; prepared in a concentration of 30 mg/mL; needed to be used with a wetting agent so Raynox was chosen	



Figure 4. Grape bunches after being treated with Biopest (left), Surround (middle) and titanium dioxide in Raynox (right)

The aqueous phenol solution consisted of guaiacol, *m*-cresol, *o*-cresol, 2,6-dimethoxyphenol (syringol) and phenol. It was prepared by dissolving 120 mg of each of the phenols in 200 mL of MilliQ water (i.e. 600 mg/L of each phenol, collectively 3 g/L). This stock solution was diluted 1 in 2.3 with MilliQ water to give a solution containing 260 mg/L of each phenol. With 120 mL of this solution used in the experiments, 20 mL in each of six pyrex dishes, approximately 31.2 mg of each phenol was contained in that 120 mL quantity, or collectively 156 mg, in a tank of 156 L capacity.

Grape bunches were randomly placed on the stainless steel platforms, arranged to ensure there was one of each treatment on each of the three platforms. A data logger recording temperature and humidity was placed inside the glass vessel. The glass lids were placed on the top of the fish tank and a small fan was used to circulate air within the tank. The grapes remained in the vessel for 60 hours. Details for each of the three experiments are given below.

Muscat Gordo experiment 1 Experiment started late on 24 March 2017 and finished at 9am on 27 March 2017. Range of bunch weights: first replicate 205-232 g; second replicate: 157-175 g; third replicate: 112-165. Temperatures in the tank ranged from 20.8°C to 35.9°C.

Muscat Gordo experiment 2 Experiment started late on 4 April 2017 and finished at 9am on 7 April 2017. Range of bunch weights: first replicate 194-255 g (12 out of 13, 224-255 g); second replicate: 150-188 g (12 out of 13, 168-188 g); third replicate: 132-157 g. Temperatures in the tank ranged from 11.5°C to 30.4°C.

Shiraz experiment Experiment started late on 23 April 2017 and finished at 9am on 26 April 2017. Range of bunch weights: first replicate 193-237 g (10 out of 13, 198-219 g); second replicate: 145-183 g (12 out of 13, 160-183 g); third replicate: 122-152 g (10 out of 13, 131-152 g). Temperatures in the tank ranged from 11.5°C to 28.1°C.

After being exposed to gaseous phenols in a fish tank for 60 hours, grape bunches underwent a semi-abrasive clean to remove as much of the coating as possible (particularly important for carbon coated grapes as presence of carbon may interfere with the analysis). It was difficult to remove the oily substances, particularly difficult for silicone oil. Tissues and wipes were used to try and wipe off

as much oil as possible but there was still some residue. Grape bunches were then placed in individual zip lock plastic bags and frozen until analysis (stored at -20°C).

All berries were removed from stalks and the number of berries counted and weighed. The grape samples were homogenised at 8,000 rpm for 20 seconds using a Retsch blender (minimum of 100 g required). Homogenate samples, 25 g and 5 g, were weighed out in 50 and 10 mL centrifuge tubes for volatile phenol and phenolic glycosides analysis, respectively.

2.2.5 Grape and wine samples

Vintage 2017

Sourcing smoke-affected juice and wine during the 2017 vintage was a considerable challenge, due to the lack of natural smoke events. A number of wines, perceived to be smoke-affected by industry, were shown not to be substantially smoke-affected by sensory and chemical analysis (free and bound phenols). The following grape juices and wines were used for initial trials with activated carbon products and glycosidases:

- 2015 Mataro juice (free run and pressings)
- 2017 'Clean' free run juice and light and heavy pressings (Sauvignon Blanc, Chardonnay and Pinot Grigio)
- 2017 Artificially smoked Cabernet Sauvignon and Merlot
- 2016 Smoke-affected Victorian Pinot Noir

<u>2015 Mataro juice (free run and pressings)</u>

Obtained previously from industry during the 2015 Sampson Flat bushfires and stored frozen at the AWRI in 20 Litre containers.

<u>2017 'Clean' free run juice and light and heavy pressings</u>

Free run juice and light and heavy pressings (approximately 18 Litres of each) of three 'clean' grape varieties (Sauvignon Blanc, Chardonnay and Pinot Grigio) were sourced from industry. This juice was only used for the evaluation of hyperoxidation for volatile phenol removal.

<u>2017 Artificially smoked Cabernet Sauvignon and Merlot</u>

Smoke treatment (1 hr in duration) of *Vitis vinifera* cultivars of Merlot and Cabernet Sauvignon grown in the University of Adelaide's Coombe vineyard took place approximately 7 days to 2 weeks post-veraison in a purpose-built smoke tent (Kennison et al. 2009). Grape bunches were hand-harvested and whole bunched pressed (approximately 2 kg at a time) using a 5.3 L stainless steel GPTPL Table Top Fruit Press, cage size 20 cm x 17 cm (Winequip, Australia). Grapes were pressed using a torque wrench to a pressure of 30 N m. Approximately 5.6 litres of Merlot juice and 4.9 litres of Cabernet Sauvignon juice was obtained from 11.7 kg and 9.6 kg of fruit, respectively. Juice was frozen at -20°C in 1 L aliquots and defrosted as required for laboratory-based experiments involving the evaluation of activated carbon products.

2016 Victorian smoke-affected Pinot Noir

In June 2017, a smoke-affected 2016 Victorian Pinot Noir wine (approximately 100 L) was sourced as bulk wine from a winery and bottled into 750 mL bottles by WIC winemaking services (Urrbrae, South Australia).

This wine was used in benchtop evaluations to assess carbon and zeolite fining and enzyme treatment and in sensory dilution studies.

Vintage 2018

Bushfires in NSW in February 2018 provided an opportunity to source 'real' smoke-affected grapes which were made into juice and wine during vintage 2018. Smoke-exposed grapes (Chardonnay, Sauvignon Blanc, Riesling and Pinot Noir) were sourced from two vineyards within 5 km of a bushfire front, with juice production and contract winemaking taking place in rural NSW during vintage 2018.

The following samples were obtained:

- 50 litres of juice for two clones of Chardonnay and a Riesling
- 250 litres of smoke-affected Sauvignon Blanc juice
- Dry white wine prepared by fermentation of 250 litres of smoke-affected Sauvignon Blanc juice
- Dry red wine prepared by fermentation of smoke-affected Pinot Noir clone MV6 (100 kg scale).

Juice samples were frozen in NSW until transport to Adelaide could be arranged. Sauvignon Blanc and Pinot Noir wines were prepared and bottled in NSW. Bottles of these wines were then shipped to Adelaide.

Vineyard variability measures

Vineyard variability amongst grapes exposed to smoke during the 2018 NSW fires was assessed across two blocks, one Chardonnay, one Pinot Noir. The Pinot Noir MV6 block consisted of 20 rows and covered approximately 1.16 hectares (approximately 57 m wide by 205 m long). The Chardonnay I10V1 block consisted of 37 rows and covered approximately 1 hectare (approximately 93 m wide with row lengths varying from 107-113 m). For each block, 30 sampling zones were randomly chosen. Within each sampling zone a single vine was chosen at random, three bunches were collected from that vine and the vine was tagged for easy identification later when GPS coordinates were collected. Grape bunches were placed in zip lock bags and frozen; samples were sent to the AWRI and remained frozen until sample preparation for analysis for volatile phenols and phenolic glycosides.

Vintage 2019

Obtaining smoke-affected juice for winery-scale remediation trials in 2017 and 2018 had been difficult. As a consequence, smoking experiments using excised grape bunches and smoking tents were undertaken in vintage 2019. In addition, fires in the Huon Valley (Tasmania) in February 2019 provided an opportunity to source smoke-affected Pinot Noir (3 tonnes) and Chardonnay (2 tonnes) grapes from natural smoke events. The juices from 2 tonnes of each of the Tasmanian Pinot Noir and Chardonnay grapes were received at the AWRI in April 2019 and were used for winery-scale

remediation treatments with activated carbon products. In addition, a full-bodied style Pinot Noir wine was produced using 1 tonne of the smoke-affected Tasmanian grapes (approximately 600 litres obtained).

2019 smoke tent experiments

Approximately 1.6 tonnes of excised Padthaway Chardonnay grapes were 'artificially' smoked in a smoke tent, generating approximately 1,000 litres of smoked Chardonnay juice for mitigation and winemaking trials. Chardonnay grapes (2.7 tonnes) were harvested on Tuesday 5 March 2019 from a commercial vineyard located at Padthaway. Smoking of excised grape bunches occurred on 6, 7 and 8 March 2019. Approximately 160 kg of the grapes were smoked at a given time, placed in a single layer on custom made mesh tables. Grapes were exposed to smoke for a duration of 1 hour, with the smoke generated by the combustion of straw inside two smokers (Char-Griller Side Fire Box, Bunnings Warehouse, Australia).

2019 smoke-affected Tasmanian juice and wine

Smoke-affected Pinot Noir (3 tonnes) and Chardonnay (2 tonnes) grapes were sourced from commercial vineyards exposed to forest fire smoke in the Huon Valley in Tasmania. This fruit was processed in Tasmania and approximately 1,300 litres (1,000 L free run juice; 300 L pressings) of each variety were sent to the AWRI for winemaking trials involving the evaluation of two activated carbon products at three dose rates (1,2 and 4 g/L).

2019 Tasmanian Pinot Noir (juice production)

Smoke-affected Pinot Noir grapes were hand-harvested on 4 April 2019 from a vineyard in the Huon Valley. The grapes were pressed on 6 April 2019, the juice was chilled to 0 degrees and the pressings were kept separate from the free-run juice. On 8 April 2019, 970 litres of clean juice were racked into a 1000 L pallecon. Total SO_2 was adjusted to 60 ppm in both free run and pressings. Juice was despatched from the Tasmanian winery on 8 April 2019, travelled under refrigerated transport and was received at the AWRI on 10 April 2019. Samples (free run and pressings) were analysed for basic chemical composition (°Baumé, TA, pH, free and total SO_2, malic acid, ammonia, alpha amino nitrogen) and extracted and analysed for volatile phenols and phenolic glycosides composition.

2019 Tasmanian Pinot Noir (full-bodied style)

Contract winemaking was performed in Tasmania on one tonne of the smoke-affected Pinot Noir fruit to produce a full style red (approximately 600 Litres). Once received at the AWRI, it was sampled and analysed for volatile phenols and phenolic glycosides composition.

2019 Tasmanian Chardonnay (juice production)

Grapes (2.195 tonnes) were hand-harvested on 18 April 2019, transported to the winery directly after harvest and processed into juice on the same day. Grapes were destemmed and crushed and allowed to soak on skins in the press for 2 hours prior to pressing. The pressing cycle was a modified white wine press program, with maximum pressure reached for free-run juice extraction at 0.4 bar and pressing fraction extraction at 1.2 bar. Free-run extraction was cut at 500 L/T and pressings at an

additional 150L/T. Sulfur dioxide was added as potassium metabisulfite (PMS) to achieve 50 ppm in both fractions.

The Chardonnay juice was despatched on 23 April 2019, travelled under refrigerated transport and was received at the AWRI on 26 April 2019. Samples (free run and pressings) were analysed for basic chemical composition (°Baumé, TA, pH, free and total SO_2, malic acid, ammonia, alpha amino nitrogen) and extracted and analysed for volatile phenols and phenolic glycosides composition.

2.2.6 Baseline concentration survey of non-smoke-exposed grape samples

During 2016 and 2017, 325 non-smoke-exposed grape samples were collected from 23 regions across Western Australia, South Australia, Victoria, New South Wales and Tasmania and analysed for the seven volatile phenol and six phenolic glycoside smoke marker compounds targeted as part of the AWRI Commercial Services' Smoke Panel Analysis (Table 3). This figure (n=325) includes 233 grape samples of the five targeted varieties (Sauvignon Blanc, Pinot Gris, Semillon, Merlot and Grenache). Fifty-five grape samples of two 'extra' varieties, Mataro and Sangiovese, were also collected, as well as 37 further samples of Riesling and Pinot Noir to increase the database numbers for these two varieties.

Table 3. Smoke exposure marker compounds analysed for the baseline concentration survey

Volatile phenols	Phenolic glycosides
Guaiacol	Guaiacol rutinoside
4-Methylguaiacol	Methylguaiacol rutinoside
Syringol	Syringol gentiobioside
4-Methylsyringol	Methylsyringol gentiobioside
o-Cresol	Cresol rutinoside
m-Cresol	Phenol rutinoside
p-Cresol	

Small-lot wines were made from the 325 non-smoke-exposed grape samples and also analysed for the volatile phenol and phenolic glycoside smoke marker compounds. The varieties and numbers collected during 2016 and 2017 are summarised in Table 4.

Table 4. Summary of grape samples collected during 2016 and 2017 for the survey of smoke exposure marker compounds in non-smoke-exposed grape samples

Variety	Number of grape samples collected
Grenache	44
Merlot	56
Pinot Gris	55
Sauvignon Blanc	39
Semillon	39
Mataro	25
Sangiovese	30
Pinot Noir	14
Riesling	23
TOTAL	**325**

The Smoke Panel Analysis results for the 325 grape samples and corresponding wines were combined with the data from the previous surveys conducted during 2010 and 2011. The background survey now contains data for 12 varieties, with volatile phenol and glycoside results for 531 and 529 grape samples respectively, and 494 wine samples. A summary of the varieties and sample numbers in the combined background concentrations database for grapes and wine is provided in Table 5.

Table 5. Summary of the varieties and sample numbers in the background concentrations database

	Number of samples			
	Volatile phenols		Phenolic glycosides	
Variety	**Grapes**	**Wine**	**Grapes**	**Wine**
Cabernet Sauvignon	44	35	44	35
Chardonnay	47	41	45	41
Pinot Noir	51	39	52	39
Riesling	38	30	38	30
Shiraz	62	61	62	61
Grenache	44	44	44	44
Merlot	56	56	56	56
Pinot Gris	55	55	55	55
Sauvignon Blanc	39	39	39	39
Semillon	39	39	39	39
Mataro	25	25	25	25
Sangiovese	30	30	30	30
Total	**531**	**494**	**529**	**494**

2.2.7 Laboratory-based experiments

Evaluation of activated carbon products

A range of commercially available activated carbon products (Table 6) were evaluated for their abilities to remove smoke taint compounds from grape juice and wine. Grape varieties included were Merlot, Cabernet Sauvignon, Pinot Noir, Mataro, Sauvignon Blanc, Riesling and Chardonnay.

General procedure: A sample of juice or wine (50 mL) was treated with a single dose of activated carbon at a dose rate between 0.5 and 4.0 g/L (addition of 25 to 200 mg) with contact times from 2 to 24 hours. Generally, unless otherwise stated, all experiments were performed with constant stirring using a magnetic bar and a multi-position magnetic stirrer. After the given contact time, samples were centrifuged (3,750 rpm for 5 minutes) and the supernatant subsampled for volatile phenol and/or phenolic glycoside analysis. Carbon-treated samples were compared against the control (i.e. no carbon treatment) to determine the amount of volatile phenols and/or phenolic glycosides remaining post-treatment.

Table 6. List of activated carbon products evaluated for their abilities to remove smoke taint compounds from grape juice and wine

Activated carbon product	**Manufacturer/ supplier**	**Information provided by manufacturers/suppliers**
Acticarb PC1000	Activated Carbon Technologies	Ex coconut – steam-activated; used in water filtration for removal of very low molecular weight pollutants where very long contact time is available i.e. 2+ hours
Acticarb PS1000	Activated Carbon Technologies	Ex coal - steam-activated; used in water filtration for removal of algal toxins and taste and odour compounds at 10+ minute contact time.
Acticarb PS1300	Activated Carbon Technologies	Ex coal - steam-activated; used in water filtration for removal of algal toxins and taste and odour compounds at sub-10-minute contact time and when concentration is very high.
CA-50	CarboChem	Mesh 325; for decolourising
P-1000	CarboChem	No information available
PC-900	CarboChem	Ex coconut, mesh 325; for food processing
Carbocromos super	Vason/IMCD Australia Ltd	Active vegetal carbon with a high level of purity, activated with phosphoric acid. It has a strong decolorising power and does not cause any organoleptic modifications in treated wine. Its adsorbent action is particularly effective towards anthocyanins and oligomers that are particularly subject to oxidation and are responsible for instability in white wines.
FPS	Vason/IMCD Australia Ltd	A vegetal powder carbon characterised by a remarkable specific adsorption surface. This is a product with a high purity, steam-activated, with a low content of releasable metals. Due to its specific characteristics and its porosity, FPS can selectively adsorb pigments and small molecules; It has a good action of removal of ethyl phenols on wines. It has a good decolourising activity and can help to remove anthocyanins and oligomers particularly subject to oxidation.
Smartvin Carb	Vason/IMCD Australia Ltd	Vegetal carbon in pellets.

Toxical	Laffort	Combination of silica sol and activated carbon; special product for removing ochratoxin by adsorption then clarification of wines
Norit D10	Cabot/IMCD Australia Ltd	A steam-activated carbon with high phenolic value that can remove taste and odour in food applications.
Norit SX Plus	Cabot/IMCD Australia Ltd	An acid-washed activated carbon that is suitable for a large range of applications such as decolourisation and purification of pharmaceuticals, food products and organic chemicals, where the use of high purity additives is required. It is also very effective as a precious metal catalyst carrier.
Bentonorit DX	Cabot/IMCD Australia Ltd	Bentonorit DX consists of approximately 87% Norit DX 10 and approximately 13% food grade calcium bentonite. The product is shaped as cylindrical 3 mm pellets, which can be dosed without having dust problems. In contact with most aqueous solutions the pellets disperse as a powder within seconds. Bentonorit DX is dedicated to adsorb undesired contaminants such as taste and odour compounds without affecting the desired natural character of the treated product.
Norit CASPF	Cabot/IMCD Australia Ltd	Norit CASPF is very suitable for the purification of highly coloured and/or foaming liquids in the food and beverage industry. Norit CASPF combines a superior adsorptive capacity with good filtration characteristics. It is especially effective in adsorbing higher molecular weight organics such as large colour bodies and proteins, at low carbon dosage rates. Norit CASPF is produced by chemical activation using the phosphoric acid process.
Deobrett	EnolTech	No information available

Evaluation of commercially available glycosidases

The enzymes investigated for their ability to cleave smoke glycosides are listed in Table 7. The general protocol used for benchtop experiments was as follows: samples of juice or wine (50 mL) were treated with the glycosidase at a given rate, typically the highest recommended dose or multiples of that (i.e. x2; x5), with contact times ranging from a few days to up to three weeks and at temperatures of either 17°C, 22°C (ambient laboratory temperature) or 28°C. All experiments were performed with constant stirring using a magnetic bar and a multi-position magnetic stirrer. After the required contact time, bentonite was added to remove the glycosidase. After allowing the bentonite to settle for 16-24 hours, samples were centrifuged (3,750 rpm for five minutes) and the supernatant was subsampled for volatile phenol and/or phenolic glycoside analysis.

Table 7. Enzymes investigated for their ability to cleave smoke glycosides, including manufacturer/supplier information, enzyme activity data and recommended dose rates

Product	**Manufacturer/ supplier**	**Information**	**Recommended dose**
Lafazym	Laffort	Recommended for use at end of fermentation; standardisation value β-glucosidase, 3000 BDG/g	2-4 g/hL
Zimarom	VASON/IMCD	Pectinase ≥25,000 AVJP/g; β-glucosidase 1-10%; polygalacturonase 5-10%	1-3 g/hL
Rapidase Revelation Aroma	DSM/Vintessential	Polygalacuronase (pectinase) ≥25,000; b-glucosidase ≥4,000 BDG	1-3 g/hL on info sheet; 1-4 g/hL on label
Lallzyme Beta	Lallemand	Polygalacuronase (pectinase) ≥9,000 nkat/g; BD glucosidase ≥1,500 BDG/g	5 g/hL
Trenolin Bouquet Plus	Erbsloh	No information available on product label or product information sheet	10-15 mL/100 L

Evaluation of other materials

Other materials were tested for their ability to scavenge phenolic glycosides from grape juice including XAD resins, zeolites, grape seed oil, cream and full cream milk. These experiments were performed as described for the activated carbon experiments, where 50 mL of juice was treated with the material being evaluated for a contact time of 24 hours. Samples were then centrifuged and the required volumes were taken for chemical analysis.

2.2.8 Winemaking trials with juice after fining with activated carbon products

The 2019 smoke-affected Tasmanian Chardonnay and Pinot Noir juice were used in winemaking trials to characterise the performance of activated carbons. For each grape variety, 14 x 50 Litre stainless steel kegs were filled with each of the juices. Two carbon products (PS1300 and CASPF) were evaluated at three dose rates (1, 2 and 4 g/L) and in duplicate. The remaining 2 x 50 Litre kegs were the controls (no carbon addition). Juices were transferred to the 50 L kegs after cold settling and racking. For activated carbon addition, a small volume of juice (1 L) was removed from each of the kegs, the carbon was suspended in that juice and it was then added back into the keg with thorough mixing. Carbon contact occurred with the kegs stored in the 0°C cool room. Individual winemaking details for each of the two varieties are described below.

Pinot Noir winemaking from carbon-fined juice: The fined Pinot Noir juices were inoculated with 250 ppm of AB Mauri PDM yeast, with 250 ppm Lallemand GoFerm added. During fermentation, two 200 ppm additions of DAP and one addition of 300 ppm of Activator Gold were made. Sulfur dioxide (80 ppm) was added after fermentation was complete and the wines remained stored at 0°C until bottling.

Chardonnay winemaking from carbon-fined juice: The fined Chardonnay juices were inoculated with 250 ppm of AB Mauri PDM yeast, with 250 ppm Lallemand GoFerm added. During fermentation two 200 ppm additions of DAP and one addition of 300 ppm of Activator Gold were made. Sulfur dioxide (80 ppm) was added after fermentation. Wines remained stored at 0°C until bottling.

Bottling: Wines were racked to cross flow filtration in the week prior to bottling but due to residual carbon and the nature of the wine, filtration was particularly slow and complicated taking 10 working days. For each variety, 60 x 375 mL was bottled.

Winery-scale remediation trials of using glycosidase enzymes and activated carbon to treat wine

The 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine (25 L) was placed in 10 x 30 Litre kegs in a temperature controlled room set at 25°C. The headspace was managed each day by being flushed with carbon dioxide by the addition of dry ice. The enzyme product Trenolin Bouquet Plus (Erbsloh, Germany) was added (3.75 mL, highest recommended dose of 15 mL/100 L) to five kegs. Samples were taken at days 0, 3, 6 and 8 to monitor the progression of glycoside cleavage over time. Eight days after enzyme addition, activated carbon (PS1300 or FPS) was added (1 or 2 g/L) to those kegs requiring carbon treatment (i.e. 8 out of 10 kegs). After 24 hours of contact with carbon at 25°C, bentonite (1 g/L) was added (including to the control) and the kegs moved to 0°C for cold settling. Wines were racked three days later into 20 L kegs and remained at 0°C until bottling.
The treatments were as follows:

- Control
- + Trenolin Bouquet Plus only
- + Trenolin Bouquet Plus + 1 g/L PS1300 carbon
- + Trenolin Bouquet Plus + 2 g/L PS1300 carbon
- + Trenolin Bouquet Plus + 1 g/L FPS carbon
- + Trenolin Bouquet Plus + 2 g/L FPS carbon
- 1 g/L PS1300 carbon

- 2 g/L PS1300 carbon
- 1 g/L FPS carbon
- 2 g/L FPS carbon

Wines were racked to cross flow filtration in the week prior to bottling and were bottled into 48 x 375 mL bottles.

2.2.9 Sensory analysis methods

Assessing sensory effects of dilution – 2016 Victorian smoke-affected Pinot Noir wine

A 2016 smoke-affected Pinot Noir wine from Victoria was sequentially diluted with unaffected Pinot Noir of a similar style sourced from the same vintage and state (2016 Coldstream Hills Pinot Noir, Yarra Valley). The unaffected wine was selected after a preliminary sensory assessment of several candidate Pinot Noir wines, together with consideration of basic chemical composition. The dilution series, resulting in six samples, was as follows: 0% (i.e. 100% smoke-affected wine); 50% (50:50 blend with the unaffected wine); 75% (25:75 blend with the unaffected wine); 87.5% (12.5:87.5) blend with the unaffected wine); 93.75% (6.25:93.75 blend with the unaffected wine) and 100% (i.e. 0% smoke-affected wine).

A panel of nine qualified and highly experienced AWRI judges (6 males and 3 females) was convened. Judges were chosen for their ability to perceive smoke flavour from phenol glycosides, and on the basis of previous experience and performance in smoke taint sensory panels. All of the judges except one were members of the AWRI technical quality panel. For this investigation, the judges were asked to rate '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' and 'band aid') and 'smoke flavour' (defined as including 'bacon', 'smoked meat', 'ashy' aftertaste and 'drying/puckering' mouth-feel after expectoration) and 'overall fruit aroma' (defined as including 'red fruit', 'red berry', 'dark berry', 'strawberry', 'raspberry', 'cherry', 'apple') and 'overall fruit flavour'.

Samples were presented to judges in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24°C, in isolated booths under colour-masking sodium lighting, with randomised presentation order - a modified Williams Latin Square design generated by Fizz sensory acquisition software (version 2.51, Biosystemes, Couternon, France). All samples were assessed in a complete block design in duplicate over two sessions, with all six samples evaluated in a session. The judges were forced to have a 45-second rest between samples and a ten-minute rest between sessions. Water was provided for palate cleansing. During the ten-minute break judges were requested to leave the booths.

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 Fizz sensory 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 post-hoc Tukey's honest significant difference (HSD) means comparison test ($p = 0.05$).

Technical quality panel

The AWRI's quality panel consists of highly experienced and trained employees who have extensive experience in wine sensory assessment and quality judgement. This panel offers an independent evaluation of a wine's attributes, indication of quality level and presence of off-flavours. Some information regarding the nature of the sample is provided to the judges, notably the grape variety, wine style (if appropriate) and vintage. The tasters are instructed to comment in detail regarding the appearance, aroma and palate attributes of a wine, and to score the wine using the 20-point quality scoring system commonly used in Australian wine show judging. The panel is also instructed to identify and rate the intensity of any perceived off-flavours on a category intensity scale from zero to nine. The quality panel was used to quantify the level of smoke taint in wines sourced or generated as part of this project.

Descriptive analysis: evaluating the sensory effect of activated carbon fining of grape juice used for making wine

The wines made from smoke-affected 2019 Pinot Noir and Chardonnay juices treated with activated carbon prior to fermentation were evaluated using the quantitative sensory descriptive analysis method. Wines for each variety included seven treatments (one control and six carbon treatments), each applied in duplicate. Preliminary sensory evaluation by a panel of expert technical wine assessors was conducted prior to the descriptive analysis and resulted in no exclusion of any fermentation replicates due to off-flavours or winemaking artefacts. Wines from the two varieties were assessed in separate studies.

Training for sensory descriptive analysis

Each wine set was evaluated separately. A panel of eleven assessors (ten females, one male) with an average age of 52 years (SD=7.3) was convened to evaluate the Pinot Noir wines and a panel of twelve assessors (nine females, three males) with an average age of 52 years (SD=11.3) was convened to evaluate the Chardonnay wines. All panellists were part of the external AWRI trained descriptive analysis panel and had extensive experience in wine sensory descriptive analysis.

Assessors attended three two-hour training sessions to determine appropriate descriptors for rating in the formal 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 mouth-feel 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 third 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 which needed adjustment were discussed and the final list of terms and standards was determined. For the Pinot Noir wine formal sessions, this list was refined to include two appearance terms, eight aroma terms (seven defined and one 'other' term) and nine palate terms (eight defined and one

'other' term). The final list of attributes, definitions/synonyms and reference standards are shown in Table 8.

Table 8. Pinot Noir wine sensory attributes, definitions and composition of reference standards.

Attribute	Definition/synonyms	Standard
Appearance		
Pink colour	The degree of intensity of the pink hue in the sample (colour intensity)	-
Hazy	The degree of intensity of the haze in the sample	-
Aroma		
Strawberry	Intensity of the aroma of strawberry and other red fruits: raspberry and cherry	1 x crushed strawberry
Confection/floral	Intensity of the aroma of roses, blossoms and confectionary	40 µL of 10 mL/L linalool
Tropical	Intensity of the aroma of melon, mango and pineapple	10 g mango juice (Admiral) and 1 x 2 cm^3 rockmelon
Fresh grass	Intensity of the aroma of fresh green grass	0.5 g fresh grass not in wine
Smoke/Band-Aid	Intensity of the aroma of smoke, medicinal, Band-Aids and bacon	50 µL of 605.3 mg/L guaiacol and 15 µL of 4.969 g/L meta-cresol
Cooked vegetable/drain	Intensity of the aroma of tinned vegetables and dirty drains	20 µL of 1 g/L dimethyl disulfide and 3 µL of 2% 2-mercaptoethanol
Pungency	Intensity of the aroma and effect of alcohol	4 mL of 95% food grade ethanol (Tarac Technologies)
Palate		
Acidity	Intensity of acid taste in the mouth including aftertaste	2 g/L L-(+)-tartaric acid (Chem-Supply) in water
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating and drying; High = puckering, lasting astringency	0.43 g/L aluminium sulfate (Ajax fine Chem Supply Pty Ltd in water
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning	8% food grade alcohol (Tarac Technologies) in water
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration	0.15 g/L quinine sulfate (Sigma Aldrich) in water
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	1.5 g/L carboxymethylcellulose sodium salt (Sigma Aldrich) in water
Strawberry	Intensity of the flavour of strawberry and other red fruits: raspberry and cherry	-
Fresh grass	Intensity of the flavour of fresh green grass	-
Smoke/Band-Aid	Intensity of the flavour of smoke, medicinal, Band-Aids and bacon.	-

All red wine standards were added to 30 mL of 2018 Yalumba premium selection bag-in-box Shiraz (2L) unless otherwise noted.

For the Chardonnay wine formal sessions, the list was refined to include one appearance term, nine aroma terms (eight defined and one 'other' term) and ten palate terms (nine defined and one 'other' term). The final list of attributes, definitions/synonyms and reference standards are shown in Table 9.

Table 9. Chardonnay wine sensory attributes, definitions and composition of reference standards.

Attribute	Definition/synonyms	Standard
Appearance		
Yellow colour	The degree of intensity of the yellow hue in the sample (colour intensity)	-
Aroma		
Tropical fruit	Intensity of the aroma of tropical fruits: pineapple, passionfruit, mango and melon	2 x pineapple pieces (Golden Circle) and 0.5 µL of 14.3 mg/L 3-mercaptohexyl acetate
Citrus	Intensity of the aroma of citrus fruits: lemon and lime	0.5 g lemon rind and 0.5 g lime rind
Fresh grass	Intensity of the aroma of fresh green grass	0.5 g fresh grass not in wine
Confection/floral	Intensity of the aroma of roses, blossoms and confectionary	40 µL of 10 mL/L linalool
Smoke	Intensity of the aroma of smoke, medicinal, Band-Aids and ash	20 µL of 605.3 mg/L guaiacol
Flint	Intensity of the aroma of struck matches and flint	15 µL of 1g/L benzyl mercaptan
Cooked vegetable/drain	Intensity of the aroma of tinned vegetables and dirty drains	20 µL of 1 g/L dimethyl disulfide and 3 µL of 2% 2-mercaptoethanol
Pungency	Intensity of the aroma and effect of alcohol	4 mL of 95% food grade ethanol (Tarac Technologies)
Palate		
Acidity	Intensity of acid taste in the mouth including aftertaste	1 g/L L-(+)-tartaric acid (Chem-Supply) in water
Astringency	The drying and mouth-puckering sensation in the mouth. Low = coating teeth; Medium = mouth coating & drying; High = puckering, lasting astringency	0.43 g/L aluminium sulfate (Ajax fine Chem Supply Pty Ltd in water
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation. Low = warm; High = hot, burning	8% food grade alcohol (Tarac Technologies) in water
Bitterness	The intensity of bitter taste perceived in the mouth, or after expectoration	0.15 g/L quinine sulfate (Sigma Aldrich) in water
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	1.5 g/L carboxymethylcellulose sodium salt (Sigma Aldrich) in water
Tropical fruit	Intensity of the flavour of tropical fruits: pineapple, passionfruit, mango and melon	-
Citrus fruit	Intensity of the flavour of citrus fruits: lemon and lime	-
Smoke	Intensity of the flavour of smoke, medicinal, Band-Aids and ash.	-
Flint	Intensity of the flavour of struck matches and flint	-

All white wine standards were added to 30 mL of 2017 Yalumba premium selection bag-in-box Unwooded Chardonnay (2L) unless otherwise noted.

Evaluation

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. Randomised presentation order was followed except in the practice sessions when there was a constant presentation order. All samples were expectorated. In the practice booth sessions, the assessors were presented with sixteen wines across four trays, with four wines per tray. In the formal sessions, for the Pinot Noir wines, assessors were presented with four trays of four wines per tray on the first day, and three trays of four wines per tray on the second day, to limit carry-over effects related to wine astringency and bitterness. For the Chardonnay wines assessors were also presented with four trays of four wines per tray on the first day, and three trays of four wines per tray on the second day. The assessors were forced to have a 60-second rest between samples and were encouraged to rinse with water, and a minimum ten-minute rest between trays. During the ten-minute break assessors were requested to leave the booths. A new bottle was used for each of the presentation days.

For both varieties, fourteen wines (seven treatments x two fermentation replicates) were presented to assessors two times, in an incomplete Williams Latin Square random block design provided by Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada).

The intensity of each attribute listed in Table 8 and Table 9 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 Compusense Cloud sensory evaluation software.

Panel performance was assessed using Compusense software and R with the SensomineR (sensominer.free.fr/) and FactomineR (factominer.free.fr/) packages. The performance assessment included analysis of variance for the effect of judge, wine and presentation replicate and their two-way interactions, degree of agreement with the panel mean, degree of discrimination across samples and the residual standard deviation of each judge by attribute. The judges were found to be performing to an acceptable standard.

Analysis of variance (ANOVA) was carried out using Minitab 18 (Minitab Inc., Sydney, NSW). The effects of the treatments, judge, judge by treatment, fermentation replicate nested into treatment, judge by fermentation replicate nested into treatment, and presentation replicate nested into treatment and fermentation replicate were assessed, treating judge as a random effect. Following ANOVA, Fisher's least significant difference (LSD) value was calculated ($P=0.05$) for the treatment effect. Principal component analysis (PCA) was conducted for the means of the treatments of the significant ($P<0.05$) and close to significant ($P<0.10$) attributes of each of the treatment fermentation replicates using the correlation matrix (XLSTAT 2017, Paris, France).

Assessing sensory effects of dilution –2019 Tasmanian smoke-affected Pinot Noir rosé

A 2019 smoke-affected Pinot Noir wine from Tasmania was sequentially diluted with unaffected Pinot Noir of a similar style sourced from the same vintage (2019 Bird in Hand rosé Pinot Noir, Adelaide Hills). The unaffected wine was selected after a preliminary sensory assessment of several candidate Pinot Noir wines, together with consideration of basic chemical composition as well as smoke- and oak-related compounds. The dilution series, resulting in six samples, was as follows: 0% (i.e. 100% smoke-affected wine); 50% (50:50 blend with the unaffected wine); 75% (25:75 blend with

the unaffected wine); 87.5% (12.5:87.5) blend with the unaffected wine); 93.75% (6.25:93.75 blend with the unaffected wine), and 100% (i.e. 0% smoke-affected wine).

A panel of eleven qualified and highly experienced AWRI judges (6 males and 5 females) was convened. Judges were chosen for their ability to perceive smoke flavour from phenol glycosides, and on the basis of previous experience and performance in smoke taint sensory panels. All of the judges were members of the AWRI technical quality panel. For this investigation, the judges were asked to rate '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', 'ashy' aftertaste and drying/puckering mouth-feel after expectoration) and 'overall fruit aroma' (defined as including 'red fruit', 'red berry', 'strawberry', 'raspberry' and 'cherry') and 'overall fruit flavour'. Judges also had an 'other' term on both aroma and palate to capture any additional noteworthy characteristics in the wines.

Samples were presented to judges in 30 mL aliquots in 3-digit-coded, covered, blackout, ISO standard wine glasses at 22–24 °C, in isolated booths under daylight-type fluorescent lighting, with randomised presentation order - a modified Williams Latin Square design generated by Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada). All samples were assessed in a complete block design in duplicate over two sessions, with all six samples evaluated in a session. The judges were forced to have a two-minute rest between samples and a ten-minute rest between sessions. Water was provided for palate cleansing. During the ten-minute break judges were requested to leave the booths.

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 Compusense Cloud 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 post-hoc Tukey's honest significant difference (HSD) means comparison test (P = 0.05).

Sensory evaluation of wines from the combined glycosidase and carbon treatment of wine trials

Ten wines were supplied to the AWRI's sensory team for assessment by the Technical Quality panel, to provide an indication of sensory characteristics and the effectiveness of carbon and/or enzyme treatments at removing smoke-related aromas and flavours.

The wines were assessed by twelve tasters: eight expert tasters from the AWRI Technical Quality panel, two members of the research team closely involved in the project, a member of the AWRI sensory group and one other smoke-sensitive taster from the AWRI. All had extensive wine sensory experience and all were sensitive to smoke compounds.

Samples (30 mL) were poured in constant order across tasters with tasting conducted blind, in clear ISO XL5 tasting glasses, with the order of the samples not disclosed to the tasters involved. The wines were presented together, and assessors were given the variety and vintage of the samples, but no other information was provided. Assessment was conducted in silence in isolated sensory booths.

The tasters were asked to write detailed comments for appearance, aroma and palate, and asked to give an intensity score (0-9) for smoke, and any additional taints or faults present.

Effect of smoke taint on consumer liking scores of Pinot Noir wines

Samples

A 2019 smoke-affected Pinot Noir rosé wine from Tasmania was sequentially diluted with unaffected Pinot Noir of a similar style sourced from the same vintage (2019 Bird in Hand rosé Pinot Noir, Adelaide Hills). The unaffected wine was selected after a preliminary sensory assessment of several candidate Pinot Noir rosé wines, together with consideration of basic chemical composition as well as smoke and oak related compounds.

In a previous study, a panel of eleven qualified and highly experienced AWRI judges (6 males and 5 females) was convened to assess six dilutions of the smoke-affected wine. Judges were chosen for their ability to perceive smoke flavour from phenol glycosides, and on the basis of previous experience and performance in smoke taint sensory panels. All judges were members of the Technical Quality panel. For this investigation, the judges were asked to rate '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'), 'smoke flavour' (defined as including 'bacon', 'smoked meat', 'ashy' aftertaste and drying/puckering mouth-feel after expectoration), 'overall fruit aroma' (defined as including 'red fruit', 'red berry', 'strawberry', 'raspberry' and 'cherry') and 'overall fruit' flavour. Judges also had an 'other' term on both aroma and palate to capture any additional noteworthy characteristics in the wines.

The dilution series of six samples, was as follows: 0% (i.e. 100% smoke-affected wine); 50% (50:50 blend with the unaffected wine); 75% (25:75 blend with the unaffected wine); 87.5% (12.5:87.5) blend with the unaffected wine); 93.75% (6.25:93.75 blend with the unaffected wine), and 100% (i.e. 0% smoke-affected wine).

This initial study showed that blending is an effective option for remediation of smoke tainted wine, with less than 25% of the affected wine, in a blend with unaffected wine of a similar style, being sufficient to lower the smoke taint intensity to be not significantly different from the unaffected wine alone. After this evaluation, it was decided that five of the six dilutions would be included in the consumer study, namely the 0%, 75%, 87.5%, 93.75% and 100% dilution wines.

Consumers

Consumers (n=82) were screened and selected from an existing database based on their drinking preferences and habits. All participants (18-65 years of age) consumed rosé wine at least once or twice per year and spent at least $10 on a bottle of rosé wine. Consumers were required to provide informed written consent prior to commencing the study and were compensated with a gift voucher at the completion of a one-hour appointment. All data were collected in isolated sensory booths at The Australian Wine Research Institute using Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada) using tablet computers.

Prior to tasting the wine samples, consumers were asked to complete an entrance questionnaire to obtain demographic information. For the tasting evaluations, samples were presented in 30 mL

aliquots in 3-digit-coded ISO black-out wine glasses, under daylight-type lighting, with balanced randomised presentation order at room temperature. Consumers rated each wine for overall liking on a nine-point hedonic scale from 'like extremely' to 'dislike extremely', with a two-minute forced break between samples, where participants were encouraged to rinse their palate with water. Following the tasting, an exit survey exploring spending behaviour, attitudes to food and wine, and likelihood of ordering a rosé at various drinking occasions was completed.

The data were analysed using a two-way analysis of variance for the effect of wine and consumer. Cluster analysis was conducted with agglomerative hierarchical clustering using Ward's method with the standardised data. All data analysis was conducted using Minitab 19.2 (Minitab Inc., Sydney, NSW). Each of the clusters identified was analysed to determine if there were trends amongst their demographic data.

2.2.10 Locations of project activities

The laboratory-based mitigation work was performed at the Australian Wine Research Institute, Urrbrae, South Australia. Grape samples were collected from the Coombe Vineyard (Urrbrae, South Australia), Alverstoke Vineyard (Urrbrae, South Australia), SARDI's Nuriootpa Viticulture Experiment Station (Barossa Valley, South Australia) and commercial vineyards located in Padthaway (South Australia), Orange (New South Wales) and the Huon Valley (Tasmania). Wines were obtained from Victoria (Ballarat), NSW (Orange), Tasmania (Huon Valley). The winery trials were performed in conjunction with WIC Winemaking Services at the Hickinbotham Roseworthy Wine Science Laboratory located on the Waite Campus of the University of Adelaide, Urrbrae, South Australia. The research findings are applicable to all wine-producing regions of Australia which can be impacted by smoke generated from bushfires, forest or stubble fires and prescribed burns.

3 Project results and discussion

3.1 Project results

3.1.1 Preventative vineyard mitigation options and strategies to reduce uptake of smoke taint compounds

Model experiments to demonstrate absorption of gaseous volatile phenols by excised grape bunches

Initial experiments focused on whether excised grape bunches (i.e. those removed from the vine) were able to absorb volatile phenols and convert them to phenolic glycosides. Previous work by Kennison et al. (2007) showed that post-harvest smoke exposure of whole Verdelho grape bunches resulted in smoke-affected wine. Wines were described as exhibiting 'smoky', 'dirty', 'earthy', 'burnt' and 'smoked meat' sensory characteristics. The presence of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol and furfural in these wines was attributed to the smoke exposure. However, this work was performed prior to researchers identifying the presence and importance of the phenolic glycosides in smoke-exposed fruit (Hayasaka et al. 2010b), so there was no previous evidence to suggest that phenolic glycosides form in post-harvest grape bunches. Therefore, the question remained whether grape enzymes responsible for conversion of volatile phenols to phenolic glycosides were active post-harvest. In order to evaluate this, table grapes were exposed to volatile phenols released from an aqueous phenol mix and the concentrations of volatile phenols and phenolic glycosides were compared to control bunches. Table grapes were used due to their availability all year round. The aqueous phenol mix, consisting of five phenols typically present in smoke, was used as source of volatile phenols rather than smoke from a smoke generator in order to achieve controlled and reproducible conditions.

Concentrations of volatile phenols and phenolic glycosides in the control and treated grapes are given in Table 10 and Table 11. As expected, the untreated table grapes contained smoke volatile phenols and phenolic glycosides below the analytical limits of detection, while elevated concentrations were observed in the grapes exposed to gaseous phenols. These results confirmed that volatile phenols evaporated from an aqueous solution can be absorbed by table grapes and converted to phenolic glycosides. The concentrations in the grapes of volatile phenols were much higher than the phenolic glycosides, suggesting that not enough time had passed for the volatile phenols to be completely converted to the phenolic glycosides. Higher concentrations of *o*-cresol and guaiacol were observed in the grape homogenates compared to syringol, which likely reflects the higher volatility of these phenols.

Table 10. Concentrations of volatile phenols in table grapes after exposure to gaseous volatile phenols (treated) or without exposure (control)

	4-methyl guaiacol (µg/kg)	guaiacol (µg/kg)	*o*-cresol (µg/kg)	*p*-cresol (µg/kg)	*m*-cresol (µg/kg)	syringol (µg/kg)	methyl syringol (µg/kg)
Control 1	<1	<1	<1	<1	<1	<2	<2
Control 2	<1	<1	<1	<1	<1	<2	<2
Treated 1	<1	114	211	<1	52	3	<2
Treated 2	<1	192	304	<1	85	8	<2

Table 11. Concentrations of phenolic glycosides in table grapes after exposure to gaseous volatile phenols (treated) or without exposure (control)

Sample	Concentration (µg/kg SyGG equivalents)					
	SyGG	MSyGG	PhRG	CrRG	GuRG	MGuRG
Control 1	<1	<1	<1	<1	<1	<1
Control 2	<1	<1	<1	<1	<1	<1
Treated 1	10	<1	4	39	19	<1
Treated 2	27	<1	7	54	24	<1

To validate the model system, the experiment was repeated with excised bunches of Chardonnay, Sauvignon Blanc and Semillon grapes harvested in 2017 from the University of Adelaide's Coombe Vineyard. Concentrations of the phenolic glycosides in the control and treated Chardonnay, Sauvignon Blanc and Semillon grapes are given in Table 12.

Table 12. Concentrations of phenolic glycosides in Chardonnay, Sauvignon Blanc and Semillon grapes after exposure to gaseous volatile phenols (Treated) or without exposure (Control)

Sample	Concentration (µg/kg SyGG equivalents)						
	SyGG	CrRG	GuRG	MGuRG	MSyGG	PhRG	Total
Chardonnay control	<1	<1	<1	<1	<1	<1	<1
Chardonnay treated 1	31	232	61	2	<1	8	334
Chardonnay treated 2	2	58	16	2	<1	3	81
Sauvignon Blanc control	<1	2	<1	<1	<1	<1	2
Sauvignon Blanc treated 1	11	894	374	<1	<1	70	1,349
Sauvignon Blanc treated 2	<1	213	120	<1	<1	18	351
Semillon control	<1	<1	<1	<1	<1	<1	<1
Semillon treated 1	4	292	77	<1	<1	18	391
Semillon treated 2	2	203	59	<1	<1	12	276

These proof-of-principle studies demonstrated that bunches of Chardonnay, Sauvignon Blanc and Semillon grapes harvested at 19.8, 19.8 and 15.4°Brix respectively were capable of absorbing gaseous volatile phenols and converting them to phenolic glycosides (mono- and disaccharides). The most abundant glycosides observed were GuPG, CrPG, GuMG and CrMG, as had been previously observed for table grapes. Some variation in glycoside concentrations was observed between replicate bunches. This is likely because each had been exposed to volatile phenols in separate desiccators; therefore, differences may be attributable to the extent of volatilisation of the phenols, as experiments were performed on separate days with different temperature conditions. Furthermore, differences between bunches of the same variety may be attributable to variation in absorption of the volatile phenols and/or differences in enzymatic activity within the berries themselves.

In summary, the results demonstrate that uptake and metabolism of volatile phenols by berries occurs even after removal of bunches from the vine. Consequently, the model system was assessed as suitable for use in screening a large number of horticultural products to identify potential candidates for subsequent in-field trials.

Evaluation of the potential of horticultural products to reduce uptake of smoke taint compounds by grapes

Experiments were conducted to investigate whether it is possible to limit the uptake of smoke volatile phenols by grapes through applying existing horticultural products that can be sprayed in the vineyard. This research was undertaken via model experiments using excised grape bunches in a controlled environment, where the grapes were exposed to a gaseous phenol mixture.

Twelve horticultural products, including oily/hydrophobic materials, pest control products, sunscreen protectants and other materials that might provide a physical barrier (silicone oil, carbon and titanium dioxide; Table 2) were evaluated for their ability to limit the absorption of volatile phenol by grapes in an upscaled version of the model system. The use of activated carbon was chosen as a positive control, rather than it being a practical option, since it's known for its ability to scavenge organic compounds including volatile phenols. Titanium dioxide was chosen due to its ability to act as a catalyst in the breakdown of phenols in the presence of UV light activation (Dougna et al. 2015).

The concentrations of volatile phenols and phenolic glycosides present in Shiraz grape homogenates after grapes had been treated with horticultural products and then exposed to volatile phenols are given in Figure 5 and Figure 6. Notably, the application of hydrophobic products (Biopest, Fruit Drying oil and Parka Plus) resulted in statistically significant increases in the total phenolic glycosides compared to those observed in the control. For volatile phenols none of the treatments were found to be significantly different from the control. Similar trends were observed when the experiment was repeated with Muscat Gordo grapes (data not shown).

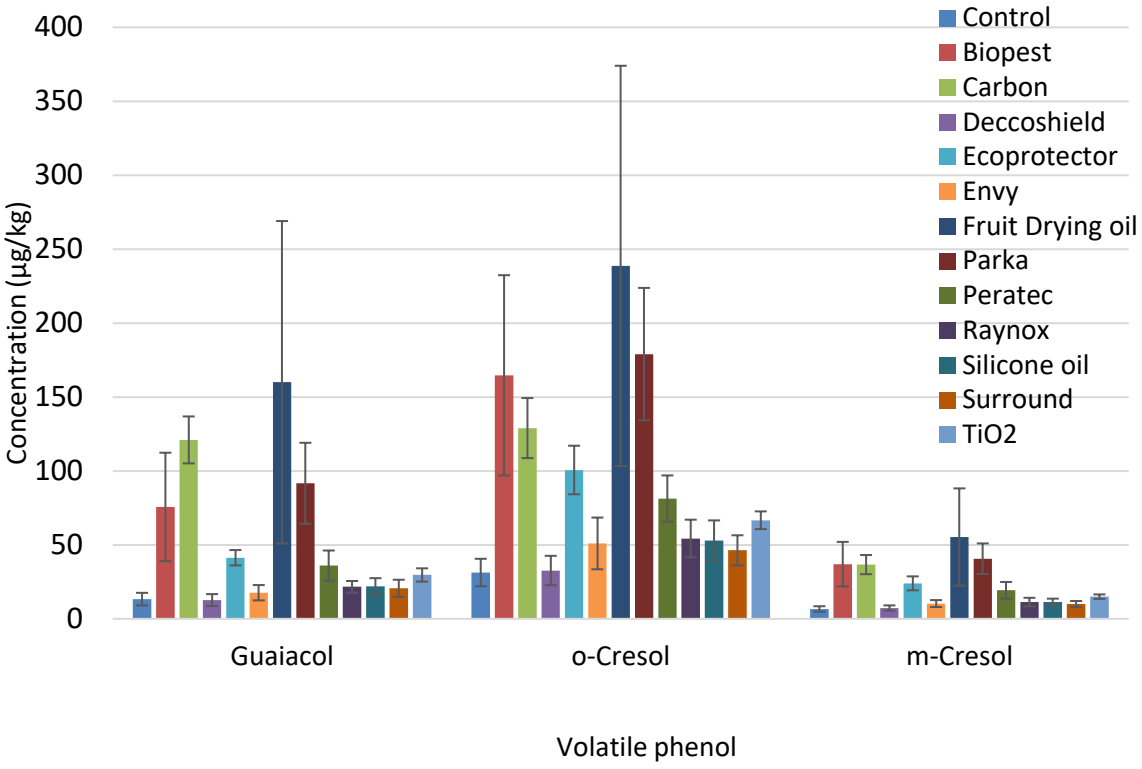


Figure 5. Concentrations of volatile phenols (guaiacol, o-cresol and m-cresol) present in Shiraz grape homogenates after treatment with horticultural products and subsequent exposure to volatile phenols

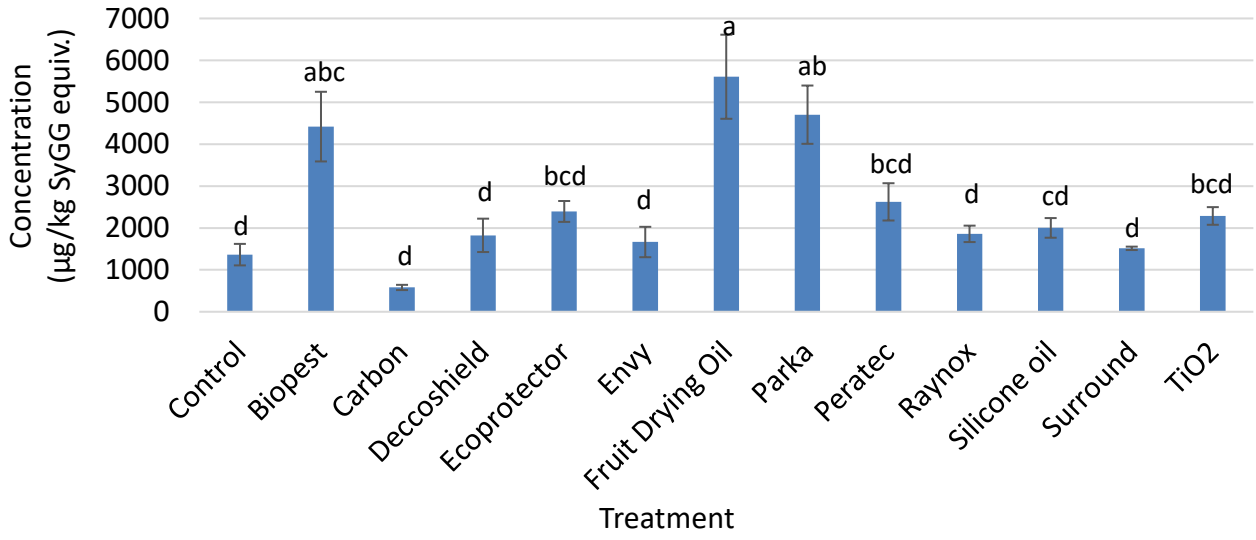


Figure 6. Concentrations of total phenolic glycosides present in Shiraz grape homogenates after treatment with horticultural products and subsequent exposure to volatile phenols

The washing of grapes after treatment and exposure was also evaluated but did not influence the results obtained (data not shown). The only exception was for grapes with activated carbon residue when homogenised for analysis, which is not surprising given that activated carbons can bind to volatile phenols and glycosides present in grape juice.

In conclusion, the model system was highly capable of evaluating the effectiveness of a range of existing agrochemicals and other materials for reducing/preventing the uptake of free phenols (guaiacol, syringol, o-cresol, m-cresol and phenol). None of the treatments tested was found to provide effective protection. The highest concentrations of volatile phenols and their glycosides (i.e. increases compared to control) were observed for the more oily/hydrophobic materials indicating that the hydrophobic nature of these materials may enhance the adsorption and/or transfer of the hydrophobic volatile phenols into the grape berries. In some experiments the activated carbon and titanium dioxide treated grapes had somewhat lower glycoside concentrations, but the differences were not significantly different from the control. As the evaluation of a range of vineyard sprays did not identify any suitable candidates to prevent uptake of smoke compounds by vines, the project focus subsequently shifted to remediation of smoke-affected juice and wine.

Evaluation of the potential of horticultural products to reduce uptake of smoke taint compounds by grapes – a case study

In 2018 the Department of Primary Industries in NSW (DPI NSW) undertook a trial of sunscreen products in a vineyard planted with Pinot Noir clones 777 and MV6. That vineyard then experienced smoke exposure from a nearby bushfire. This situation provided the opportunity to source grape samples that had been treated with either VaporGard or Surround prior to smoke exposure, as well as controls which were untreated. VaporGard and Surround had been applied pre-veraison at rates of 1 L/1000 L/ha and 5 kg/100 L/ha, respectively. Grapes were harvested in March 2018, frozen and then sent to the AWRI for smoke glycoside analysis (Table 13 and Table 14).

Table 13. Concentration of phenolic glycosides in Pinot Noir 777 grapes (control or treated with sunscreen protectants, i.e. VaporGard or Surround) after exposure to smoke from a bushfire

	Concentration (µg/kg SyGG equivalents)						
	SyGG	**CrRG**	**GuRG**	**MGuRG**	**MSyGG**	**PhRG**	**Total**
Control rep 1	11.5	4.0	1.6	2.8	1.9	<1	21.8
Control rep 2	13.8	4.2	1.6	3.0	2.1	<1	24.8
Control rep 3	14.8	3.7	1.4	2.8	2.3	<1	25.0
Average ± SE	13.4 ± 1.0	4.0 ± 0.2	1.5 ± 0.1	2.9 ± 0.1	2.1 ± 0.1	<1	23.9 ± 1.0
VaporGard rep 1	10.5	3.4	1.2	2.5	1.3	<1	18.9
VaporGard rep 2	11.6	4.3	1.2	2.6	1.5	<1	21.2
VaporGard rep 3	10.2	2.8	0.9	2.1	1.4	<1	17.3
Average ± SE	10.7 ± 0.4	3.5 ± 0.4	1.1 ± 0.1	2.4 ± 0.2	1.4 ± 0.05	<1	19.1 ± 1.1
Surround rep 1	10.0	6.1	1.6	4.1	1.3	1.0	24.1
Surround rep 2	11.3	4.4	1.5	3.7	1.4	1.0	23.2
Surround rep 3	9.6	3.4	1.2	2.5	1.2	<1	17.8
Average ±SE	10.3 ± 0.5	4.6 ± 0.8	1.4 ± 0.1	3.4 ± 0.5	1.3 ± 0.1	<1	21.0 ± 1.6

SE = Standard Error

Table 14. Concentration of phenolic glycosides in Pinot Noir MV6 grapes (control or treated with sunscreen protectants, i.e. VaporGard or Surround) after exposure to smoke from a bushfire

	Concentration (µg/kg SyGG equivalents)						
	SyGG	**CrRG**	**GuRG**	**MGuRG**	**MSyGG**	**PhRG**	**Total**
Control rep 1	15.6	2.9	1.3	2.1	2.4	<1	24.3
Control rep 2	12.7	3.4	1.8	2.6	1.9	<1	22.5
Control rep 3	12.4	3.4	1.9	2.7	1.9	<1	22.3
Average ± SE	13.6 ± 1.0	3.3 ± 0.2	1.6 ± 0.2	2.5 ± 0.2	2.1 ± 0.2	<1	23.0 ± 0.6
VaporGard rep 1	9.5	3.5	1.4	2.9	1.5	<1	18.8
VaporGard rep 2	10.3	3.5	1.5	3.4	1.5	<1	20.1
VaporGard rep 3	7.1	3.0	1.2	2.5	1.0	<1	14.7
Average ± SE	8.9 ± 1.0	3.3 ± 0.2	1.4 ± 0.1	2.9 ± 0.3	1.3 ± 0.2	<1	17.9 ± 1.6
Surround rep 1	10.4	4.8	2.2	3.9	1.6	1.0	23.9
Surround rep 2	8.8	5.0	2.0	3.8	1.2	1.3	22.1
Surround rep 3	9.5	4.7	2.0	3.9	1.2	1.2	22.4
Average ±SE	9.5 ± 0.5	4.8 ± 0.1	2.1 ± 0.1	3.9 ± 0.03	1.3 ± 0.1	1.2 ± 0.1	22.8 ± 0.6

SE = Standard Error

The application of VaporGard and Surround to grapes in the vineyard did not significantly reduce the levels of phenolic glycosides compared to the untreated control. Also, no clonal effect was observed. Notably, the overall concentrations of glycosides in the samples were relatively low, indicating low smoke exposure, and it would be warranted to repeat this experiment with VaporGard and Surround treated grapes and after severe smoke exposure.

3.1.2 Vineyard sampling strategies for the detection of smoke exposure markers

Vineyard variability among grapes exposed to smoke was assessed across two blocks, one Chardonnay, one Pinot Noir, after a bushfire in 2018. Field variability of volatile phenols and smoke glycosides was typically within 20%. Importantly all samples, independent of their location within the vineyard, had post-exposure concentrations of smoke markers above concentrations for non-exposed grapes as indicated from comparison to baseline data. The consistency across the vineyard (absence of false negatives) is notable and has been used to inform sampling strategies recommended to growers following a smoke event.

The results for the phenolic glycosides for each of the grape bunches collected for Chardonnay and Pinot Noir are summarised in Table 15 and Table 16. The distribution of the data is visually presented in box plots in Figure 7 and Figure 8.

Table 15. Concentrations of phenolic glycosides in Chardonnay grapes sampled throughout a single vineyard exposed to smoke from a bushfire

Chardonnay grapes (n=17)	Concentration (µg/kg SyGG equivalents)						
	SyGG	**CrRG**	**GuRG**	**MGuRG**	**MSyGG**	**PhRG**	**Total**
Average	45.7	6.8	3.3	6.9	8.4	2.7	73.8
Minimum	32.8	5.0	2.4	4.7	6.1	2.0	56.3
Maximum	58.9	10.3	5.4	10.8	13.5	3.9	95.2

Table 16. Concentrations of phenolic glycosides in Pinot Noir grapes sampled throughout a single vineyard exposed to smoke from a bushfire

Pinot Noir grapes (n=30)	Concentration (µg/kg SyGG equivalents)						
	SyGG	**CrRG**	**GuRG**	**MGuRG**	**MSyGG**	**PhRG**	**Total**
Average	61.2	21.4	13.5	25.0	8.0	9.4	138.5
Min.	40.8	11.9	7.0	12.8	4.2	6.2	85.5
Max.	137.1	60.6	24.9	48.9	22.8	15.2	291.0

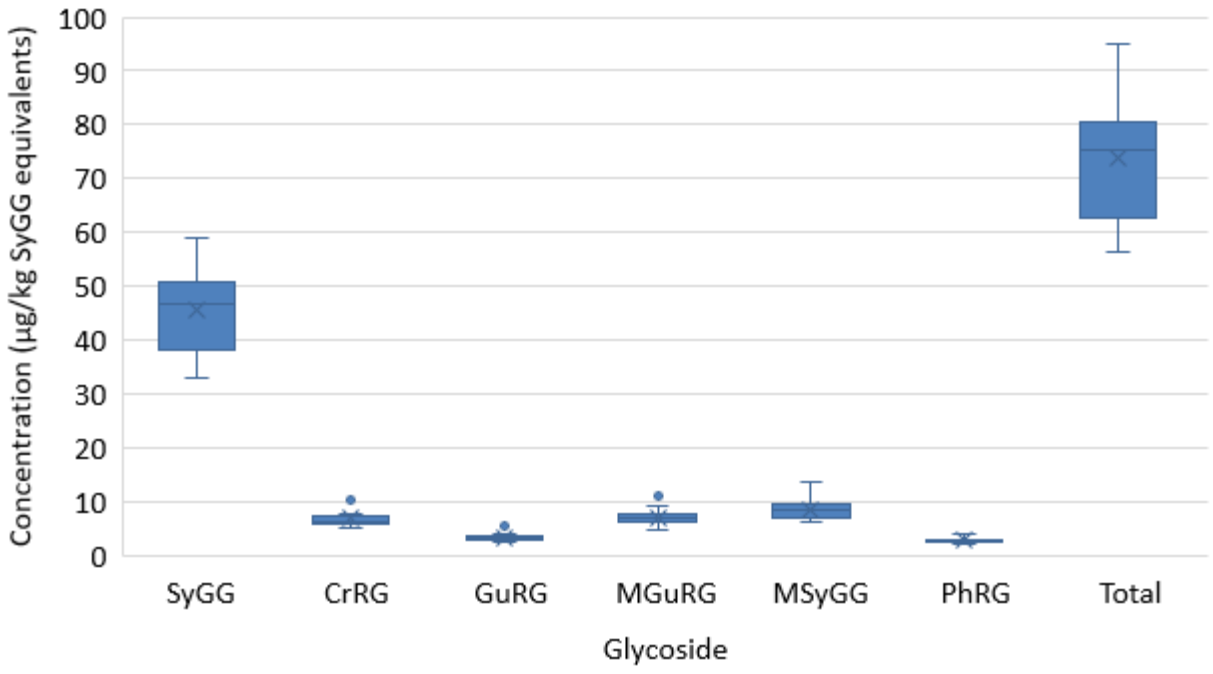


Figure 7. Box plot representation of the concentrations of phenolic glycosides in Chardonnay grapes (n=17) sampled throughout a vineyard following exposure to smoke from a bushfire

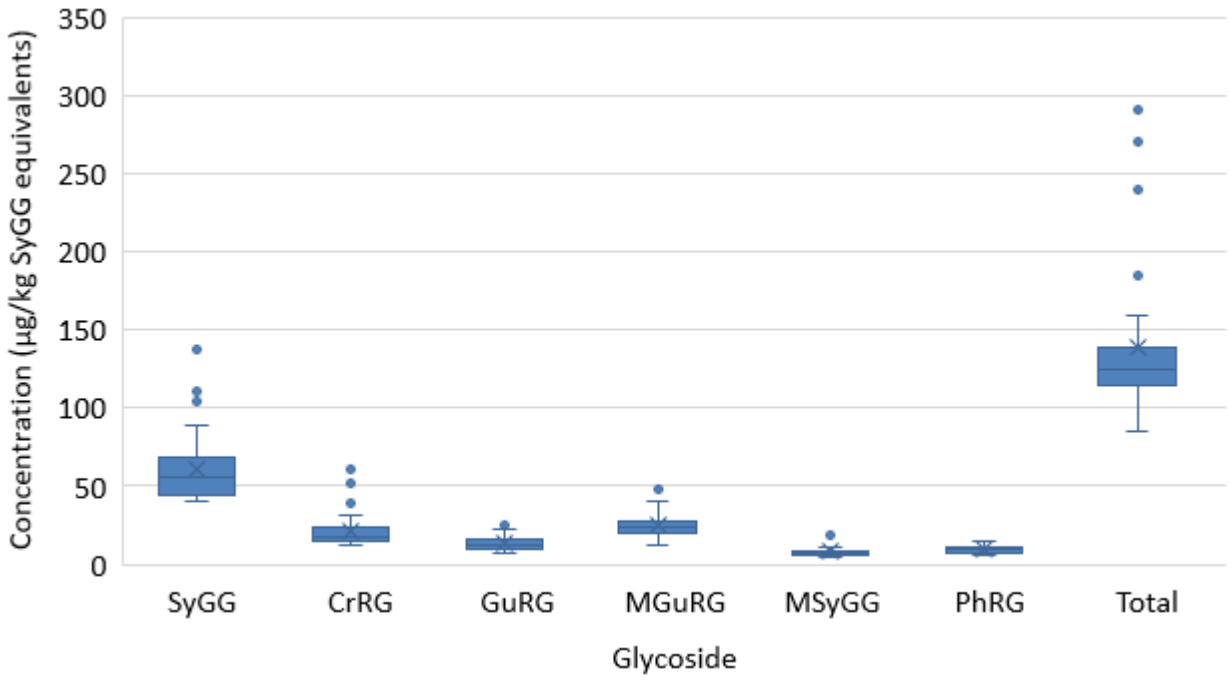


Figure 8. Box plot representations of the concentrations of phenolic glycosides in Pinot Noir grapes (n=30) sampled throughout a vineyard following exposure to smoke from a bushfire

3.1.3 Baseline concentrations of volatile phenols and phenolic glycosides in non-smoke-exposed fruit

Since volatile phenols and phenolic glycosides occur naturally in grapes at low concentrations, it is important to understand background concentrations for non-smoke-exposed grapes as a point of comparison when assessing if suspect samples have been exposed to smoke. Some baseline data already exists, and through this project an additional 325 non-smoke-exposed grape samples (Table 4) were collected from 23 regions across Western Australia, South Australia, Victoria, New South Wales and Tasmania. Small-lot wines were made from the grapes, and both grapes and wines were analysed for smoke volatile phenols and phenolic glycosides.

Table 17 and Table 18 show the 99th percentile values (i.e. 99% of the data collected for non-smoke-exposed samples are at or below this concentration) for the 13 compounds targeted as part of the smoke marker analysis for red and white grapes across all samples (Table 5**Table 4**) respectively, while Table 19 and Table 20 show the 99th percentile values for red and white wines, respectively. Note that the values shown in the table have been adjusted, where necessary, to take into account the uncertainty of measurement.

Table 17. The 99th percentile values for the 13 compounds targeted for smoke exposure marker analysis of red grapes

Compound	99th percentile values (µg/kg)						
	Shiraz	Cabernet	Pinot Noir	Merlot	Grenache	Mataro	Sangiovese
Glycosides							
Syringol gentiobioside	12.5	7.6	5.0	9.1	8.5	7.3	2.1
Methylsyringol gentiobioside	4.5	1.1	0.9	1.1	1.0	1.0	1.0
Phenol rutinoside	2.2	2.5	1.1	1.1	2.4	1.1	1.0
Cresol rutinoside	5.3	3.6	4.1	3.9	4.7	5.0	3.7
Guaiacol rutinoside	8.8	2.2	1.4	1.2	1.8	1.3	1.0
Methylguaiacol rutinoside	4.7	3.9	2.6	1.5	1.0	4.7	1.0
Volatile phenols							
Methyl guaiacol	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Guaiacol	7.2	3.4	3.6	3.1	2.0	2.4	2.8
m-Cresol	1.0	1.3	1.0	1.0	1.0	1.0	1.0
Methyl syringol	2.0	2.0	2.0	2.0	2.0	2.0	2.0
o-Cresol	5.8	2.8	3.4	1.0	2.0	1.9	1.6
p-Cresol	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Syringol	2.0	2.0	2.0	2.0	2.0	2.0	2.0

Table 18. The 99th percentile values for the 13 compounds targeted for smoke exposure marker analysis of white grapes

Compound	99th percentile values (µg/kg)				
	Chardonnay	Riesling	Semillon	Pinot Gris	Sauvignon Blanc
Glycosides					
Syringol gentiobioside	1.3	3.6	1.4	1.0	1.5
Methylsyringol gentiobioside	1.0	1.0	1.0	1.0	1.0
Phenol rutinoside	1.0	1.0	1.5	1.0	1.6
Cresol rutinoside	2.4	3.6	3.8	1.6	3.5
Guaiacol rutinoside	1.0	1.0	1.1	1.0	1.1
Methylguaiacol rutinoside	1.5	1.5	1.5	1.0	1.5
Volatile phenols					
Methyl guaiacol	1.0	1.0	1.0	1.0	1.0
Guaiacol	1.0	1.3	1.2	1.2	1.0
m-Cresol	1.0	1.0	1.0	1.0	1.0
Methyl syringol	2.0	2.0	2.0	2.0	2.0
o-Cresol	2.0	1.8	1.9	2.3	2.6
p-Cresol	1.0	1.0	1.0	1.0	1.0
Syringol	2.0	2.0	2.0	2.0	2.0

Table 19. The 99th percentile values for the 13 compounds targeted for smoke exposure marker analysis of small-lot red wines made from non-smoke-exposed grapes

Compound	99th percentile values (µg/L)						
	Shiraz	Cabernet	Pinot Noir	Merlot	Grenache	Mataro	Sangiovese
Glycosides							
Syringol gentiobioside	8.9	3.9	5.3	9.9	7.2	8.1	2.3
Methylsyringol gentiobioside	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Phenol rutinoside	2.7	2.0	1.0	2.7	4.8	3.1	1.3
Cresol rutinoside	7.2	3.3	3.6	6.1	8.3	6.6	3.4
Guaiacol rutinoside	12.6	1.7	1.8	1.7	3.4	2.2	1.4
Methylguaiacol rutinoside	6.4	1.6	2.8	2.6	2.2	8.2	1.5
Volatile phenols							
Methyl guaiacol	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Guaiacol	12.0	3.7	3.4	5.2	2.1	3.6	2.8
m-Cresol	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Methyl syringol	1.0	1.0	1.0	1.0	1.0	1.0	1.0
o-Cresol	1.0	2.0	4.6	1.0	2.2	2.4	1.3
p-Cresol	1.0	2.0	1.0	1.0	1.0	1.0	1.0
Syringol	9.6	5.4	2.3	4.0	4.6	3.0	3.0

Table 20. The 99th percentile values for the 13 compounds targeted for smoke exposure marker analysis for small-lot white wines made from non-smoke-exposed grapes

Compound	99th percentile values (µg/L)				
	Chardonnay	Riesling	Semillon	Pinot Gris	Sauvignon Blanc
Glycosides					
Syringol gentiobioside	2.2	2.5	1.5	1.6	2.4
Methylsyringol gentiobioside	1.0	1.0	1.0	1.0	1.0
Phenol rutinoside	1.0	1.4	2.2	1.0	3.0
Cresol rutinoside	1.5	5.8	7.4	2.7	6.5
Guaiacol rutinoside	1.0	1.0	1.2	1.0	1.7
Methylguaiacol rutinoside	1.0	1.0	1.7	1.1	2.0
Volatile phenols					
Methyl guaiacol	1.0	1.0	1.0	1.0	1.0
Guaiacol	1.0	1.8	1.1	1.4	1.4
m-Cresol	1.0	1.0	1.0	1.0	1.0
Methyl syringol	1.0	1.0	1.0	1.0	1.0
o-Cresol	1.0	1.1	1.3	2.1	3.1
p-Cresol	1.0	1.0	1.0	1.0	1.0
Syringol	2.0	1.3	1.1	1.3	1.0

3.1.4 Carbon fining as a remedial management option for dealing with smoke-affected grapes and wine

Activated carbon is a highly porous carbonaceous material produced from pyrolysis of carbon-rich sources such as coal, coconut shells, hard woods, fruit stones and synthetic materials. The high porosity of activated carbon (Figure 9) furnishes a material with a high surface area and adsorptive properties suitable for removing undesirable contaminants. For this reason, it has been used for many applications including air, gas and chemical purification; groundwater remediation; drinking water filtration; and medical and pharmaceutical applications. In the wine industry, activated carbon has been used for decolourising wine, removing ochratoxin A from in red wines (Castellari et al. 2001), to reduce the concentrations of *Brettanomyces* metabolites 4-ethylphenol and 4-ethylguaiacol in wine (Lisanti et al. 2008, Milheiro et al. 2017, Filipe-Ribeiro et al. 2017), to reduce fungicidal residues in white wines via addition during fermentation (Nicolini et al. 2016) and for amelioration of smoke taint in wine (Fudge et al. 2012). Studies on remediation of smoke taint using activated carbon have been limited and have often focused on treating wine rather than grape must and/or juice. In addition, earlier studies focused solely on the fate of volatile phenols as the discovery of phenolic glycosides occurred later, first reported by Hayasaka and colleagues in 2010 (Hayasaka et al. 2010b).

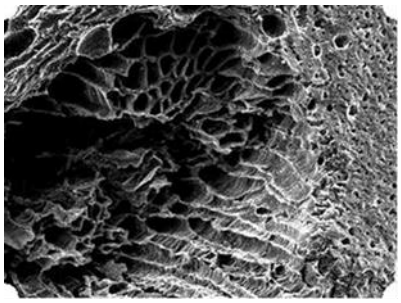


Figure 9. Microscopic image of an activated carbon taken on a scanning electron microscope showing its highly porous properties

Activation is complex, and little is known about how the activated carbon materials in market are prepared. Not all activated carbon products are the same; they can be derived from various raw materials (i.e. vegetables, coal, coconut) and depending on the activation process, which can be either chemical or physical, the resulting activated carbon product will have varying characteristics (Marsh and Rodríguez-Reinoso, 2006). The removal of organic compounds by activated carbon works by the compound being adsorbed within a pore about the same size as itself, therefore, the size of the pores is important. The surface is mainly hydrophobic, contains oxygen and other heteroatoms and can have a positive or negative charge. The internal pore structure and the surface characteristics affect the adsorption properties. The speed at which a compound adsorbs depends on how many sites are available for the adsorption and how easily the compound can get to the site. A high volume of larger pores typically results in fast transport to the adsorption site. The strength at which a compound adsorbs also depends on surface chemistry. A hydrophobic compound will prefer a hydrophobic surface and a positively charged compound will prefer a negatively charged surface (and vice versa). In summary, different compounds have different chemical and physical properties

so they will adsorb to different extents and depending on the characteristics of the activated carbon being used, the removal of the smaller free volatile phenols may vary compared to the larger phenolic glycosides. In other words, an activated carbon that is effective in removing volatile phenols may not necessarily be suitable for removing larger and more hydrophilic molecules such as phenolic glycosides. The aim of this study was therefore to screen a range of commercially available activated carbon products for their abilities to remove smoke volatile phenols and their phenolic glycosides from smoke-affected juices and wines. The following factors were investigated:

- matrix effects (juice versus wine; white wine versus red wine)
- dose rate
- contact time
- contact temperature
- continuous stirring versus no stirring
- comparison between different batches of the same activated carbon product.

Influence of activated carbon type on the removal of smoke-related compounds from juice

Over the course of the project a total of 15 commercially available activated carbon products were evaluated for their ability to remove volatile phenols and phenolic glycosides from smoke-affected juice and wine. The experiments performed are listed in Table 21, with a total of nine grape juice and pressing samples and five wines studied. In regards to volatile phenols, there was limited ability to study their removal from juice matrices as most had been converted to their glycosidic forms as part of normal grape metabolism and therefore their concentrations in juices were low. Consequently, the two experiments that were performed on the removal of volatile phenols required juice to be spiked with volatile phenols.

Table 21. List of experiments performed investigating the influence of various activated carbon products on phenolic glycoside removal

Matrix	Number of carbon products evaluated	Dose rate(s) (g/L)	Contact time(s) (hrs)	Temp.
2015 Mataro juice pressings	13	0.5	2	22°C
2017 Merlot juice	13	0.5	2 & 24	22°C
2017 Cabernet Sauvignon juice	13	0.5	2	22°C
2017 Merlot juice*	13	0.5	24	22°C
2018 NSW Sauvignon Blanc juice	14	0.5, 2.0	24	22°C
2018 NSW Riesling juice	14	0.5, 2.0	24	22°C
2018 NSW Chardonnay juice	14	0.5, 2.0	24	22°C
2019 Tasmanian Pinot Noir free run juice	14	2.0	24	22°C
2019 Tasmanian Chardonnay free run juice	14	2.0	24	22°C
2016 Victorian Pinot Noir wine	13	0.5, 2.0	24	22°C
2018 NSW Sauvignon Blanc wine	14	2.0	24	22°C
2018 NSW Pinot Noir wine	14	2.0	24	22°C
2019 Tasmanian full-bodied Pinot Noir wine	15	2.0	24	22°C
2019 Tasmanian rosé Pinot Noir wine	15	2.0	24	22°C

*additional VPs dosed into juice

Initial experiments demonstrated that the removal efficiency of phenolic glycosides from smoke-affected juice and wine was dependent on the activated carbon product used. Using 2017 Merlot and Cabernet Sauvignon juices (obtained from artificially smoked grapes) and 2015 Mataro juice (from grapes affected by the Adelaide Hills Sampson Flat bushfires) highlighted that even at a low dose rate of 0.5 g/L and a short contact time of two hours, differences between activated carbon products could be observed (Figure 10). For instance, PS1300, CA50 and Smartvin were amongst the best performers, reducing total phenolic glycosides by up to 40%. However, the reduction was not as prominent in the 2015 Mataro juice, which was obtained from a natural bushfire event, compared to the juices obtained from artificially smoking Merlot and Cabernet Sauvignon grapes. The results also indicated that the presence of volatile phenols dosed into the 2017 Merlot juice did not significantly influence the removal of the phenolic glycosides, with the percentage reductions being similar for the Merlot juice with or without the added volatile phenols. The removal of glycosides is likely independent of the free phenols present in this case (they don't seem to compete for the same sites in the activated carbon, or the binding of the phenolic glycosides is much stronger).

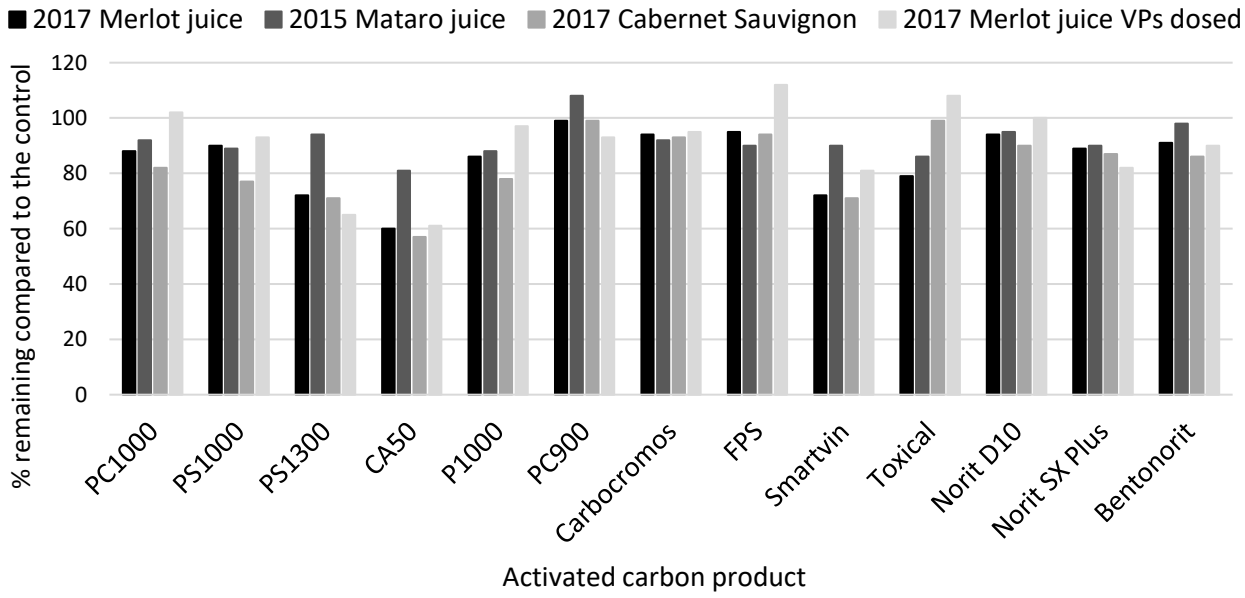


Figure 10. Comparison of the ability of several activated carbon products (dose rate 0.5 g/L, contact time two hours) to remove total phenolic glycosides from several juice matrices. Results are presented as percentage of phenolic glycosides remaining compared to the control (i.e. juice without carbon treatment). Starting concentrations of total phenolic glycosides in the Merlot, Mataro and Cabernet Sauvignon juices were 100, 74 and 52 µg/L SyGG equivalents, respectively.

The greater affinity of some activated carbon products for phenolic glycosides was further highlighted when the 2019 smoke-affected Tasmanian Chardonnay and Pinot Noir juices were treated with various carbons at a dose rate of 2 g/L and a contact time of 24 hours (Figure 11). Confirming earlier results, activated carbon products PS1300, CA50 and Smartvin were amongst the best performers, as too was CASPF which had not been highlighted in the previous study. P1000 also showed similar performance to Smartvin.

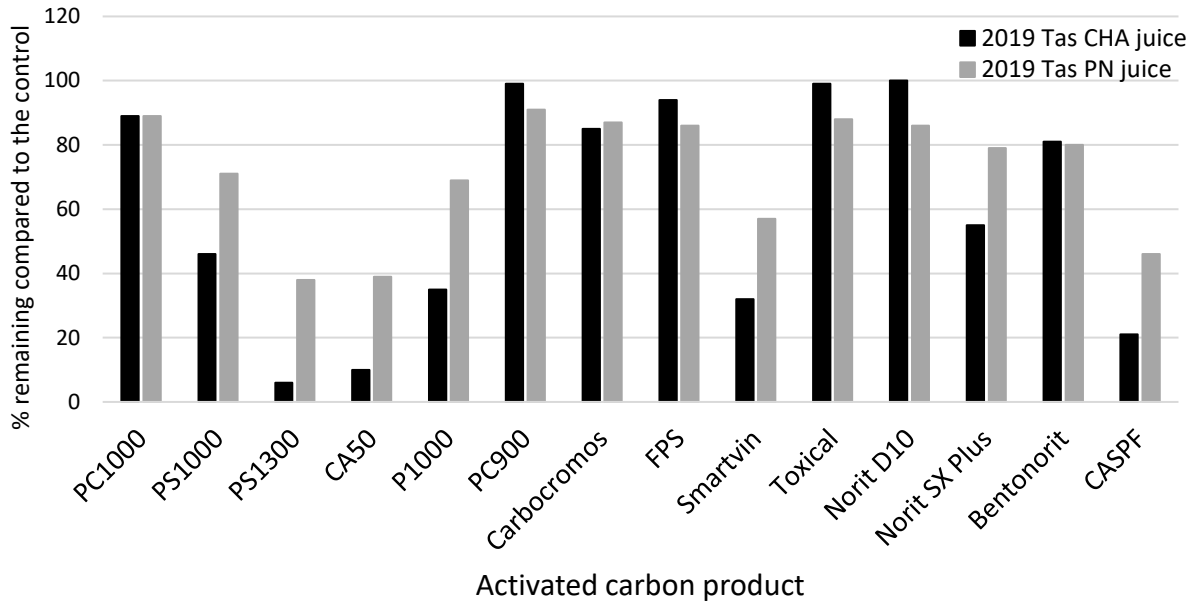


Figure 11. Comparison of the ability of several activated carbon products (dose rate 2 g/L, contact time 24 hours) to remove total phenolic glycosides from two 2019 smoke-affected Tasmanian juices (CHA = Chardonnay; PN = Pinot Noir). Results are presented as percentage of phenolic glycosides remaining compared to the control (i.e. juice without carbon treatment). Starting concentrations of total glycosides for the CHA and PN juices were 251 and 335 µg/L SyGG equivalents, respectively.

The superior performance of PS1300, CA50, Smartvin and CASPF in the removal of phenolic glycosides was further corroborated with juice from 2018 smoke-affected Sauvignon Blanc, Chardonnay and Riesling grapes (data not shown). In summary, similar performance trends were observed amongst the activated carbon products regardless of the juice matrix treated.

Influence of activated carbon type on the removal of smoke-related glycosides from wine

Differences in performance among activated carbon products were also seen in their ability to remove phenolic glycosides from wine (Figure 12 and Figure 13). However, the overall reduction in phenolic glycosides from wine at a dose rate of 2 g/L and contact time of 24 hours was much lower than that observed for juice. All carbon products struggled to remove phenolic glycosides from the 2018 NSW smoke-affected Pinot Noir wine, while there was slightly better performance when treating a 2018 NSW smoke-affected Sauvignon Blanc wine (Figure 12). In this white wine, PS1300 and CA50 continued to exhibit the best performance, and PS1000 and P1000 also exhibited some effectiveness, removing approximately 40-50% of the total glycosides from the Sauvignon Blanc wine. Similar trends were observed for the 2019 smoke-affected Tasmanian Pinot Noir rosé wine, where PS1300, CA50, PS1000 and P1000 were amongst the best performers, removing approximately 30-40% of the total phenolic glycosides (Figure 13). The various carbon products also removed little to none of the phenolic glycosides from the 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine. As a general rule, activated carbon products are not very effective at removing phenolic glycosides from wine and perform particularly poorly in red wine. Therefore, the most promising approach for fining with activated carbon to reduce smoke taint is to target removal of phenolic glycosides from juices rather than from wine, and perhaps consider a second carbon fining step in white wine.

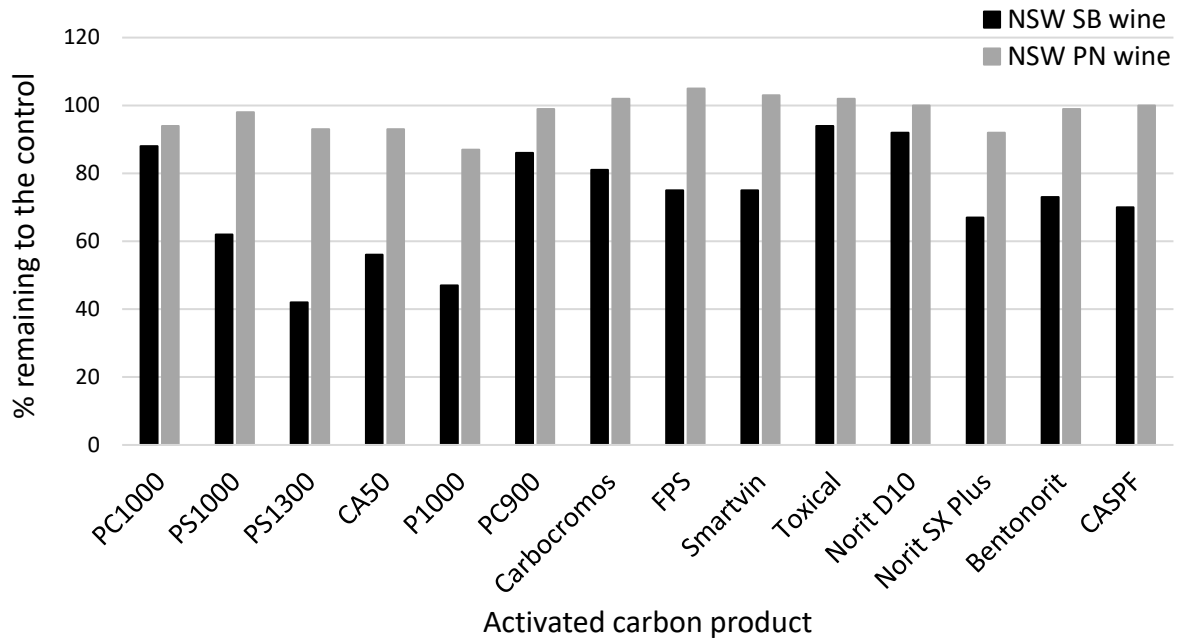


Figure 12. Comparison of the ability of several activated carbon products (dose rate 2 g/L, contact time 24 hours to remove total phenolic glycosides from two 2018 smoke-affected wines (SB = Sauvignon Blanc; PN = Pinot Noir). Results are presented as percentage of phenolic glycosides remaining compared to the control (i.e. wine without carbon treatment). Starting concentrations of total glycosides for the SB and PN wines were 96 and 57 µg/L SyGG equivalents, respectively.

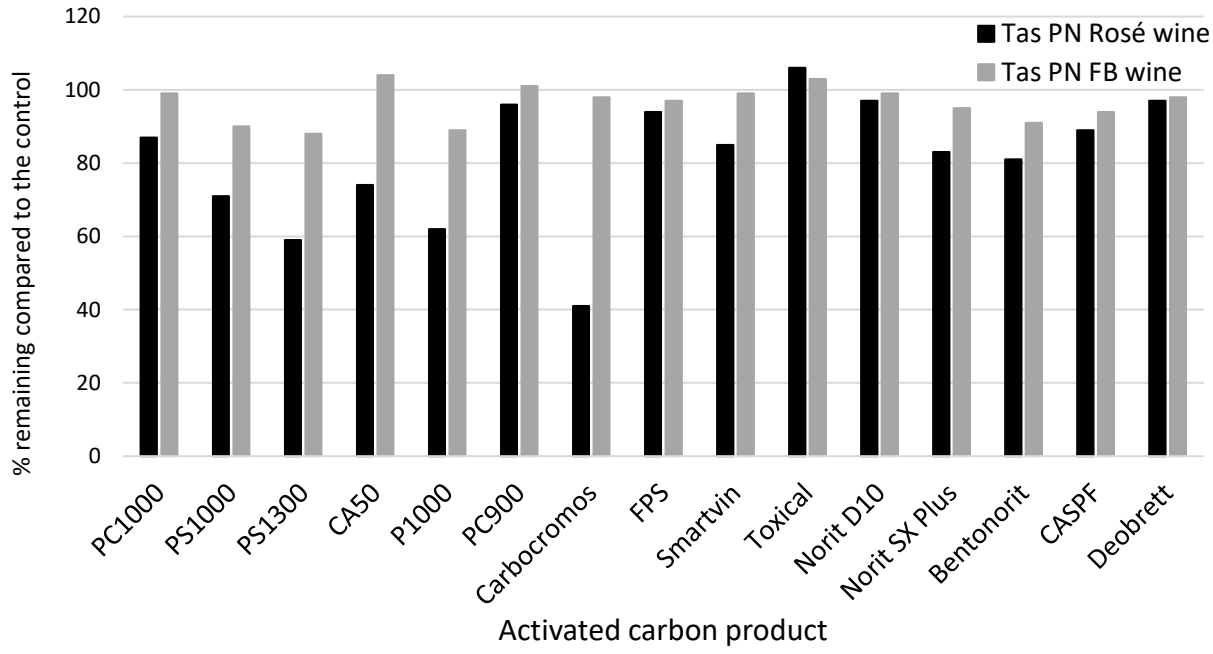


Figure 13. Comparison of the ability of several activated carbon products (dose rate 2 g/L, contact time 24 hours) to remove total phenolic glycosides from two 2019 smoke-affected Tasmanian wines (PN = Pinot Noir rosé wine; FB = full-bodied dry red wine). Results are presented as percentage of phenolic glycosides remaining to the control (i.e. wine without carbon treatment). Starting concentrations of total glycosides for the PN rosé and PN FB wines were 201 and 243 µg/L SyGG equivalents, respectively.

Influence of activated carbon type on the removal of smoke-related volatile phenols from wine

Typically, grape juice samples are low in volatile phenols as they have been converted to their glycosidic metabolites. Therefore, in order to evaluate the removal of volatile phenols from a juice matrix, juice samples were dosed with additional volatile phenols. Due to the lack of a smoke-affected white wine, volatile phenols were also dosed into a commercial Sauvignon Blanc wine. In addition, volatile phenol removal was studied in a 2016 Victorian smoke-affected Pinot Noir wine, as well as from two wines (a rosé wine and full-bodied dry red wine) produced from smoke-affected Tasmanian Pinot Noir grapes in 2019, which had elevated concentrations of volatile phenols after severe smoke exposure of the corresponding vineyards (Table 22).

Table 22. Concentrations of volatile phenols in juices and wines prior to carbon fining experiments

Matrix	4-MG** (µg/L)	Guaiacol (µg/L)	*o*-Cresol (µg/L)	*p*-Cresol (µg/L)	*m*-Cresol (µg/L)	Syringol (µg/L)	MSy** (µg/L)	Total (µg/L)
2017 Merlot juice*	34	29	27	<1	10	3	3	106
2018 Sauvignon Blanc Juice*	78	85	<1	1	72	<1	<1	236
2017 Sauvignon Blanc wine*	80	63	66	<1	23	82	86	400
2016 Vic Pinot Noir wine	8	23	6	1	2	27	11	78
2019 Tas Pinot Noir rosé wine	6	29	9	10	10	11	2	77
2019 Tas Pinot Noir full-bodied wine	12	54	16	25	23	16	3	149

*Matrices spiked with VPs; **4-MG = 4-methylguaiacol; MSy = methylsyringol;

The percentage of total volatile phenols remaining in four matrices, two juice, two wine, after treatment with various activated carbons is provided in Table 23. Three out of the four matrices were treated at a dose of 0.5 g/L, the other was treated with 1 and 2 g/L. As observed for removal of smoke glycosides by activated carbon products, there were differences in the removal of volatile phenols depending on the carbon product used and in general activated carbons were more effective in removing volatile phenols from juice compared to wine.

Table 23. Percentage of total volatile phenols remaining in four matrices after treatment with a range of activated carbon products at different doses

Activated carbon product	2016 Vic PN wine 0.5 g/L activated carbon	2017 SB wine with added VPs 0.5 g/L activated carbon	2017 Merlot juice with added VPs 0.5 g/L activated carbon	2018 NSW SB juice with added VPs 1 g/L activated carbon	2018 NSW SB juice with added VPs 2 g/L activated carbon
PC1000	65%	-	8%	-	
PS1000	69%	-	13%	-	
PS1300	74%	42%	18%	7%	1%
CA50	90%	49%	47%	-	
P1000	73%	-	12%	-	
PC900	77%	-	10%	-	
Carbocromos	58%	19%	8%	-	
FPS	63%	19%	8%	5%	5%
Smartvin	88%	-	51%	-	-
Toxical	97%	57%	91%	-	-
Norit D10	91%	46%	21%	-	-
Norit SX Plus	73%	-	8%	-	-
Bentonorit	85%	-	17%	-	-
CASPF	-	-	-	39%	17%

Higher relative removals were observed for the white wine compared to the red wine, suggesting that other compounds typically found in red wine such as anthocyanins or tannins might compete for binding sites in the activated carbon, making it less effective. This observation was further corroborated through comparison of the removal of volatile phenols through carbon fining of a Pinot Noir red wine and Pinot Noir rosé wine (Figure 14).

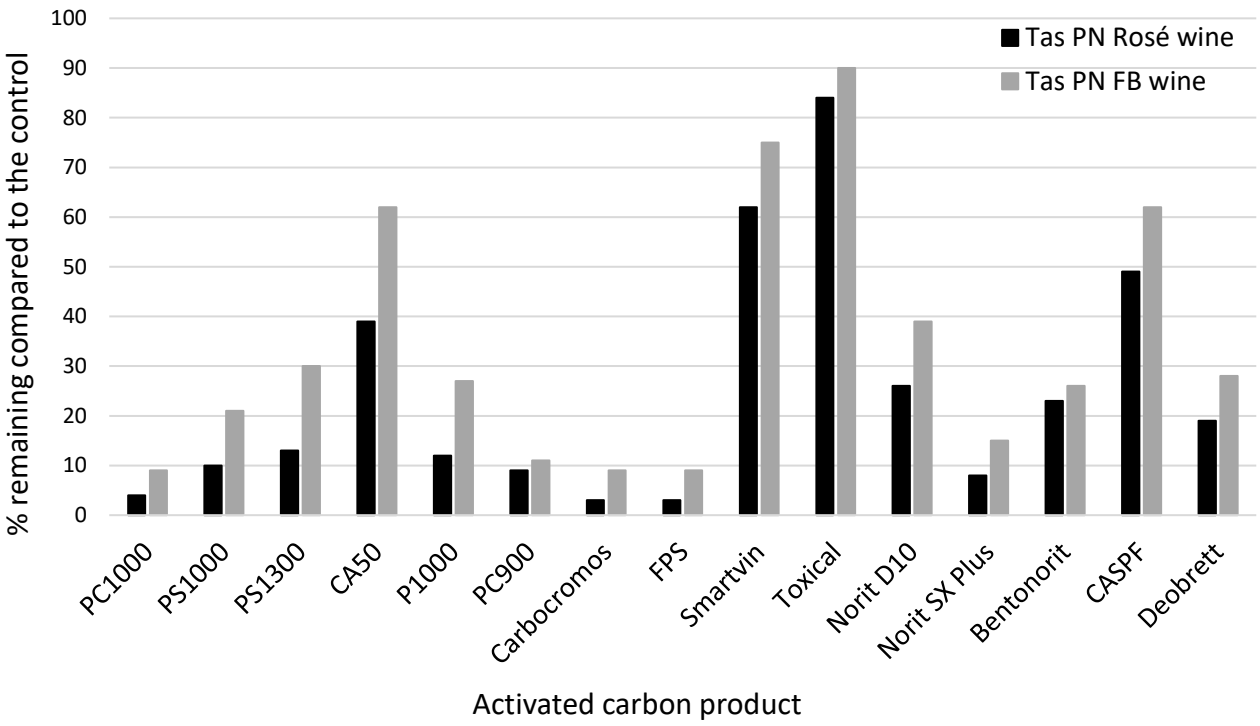


Figure 14. Comparison of the ability of several activated carbon products (2 g/L addition, contact time 24 hours) to remove total volatile phenols (VPs) from a 2019 smoke-affected Tasmanian Pinot Noir (PN) rosé wine and a full-bodied (FB) red wine. Results are presented as percentage of VPs remaining compared to the control (i.e. wine without carbon treatment). Starting total VPs for the rosé and FB PN wines were 77 and 149 µg/L, respectively.

In summary, the removal efficiency of phenolic glycosides and volatile phenols from smoke-affected juice and wine was dependent on the activated carbon product. In these experiments the superior performance of PS1300, CA50, Smartvin and CASPF for removing phenolic glycosides was observed in several smoke-affected juices and wine. On the other hand, Carbocromos and FPS were the best performers for removing volatile phenols. Generally, activated carbon products are more effective at removing phenolic glycosides in juice than wine. Certain carbons could reduce the concentration of phenolic glycosides in juice by 90% or more. For two full-bodied Pinot Noir wines, the reduction in phenolic glycosides was less than 20%, even at a dose rate of 2 g/L. The activated carbon products fared slightly better in white and rosé wines, with some removing between 50 and 60% of the phenolic glycosides. Based on these results, activated carbons PS1300 and CASPF were selected for small-scale (50 L) winemaking trials treating smoke-affected Chardonnay and Pinot Noir juices prior to fermentation.

Prior to the winemaking trials, the effects of activated carbon dose and contact time on removal of volatile phenols and phenolic glycosides were evaluated in series of fining experiments. The effectiveness of activated carbon fining at lower temperatures and with or without stirring was also evaluated.

A 2018 smoke-affected Sauvignon Blanc juice was treated with four activated carbons (PS1300, CA50, CASPF, FPS) at two doses rates (1 and 2 g/L) with 24 hours contact time, either with or without stirring, and at either 4°C or 22°C and no significant temperature or mixing effects were observed.

For contact time, there were only small differences in the relative ability of activated carbons to remove phenolic glycosides from a Merlot juice (137 µg/L total glycosides as SyGG equivalents prior to carbon treatment) when treated with 500 mg/L activated carbon for 2 hours or 24 hours. Similar results were obtained with a 2019 Tasmanian Pinot Noir rosé juice after contact with 2 g/L of various activated carbon products for 2 or 24 hours contact time. Overall, a small increase in the removal of phenolic glycosides after extended exposure was observed for some activated carbon products and carbon benchmarking experiments were undertaken at a carbon contact time of 24 hours, noting that some manufacturers advise that contact times as short as 10 minutes can be sufficient.

A number of of experiments were also conducted to assess the influence of the dose of activated carbon addition, using the removal of phenolic glycosides as an analytical proxy for likely reduction of smoke taint in wine.

Influence of activated carbon dose on the removal of smoke-related phenolic glycosides from juice and wine

The experiments performed to evaluate the influence of activated carbon dose on phenolic glycoside removal are listed in Table 24. A typical example is given in Figure 15, which shows result from treating a 2018 smoke-affected Sauvignon Blanc juice 24 hours with six activated carbon products at four dose rates. The products which were effective in removing phenolic glycosides clearly performed in a dose-dependent manner, with increasing additions of activated carbon resulting in decreasing concentrations of phenolic glycosides in the treated juice. Similar results were obtained for the 2019 smoke-affected Tasmanian Chardonnay juice (Figure 16) and the 2019 smoke-affected Tasmanian Pinot Noir rosé juice (Figure 17). Typically, a linear relationship can be observed between increased additions of activated carbon and the concomitant reduction in phenolic glycosides (Figure 18).

Table 24. List of experiments performed investigating the influence of carbon dose rate on phenolic glycoside removal

Matrix	Carbons evaluated	Dose rate (g/L)	Contact time (hrs)	# of reps	Temp.
2015 Mataro juice pressings	PS1300, CA50, Carbocromos, FPS	0.5, 2.0		2	22°C
2017 Merlot juice	PS1300, CA50, Carbocromos, FPS, CASPF	0.5, 2.0	24	2	22°C
2018 NSW Sauvignon Blanc juice	PC1000, PS1000, PS1300, CA50, P1000, PC900, Carbocromos, FPS, Smartvin, Toxial, Norit D10, Norit SX Plus, Bentonorit, CASPF	0.5, 2.0	24	1	22°C
2018 NSW Sauvignon Blanc juice	PS1300, CA50, CASPF, Smartvin, Carbocromos, FPS	0.5, 1.0, 1.5, 2.0	24	3	22°C
2018 NSW Sauvignon Blanc juice, dosed with VPs and also analysed for VPs	PS1300, CASPF, FPS	1.0, 2.0	24	3	22°C
2018 NSW Sauvignon Blanc juice	PS1300, CA50, CASPF, FPS	1.0, 2.0	24	3	4°C
2018 NSW Riesling juice	PC1000, PS1000, PS1300, CA50, P1000, PC900, Carbocromos, FPS, Smartvin, Toxial, Norit D10, Norit SX Plus, Bentonorit, CASPF	0.5, 2.0	24	1	22°C
2018 NSW Chardonnay juice	PC1000, PS1000, PS1300, CA50, P1000, PC900, Carbocromos, FPS, Smartvin, Toxial, Norit D10, Norit SX Plus, Bentonorit, CASPF	0.5, 2.0	24	1	22°C
2019 Tasmanian Pinot Noir free run juice	PS1300, CA50, CASPF	0.5, 1.0, 1.5, 2.0, 4.0	24	1	22°C
2019 Tasmanian Chardonnay free run juice	PS1300, CA50, CASPF	1.0, 2.0, 4.0	24	1	22°C
2019 Tasmanian Chardonnay free run juice diluted with Padthaway Chardonnay juice	PS1300, CA50, CASPF	0.5, 1.0, 1.5, 2.0	24	1	22°C
2016 Victorian Pinot Noir wine	PS1300, CA50, Toxical, Norit D10, Carbocromos, FPS	0.5, 2.0	24	2	22°C
2018 NSW Sauvignon Blanc wine	PS1300, CA50, CASPF	1.0, 2.0, 5.0	24	3	22°C
2019 Tasmanian rosé Pinot Noir wine	PS1300, CA50, CASPF, FPS, Carbocromos	1.0, 2.0, 4.0	24	2	22°C
2019 Tasmanian full-bodied Pinot Noir wine	PS1300, CA50, CASPF, FPS, Carbocromos	1.0, 2.0, 4.0	24	2	22°C

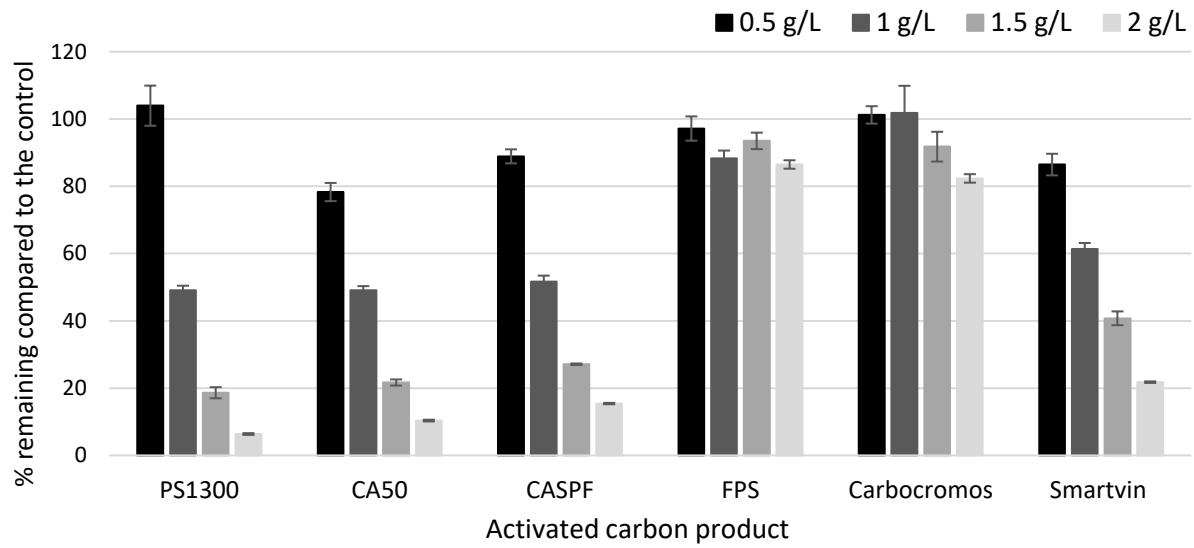


Figure 15. The ability of six activated carbon products at four doses (0.5, 1.0, 1.5 and 2.0 g/L; contact time of 24 hours) to remove total phenolic glycosides from a 2018 smoke-affected Sauvignon Blanc juice. Results are presented as the percentage of phenolic glycosides remaining compared to the control (i.e. juice without carbon treatment), average of three treatment replicates. Starting concentration of total glycosides was 106 µg/L SyGG equivalents.

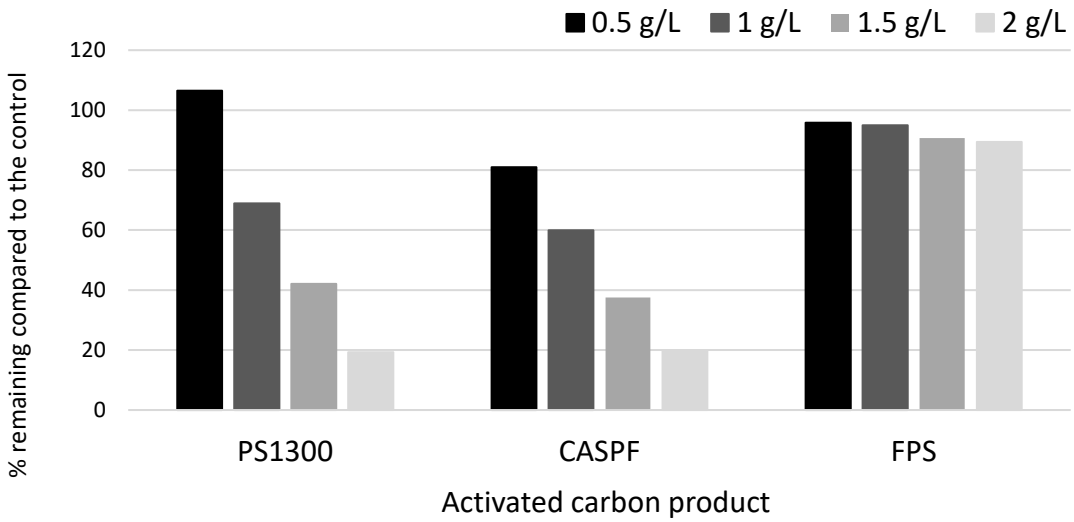


Figure 16. The ability of three activated carbon products at four doses (0.5, 1.0, 1.5 and 2.0 g/L; contact time of 24 hours) to remove total phenolic glycosides from a 2019 Tasmanian smoke-affected Chardonnay juice. Results are presented as the percentage of phenolic glycosides remaining compared to the control (i.e. juice without carbon treatment). Starting concentration of total glycosides was 251 µg/L SyGG equivalents.

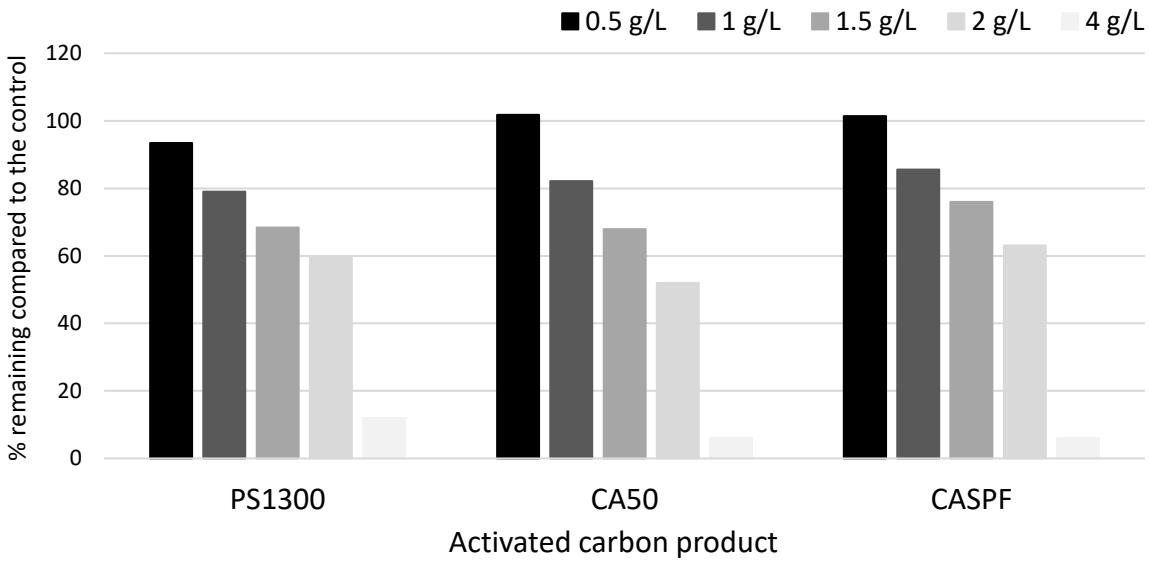


Figure 17. The ability of three activated carbon products at five doses (0.5, 1.0, 1.5, 2.0 and 4.0 g/L; contact time of 24 hours) to remove total phenolic glycosides from a 2019 Tasmanian smoke-affected Pinot Noir rosé juice. Results are presented as the percentage of phenolic glycosides remaining compared to the control (i.e. juice without carbon treatment). Starting concentration of total glycosides was 331 µg/L SyGG equivalents.

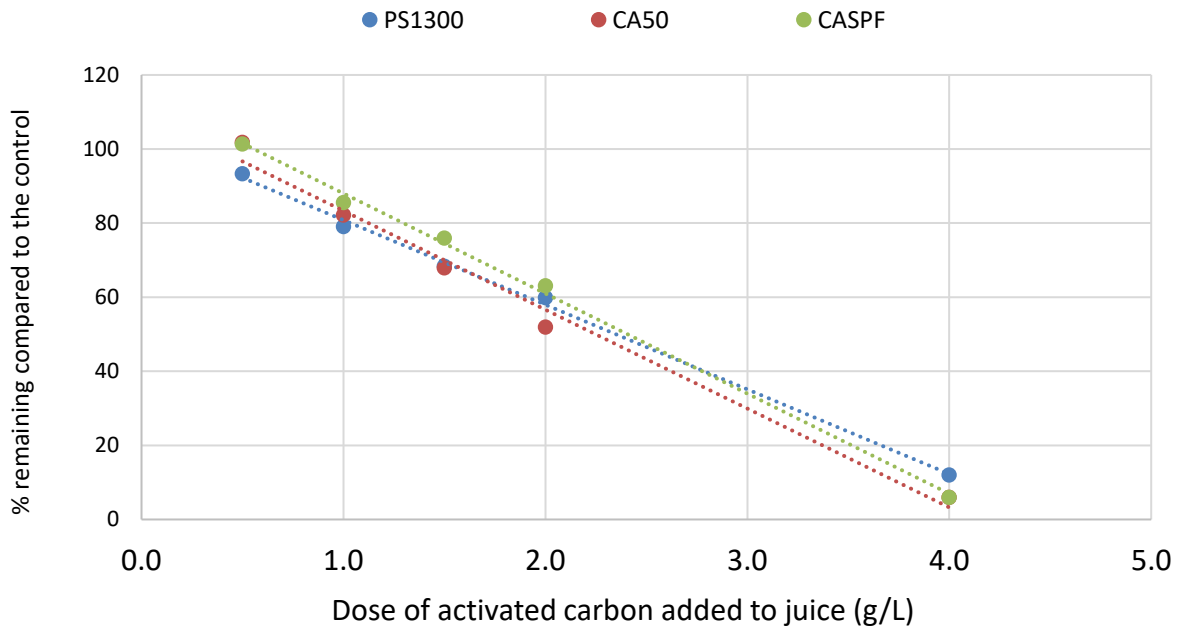


Figure 18. The relationship between the dose of activated carbon added (0.5-4.0 g/L) and percentage removal of phenolic glycosides from a 2019 Tasmanian smoke-affected Pinot Noir rosé juice for three activated carbon products (PS1300, CA50, CASPF)

The efficacy of the activated carbon products tested was influenced by the starting concentration of phenolic glycosides and other compounds present in the juice matrix. As expected, at a given dose, the carbon products' performance was best for the Sauvignon Blanc juice with a low starting concentration of total glycosides (106 µg/L SyGG equivalents). Results showed that a 2 g/L activated carbon addition could remove up to 90% of phenolic glycosides from this matrix. Treating the 2019 Tasmanian smoke-affected Chardonnay juice with 2 g/L activated carbon could remove up to 80% of phenolic glycosides from a starting concentration of 251 µg/L SyGG equivalents, and fining of the 2019 smoke-affected Tasmanian Pinot Noir rosé juice with 2 g/L activated carbon removed around 40% of the phenolic glycosides from a starting concentration of 331 µg/L SyGG equivalents. This indicates that to achieve an optimum outcome from fining smoke-affected grape juice with activated carbon to remove phenolic glycosides it is important to select an appropriate fining material and also to optimise its dose as a function of juice composition and level of contamination.

Influence of activated carbon dose on the removal of smoke-related volatile phenols from wine

As most smoke-affected juice samples contained low or no concentrations of volatile phenols, the effect of increasing additions of activated carbon on the removal of volatile phenols was only evaluated in two wine samples, the 2019 smoke-affected Tasmanian Pinot Noir rosé and full-bodied wines. These contained total volatile phenol concentrations of 63 and 123 µg/L, respectively. Increasing carbon additions increased the removal of the volatile phenols from wine, with FPS and Carbocromos showing superior performance in these experiments (Figure 19 and Figure 20).

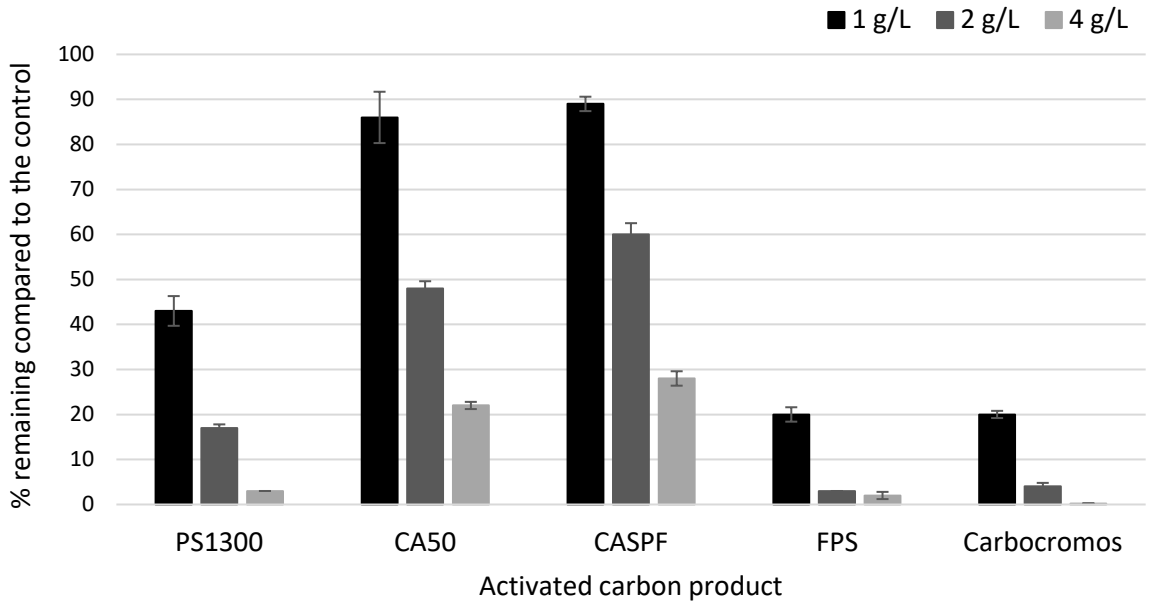


Figure 19. The ability of five activated carbon products at three doses (1, 2 and 4 g/L; contact time of 24 hours) to remove total volatile phenols from a 2019 smoke-affected Tasmanian Pinot Noir rosé wine. Results are presented as percentage of phenols remaining compared to the control (i.e. wine without carbon treatment) and are the average of two treatment replicates. Starting concentrations of total VPs was 63 µg/L.

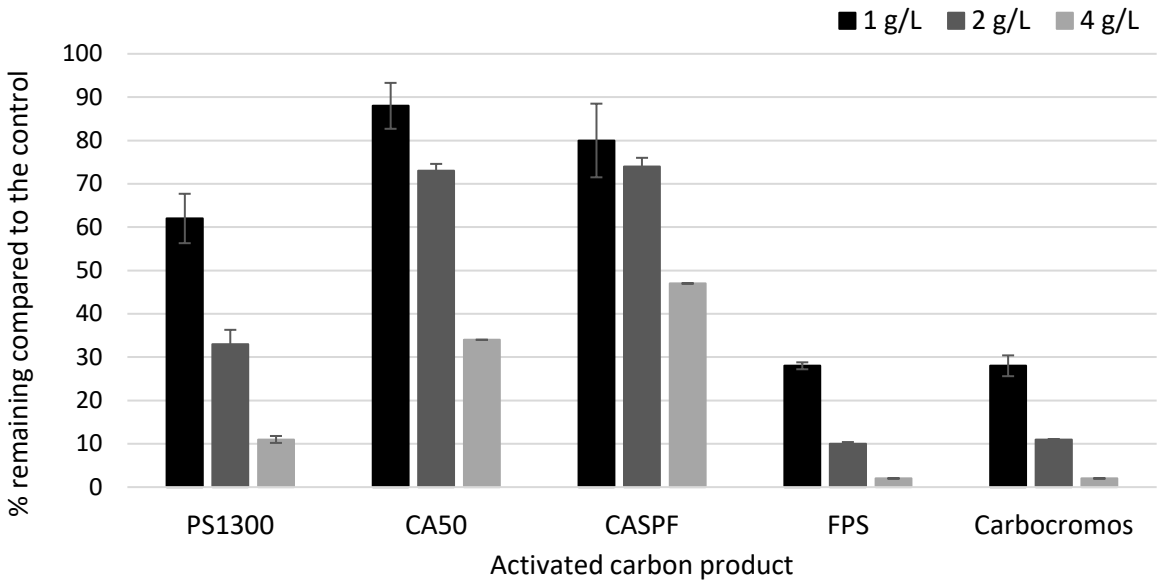


Figure 20. The ability of five activated carbon products at three dose rates (1, 2 and 4 g/L; contact time of 24 hours) to remove total volatile phenols from a 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine. Results are presented as the percentage of phenols remaining compared to the control (i.e. wine without carbon treatment) and represent the average of two treatment replicates. Starting concentration of total VPs was 123 µg/L.

3.1.5 Potential of glycosidase enzymes to reduce concentrations of smoke-related phenolic glycosides in wine

Phenolic glycosides are formed after volatile phenols from smoke are absorbed into the skin and flesh of grapes and grape enzymes add sugar units. During winemaking, only some of these glycosides are cleaved. Therefore, wine produced from smoke-exposed fruit is likely to contain phenolic glycosides as well as volatile phenols, the amount dependent on the level of smoke exposure. This can be problematic as phenolic glycosides are stable during storage and can gradually hydrolyse to release the volatile phenols. In addition, there is potential for these phenolic glycosides to undergo in-mouth release during consumption due to the presence of glycosidases in human saliva (Parker et al. 2012; Parker et al. 2019).

The ability of five commercially available glycosidases to cleave smoke glycosides in smoke-affected juice and wine was investigated under various temperatures, contact times and dosage rates. The glycosidases were not effective in cleaving the phenolic glycosides in a juice matrix (data not shown), most likely a consequence of the high concentration of sugars inhibiting the enzyme. This is in line with manufacturer recommendations for glycosidase addition at the end of fermentation when sugar concentrations are low. Therefore, the focus was on evaluating glycosidases as a remediation treatment for wine.

Studies were undertaken with several smoke-affected wines including:

- 2016 Victorian smoke-affected Pinot Noir wine
- two smoke-affected US Cabernet Sauvignon wines
- 2018 NSW smoke-affected Pinot Noir wine
- 2018 NSW smoke-affected Sauvignon Blanc wine
- 2019 Tasmanian smoke-affected Chardonnay wine
- 2019 Tasmanian smoke-affected Pinot Noir rosé wine
- 2019 Tasmanian smoke-affected full-bodied Pinot Noir wine

Table 25 lists the various experiments performed to evaluate the influence of glycosidase treatment on the fate of phenolic glycosides and the influence of dose rate, contact time and temperature.

Table 25. List of experiments performed investigating the influence of dose rate, contact time and temperature on the cleavage of phenolic glycosides by glycosidases in various matrices

Matrix	Glycosidases evaluated	Dose rate*	Contact time	# of reps	Temp.
2016 Victorian Pinot Noir wine	Lafazym, Zimarom, Revelation, Lallzyme	1 x HRD	3 weeks	3	17°C
2015 Mataro juice pressings	Lafazym, Zimarom, Revelation, Lallzyme, Trenolin Bouquet Plus	1 x HRD 5 x HRD	24 hr and 96 hr	1	17°C
2016 Victorian Pinot Noir wine	Lafazym, Zimarom, Revelation, Lallzyme, Trenolin Bouquet Plus	1 x HRD 5 x HRD	24, 96 and 168 hr	1	17°C and 28°C
2016 Victorian Pinot Noir wine	Lallzyme, Revelation, Trenolin Bouquet	5 x HRD	115 hr	3	28°C
2017 US Cabernet Sauvignon wine	Lafazym, Zimarom, Revelation, Lallzyme, Trenolin Bouquet Plus	1 x HRD	96 hr	1	28°C
2017 US Cabernet Sauvignon wine	Revelation, Trenolin Bouquet Plus	1 x HRD 5 x HRD	72, 96 and 168 hr	3	28°C
2018 NSW Pinot Noir wine	Revelation, Trenolin Bouquet Plus	5 x HRD	72 hr	3	28°C
2018 NSW Sauvignon Blanc wine	Revelation, Trenolin Bouquet Plus	5 x HRD	72 hr	3	28°C
2019 Tasmanian Pinot Noir rosé wine	Revelation, Trenolin Bouquet Plus	5 x HRD	72 hr	2	29.7°C
2019 Tasmanian Chardonnay wine	Revelation, Trenolin Bouquet Plus	5 x HRD	72 hr	2	29.7°C
2019 Tasmanian full-bodied Pinot Noir wine	Revelation, Trenolin Bouquet Plus	5 x HRD	72 hr	2	29.7°C
2019 Tasmanian full-bodied Pinot Noir wine**	Revelation, Trenolin Bouquet Plus	5 x HRD	72 hr	2	29.7°C
2019 Tasmanian full-bodied Pinot Noir wine	Trenolin Bouquet Plus	1 x HRD 2 x HRD 5 x HRD	120, 168 hr	2	17°C

*HRD = highest recommended dose by the manufacturer; ** 2 g/L of PS1300, CASPF or FPS added after three days of contact with glycosidase, carbon contact for 24 hours then addition of 1 g/L of 5% v/v bentonite; Rapidase Revelation Aroma; Lallzyme beta

Figure 21 summarises the results from an experiment evaluating the ability of four commercially available glycosidases to cleave the phenolic glycosides in a 2016 smoke-affected Pinot Noir wine at 17°C. The enzymes were evaluated at the highest recommended dose rate and a contact time of three weeks. All four enzymes gave similar results; that is, they removed some phenolic glycosides almost completely (for example, SyGG) and reduced the concentration of others such as GuPG to some degree.

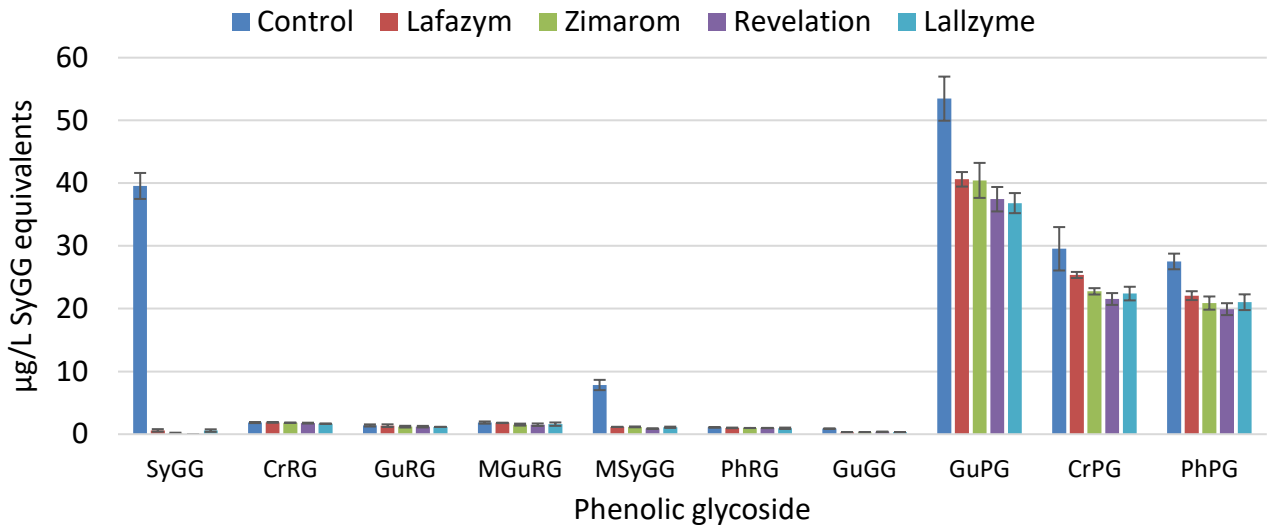


Figure 21. Concentrations of phenolic glycosides remaining in a 2016 smoke-affected Pinot Noir wine after treatment with various glycosidases at their highest recommended doses for three weeks at 17°C

Subsequently, a fifth enzyme (Erbsloh's Trenolin Bouquet Plus) was sourced and the influence of factors such as dose rate, temperature and contact time on phenolic glycoside cleavage was evaluated in the 2016 Victorian smoke-affected Pinot Noir wine. All five enzymes were evaluated at contact times of 24 hours, 96 hours and 168 hours, with the highest recommended dose and five times the highest recommended dose at two temperatures (17°C and 28°C). Similar cleavage patterns were observed for all glycosidases (data not shown), with preferential cleavage of the GGs, a slight increase in monoglucosides (MGs), generally no change in rutinosides (RGs) or a slight reduction in RGs for the Trenolin Bouquet Plus enzyme, and up to 20% reduction in the PGs.

In another experiment, two smoke-affected Cabernet Sauvignon wines were subject to glycosidase treatment, with five enzymes at their highest recommended dose for 96 hours at 28°C. Similar results were obtained for both wines and the relative glycoside hydrolysis patterns were similar to those observed for the 2016 Pinot Noir wine. There was a reduction in total GGs of 60-75%, no change in PGs, RGs were reduced by approximately half but only by one enzyme (Trenolin Bouquet Plus) and there was a 15-30% reduction in MGs. In summary the enzymes showed similar activity, the only exception being Trenolin Bouquet Plus which seemed to have some ability to cleave RGs (Figure 22).

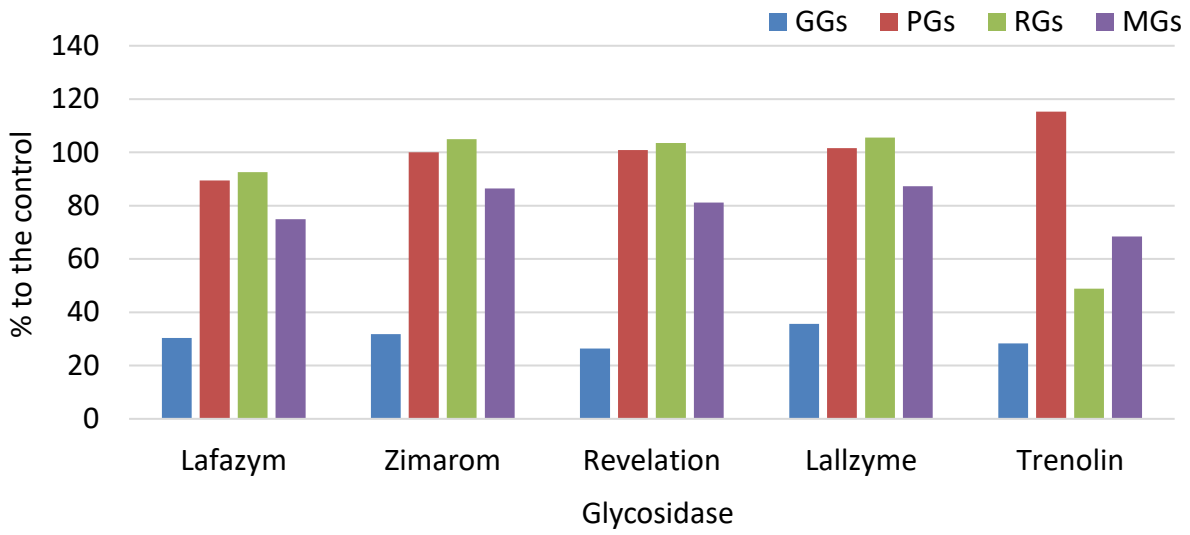


Figure 22. Total gentiobiosides (GGs), pentosylglucosides (PGs), monoglucosides (MGs) and rutinosides (RGs) remaining in a smoke-affected Cabernet Sauvignon wine after treatment with various glycosidase enzymes at their highest recommended dose rates for 96 hours at 28°C

As a consequence of treating wine with glycosidases, an increase in total volatile phenols is typically observed post-treatment (Table 26) which might lead to an increase in smoky aroma attributes. However, the focus here is on removing the glycosides which can result in phenols being released in the mouth when the wine is tasted.

Table 26. Volatile phenols present in Tasmanian smoke-affected Chardonnay and Pinot Noir rosé wines after treatment with five times the highest recommended dose for Rapidase Revelation Aroma and Trenolin Bouquet Plus at 29.7°C for 3 days.

Treatment	4-MG* (µg/L)	Guaiacol (µg/L)	o-cresol (µg/L)	p-cresol (µg/L)	m-cresol (µg/L)	Syringol (µg/L)	MSy* (µg/L)	Total (µg/L)
CHA control	1	3	<1	6	2	4	<1	16
CHA + Revelation	3	6	1	12	6	6	<1	34
CHA + Trenolin	7	9	1	11	5	8	1	42
rosé PN control	4	20	6	9	8	7	1	55
Rosé PN + Revelation	6	25	7	15	12	8	2	75
Rosé PN + Trenolin	10	27	6	16	12	10	2	83

*4-MG = 4-methylguaiacol; MSy = methylsyringol

In summary, all five commercially available glycosidases evaluated preferentially and effectively cleaved the phenolic gentiobiosides in wine. As expected, longer contact time, higher additions of enzymes and treatment at higher temperature influenced the hydrolysis of phenolic glycosides. Independent of treatment conditions, the PGs remained relatively intact, while there was either a slight increase or decrease in the concentrations of MGs. Glycosidase treatment will result in higher

concentrations of volatile phenols and therefore should be used in combination with carbon fining of wine, as a secondary measure to remove the volatile phenols generated by cleavage of the phenolic glycosides. Trenolin Bouquet Plus was the only enzyme evaluated which demonstrated an ability to cleave RGs, and for this reason it was chosen for winery-scale trials.

3.1.6 Other potential materials and methods for the remediation of smoke-affected juice and wine

A range of other materials and methods were evaluated for the remediation of smoke-affected juice and wine through removing volatile phenols and/or phenolic glycosides. However, none were shown to effectively reduce the concentrations of smoke compounds. Treating juice samples with XAD resins, zeolites, grape seed oil, cream or full cream milk did not reduce phenolic glycosides. In addition, juice samples spiked with volatile phenols were subjected to hyperoxidation (via bubbling of oxygen through the juice) or UV light in the presence of titanium dioxide (a method used to purify water) but neither method reduced the volatile phenols in these model experiments.

3.1.7 Winemaking trials with grape juice after fining with activated carbon

To obtain wine for sensory studies and validate the laboratory-based experiments at a larger scale, activated carbons PS1300 and CASPF were evaluated in small-scale (50 L) winemaking trials with smoke-affected 2019 Tasmanian Chardonnay and Pinot Noir rosé juice. Juices were treated with the activated carbon products (1, 2 or 4 g/L) prior to fermentation.

In agreement with benchtop studies, chemical analysis indicated that activated carbon treatment of smoke-affected juices removed phenolic glycosides, to an extent dependent on the dose added. Furthermore, when considering the same dose rates, the activated carbons were more effective at removing phenolic glycosides from the Chardonnay juice than the Pinot Noir rosé juice. As expected, the use of activated carbon reduced wine colour, with CASPF having a greater impact on colour than PS1300. Overall, CASPF was slightly more effective than PS1300 in removing the smoke phenolic glycosides, although depending on dose rate the observed differences between the two activated carbon products were not always statistically significant.

The data in Table 27 for volatile phenols and Table 28 for phenolic glycosides demonstrate that grape processing can significantly affect the concentrations of smoke taint marker compounds, with substantially lower concentrations in free run juice compared to grapes and the highest concentrations in pressings.

Table 27. Volatile phenols present in Pinot Noir and Chardonnay grapes (at harvest), free run juice and juice pressings prior to carbon treatment and fermentation

Treatment	4-MG* (µg/L‡)	Guaiacol (µg/L‡)	o-cresol (µg/L‡)	p-cresol (µg/L‡)	m-cresol (µg/L‡)	Syringol (µg/L‡)	MSy* (µg/L‡)	Total (µg/L‡)
Chardonnay grapes	9	31	17	<1	4	<2	<2	61
Chardonnay free run juice	<1	2	<1	2	<1	<1	<1	4
Chardonnay juice pressings	2	8	3	3	3	<1	<1	19
Pinot Noir grapes	5	23	12	<1	4	<2	<2	44
Pinot Noir free run juice	2	17	5	<1	5	6	<1	35
Pinot Noir juice pressings	4	25	8	<1	7	3	<1	47

*4-MG = 4-methylguaiacol; MSy = methylsyringol; ‡ In the case of grape samples, concentrations are in µg/kg

Table 28. Phenolic glycosides present in Pinot Noir and Chardonnay grapes (at harvest), free run juice and juice pressings prior to carbon treatment and fermentation

	Concentration (µg/L* SyGG equivalents)						
Treatment	**SyGG**	**CrRG**	**GuRG**	**MGuRG**	**MSyGG**	**PhRG**	**Total**
Chardonnay grapes	322	28	32	86	32	7	507
Chardonnay free run juice	142	15	23	57	13	3	253
Chardonnay juice pressings	311	23	29	79	29	6	477
Pinot Noir grapes	200	38	39	78	29	9	392
Pinot Noir free run juice	193	31	28	48	22	13	335
Pinot Noir juice pressings	257	34	34	60	29	16	431

* In the case of grape samples, concentrations are in µg/kg SyGG equivalents

Prior to fermentation, Pinot Noir and Chardonnay free run juices (50-L scale) were treated with various carbons at either 1, 2 or 4 g/L. As expected, the use of carbon reduced the concentrations of phenolic glycosides in juice for both Pinot Noir and Chardonnay (Figure 23 and Figure 24). For Chardonnay, additions of 1, 2 and 4 g/L of PS1300 reduced the total phenolic glycosides by 29%, 73% and 99%, respectively, in comparison to 44%, 82% and 99% for treatment with CASPF. For Pinot Noir, additions of 1, 2 and 4 g/L of PS1300 reduced the total phenolic glycosides by 22%, 57% and 76%, respectively, in comparison to 23%, 53% and 93% for treatment with CASPF. In summary, the removal efficacy for both activated carbon products for juice fining was similar at the two lower dose rates, but CASPF was slightly superior at removing phenolic glycosides at 4 g/L.

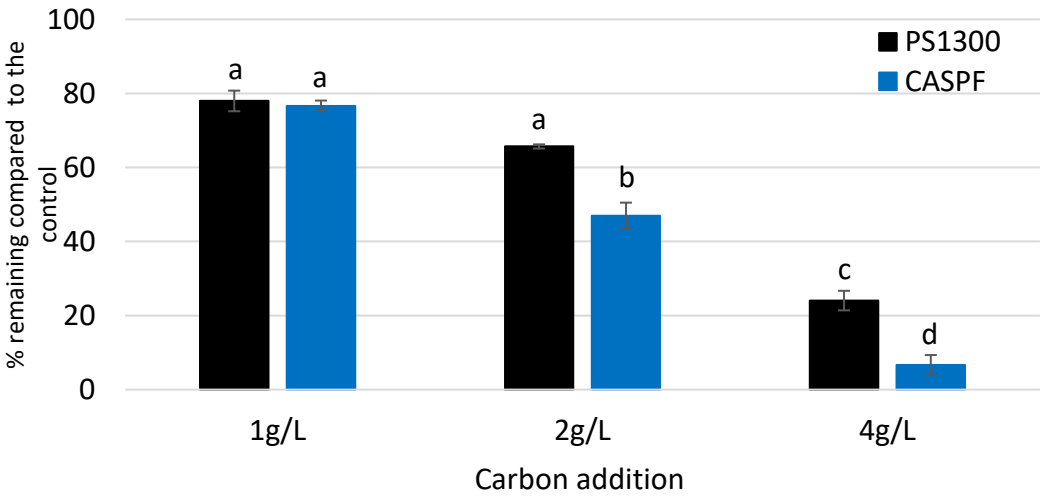


Figure 23. Percentage removal of total phenolic glycosides from Pinot Noir juice (average of two treatment replicates) by two activated carbon products at three dose rates

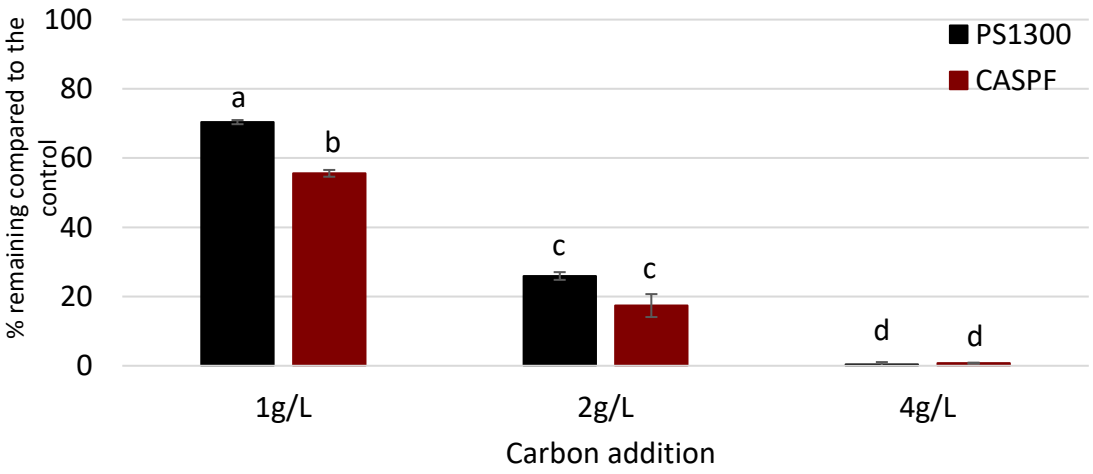


Figure 24. Percentage removal of total phenolic glycosides from Chardonnay juice (average of 2 treatment replicates) by two activated carbon products at three dose rates

Post-bottling, the Pinot Noir and Chardonnay wines were analysed for volatile phenols and phenolic glycosides. Compared to the free run juice, the total volatile phenols for both the Pinot Noir and Chardonnay control wines (i.e. no carbon treatment) increased from 35 to 54-57 µg/L and from 4 to 14 µg/L, respectively. This is expected as phenolic glycosides can be cleaved during fermentation, releasing volatile phenols into the wine and is supported by a comparison of the phenolic glycoside data for the free run juice and the control wines for both Pinot Noir and Chardonnay. Total phenolic glycosides in the Pinot Noir free run juice decreased by approximately 40% (from 335 to an average of 197 µg/L SyGG equivalents), and were reduced by approximately 35% for Chardonnay (from 253 to an average of 165 µg/L SyGG equivalents). If considering all the treatments and controls, on average total glycosides reduced by 45% during fermentation from juices post-carbon treatment to bottled wine (Table 29).

Table 29. Comparison of total glycosides in juices post-carbon treatment and wine post-bottling and the relative reduction between total glycosides pre- and post-fermentation

	Pinot Noir – total glycosides*			Chardonnay – total glycosides		
Treatment	**Juice**	**Wine post-bottling**	**% reduction**	**Juice**	**Wine post-bottling**	**% reduction**
Control not fined	333	197	41	242	165	32
	Juice post-carbon			**Juice post-carbon**		
PS1300 1 g/L	260	153	41	171	95	45
PS1300 2 g/L	219	106	52	64	26	60
PS1300 4 g/L	80	34.5	57	2	1	33
CASPF 1 g/L	256	139	46	135	71	47
CASPF 2 g/L	157	74	53	43	16	64
CASPF 4 g/L	22	15.5	30	2	1	33

*Total glycosides expressed as µg/L SyGG equivalents

3.1.8 Winemaking trials with Pinot Noir – A case study in monitoring the concentrations of volatile phenols and phenolic glycosides from grape to wine

In the context of the experiments fining grape juice with activated carbon described above, a significant decrease in the concentrations of phenolic glycosides was observed between grapes, free run juice and bottled wine, even in the absence of carbon fining. Following the 2019 Tasmanian bushfires and concomitant smoke exposure of vineyards, a case study was conducted to monitor the concentrations of phenolic glycosides (Table 30) and volatile phenols (Table 31) from Pinot Noir grapes to juice to wine produced by various methods. The results described below allow for a detailed understanding of the fate of volatile phenols from smoke and their grape metabolites, the

phenolic glycosides, and demonstrate how winery management practices and techniques may influence their extraction.

The concentrations of phenolic glycosides were broadly similar for Pinot Noir grape samples collected in February 2019 compared to grapes from the same region at harvest in April 2019 (data not shown). Lower concentrations of phenolic glycosides were seen in the Pinot Noir rosé free run juice compared to the juice pressings (Table 30). This indicates that pressing the skins will result in increased extraction of phenolic glycosides (total of 431 µg/L in pressings, compared to 335 µg/L SyGG equivalents in free run juice). Leading on from this observation, it is not surprising to see higher concentrations of phenolic glycosides in the full-bodied red wine compared to the rosé wines (total of 259 µg/L, compared to 209 and 221 µg/L SyGG equivalents). It is interesting to note that the whole bunch ferment contained similar concentrations of phenolic glycosides compared to the rosé style wines, though this result would warrant a repeat before the findings can be generalised. As expected, treatment of the juice with activated carbon to create a rosé style wine reduced the final concentrations of phenolic glycosides in wine substantially, even when compared to the rosé wine made from free run juice.

For the volatile phenols (Table 31), differences between grapes, free run juice and pressings were less pronounced; the concentrations of volatile phenols in the rosé style wine from juice fined with activated carbon was substantially lower than volatile phenols in rosé style wine made from free run juice; and total volatile phenols were notably elevated in both the full-bodied red wine and red wine made by whole bunch fermentation.

Table 30. Concentrations of phenolic glycosides in 2019 Tasmanian smoke-affected Pinot Noir grapes, juice (free run and pressings) and wine produced using various winemaking techniques

Description	Concentration (µg/L* SyGG equivalents)						
	SyGG	CrRG	GuRG	MGuRG	MSyGG	PhRG	Total
Grapes at harvest – April 2019	200	29	9	38	39	78	392
Rosé free run juice	193	22	13	31	28	48	335
Rosé juice pressings	257	29	16	34	34	60	431
Rosé wine - 50 L ferment	94	4	15	26	27	44	209
Rosé wine - bulk ferment (250 L)	100	6	15	25	30	44	221
Full bodied red wine	148	13	14	16	26	42	259
Whole bunch ferment**	103	6	18	25	31	42	223
Rosé wine** – after carbon treating juice	48	3	7	11	12	16	97

*Results are in µg/kg for grapes; **winemaking performed by a winery in Tasmania, carbon type and dose rate unknown

Table 31. Concentrations of volatile phenols in 2019 Tasmanian smoke-affected Pinot Noir grapes, juice (free run and pressings) and wine produced using various winemaking techniques

Description	4-MG* (µg/L)	Guaiacol (µg/L)	o-cresol (µg/L)	p-cresol (µg/L)	m-cresol (µg/L)	Syringol (µg/L)	MSy* (µg/L)	Total (µg/L)
Grapes at harvest – April 2019	5	23	12	<1	4	<2	<2	44
rosé free run juice	2	17	5	<1	5	6	<1	35
rosé juice pressings	4	25	8	<1	7	3	<1	47
rosé wine - 50 L ferment	5	27	10	2	8	10	9	71
rosé wine - bulk ferment (250 L)	4	21	7	1	6	9	7	55
Full bodied red wine	10	45	19	3	12	22	12	123
Whole bunch ferment**	10	50	18	3	15	15	15	126
rosé wine** –after carbon treating juice	2	3	1	2	<1	2	7	17

*4-MG = 4-methylguaiacol; MSy = methylsyringol; ** winemaking performed by a winery in Tasmania

3.1.9 Combining enzyme treatment and carbon fining of wine as a strategy to manage smoke-damaged grapes

To obtain wine for sensory studies and validate the laboratory-based experiments for glycosidase treatments at a larger scale, a smoke-affected full-bodied Pinot Noir wine was used for winery trials with the glycosidase enzyme, Trenolin Bouqet, combined with subsequent fining of wine with activated carbons to reduce volatile phenols in wine.

The impact of the enzyme treatment of wine on phenolic glycosides is summarised in Figure 25. As expected, individual and total phenolic glycosides decreased with enzyme treatment, to approximately half the concentration in the control wine (i.e. total phenolic glycosides decreased from 220 to 117 µg/L SyGG equivalents). Mirroring the results from laboratory benchtop trials, fining wine with activated carbons alone failed to remove phenolic glycosides from wine. Glycosidase enzyme treatment of wine, followed by carbon fining, led to concentrations of phenolic glycosides which were comparable to concentrations obtained by enzyme treatment alone (Figure 25).

Carbon fining of wine decreased the concentrations of individual volatile phenols and total volatile phenols (Figure 25).The enzyme treatment increased the total volatile phenols compared to the control wine from 132 to 146 µg/L; however, the volatile phenol concentration could be reduced by carbon fining in a dose-dependent manner. Combining glycosidase treatment with carbon fining reduced volatile phenols compared to enzyme treatment alone, but led to increased concentrations of volatile phenols in wine when compared to the wine treated by carbon alone, which is consistent with a release of volatile phenols by the added glycosidase enzyme (Figure 26). Increased volatile phenols is a trade-off in this case for reducing levels of glycosides, which may release smoky phenols when the wine is tasted (see discussion below; Figure 27).

The various treatments had some impact on the colour of the Pinot Noir wines. CIELab measurements (data not shown) demonstrated that red colour was slightly enhanced with enzyme treatment alone. Carbon fining the wine with FPS, whether it was added exclusively or post-enzyme treatment, also resulted in wines with slightly enhanced red colour. In contrast, wines treated with PS1300, regardless of whether it was added solely or in conjunction with enzyme, showed less red colour.

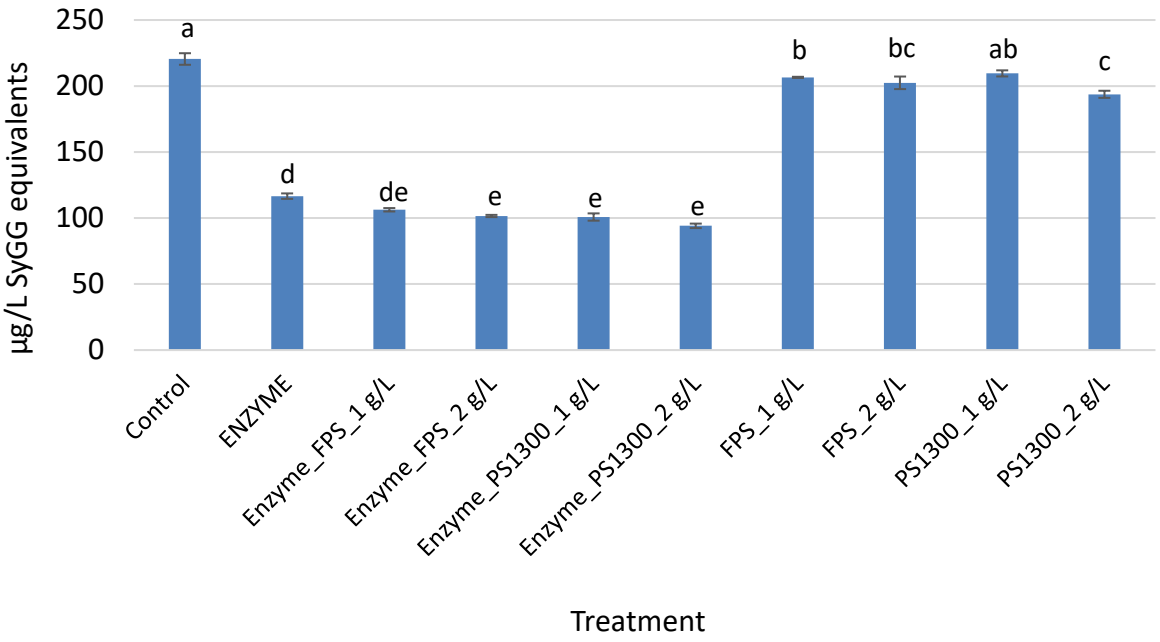


Figure 25. Total phenolic glycosides (n=6) in the 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine after treatment with either enzyme (Trenolin Bouquet Plus), activated carbon (PS1300 or FPS) or a combination of both. Results are post-bottling and an average of three analytical replicates. Different letters indicate statistical differences among treatments ($p<0.05$).

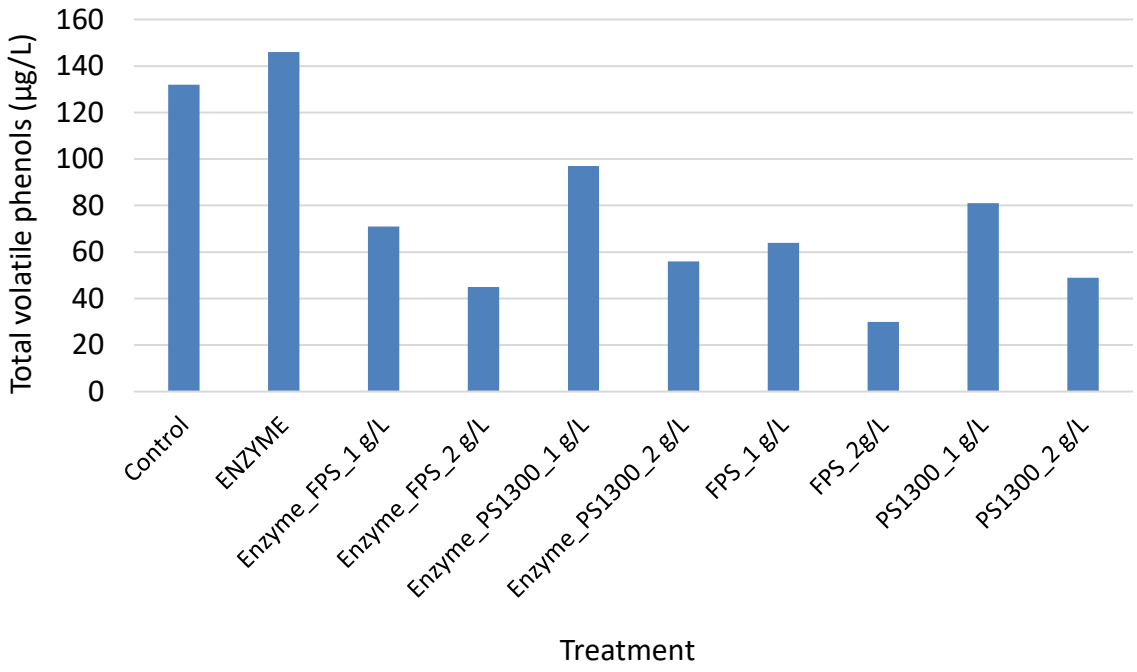


Figure 26. Total volatile phenols (n=7) in the 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine after treatment with either enzyme (Trenolin Bouquet Plus), activated carbon (PS1300 or FPS at 1 or 2 g/L) or a combination of both. Results are post-racking after clarification with bentonite.

Sensory effects of combined enzyme treatment and carbon fining of Pinot Noir wine

Mean smoke taint sensory scores from 12 judges for the 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine after treatment with either enzyme (Trenolin Bouquet Plus), carbon (PS1300 or FPS at 1 or 2 g/L) or a combination of both demonstrated that the carbon treatments (with or without prior glycosidase enzyme addition) were more effective in reducing smoke taint ratings than the enzyme treatment alone. Wines that had been treated with the enzyme were still reported as having obvious 'smoke' aroma/flavours ('campfire', 'ash', 'bitterness', etc.). While for many of the treatments a decrease of 'smoke' intensity was observed, wines were also described as appearing very stripped and lacking positive characters ('fruit' aroma/flavours), which is consistent with previous knowledge about sensory effects of carbon fining.

Notably, the pattern observed for the average smoke taint score for each of the treatments in Figure 27 mirrors that observed for the total volatile phenols in Figure 26 which suggests that the very high volatile phenol concentrations in this particular red wine (which reflect the volatile phenol concentrations in the grape juice prior to fermentation) are having an obvious influence on smoke taint scores. The increase in volatile phenols in enzyme-treated wine would also explain the increase of the smoke taint scores after the enzyme-only treatment compared to the control wine; this suggests that enzyme treatment would be best suited to red wine with elevated phenolic glycosides but otherwise low or only slightly enhanced free volatile phenol concentrations.

As a side aspect, the variation in smoke taint ratings amongst the group of judges is indicative of the variability amongst individuals in rating smoke-related sensory attributes, with some tasters being more sensitive than others. This variation amongst judges is also reflected in the large standard errors of the means (Figure 27).

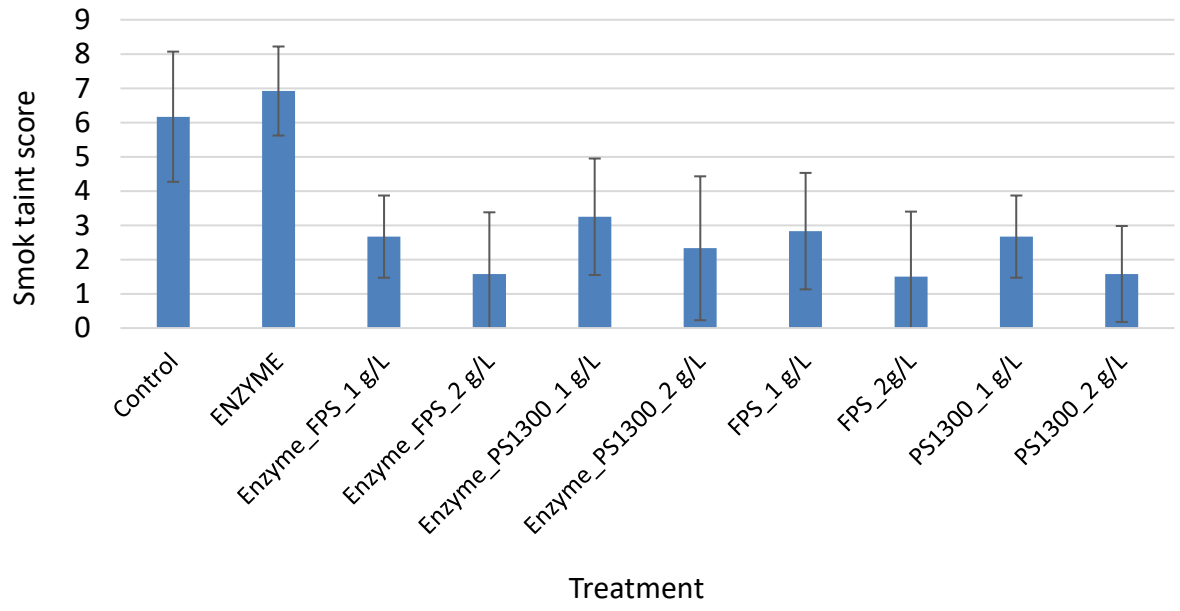


Figure 27. Mean smoke taint sensory scores from 12 judges for the 2019 smoke-affected Tasmanian full-bodied Pinot Noir wine after treatment with either enzyme (Trenolin Bouquet Plus), activated carbon (PS1300 or FPS at 1 or 2 g/L) or a combination of both

3.1.10 Sensory effects in wines made from Pinot Noir rosé or Chardonnay juice after carbon fining

A quantitative descriptive analysis was conducted to assess the sensory effects of removing smoke taint compounds from juice prior to fermentation. From the nested ANOVA for the Pinot Noir wines in the study, eleven attributes rated by the panel differed significantly (P<0.05) among the treatments (Table 32). These attributes included pink colour (colour intensity), 'hazy', 'strawberry' aroma, 'confection/floral' aroma, 'tropical' aroma, 'smoke/Band-Aid' aroma, 'cooked vegetable/drain' aroma, acidity, 'strawberry' flavour, 'fresh grass' flavour and 'smoke/Band-Aid' flavour. There was a significant fermentation replicate nested in treatment effect for pink colour and approaching significant effect for acidity. These significant and close to significant effects indicate variation between some fermentation replicates within the treatments. Notably, the CASPF 2 g/L treatment and the PS1300 4 g/L treatments were each found to have one variable replicate in each treatment (Figure 28). There were significant (P<0.05) judge by treatment interaction effects for several attributes indicating some concept alignment issues between some judges. Some judge and fermentation replicate variation is expected within sensory panels and fermentation replicates.

Table 32. F-ratios, probability values[†], degrees of freedom (df) and mean square error (MSE) from the analysis of variance of the Pinot Noir wines.

Attribute	Treatment	FRep (Treatment)	J* Treatment	J*FRep (Treatment)	PRep (Treatment, FRep)	MSE
Pink colour	169.5***	11.0***	4.14***	0.69	0.89	0.440
Hazy	13.4***	0.85	0.65	1.03	0.44	1.876
Strawberry A	6.96***	0.49	3.98***	0.72	1.42	1.644
Confection/floral A	2.37*	1.33	2.39***	0.71	1.09	2.111
Tropical A	4.55**	1.03	2.18**	1.12	1.11	1.452
Fresh grass A	1.75	0.87	2.13**	0.83	0.86	1.020
Smoke/Band-Aid A	76.5***	0.22	2.15**	1.22	1.14	1.529
Cooked vegetable/ drain A	13.0***	1.62	2.44***	1.09	0.87	2.046
Pungency	0.89	0.40	1.03	1.56*	0.86	0.751
Acidity	2.80*	1.83‡	2.37***	0.84	0.88	0.626
Astringency	1.05	1.25	1.97**	1.11	0.86	0.585
Hotness	0.62	0.51	1.43‡	1.10	0.88	1.302
Bitterness	0.70	1.59	1.82**	0.98	0.45	1.125
Viscosity	0.18	0.37	1.69*	1.99***	0.95	0.304
Strawberry F	4.67**	0.98	3.46***	0.99	2.05*	1.121
Fresh grass F	3.11*	0.36	1.69*	1.09	0.75	0.952
Smoke/Band-Aid F	56.9***	1.15	3.10***	0.82	0.79	1.671
df	6	7	60	70	14	140

A: aroma, F: Flavour. [†]Significance levels are as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ‡ $P \leq 0.10$. df = degrees of freedom. Judge effect was significant for all attributes ($P<0.05$), FRep = Fermentation replicate, PRep = Presentation replicate, J = Judge.

From the nested ANOVA for the Chardonnay wines from the study, seven attributes rated by the panel differed significantly (P<0.05) among the carbon treatments (Table 33). These attributes

included yellow (colour intensity), citrus aroma, smoke aroma, cooked vegetable/drain (reduction) aroma, viscosity, smoke flavour and flint flavour. The attribute confection/floral aroma was close to being significant (P<0.10). There were some significant fermentation replicate nested in treatment effects for pink colour, tropical aroma, confection/floral aroma and citrus flavour and approaching significance for Cooked Veg/Drain (reduction) aroma and hotness (alcohol sensation). These significant and close to significant effects indicate variation between some fermentation replicates within the treatments, which can be assessed in Figure 29. There were significant (P<0.05) judge by treatment interaction effects for several attributes indicating some concept alignment issues between some judges.

Table 33. F-ratios, probability values[†], degrees of freedom (df) and mean square error (MSE) from the analysis of variance of the Chardonnay wines.

Attribute	Treatment	FRep (Treatment)	Judge	J* Treatment	J*FRep (Treatment)	PRep (Treatment, FRep)	MSE
Yellow	33.67***	10.90***	26.69***	2.49***	0.84	0.91	0.384
Tropical A	1.53	2.44*	17.61***	1.72*	1.04	0.96	1.289
Citrus A	3.41**	0.68	45.93***	1.15	1.17	0.74	0.675
Fresh Grass A	1.25	0.92	13.48***	2.05**	0.79	1.40	1.286
Confection/Floral A	1.92ǂ	2.23*	1.57	2.31***	1.07	1.27	2.030
Smoke A	6.27***	0.58	4.88***	2.98***	0.80	0.91	2.729
Flint A	1.40	1.12	12.11***	1.31	1.00	0.37	2.245
Cooked Veg/Drain A	6.07***	2.04ǂ	16.57***	1.02	1.20	2.43**	2.241
Pungency	0.58	1.59	45.25***	1.22	0.91	1.42	0.884
Acidity	1.38	0.92	53.19***	1.12	0.94	0.86	0.670
Astringency	1.80	0.89	70.98***	1.29	0.72	1.25	0.793
Hotness	1.56	2.04ǂ	21.13***	1.94**	0.77	0.79	1.155
Bitterness	1.40	1.09	18.53***	1.26	0.70	1.23	1.604
Viscosity	3.62**	1.22	42.28***	0.88	0.94	0.92	0.618
Tropical F	1.05	1.75	15.72***	2.22***	0.94	0.78	0.950
Citrus F	1.21	2.21*	18.41***	2.29***	0.75	0.71	0.806
Smoke F	6.49***	0.36	8.44***	2.13**	1.23	1.06	1.831
Flint F	2.52**	0.30	19.96***	0.81	1.57**	0.86	1.635
df	6	7	11	66	77	14	154

A: aroma, F: Flavour. [†]Significance levels are as follows: * *P*≤0.05; ** *P*≤0.01; *** *P*≤0.001; ǂ *P*≤0.10. df = degrees of freedom., FRep = Fermentation replicate, PRep = Presentation replicate, J = Judge.

For the Pinot Noir wines the Principal Component Analysis (PCA) for both the treatments and the treatment fermentation replicates (Figure 28 and Figure 29) was created using the attributes that were significantly different (P<0.05) and close to significant (P<0.10) across the seven treatments within each variety.

For the sensory analysis of Pinot Noir wine in Figure 28, PC1 and PC2 explained 73.3% and 16.9% of the variation in the data. Beyond the second PC, eigenvalues were below 1 and explained little variability in the data.

Wines with the highest concentration of carbon treatments were plotted to the right of Figure 28, being rated highly in those attributes highly positively loaded on PC1, notably 'cooked veg/drain' (reduction) aroma, acidity and hazy appearance. These wines were also rated as low in the attributes 'strawberry' aroma and flavour and 'confection/floral' aroma. Conversely, wines from the control treatment were plotted to the left of the figure, being rated low in 'cooked veg/drain' (reduction) aroma, acidity and hazy appearance and rated highly in the attributes pink colour and 'smoke/band-aid' aroma.

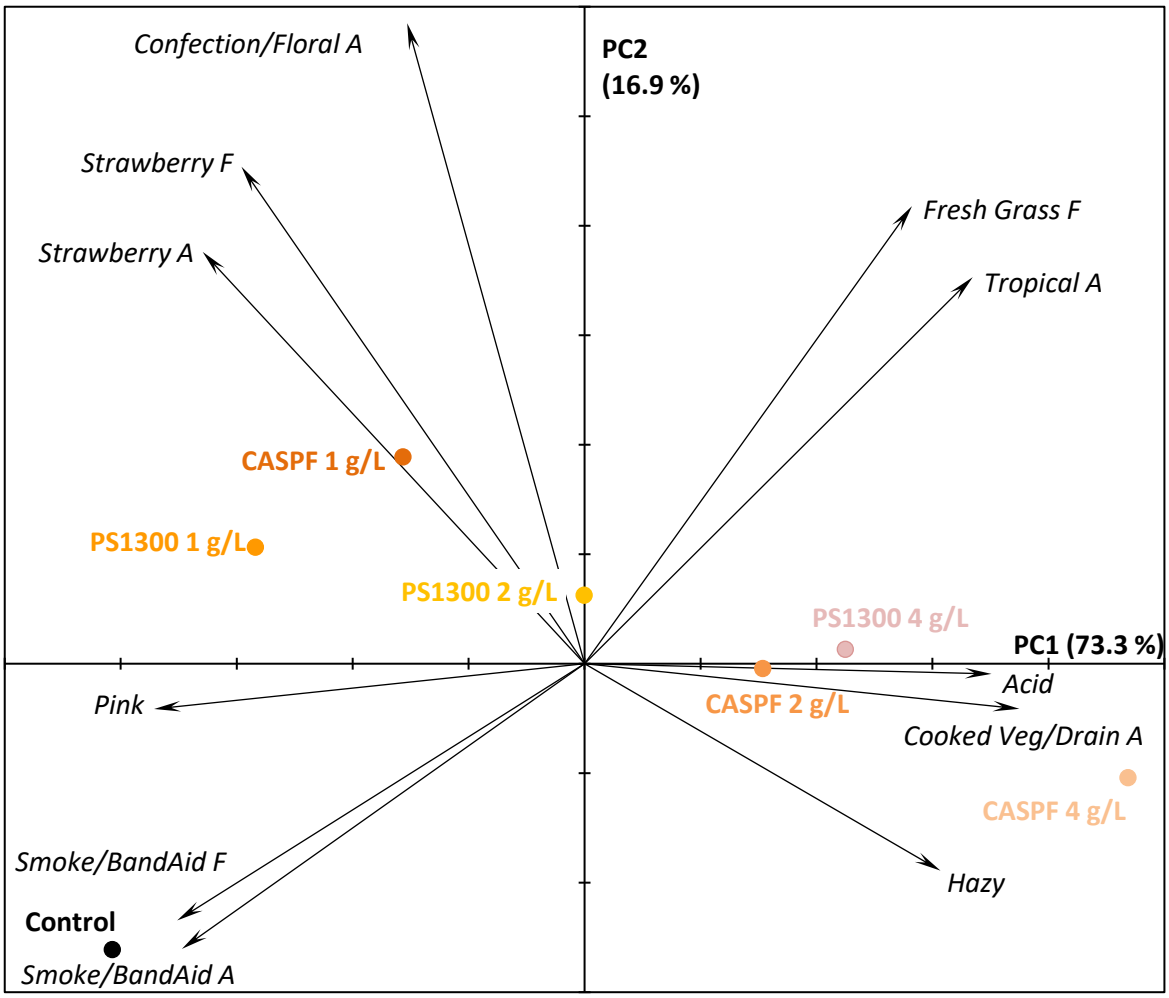


Figure 28. Principal component biplot of significant (P<0.05) and close to significant sensory attributes (P<0.10) from the Pinot Noir wine treatment means

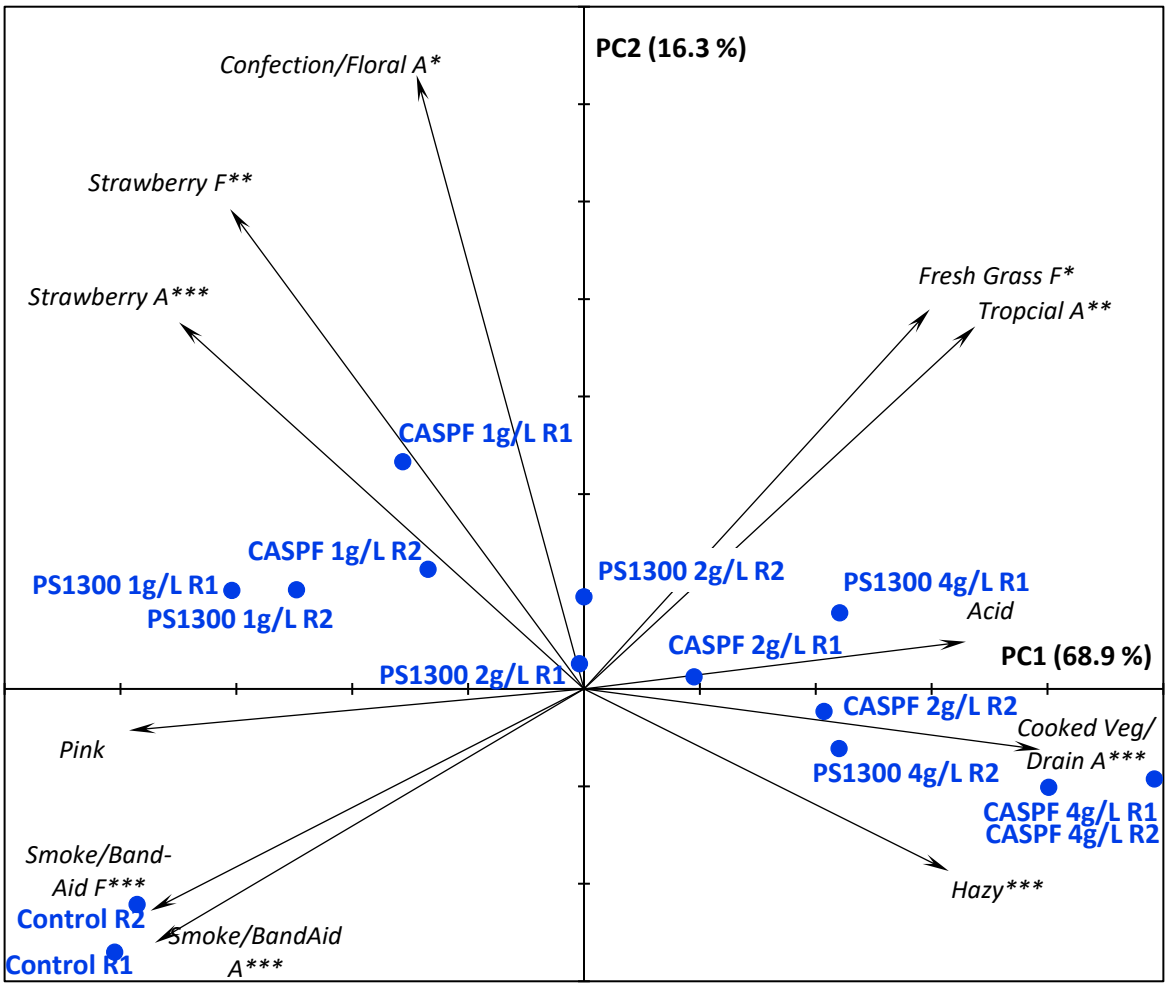


Figure 29. Principal component biplot of significant (P<0.05) and close to significant sensory attributes (P<0.10) for the Pinot Noir wine treatment replicates

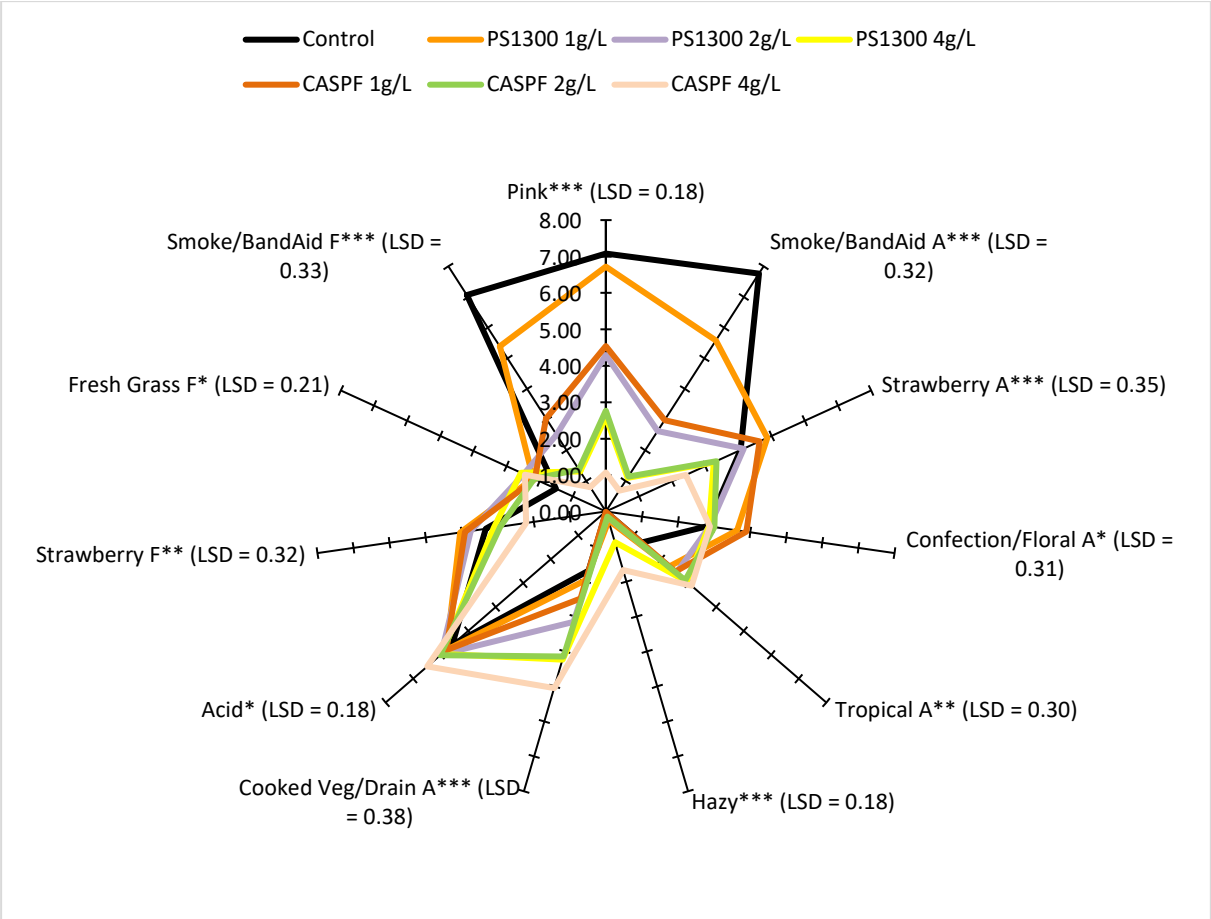


Figure 30. Mean attribute intensity scores for significant (*P<0.05; **P<0.01; ***P<0.001) and approaching significance (ǂ P< 0.10) attributes for the seven Pinot Noir wines. LSD (P=0.05) values included for the significant attributes (P<0.05)

Figure 30 shows that the lowest concentration of activated carbon used for juice fining had a significant effect, while the higher carbon additions were clearly different from the control in each of the attributes presented. While the highest dose treatments were able to reduce the severity of 'smoke' aromas and flavours in the wine, many of the pleasant 'fruit/floral' attributes also decreased. In addition, the intensity of 'cooked veg/drain' aroma increased with the higher carbon treatments. Only the CASPF 4 g/L treatment had 'smoke/band aid' aroma and flavour rating of less than 1.0 (i.e. no such taint noticeable).

For the Chardonnay wines in Figure 31 PC1 and PC2 explained 65.2% and 21.6% of the variation in the data. Beyond the second principal component eigenvalues were below 1 and explained little variability in the data.

Wines from the high carbon treatments were again plotted to the right of Figure 31, being rated highly in those attributes highly positively loaded on PC1, notably 'flint' flavour and 'cooked veg/drain' (reduction) aroma. Conversely, wines from the control treatment were plotted to the left of the figure, being rated low in these attributes and rated highly in attributes negatively loaded on PC1, notably yellow colour and 'smoke' aroma and flavour. Wines from the low treatment were scored moderately high in these negatively loaded attributes and low in positively loaded attributes,

resulting in a central positioning. The vertical separation of the treatment replicates was determined by the intensity scoring of 'citrus' aroma and 'confection/floral' aroma attributes, loaded negatively and positively on PC2, respectively.

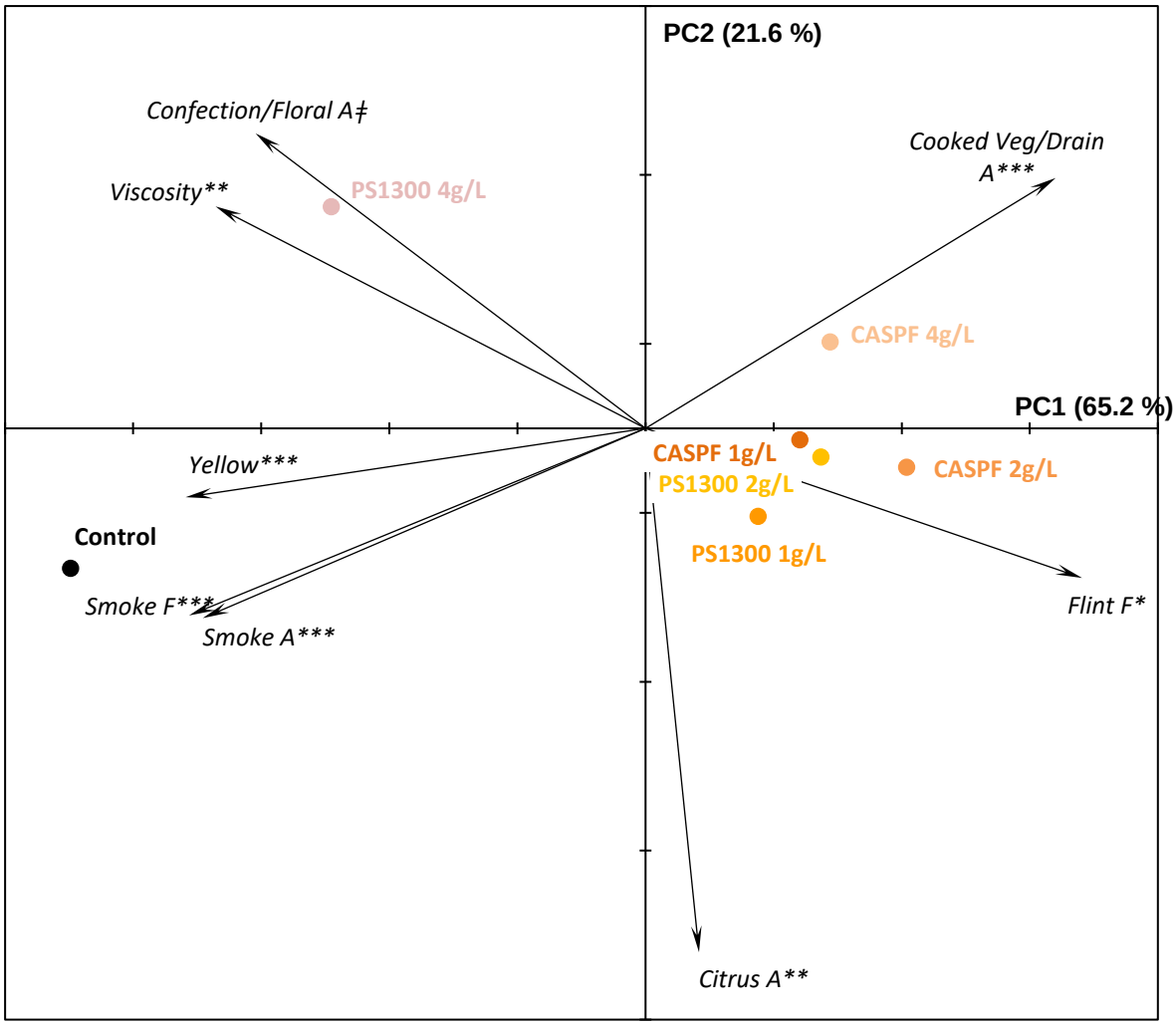


Figure 31. Principal component biplot of significant (P<0.05) and close to significant sensory attributes (P<0.10) for the Chardonnay treatments

Figure 32 shows some similar trends for the Chardonnay wines made after carbon fining Chardonnay juice as were seen in the Pinot Noir wines. The highest carbon dose treatments were able to reduce the severity of 'smoke' aromas and flavours in the wine, with many of the pleasant 'fruit/floral' attributes also decreasing. However, in the Chardonnay treatments, the differences were mostly significant between the control and the highest dose treatments, with the lower dose treatments not significantly different from one another. None of the treatments reduced the 'smoke' attributes to a score below 1.0, indicating some 'smoke' flavour remained in the treated wines.

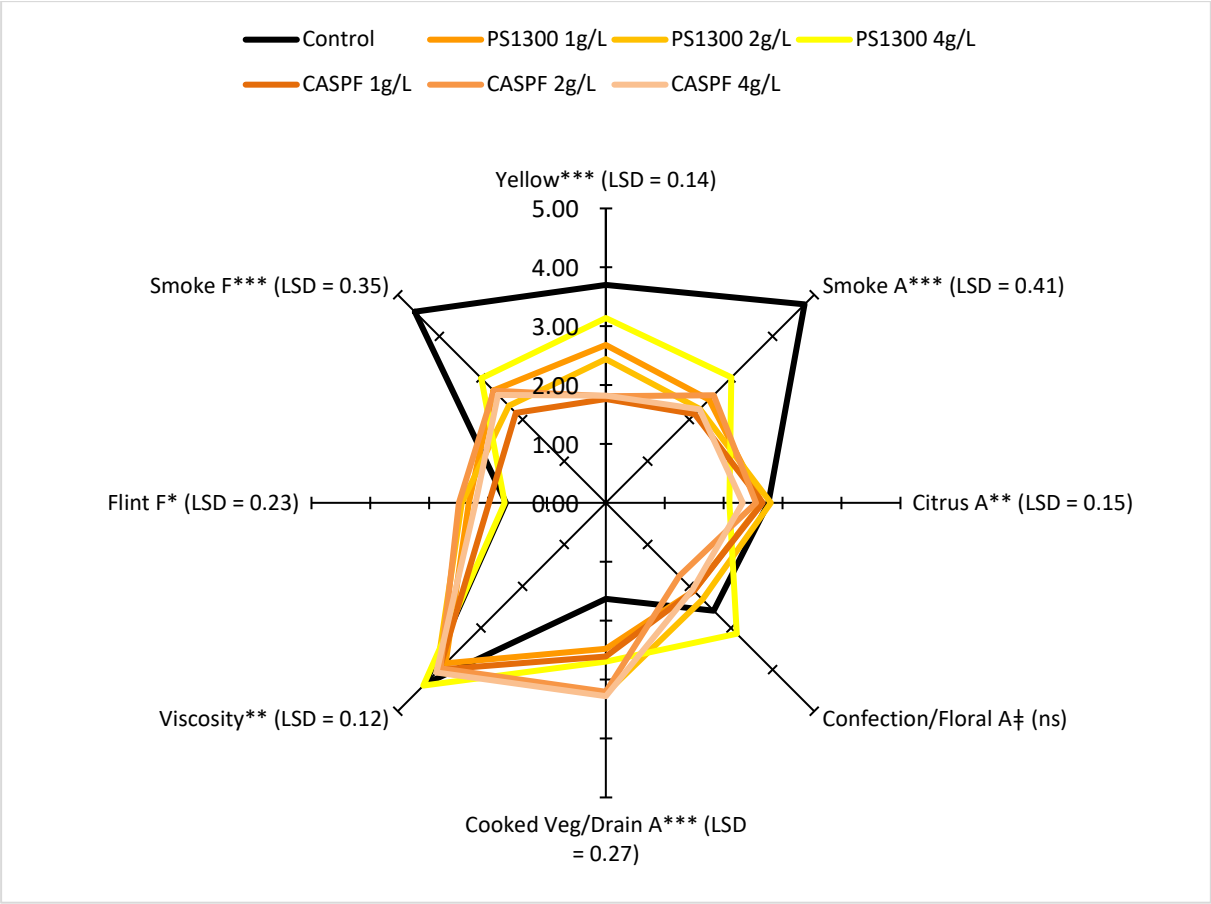


Figure 32. Mean attribute intensity scores for significant (*P<0.05; **P<0.01; ***P<0.001) and approaching significance (‡ P< 0.10) attributes for the seven treatments in the Chardonnay samples. LSD (P=0.05) values included for the significant attributes (P<0.05). ns = not significant

3.1.11 The impact of blending on sensory characteristics of a 2016 smoke-affected Pinot Noir wine

A sensory study was conducted to evaluate the effects of blending (i.e. dilution of smoke-affected wine with unaffected wine) to diminish or eliminate the sensory impact of smoke taint in a Pinot Noir wine. A 2016 smoke-affected Pinot Noir wine was sequentially diluted with unaffected Pinot Noir of a similar style sourced from the same vintage. The unaffected wine was selected after a preliminary sensory assessment of several candidate Pinot Noir wines, together with consideration of basic chemical composition.

Analysis of variance demonstrated significant differences ($p<0.05$) among wine blends for all four attributes (Table 34): overall fruit aroma, smoke aroma, 'overall fruit' flavour and 'smoke' flavour. Judges were a significant source of variation ($p<0.01$) for 'overall fruit' aroma and flavour. The Judge effect was not significant for 'smoke' aroma and flavour indicating the expert panel was well trained to scale and rank these taint attributes. Presentation replicate was a significant source of variation ($p < 0.05$) for 'overall fruit' flavour. Judge*Wine interaction was a significant source of variation for 'overall fruit' aroma ($p < 0.05$) and 'smoke' flavour ($p < 0.01$), indicating that individual judges have different levels of sensitivity to the compounds responsible for these attributes or lack of concept alignment. Judge*PRep interaction was a significant source of variation for 'smoke' aroma ($p<0.05$) and 'smoke' flavour ($p < 0.01$), indicating some judges changed the way they were rating these attributes between repetitions.

Table 34. Probability values[†], degrees of freedom (df) and mean square error from analysis of variance for smoke taint dilution wines.

Attribute	Wine	Judge	PRep	Judge* Wine	Judge* PRep	Wine* PRep	MSE
Overall fruit A	0.008**	0.001***	0.201	0.02*	0.677	0.473	0.93
Smoke A	0.000***	0.636	0.178	0.139	0.036*	0.751	1.49
Overall fruit F	0.008**	0.003**	0.043*	0.094ǂ	0.978	0.335	0.93
Smoke F	0.000***	0.478	0.464	0.002**	0.007**	0.604	0.88
df	5	8	1	40	8	5	40

A: aroma, F: Flavour. [†]Significance levels are as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ǂ $p < 0.10$. df = degrees of freedom. PRep = Presentation Replicate, df = degrees of freedom, MSE = Adjusted Mean Square Error.

The mean data for each wine is shown in Table 35. The 100% smoke-affected wine was scored significantly higher ($p < 0.05$) in 'smoke' aroma and flavour than the unaffected wine and was the lowest scoring wine for 'overall fruit' aroma and flavour. The blends with 75% or greater unaffected wine were not significantly different from the unaffected wine for any of the attributes. Interestingly, the blends 3 and 4 had higher 'overall fruit' aroma and flavour scores than the unaffected wine, although not by a statistically significant margin. This may allude to a synergistic ability of some smoke-affected wine compounds to enhance overall intensity perception.

Table 35. Attribute mean scores for each wine blend

Blend	Overall fruit aroma	Smoke aroma	Overall fruit flavour	Smoke flavour
100% affected wine	1.77	5.52	1.88	5.42
Blend 1 - 50% NA	2.28	2.78	2.68	2.51
Blend 2 - 75% NA	2.88	1.17	2.77	1.03
Blend 3 - 87.5% NA	3.48	0.58	3.39	0.61
Blend 4 - 93.75% NA	2.98	0.66	3.27	0.38
100% not affected wine (NA)	2.98	0.47	2.70	0.67
HSD[†]	*0.96*	*1.22*	*0.96*	*0.93*

[†]Post-hoc Tukey HSD values denote the numerical difference between means needed to attribute significant difference (p=0.05).

The relationship between the percentage of unaffected wine added to the smoke-affected wine and the mean sensory scores, together with the concentration of smoke taint-related free phenols (methylguaiacol, guaiacol, methyl syringol, *o*-cresol, *p*-cresol, *m*-cresol, syringol) and glycosides (syringol gentiobioside, methylsyringol gentiobioside, phenol rutinoside, cresol rutinoside, guaiacol rutinoside, methylguaiacol rutinoside) for each wine can be seen in Figure 33. Mean smoke taint attribute trendlines for the affected wine and blends 1, 2 and 3 appear parallel to the calculated and measured phenol and glycoside value trendlines. This parallel relationship of chemical and sensory data strongly reinforces the link between the measured compounds as indicators of smoke taint. The lower range of mean smoke taint attribute scores trendlines appear to plateau after 87.5% of the blend contained unaffected wine, reinforcing that there were no significant sensory differences between these blends and the unaffected wine.

Overall, this study demonstrated that blending is an effective option for remediation of smoke-affected wine, with 25% or less of the affected wine in a blend with unaffected wine of a similar style, sufficient to lower the smoke taint intensity to be not significantly different from the unaffected wine alone. Depending on the commercial considerations of individual wine producers, some companies might prefer not to risk the 25% blend being released to the market, given the somewhat elevated 'smoke' aroma and flavour score above the unaffected wine. Consumer testing would provide more confidence regarding the limit, although as discussed above, the perceived taint varies widely between individuals.

It should be noted that further sample sets should be investigated to assess whether this result might be applicable more generally. The intensity of smoke taint, as well as the sensory properties of the unaffected wine used as the blending wine, will both influence the amount of unaffected wine required to dilute the taint sufficiently.

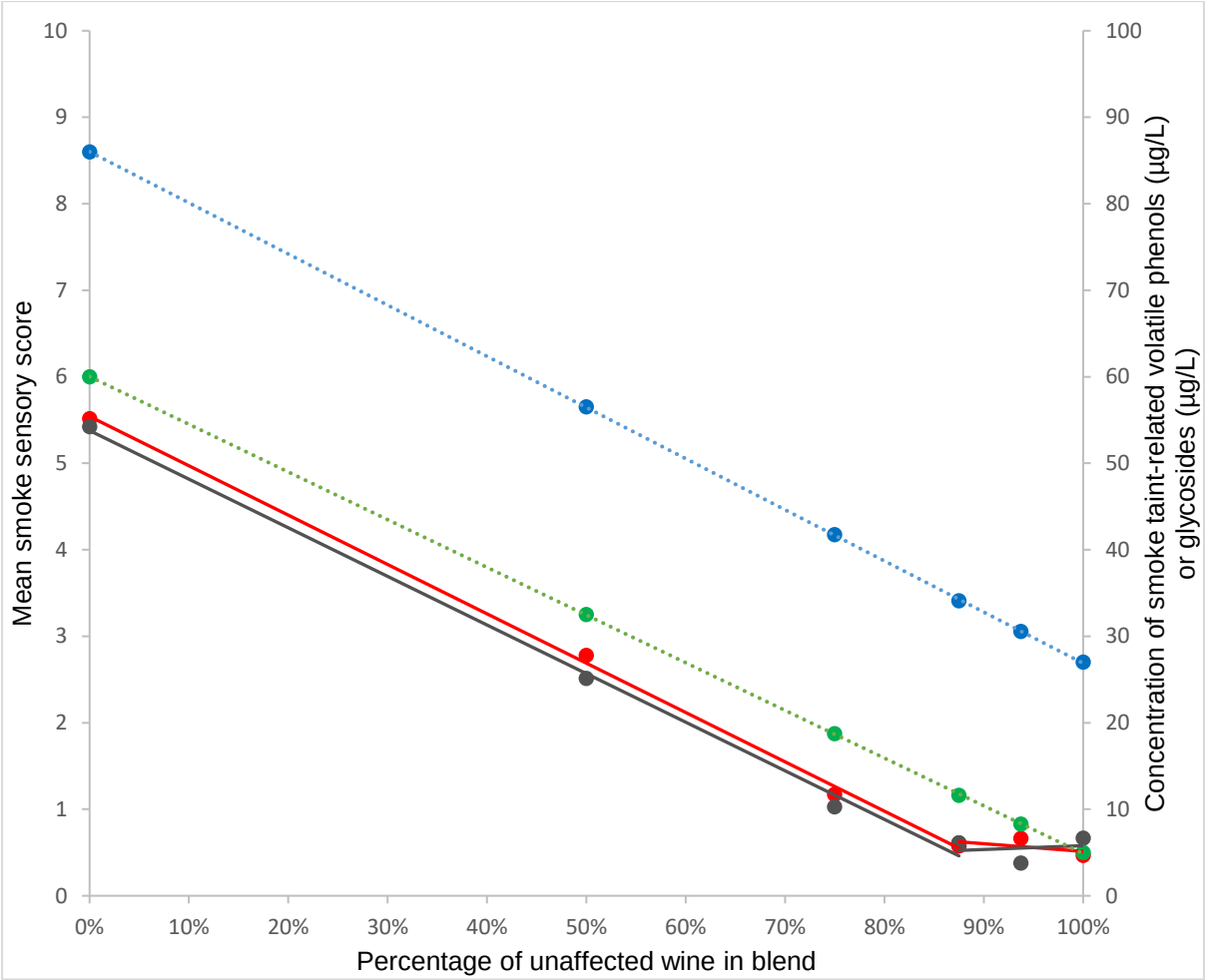


Figure 33. The relationship between the percentage of unaffected Pinot Noir wine added to the smoke-affected wine and the mean 'smoke' sensory aroma (●) and flavour (●) scores together with the concentration of smoke taint-related phenols (●) and glycosides (●) within each wine. Bivariate fit lines for 'smoke' aroma (—) upper concentration range [y = -5.70x + 5.54, R^2 = 0.9988] and lower concentration range [y = -0.89x + 1.40, R^2 = 0.3243]. 'Smoke' flavour (—) upper concentration range [y = -5.62x + 5.38, R^2 = 0.9968] and lower concentration range [y = -0.44x + 0.14, R^2 = 0.0328]. Linear trends for smoke taint-related phenols (.....) and glycosides (.....) [y = -59x + 86] and [y = -55x + 60], respectively.

3.1.12 The impact of blending wine on sensory characteristics of a smoke-affected 2019 Pinot Noir rosé wine

A further blending study evaluated the practical dilution of smoke-affected wine with unaffected wine to diminish or eliminate the sensory impact of smoke taint in a Tasmanian rosé Pinot Noir. It also compared the sensory effects of blending between ratings from an expert panel and consumer feedback.

For this study, a 2019 smoke-affected Pinot Noir rosé wine was sequentially diluted with unaffected Pinot Noir rosé of a similar style sourced from the same vintage. The unaffected wine was selected after a preliminary sensory assessment of several candidate Pinot Noir wines, together with consideration of basic chemical composition as well as smoke- and oak-related compounds.

Analysis of variance for the sensory data demonstrated that there were significant differences (p<0.001) among wine blends for all four attributes (Table 36): 'overall fruit' aroma, 'smoke' aroma, 'overall fruit' flavour and 'smoke' flavour. Judges were a significant source of variation (p<0.05) for 'overall fruit' aroma and flavour. Presentation replicate was not a significant source of variation (p < 0.05) for any of the attributes indicating consistency between sessions. Judge*Wine interaction was a significant source of variation for 'overall fruit' aroma (p<0.01), 'smoke' aroma (p< 0.01) and 'overall fruit' flavour (p<0.05), indicating that individual judges have different levels of sensitivity to the compounds responsible for these attributes or lack of concept alignment.

Table 36. Probability values[†], degrees of freedom (df) and mean square error from analysis of variance for smoke taint dilution wines.

Attribute	Wine	Judge	PRep	Judge* Wine	Wine* PRep	MSE
Overall fruit A	0.000***	0.000***	0.310	0.003**	0.995	1.047
Smoke A	0.037*	0.000***	0.721	0.006**	0.058‡	2.009
Overall fruit F	0.000***	0.000***	0.795	0.010*	0.320	0.935
Smoke F	0.002**	0.000***	0.815	0.119	0.371	2.765
df	5	10	1	50	5	60

A: aroma, F: Flavour. [†]Significance concentrations are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ‡ $P < 0.10$. df = degrees of freedom. PRep = Presentation Replicate, df = degrees of freedom, MSE = Adjusted Mean Square Error.

The mean data for each rosé wine is shown in Table 37. The 100% smoke-affected wine was scored significantly higher (p < 0.05) in smoke aroma and flavour than the unaffected wine and was the lowest scoring wine for overall fruit aroma and flavour. The blend with 75% unaffected wine was significantly different from the unaffected wine for smoke aroma and flavour only. The blends with greater than 75% unaffected wine were not significantly different from the unaffected wine for any of the attributes. Interestingly, blend 4 had higher overall fruit aroma and flavour scores than the unaffected wine, although not by a statistically significant margin. This may allude to a synergistic ability of some smoke-affected wine compounds to enhance overall intensity perception.

Table 37. Attribute mean scores for each wine blend

Blend	Overall fruit aroma	Smoke aroma	Overall fruit flavour	Smoke flavour
100% affected wine	2.16	6.65	1.84	7.01
Blend 1 - 50% unaffected	2.65	4.47	2.41	4.88
Blend 2 - 75% unaffected	3.63	2.38	3.38	2.30
Blend 3 - 87.5% unaffected	3.66	1.24	3.97	1.43
Blend 4 - 93.75% unaffected	4.31	0.53	4.15	0.73
100% unaffected wine	4.17	0.95	4.02	0.86
LSD[†]	*0.90*	*1.20*	*0.80*	*1.18*

[†]Post-hoc Fisher's least significant difference (LSD) values denote the numerical difference between means needed to attribute significant difference (p=0.05).

Overall, the study with rosé wine confirmed that blending is an effective option for the remediation of smoke-tainted wine and can reduce smoke aroma scores in a linear manner associated with the volume of tainted wine in the blend (Figure 34). As already seen for the dry Pinot Noir red wine above, a blend of 25% or less of the affected rosé wine with unaffected wine of a similar style was sufficient to lower the smoke taint intensity to be not significantly different from the unaffected wine alone. Depending on the commercial considerations of individual wine producers, some companies might prefer not to risk the 25% blend being released to the market, given the somewhat elevated smoke aroma and flavour score above the unaffected wine. Consumer testing would provide more confidence regarding the limit.

As with the dry Pinot Noir red wine above, further sample sets should be investigated to assess whether this result might be applicable more generally. It is likely that the intensity of smoke taint as well as the sensory properties of the unaffected wine used as the blending wine will both influence the amount of unaffected wine required to dilute the taint sufficiently.

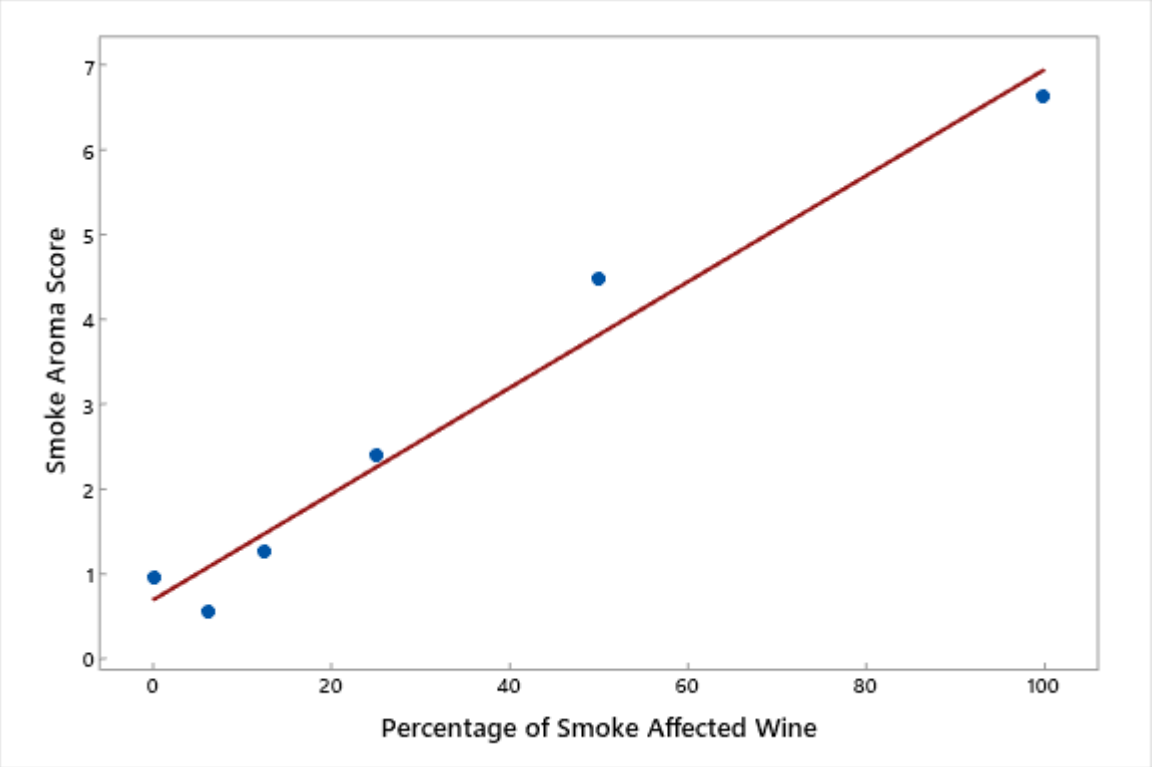


Figure 34. The relationship between the percentage of unaffected Pinot Noir wine added to the smoke-affected wine and the mean smoke sensory aroma

3.1.13 Consumer liking scores for smoke-affected and blended Pinot Noir rosé wines

The blended rosé wines made in the study described above were also used in a consumer preference study. The aim of this study was to complement the results from expert's sensory assessments and evaluate consumers' liking for a smoke-affected Pinot Noir rosé-style wine compared to the wine blended with varying proportions of an unaffected wine. A highly significant difference ($P<0.001$) was

found for the mean liking scores of the wines for the 82 consumers (Figure 35). That is, when all 82 consumers were considered as a single group, all diluted wines were significantly higher rated in liking compared to the undiluted smoke tainted wine. Overall, liking scores decreased with increasing proportion of smoke-affected wine. The least liked wine was the smoke-affected wine, with the blend of 25% of the unaffected wine also less liked than the unaffected wine. There were similar liking scores for the 0% wine and the 6.25% wine, with a slightly lower liking score for the 12.5% wine.

There was also strong evidence that clusters of consumers existed amongst the 82 participants. Cluster analysis identified three groups of consumers with significantly different liking scores (P<0.05) within each of the clusters.

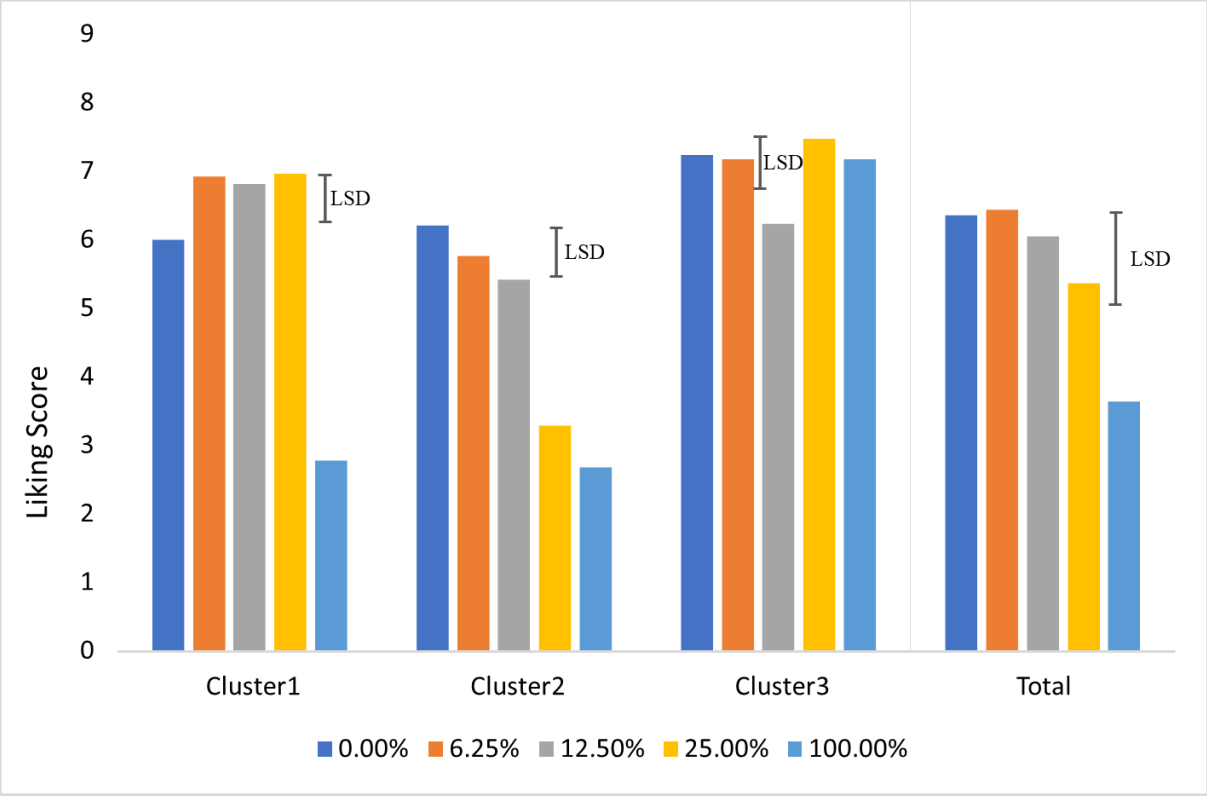


Figure 35. Mean consumer liking scores of 82 consumers. Cluster 1 (33%), Cluster 2 (46%), Cluster 3 (21%) and Total (100%) liking scores

Cluster 1 (33% of consumers) showed a significant difference in liking among the wines, with most of the wines being well liked (mean scores above 6.0), except for the 100% treatment sample which was significantly disliked. The 6.25%, 12.5% and 25% treatments were liked significantly more than the 0% clean wine (P=<0.001).

Consumers in the largest cluster (Cluster 2, 46% of consumers) most liked the clean wine, and this cluster had progressively lower liking scores for the wines with a greater percentage of smoke-affected wine. There was no significant difference between the 0% and 6.25% treatments, although the 12.5% treatment was liked significantly less than the 0% wine. The 25% and 100% treatments were both

significantly less liked than the 0%, 6.25% and 12.5% treatments. This cluster had the highest percentage of consumers with less than 10 years of drinking experience (24% of the cluster). The results from the total sample and for the largest cluster provide evidence that even a small proportion of smoke-affected wine in a blend can affect consumer preference.

Cluster 3 (21% of consumers) liked all the wines, suggesting that they were either not sensitive to smoke or enjoy the sensory characteristics it provides. There was a significant drop in liking for the 12.5% sample, but this was still well-liked. This cluster had the highest percentage of consumers with 20 or more years of drinking experience, and they were more likely to select a rosé wine as their first choice in different drinking scenarios.

Overall, the studies conducted in this project show that consumer perception of smoke taint varies widely. Whilst some smokiness will always be palatable to some consumers, it won't be at all palatable to others. This highlights the difficulty with defining a universally accepted, objective measure for smoke taint that can be used to accept or reject a wine, even for a rather simplistic model using dilution. In other words, any decision-making matrix will always lead to false positives (wines classified as 'tainted' but taste fine for some) and false negatives (wines classified as 'untainted', but not enjoyed by sensitive individuals). It will be challenging for the research community, industry and consumers to agree on a single objective measure for predicting smoke taint in wine.

3.2 Project level achievements

The project achieved or exceeded the activities, KPIs and outputs as specified in the agreement. Further detail is provided in 8.5 project evaluation report.

3.3 Contribution to program objectives

This project addressed the objectives of the Rural R&D for Profit program through collaborative research, development and extension, in support of continued innovation in Australia's primary industries. Specifically, the project successfully addressed the following program outcomes:

Generating knowledge, technologies, products or processes that benefit primary producers

Provide primary producers of wine grapes with a measurement of the risk of smoke exposure:

- Primary producers now have improved options for measurement of the risk of smoke exposure, due to the increased data available in the baseline database. The improved risk assessment is based on analysis of smoke exposure markers in suspect grapes and comparison to data for baseline concentrations in grapes from vineyards and multiple growing seasons without known exposure to smoke.

In the event that smoke exposure is unavoidable, provide growers with practical mitigation strategies to prevent smoke compounds from entering the grapes

- While no horticultural products could be identified in this project that provided effective protection to grapes, a detrimental role of some products in exacerbating uptake by grapes

of taint compounds from smoke was identified. This knowledge will be used to reduce the use of potential problem products during periods of smoke exposure of vineyards, and in the long term might be applied in the re-formulation of essential plant protection products for spray application.

- Fining of grape juice or wine with activated carbon was found to provide a practical option for the remediation of smoke-affected juice and wine at the winery, thereby minimising the need to reject smoke-affected fruit, increasing returns and lowering crop losses.
- The economic analysis identified which processing strategies and readily available products were most efficient and applicable.
- Together, the project's findings will improve certainty of supply and resilience of the Australian wine sector to bushfire impacts, protect Australia's wine reputation, enhance grower/business/industry profitability and Australia's competitiveness in global markets and contribute to the profitability of grapegrowers and wine producers, with associated economic and social benefits for rural communities.

Strengthening pathways to extend the results of rural R&D, including understanding the barriers to adoption

- The project team worked closely with industry partners from the start of the project and an Industry Reference group established at the beginning of the project was consulted regularly, to aid experimental design and guide project progress.
- Laboratory-based model studies were corroborated through field work in commercial vineyards (for example, in NSW in 2018), and grape, juice and wine samples were sourced from commercial producers (for example, in 2019 from Tasmania and Padthaway).
- Project findings have been shared with members of the Industry Reference Group, who acted as early adopters. In addition, key findings were communicated and discussed at industry briefing events in January 2020, for example with major producers such as Treasury Wine Estates, Accolade Wines and Pernod Ricard Winemakers. Wines made for this project have been used in multiple sensory training sessions, as part of the AWRI's extension efforts to support winemakers managing the consequences of the 2019/20 bushfire season.
- Well-established extension platforms have been effectively used, for example through a poster and short plenary presentation at the 17th Australian Wine Industry Conference in 2019, production of a fact sheet on carbon fining and publishing of associated articles in industry journals.
- Qualitative economic analyses assessed costs and benefits for growers, with reductions in costs following a smoke event achieved through the discontinuation of non-effective but costly practices (such as application of horticultural sprays in vineyards; enzyme application in grape juice) and an increased ability to reduce taint levels and reduce quality defects in wine.

Establishing and fostering industry and research collaborations that form the basis for ongoing innovation and growth of Australian agriculture

- This project attracted significant industry support at the individual business level with commercial vineyards and wine businesses included in the project as partners and collaborators. These partnerships have significant potential to contribute to ongoing innovation.
- The success of this project was made possible through close collaboration between Wine Australia and the AWRI and with researchers at Agriculture Victoria, University of South

Australia, University of Adelaide and grape and wine industry partners in Australia and wine industry suppliers in Australia and overseas.

- Many of these are new collaborations, with some organisations working together for the first time. Based on previous experience, it is expected that a number of productive collaborations consolidated through this project with 'new' partners such as Metabolomics Australia and CSIRO Atmospheric Chemistry will continue past the project end date.

3.4 Productivity and profitability

The economic evaluation of remediation activities focused on the application of activated carbon for fining of grape juice to reduce smoke taint in wine, as the other treatment strategies (horticultural sprays for protection of vines and use of enzymes to degrade smoke taint compounds in wine) did not result in obvious improvements. The cost/benefit analysis of mitigation options for smoke taint at the winery is attached to this report.

Overall, the increase in value, reduction in losses and improvements to profitability that are achievable will be a function of many variables, including: severity of smoke exposure; grape varieties and wine styles produced; and distribution of grape and wine prices. At the same time any value recoverable through fining of grape juice or wine (or through other mitigation techniques) may be offset by money spent on buying or producing grapes and other sunk costs prior to the mitigation treatment.

For this evaluation it is estimated that exposure of vineyards to smoke could result in recognisable contamination of grapes and/or tainted wine that is unfit for sale and has an impact on approximately 2% to 10% of the national harvest in each year. This equates to approximately 34,570 to 172,845 tonnes of grapes which might not be harvested, cannot be sold or result in tainted wine (11.3 ML white wine and 9.4 ML red wine under the 2% exposure scenario). Based on average 2019 grape prices ($845/tonne red grapes, $462/tonne white grapes) economic losses from value of grapes that cannot be realised would range from $23.2 million pa to $115.9 million pa. These estimates exclude additional costs associated with analytical testing, winemaking steps needed when dealing with at risk grapes, damage to brand image etc. As such, total losses will very likely exceed the above estimates based on average grape prices.

As demonstrated in the sensory studies described in section 3.1, fining of tainted wine with a low dose of a suitable activated carbon can reduce smoke taint, but might not always fully eliminate quality defects from wine. Hence this analysis explored three quality improvement scenarios together with blending options:

A) quality improvements to a wine that is otherwise unfit for sale leading to production of a dry bulk wine that can be sold at a 30% discount

B) quality improvements to a wine that is otherwise unfit for sale leading to a varietal bulk wine that can be sold at a 30% discount

C) quality improvements to a tainted dry wine leading to a varietal bulk wine that can be sold at a 30% discount

D) blending of improved wine made under B) or C) with clean varietal bulk wine. Note the blending option requires large volumes of blending wine and may not be suitable for many producers and styles.

The cost of the materials required for fining is very low ($3.70 to $17.00/1,000L wine, depending on dose rate and choice of product) and the impact of wine volume losses (typically 2%) is also marginal, given the low value of the wine prior to carbon fining. Addition of fining agents and their removal are standard operations in winery, do not require specialised equipment or significant extra labour inputs.

Another key variable that impacts on value recovered is the extraction rate, or in other words the volume of wine produced from 1 kg of grapes. For this analysis the extraction rate was assumed to be 0.6 L wine/kg grapes, which is a conservative assumption for achievable extraction rates for smoke-tainted grapes and is positioned between extraction rates of 0.5 L wine/kg grapes (where free run juice only is used which may be lower in some taint compounds) and common extraction rates of 0.75 L wine/kg grapes (free run juice plus pressings which are likely to be higher in taint compounds and more difficult to improve).

Key risks to producers after carbon fining (as for any other mitigation option) include that the resulting wine cannot be sold - despite suitable quality attributes - because of damage to brand image and/or negative perception associate with smoke exposure, and a lack of market demand or pre-existing oversupply in the bulk market. There is therefore a risk that producers might incur additional costs which cannot be recovered and would further increase their losses.

Where there is a sufficient market for bulk wine, wine made after carbon fining of grape juice will contribute to cashflow and assist in regaining some of the value otherwise lost. The relative reduction in losses (or value recovered) can be in the range of 38 to 96% when based on the cost of wine which is otherwise not fit for sale.

Finally, the overall increase in value, reduction in losses and improvements to profitability that are achievable will also be a function of prize paid for grapes (or cost of producing grapes) which varies widely between < $300 to $2000+ for grapes from different varietals, for different quality brackets, and between warm and cool/temperate regions (Wine Australia, National Vintage Report 2019). Not surprisingly, the biggest gains can be anticipated from carbon fining of juice from white varietals, given that 71% of fruit from white varieties sold in 2019 was in the $300 to $600 category. Considering the overall higher grape prices for red varietals (62% in the $600 -$1500 category; 12% in the $1500-$2000+ category), together with the detrimental impact carbon fining has on red wine colour, it is estimated that the aggregate value recoverable from ameliorating smoke-tainted red wine would be similar to value recovered from smoke-exposed white grapes and smoke tainted white wine, but the relative reduction in losses is likely significantly smaller for most red wine compared to white wine.

4 Collaboration

The success of this project was made possible through many collaborations with researchers at Agriculture Victoria, DPI NSW, University of South Australia, University of Adelaide, Metabolomics Australia, the University of Nottingham and grape and wine industry partners in Australia and wine industry suppliers in Australia and overseas.

Agriculture Victoria
Researchers at Agriculture Victoria supported the model studies of horticultural sprays as part of investigations of vineyard mitigation options and strategies to reduce the uptake of smoke taint compounds in the vine and grapes.

DPI NSW
The Department of Primary Industries in NSW (DPI NSW) provided grape samples from a field trial of sunscreen products in a vineyard planted with Pinot Noir clones 777 and MV6. After the vineyard unexpectedly experienced smoke exposure from a nearby bushfire, the effects of smoke exposure on grapes that had been treated with the horticultural protectants VaporGard or Surround could be assessed.

UniSA
A range of zeolites were supplied by Professor Krasimir Vasilev, School of Engineering, University of South Australia. These zeolites were evaluated for their ability to remove smoke phenolic glycosides from juices; however, none was found to be effective.

University of Adelaide
The AWRI collaborates with the University of Adelaide's Industrial Transformation Training Centre on smoke taint research. Assistance has been provided for two PhD projects, one investigating whether in-canopy misting can reduce the uptake of smoke molecules in grapes and a second investigating the use of cyclodextrins for mitigating smoke taint in juice and wine.

In addition, researchers from the University of Adelaide assisted project staff with model smoking experiments in 2018 and 2019, and the model studies on excised grape bunches.

A new collaboration with the University of Adelaide, PIRSA, Grains Producers SA, SA Grains Industry Trust and La Trobe University commenced in 2019 with funding obtained from the South Australian Wine Industry Development Scheme to assess the potential impact of smoke from stubble burns on grapes and wine.

Metabolomics Australia
The project provided Dr Natoiya Lloyd (Metabolomics Australia) with a range of fruit, juice and wine samples for analysis using mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. Dr Lloyd's research was funded through the '2017 Science and Innovation Awards for Young People in Agriculture, Fisheries and Forestry' and aimed to explore metabolomics approaches for identifying additional unknown components that might be contributing to smoke taint.

CSIRO Atmospheric Chemistry
Sensing techniques for establishing spatial variability of smoke and risk profiles for vineyards within a smoke-exposed region.

University of Nottingham
A visiting student from the University of Nottingham spent 4.5 months at the AWRI. As part of her PhD research, two novel glycosidases, (i) *Halothermothrix orenii* (Hal) β-glycosidase; and (ii) *Alicyclobecillun Acidiphilas* (Aci) β-glycosidase were evaluated for their abilities to cleave smoke glycosides in two smoke-affected Pinot Noir wines. Results showed that these glycosidases were not able to cleave smoke glycosides in real wine, perhaps a consequence of them being inhibited by natural wine pH.

Industry partners
A number of industry partners participated in the projects's industry reference group, contributed grape and wine samples for analysis and testing, and provided contract winemaking services to the project.

The AWRI assisted a large winery during the 2019 vintage by providing some insights from laboratory-scale experiments which enabled the winery to treat 400,000 Litres of juice, made from grapes where there was concern about smoke impact. The resulting wine was not smoke tainted and was usable in the winery's larger blend.

Wine industry suppliers in Australia and overseas
Suppliers provided access to commercially available activated carbon materials and glycosidase enzymes.

5 Extension and adoption activities

The extension activities over the life of the project are captured in the tables below summarising (i) external presentations, workshops and talks; (ii) industry reference group meetings; and (iii) articles, posters and factsheets.

In brief, presentations were given in regions affected by smoke events, such as those in NSW during vintage 2018, Tasmania during vintage 2019 and the most recent bushfires in 2020 which affected multiple grapegrowing regions throughout Australia. Workshops were also presented to educate wine industry personnel on smoke taint (what is it and how to identify it). Many of these educational workshops involved tasting smoke tainted wines sourced or produced from this project. These workshops also provided a forum to disseminate information on options for remediating smoke taint in juice and wine.

Individual feedback and commentary from industry debriefings conducted post-vintage in 2020 (Hunter Valley, Adelaide Hills, N-W Victoria) highlighted the usefulness of the carbon fining and carbon factsheets as remediation tools.

Dissemination of information to industry was also achieved via the industry reference group, consisting of representatives from major wine companies, who met annually with the research team. Presentations at the 17th Australian Wine Industry Technical conference also provided an opportunity to disseminate information of research outcomes to a large audience. The AWRI's helpdesk has also received a large number of queries about smoke taint, particularly in regard to remediation of smoke tainted juice and wine using activated carbon. Information generated from the project has been relayed to industry personnel via the helpdesk.

Further adoption of project outcomes will be facilitated via publication in peer-reviewed journals, industry journals and additional roadshows and workshops.

External presentations, workshops and talks

Staff	Title of presentation or event	Presented to and where	Date
Mark Krstic	Impact of smoke exposure to vineyards	Wine Victoria/Department of Environment, Land, Water and Planning (DELWP) Annual Planned Burning Forum, Melbourne, Vic	14 Dec 2016
Mark Krstic	Insights into vineyard and winery smoke taint mitigation options	Yarra Valley smoke forum, Yarra Valley, Vic	8 Dec 2017
Mark Krstic Ian Porter	Outline of smoke taint project	Wine Victoria/Department of Environment, Land, Water and Planning (DELWP) Annual Planned Burning Forum, presented to 25 industry personnel and Victorian Government/DELWP managers, Melbourne, Vic	14 Dec 2017
Julie Culbert Geoff Cowey	Smoke taint Q&A including sensory training to assist in detecting smoke taint	Smoke taint Q&A workshop, Orange, NSW	20 Feb 2018
Julie Culbert Geoff Cowey	Smoke taint Q&A including sensory training to assist in detecting smoke taint	Smoke taint Q&A workshop, Mudgee, NSW	21 Feb 2018
Mark Krstic Geoff Cowey Con Simos Katie Dunne Ian Porter	Smoke taint Q&A	Local wine producers and DELWP regional planned burn managers, Macedon Ranges, Vic	27 Jun 2018

Staff	Title of presentation or event	Presented to and where	Date
Tim Plozza			
Con Simos Mark Krstic	Smoke taint Q&A	Smoke taint Q&A workshop, Cambridge, Tas	13 Feb 2019
		Smoke taint Q&A workshop, Hobart, Tas	
Julie Culbert	Evaluating activated carbons for removal of phenols and their glycoconjugates from smoke-affected juice and wine	Fresh Science session at the 17th Australian Wine Industry Technical conference, Adelaide, SA	23 Jul 2019
Julie Culbert Eleanor Bilogrevic Damian Espinase Nandorfy Desiree Likos Leigh Francis	Summary of outcomes of R&D4P smoke taint project followed by a sensory evaluation session on smoke tainted wines	13 winemakers, held at the AWRI, Urrbrae, SA	13 Jan 2020
Markus Herderich	Adelaide Hills bushfire recovery meeting	Grape growers and winemakers, Adelaide Hills, SA	8 Jan 2020
Markus Herderich Mark Krstic	Smoke taint Q&A	Pernod Ricard Winemakers, SA	22 Jan 2020
Markus Herderich Mark Krstic Eric Wilkes Con Simos	Smoke taint and testing Q&A	Wine Australia, SA	23 Jan 2020

Staff	Title of presentation or event	Presented to and where	Date
Markus Herderich Mark Krstic	Smoke taint Q&A	Accolade Wines, SA	24 Jan 2020
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Wine industry personnel, Pokolbin, NSW	24 Jan 2020
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Wine industry personnel, Adelaide Hills, SA	29 Jan 2020
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Treasury Wine Estates, SA	31 Jan 2020
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Wine industry personnel, Mudgee, NSW	4 Feb 2020
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Wine industry personnel, Orange, NSW	5 Feb 2020
Julie Culbert	Smoke taint remediation in the winery (major findings from the smoke taint R&D4P project)	Managing Bushfire Risk 2020 Forum held by Wine Tasmania, Hobart, Tas	11 Feb 2020
Julie Culbert	Smoke taint remediation in the winery (major findings from the smoke taint R&D4P project)	Managing Bushfire Risk 2020 Forum held by Wine Tasmania, Launceston, Tas	12 Feb 2020

Staff	Title of presentation or event	Presented to and where	Date
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Wine industry personnel, Yarra Valley, Vic	14 Feb 2020
Roadshow team	Smoke taint screening tasting – wines obtained or produced during the R&D4P smoke taint project were used for training purposes	Wine industry personnel, Canberra, ACT	19 Feb 2020

Industry reference group meetings

Staff	Title of event	Held	Date
Julie Culbert Mark Krstic Markus Herderich Ian Porter Tim Plozza	Project update to industry representatives on project progress and future directions	Teleconference – multiple locations	6 Dec 2017
Julie Culbert WenWen Jiang Mark Krstic Markus Herderich Ian Porter Tim Plozza	Project update to industry representatives on project progress and future directions	Teleconference – multiple locations	29 Oct 2018
Julie Culbert WenWen Jiang Mark Krstic Markus Herderich Ian Porter Tim Plozza	Final update on smoke taint project outcomes	Teleconference – multiple locations	10 Dec 2019

Industry representatives: Paul Smart (Wine Tasmania); Alex Sas (Accolade Wines); Louisa Rose (Yalumba); Brett McClen (Brown Brothers); Adrian Coulter (AWRI); Gioia Small (TWE); Sharon Harvey (Wine Australia); Damien Sheehan (Mt Langi/Wine Victoria); Larry Jorgensen (Wines of WA); Mandy Gerhardy (TWE); Warren Birchmore (Accolade) and Matt Holdstock (AWRI helpdesk).

Articles, posters and fact sheets

Staff	Title of poster/article/factsheet	Presented at	Date
Julie Culbert Mark Krstic Markus Herderich	Article: Smoke taint remediation – R&D update	Published in Technical Review No. 230, pages 7-9	Oct 2017
Adrian Coulter	Article: Smoke taint: analysis and interpretation	Published in Technical Review No. 234, pages 511	Jun 2018
Julie Culbert WenWen Jiang Mark Krstic Markus Herderich	Poster: Does the application of horticultural products to grapes form a protective barrier against smoke taint?	17th Australian Wine Industry Technical conference, Adelaide, SA	21-24 Jul 2019
Julie Culbert WenWen Jiang Mark Krstic Markus Herderich	Poster: Evaluating activated carbons for removal of phenols and their glycoconjugates from smoke-affected juice and wine	17th Australian Wine Industry Technical conference, Adelaide, SA	21-24 Jul 2019
Julie Culbert Mark Krstic Markus Herderich Ella Robinson	Fact sheet: Treating smoke-tainted must or wine with activated carbon	Available on the AWRI website	Feb 2020
Con Simos Mark Krstic	Article: I can smell smoke – now what?	Published in Aust. N.Z. Grapegrower & Winemaker, Issue 672, 28-31	Jan 2020

6 Lessons learnt

There were significant delays to the contracting at the initiation of this project. This placed pressure on the project team, especially during the first year of the project. Despite the delay in the start phase it was not possible to extend the project end date beyond January 2020; this meant that no field work and only a small number of extension activities could be conducted under this project in the 2019/2020 bushfire season.

7 Recommendations

Future research is warranted:

- to assess consequences of early-season smoke exposure on mature grapes and wine, to support producers in earlier decision-making
- to continue the evaluation of commercially available enzymes, activated carbons and other emerging mitigation technologies
- to identify drivers of selectivity and binding capacity of activated carbons and further enhance the performance of best-performing carbons for removal of taint compounds
- to identify transport form(s) of taint compounds in air and use this knowledge to adopt remote sensing techniques and rapid analytical technologies for establishing spatial variability and risk profiles for vineyards within a smoke-exposed region.

Additional adoption and extension activities are also encouraged, including establishing regional sensory panels and improving the sensory training of winemakers through hands-on experiences with smoke-affected wines and wines from winery-based mitigation experiments.

8 Appendix - additional project information

8.1 Project, media and communications material and intellectual property

Media interviews

17 Jan 2017	M.P. Krstic, C.A. Simos Smoke taint and the sensory impact on wine Sally Davies, ABC Country Hour, Tas
29 Sep 2017	M.J. Herderich Glucosylation of smoke-derived volatiles in grapevine (Vitis vinifera) by a promiscuous resveratrol/guaiacol glucosyltransferase Doug Main, Scientific American
14 Oct 2017	M.P. Krstic Smoke taint in wine Jancis Robinson, Financial Times and JancisRobinson.com
18 Oct 2017	M.J. Herderich Smoke taint threat of wildfires to California wineries Jesse B. Staniforth, Food Quality & Safety, USA
30 Oct 2017	M.P. Krstic Smoke taint in wine Lexi Williams, Wine Spectator; Amanda Gallagher, Western Fruit Grower, USA
7 Dec 2017	M.P. Krstic Smoke taint in wine Danielle Beurteaux, NPR 'The Salt' blog, USA
21 Mar 2018	M.J. Herderich Smoke taint management Lauren Wolf, ACS C&EN news
13 Feb 2019	M.P. Krstic, C.A. Simos Smoke taint Hugh Hogan, ABC Tasmania; Mark Smith, TasmanianTimes.com
20 Feb 2019	M.P. Krstic Smoke taint in Tasmania Emily Jarvie, Australian Community Media
22 Feb 2019	M.P. Krstic Smoke in Tasmania Huon Hooke, huonhooke.com
6 Mar 2019	M.P. Krstic Stubble burning collaborative project Stuart Taverner, Barossa and Light Herald
8 Mar 2019	M.P. Krstic Smoke taint in Tasmania Amanda Ducker, News Corp Australia
22 Mar 2019	M.P. Krstic Smoke taint in Victorian wineries Sumeyya Ilanbey, The Age
26 Mar 2019	M.P. Krstic Smoke taint/stubble burning project Richard Whitehead, Beverage Daily
23 Oct 2020	M.P. Krstic Transfer of volatiles from plants near vineyards Kerana Todorov, Wine Business Monthly
14 Jan 2020	M.P. Krstic Smoke research at the AWRI Mike Cherney, Wall Street Journal
16 Jan 2020	M.J. Herderich New research on early-season smoke exposure with Adelaide Hills producers Andrew Spence, The Lead SA
22 Jan 2020	M.P. Krstic Smoke research at the AWRI Mike Cherney, Wall Street Journal
22 Jan 2020	M.P. Krstic Smoke research at the AWRI Richard Whitehead, Beverage Daily

In addition, a number of presentations, articles, posters and fact sheets have been prepared and disseminated as described above in section 5. Extension and adoption activities.

8.2 Equipment and assets

Equipment: None acquired.

Assets: Smoke-tainted and treated wine from the 2019 vintage which will be used for industry sensory training purposes.

8.3 References

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8.4 Staff

Project staff: Gayle Baldock, Adrian Coulter, Dr Julie Culbert, Dr Markus Herderich, WenWen Jiang, Dr Mark Krstic

AWRI sensory team: Dr Leigh Francis, Damian Espinase Nandorfy, Eleanor Bilogrevic, Desiree Likos, members of the sensory panels

8.5 Evaluation report

Has the project addressed the Rural R&D for Profit outcomes and measures?

This project addressed the objectives of the Rural R&D for Profit program through collaborative research, development and extension, in support of continued innovation in Australia's primary industries. Specifically, the project successfully addressed the following objectives:

Provide primary producers of wine grapes with a measurement of the risk of smoke exposure

- Primary producers now have improved options for measurement of the risk of smoke exposure, due to the increased data available in the baseline database. The improved risk assessment is based on analysis of smoke exposure markers in suspect grapes and comparison to data for baseline concentrations in grapes from vineyards and multiple growing seasons without known exposure to smoke.

In the event that smoke exposure is unavoidable, provide growers with practical mitigation strategies to prevent smoke compounds from entering the grapes

- While no horticultural products could be identified in this project that provided effective protection to grapes, a detrimental role of some products in exacerbating uptake by grapes of taint compounds from smoke was identified. This knowledge will be used to reduce the use of potential problem products during periods of smoke exposure of vineyards, and in the long term might be applied in the re-formulation of essential plant protection products for spray application.
- Fining of grape juice or wine with activated carbon was found to provide a practical option for the remediation of smoke-affected juice (and wine to a lesser degree) at the winery, thereby minimising the need to reject smoke-affected fruit, increasing returns and lowering crop losses.
- The economic analysis identified which processing strategies and readily available products were most efficient and applicable.
- Together, the project's findings will improve certainty of supply and resilience of the Australian wine sector to bushfire impacts, protect Australia's wine reputation, enhance grower/business/industry profitability and Australia's competitiveness in global markets and contribute to the profitability of grapegrowers and wine producers, with associated economic and social benefits for rural communities.

Strengthening pathways to extend the results of rural R&D, including understanding the barriers to adoption

- The project team worked closely with industry partners from the start of the project and an Industry Reference group established at the beginning of the project was consulted regularly, to aid experimental design and guide project progress.
- Laboratory-based model studies were corroborated through field work in commercial vineyards (for example, in NSW in 2018), and grape, juice and wine samples were sourced from commercial producers (for example, in 2019 from Tasmania and Padthaway).
- Project findings have been shared with members of the Industry Reference Group, who acted as early adopters. In addition, key findings were communicated and discussed at industry briefing events in January 2020, for example with major producers such as Treasury Wine Estates, Accolade Wines and Pernod Ricard Winemakers. Wines made for this project have

been used in multiple sensory training sessions, as part of the AWRI's extension efforts to support winemakers managing the consequences of the 2019/20 bushfire season.

- Well-established extension platforms have been effectively used, for example through a poster and short plenary presentation at the 17th Australian Wine Industry Conference in 2019, production of a fact sheet on carbon fining and publishing of associated articles in industry journals.
- Qualitative economic analyses assessed costs and benefits for growers, with reductions in costs following a smoke event achieved through the discontinuation of non-effective but costly practices (such as application of horticultural sprays in vineyards; enzyme application in grape juice) and an increased ability to reduce taint levels and reduce quality defects in wine.

Establishing and fostering industry and research collaborations that form the basis for ongoing innovation and growth of Australian agriculture

- This project attracted significant industry support at the individual business level with commercial vineyards and wine businesses included in the project as partners and collaborators. These partnerships have significant potential to contribute to ongoing innovation.
- The success of this project was made possible through close collaboration between Wine Australia and the AWRI and with researchers at Agriculture Victoria, University of South Australia, University of Adelaide and grape and wine industry partners in Australia and wine industry suppliers in Australia and overseas.

- Many of these are new collaborations, with some organisations working together for the first time. Based on previous experience, it is expected that a number of productive collaborations initiated through this project with 'new' partners such as Metabolomics Australia and CSIRO Atmospheric Chemistry will continue past the project end date.

8.6 Attachments

8.6.1 Factsheet: Treating smoke-tainted must or wine with activated carbon

8.6.2 Factsheet: Remediation of smoke-affected wine by dilution

8.6.3 Cost/benefit analysis of mitigation options for smoke taint at the winery

The Australian Wine Research Institute

Fact Sheet
WINEMAKING

Treating smoke-affected juice or wine with activated carbon



Background

When grapes are exposed to smoke, they can absorb volatile phenols, which bind to sugars in the grapes forming non-volatile phenolic glycosides. In juice and wine, both volatile phenols and their glycosides can cause unpleasant 'ashy' and 'smoky' sensory sensations and a lingering aftertaste, commonly described as 'smoke taint'.

What is activated carbon and how can it be used to mitigate smoke taint?

Activated carbon products are highly porous carbon-rich materials used in applications such as filtration and water treatment. They are known to adsorb organic compounds, including undesirable contaminants such as the volatile phenols and phenolic glycosides associated with smoke taint. Activated carbon products can be used to treat smoke-affected juices (white or rosé) or wines; however, they also remove positive colour, aroma and flavour compounds.

Generally, phenolic glycosides are more prevalent than volatile phenols in juices or musts, whereas smoke-tainted wines often contain a combination of both volatile phenols and phenolic glycosides. It is important to select the right carbon product for the desired application (e.g. one product might be better at removing phenolic glycosides and another at removing volatile phenols).

Performance differences among different carbon products

The efficiency of activated carbon products in removing smoke taint compounds is highly dependent on:

(i) the type of carbon product used

(ii) the matrix (e.g. juice versus wine; red versus rosé or white)

(iii) the dose added.

Some activated carbon products are better at removing phenolic glycosides (Figure 1), and the removal occurs better in juice than wine. Other carbons preferentially remove volatile phenols in wine (Figure 2).

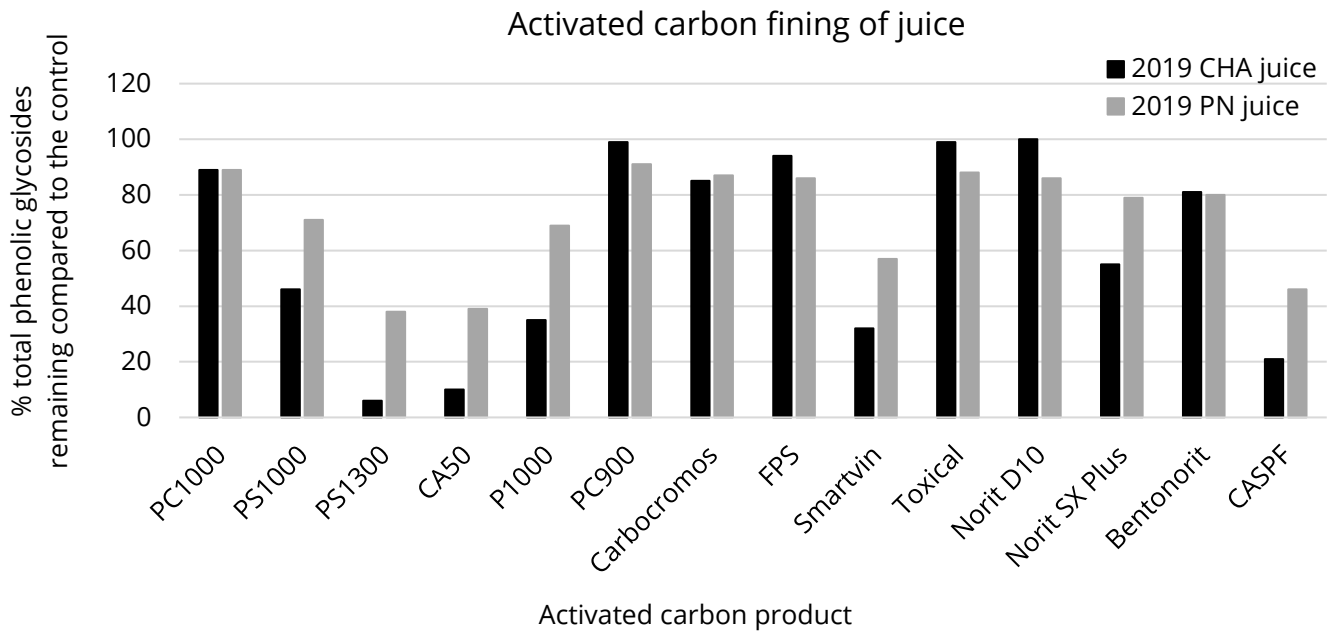


Figure 1. Comparison of the ability of 14 activated carbon products (dose 2 g/L, contact time 24 hours) to remove phenolic glycosides from two 2019 smoke-affected juices (CHA = Chardonnay; PN = Pinot Noir). Results are presented as percentage of phenolic glycosides remaining compared to the control (i.e. juice without carbon treatment). Starting concentrations of phenolic glycosides (sum of six common phenolic glycosides) for the CHA and PN juices were 251 and 335 µg/L SyGG equivalents, respectively.

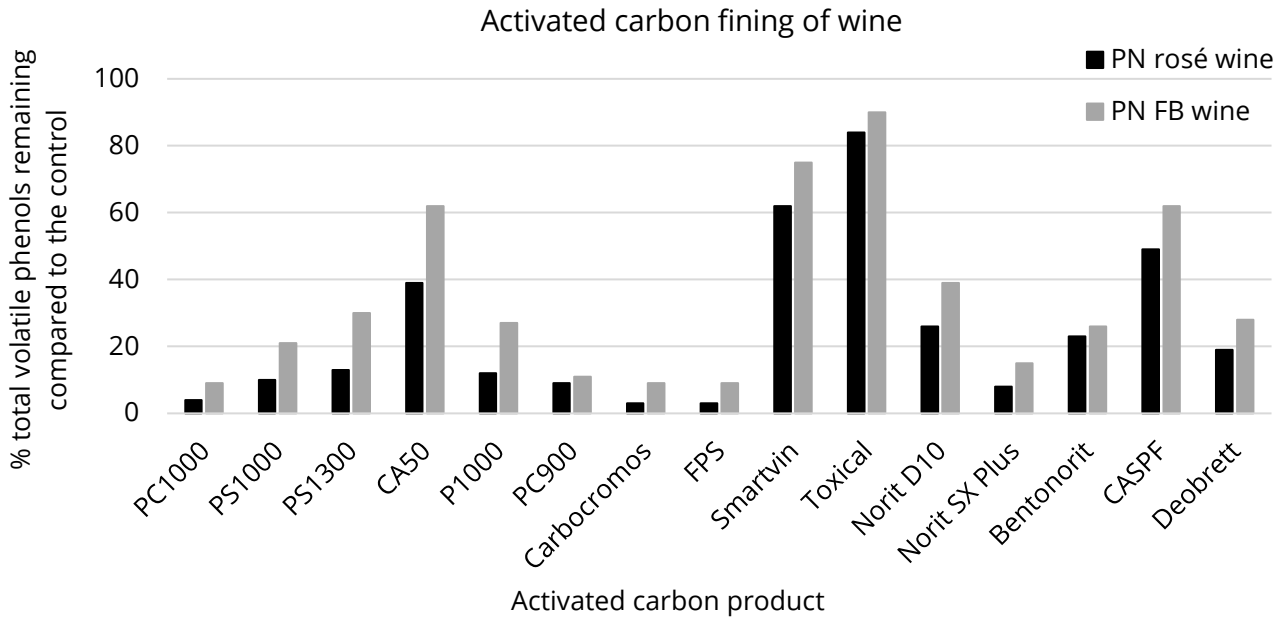


Figure 2. Comparison of the ability of 15 activated carbon products (dose 2 g/L, contact time 24 hours) to remove total volatile phenols (VPs) from 2019 smoke-affected Pinot Noir (PN) rosé and full-bodied (FB) wines. Results are presented as percentage of volatile phenols remaining compared to the control (i.e. wine without carbon treatment). Starting total volatile phenols (sum of guaiacol, 4-methylguaiacol, m-, o- and p-cresols, syringol and methylsyringol) for the rosé and full-bodied PN wines were 77 and 149 µg/L, respectively.

Assessing performance

The evaluation of the different activated carbon products was based on measurement of their ability to remove volatile phenols and phenolic glycosides from wine and juice.

For removal of glycosides from juice, the products PS1300 (Activated Carbon Technologies), CA50 (Carbochem), smartvin (Vason) and Norit CASPF (Cabot) performed best, removing 90% of the phenolic glycosides or more.

For removal of volatile phenols from both juice and wine, Carbocromos (Vason) and FPS (Vason) were among the best performers.

Overall, the activated carbon products were more effective at removing phenolic glycosides from juice than wine. For example, for two full-bodied Pinot Noir red wines, the reduction in phenolic glycosides, at best, was less than 20%, even at a dose of 2 g/L (data not shown). The activated carbon products fared slightly better in white and rosé wines, with some removing between 50 and 60% of the phenolic glycosides.

Greater success was observed when using activated carbons to remove volatile phenols from wine.

Impact of activated carbon treatment on sensory characteristics of wine

Carbon treatment of juice

In an experiment assessing the sensory effects of carbon fining, 2019 smoke-affected Chardonnay and Pinot Noir rosé juices were treated, in duplicate, with one of two carbon products (PS1300 and CASPF) at 1, 2 and 4 g/L prior to fermentation. Carbon contact time was 48 hours and inoculation occurred after juices were clarified with the assistance of bentonite. For the Pinot Noir rosé wines, eleven attributes were rated by the panel as being significantly different (P<0.05, Figure 3).

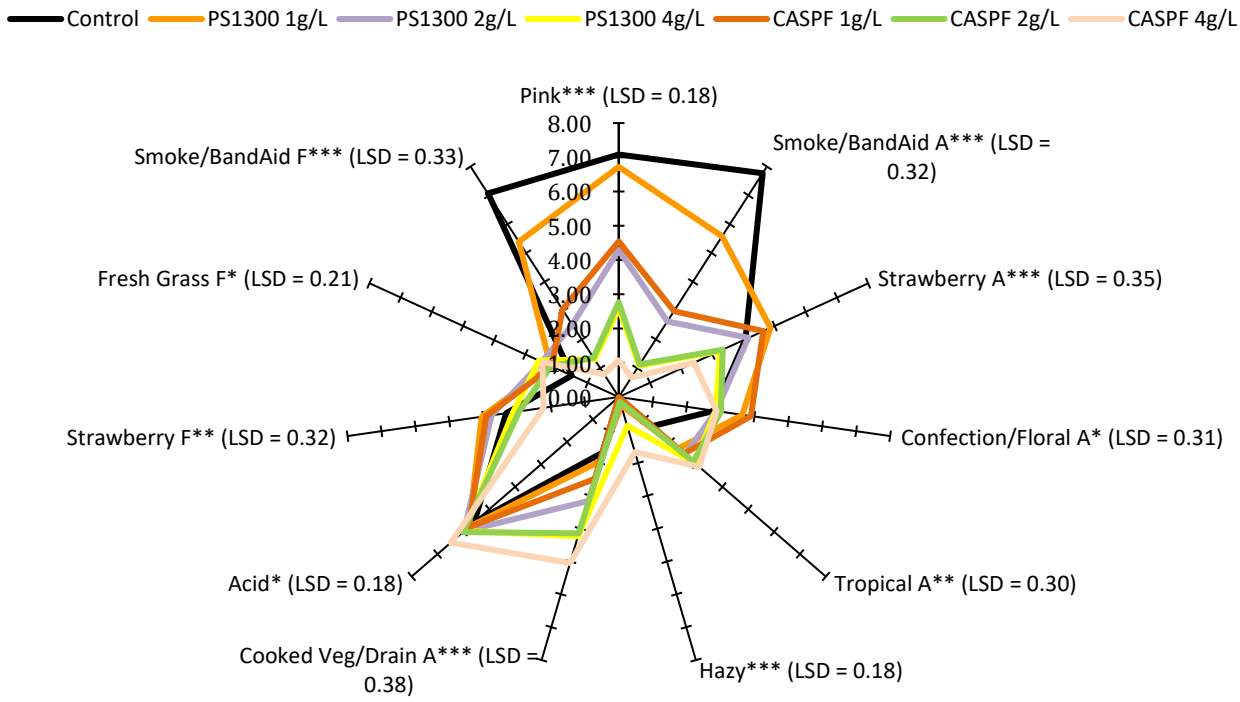


Figure 3. Mean attribute intensity scores for significant (*P<0.05; **P<0.01; ***P<0.001) and approaching significance (‡ P< 0.10) attributes for six Pinot Noir rosé wines made from carbon-treated juice and one control wine. LSD (P=0.05) values included for the significant attributes (P<0.05)

All activated carbon treatments in the trial decreased the intensity of smoke-related characters in the final wines to some extent. However, particularly at the higher doses, the carbon treatments also reduced the intensity of many of the pleasant 'fruit/floral' attributes. The intensity of 'cooked veg/drain' aroma also increased with the higher carbon treatments. The Pinot Noir rosé wines made from juices treated with 1 g/L of activated carbon had enhanced 'strawberry' aroma and flavour compared to the control while also having a reduction in smoke aroma and flavour. Note that the OIV recommends using carbon additions of less than 1 g/L for both juices and wine, with fining rates of 50 to 500 mg/L typically used for removal of odours. These results indicate that using activated carbons to remove smoke characters from wine will always be a balancing act between reducing smoky aromas and flavours, while retaining some desirable sensory attributes.

Carbon treatment of wine

A 2019 smoke-affected full-bodied Pinot Noir wine was treated with PS1300 or FPS at doses of 1 and 2 g/L. Carbon contact time was 48 hours and wines were clarified with the assistance of bentonite. Wine treated with activated carbon showed a reduction in smoke taint sensory ratings (rated on a scale of 0 to 9, Table 1), with a more pronounced effect seen at higher carbon doses.

Table 1. Smoke taint ratings for 2019 smoke-affected Pinot Noir wine with no treatment (control) and treated with activated carbon products (FPS or PS1300) at two doses (1 and 2 g/L)

Control (no carbon)	FPS 1 g/L	FPS 2 g/L	PS1300 1 g/L	PS1300 2 g/L
6.2	2.8	1.5	2.7	1.6

Similarly to smoke-affected juices, treating wine with activated carbon requires a balancing act between reducing smoky attributes while retaining desirable sensory attributes. Additions of activated carbons at less than 1 g/L might be appropriate in some wines for achieving this balance.

Recommendations/conclusions

Smoke-affected juices (especially white and rosé styles) can be effectively treated with activated carbon products prior to fermentation to reduce the intensity of smoke characters in the wines made from treated juices. The impact of carbon fining must be closely considered as the intensity of positive sensory attributes will also be reduced.

The selectivity and tendency to protect desirable aromas and flavours might differ between carbon products and needs to be considered prior to carbon fining of juice or wine.

The appropriate carbon addition will be dependent on the level of taint compounds in the juice. Chemical analysis for volatile phenols and phenolic glycosides is therefore recommended. Where possible, fruit should be analysed approximately two weeks before harvest, while undertaking in parallel mini-ferments of juice and/or carbon-fined juices followed by sensory assessment of the mini-ferments.

In respect to carbon fining of wine, it is recommended that evaluations of carbon treatments be performed on small volumes of wine to determine sensory impacts before treating larger quantities of wine. Further blending of carbon-treated wine with non-smoke-affected wine is another option to obtain a final wine with suitable sensory characteristics.

While all activated carbon products evaluated in this work were commercially available, it is also recommended that wineries contact product manufacturers to ensure that their product adheres to the relevant food standards code for use in wine production. Note that the OIV recommends carbon additions should be less than 1 g/L for both juices and wine. Carbon fining rates are more typically around 500 mg/L. The addition of larger quantities of carbon (>1 g/L) to juice and wine may remove excessive colour and flavours, making the sensory characteristics of the resulting wine

appear un-wine-like. In addition, for some carbon types, the addition of larger quantities could result in the release of metal compounds into the juice or wine.

Acknowledgement

This work was supported by the AWRI, and through funding from the Australian Government Department of Agriculture, Water and the Environment as part of its Rural R&D for Profit program and Wine Australia.

The AWRI is a member of the Wine Innovation Cluster.

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The Australian Wine Research Institute

Fact Sheet
WINEMAKING

Remediation of smoke-affected wine by dilution



Background

When grapes are exposed to smoke, they can absorb volatile phenols, which bind to sugars in the grapes, forming non-volatile phenolic glycosides. In juice and wine, both volatile phenols and their glycosides can cause unpleasant 'ashy' and 'smoky' sensory sensations and a lingering aftertaste, commonly described as 'smoke taint'.

Dilution studies on smoke-affected wine

Dilution with an unaffected wine is one option for managing a smoke-affected wine, with the goal of diminishing or eliminating smoke-related sensory characters. This option was evaluated in a trial using a smoke-affected 2019 Pinot Noir rosé wine. The smoke-affected wine was blended with an unaffected Pinot Noir wine of a similar style sourced from the same vintage, to produce a dilution series of six samples: 100% smoke-affected wine, 50%, 25%, 12.5%, 6.25% and 0% (equivalent to 100% unaffected wine). Wines were assessed by members of the AWRI's technical quality panel for 'smoke' aroma and flavour and 'overall fruit' aroma and flavour (Table 1).

As expected, the 100% smoke-affected wine was scored significantly higher ($P < 0.05$) in 'smoke' aroma and flavour than the unaffected wine and was the lowest scoring wine for 'overall fruit' aroma and flavour. Dilutions of the affected wine with 75% or more unaffected wine resulted in 'smoke' aroma and flavour scores not significantly different from the unaffected wine. The reduction in 'smoke' aroma and flavour with increased dilution followed a linear pattern (Figure 1).

Table 1. Attribute mean scores for each wine blend. 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.

Blend	Overall fruit aroma	Smoke aroma	Overall fruit flavour	Smoke flavour
100% smoke-affected wine	2.16	6.65	1.84	7.01
Blend 1 - 50% smoke-affected wine	2.65	4.47	2.41	4.88
Blend 2 - 25% smoke-affected wine	3.63	2.38	3.38	2.30
Blend 3 - 12.5% smoke-affected wine	3.66	1.24	3.97	1.43
Blend 4 - 6.25% smoke-affected	4.31	0.53	4.15	0.73
100% unaffected wine	4.17	0.95	4.02	0.86
LSD†	*0.90*	*1.20*	*0.80*	*1.18*

†Post-hoc Fisher's least significant difference (LSD) values denote the numerical difference between means needed to attribute significant difference (p=0.05).

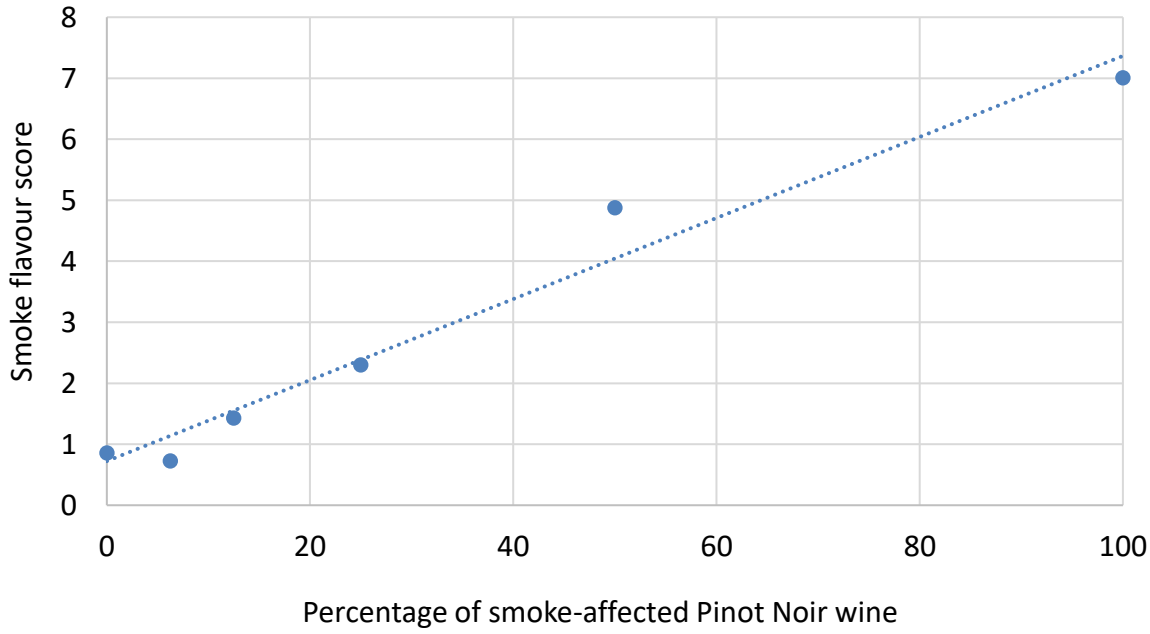


Figure 1. The relationship between the percentage of smoke-affected wine in the blend and the mean score for 'smoke' flavour

Comparison of smoke compounds in diluted wine to background levels

The AWRI maintains a database of the background levels of volatile phenols and phenolic glycosides that occur naturally in grapes that have not been exposed to smoke (and wines made from those grapes). This background data can be compared to analytical results from potentially smoke-affected fruit and wine to determine the likelihood of the fruit or wine being affected by smoke (assuming no oak contact for wine).

The concentrations of volatile phenols and phenolic glycosides in the wines in this dilution study (100%, 50%, 25%, 12.5% and 6.25% smoke-affected wine) were compared against those in the background database (Figures 2 and 3, respectively). The grey bars in Figure 2 and Figure 3 represent the 99th percentile values (i.e. 99% of the data collected for non-smoke-exposed samples are at or below this concentration). For the volatile phenols in this particular wine, 75% or more of the unaffected Pinot Noir rosé wine in a blend resulted in concentrations within or just above the background levels. A similar result was observed for the phenolic glycosides. If smoke compounds are diluted in an affected wine to concentrations equivalent to those in the background database for that particular variety, then it is likely that the perception of smoke characters will be greatly reduced and more desirable 'fruit' aroma and flavours may be enhanced.

Recommendations and conclusions

Blending can be an effective option for remediation of smoke-affected wine. In this study, dilution with 75% or more of an unaffected Pinot Noir rosé wine of a similar style was enough to lower the smoke taint intensity to levels not significantly different from the unaffected wine alone. Similar results were obtained for a smoke-affected 2016 full-bodied Pinot Noir wine (data not shown). However, further sample sets should be investigated to assess whether this result is applicable more broadly.

The level of dilution required to dilute smoke characters sufficiently will depend on the level of smoke compounds in the wine and the sensory properties of the unaffected wine used as the blending wine. It is therefore recommended that blending trials are conducted on small volumes of wine to determine a final wine blend with suitable sensory characteristics.

As with all remediation treatments for smoke-affected grapes and wines, producers should consider the costs and benefits of this approach and their own individual circumstances and wine characteristics.

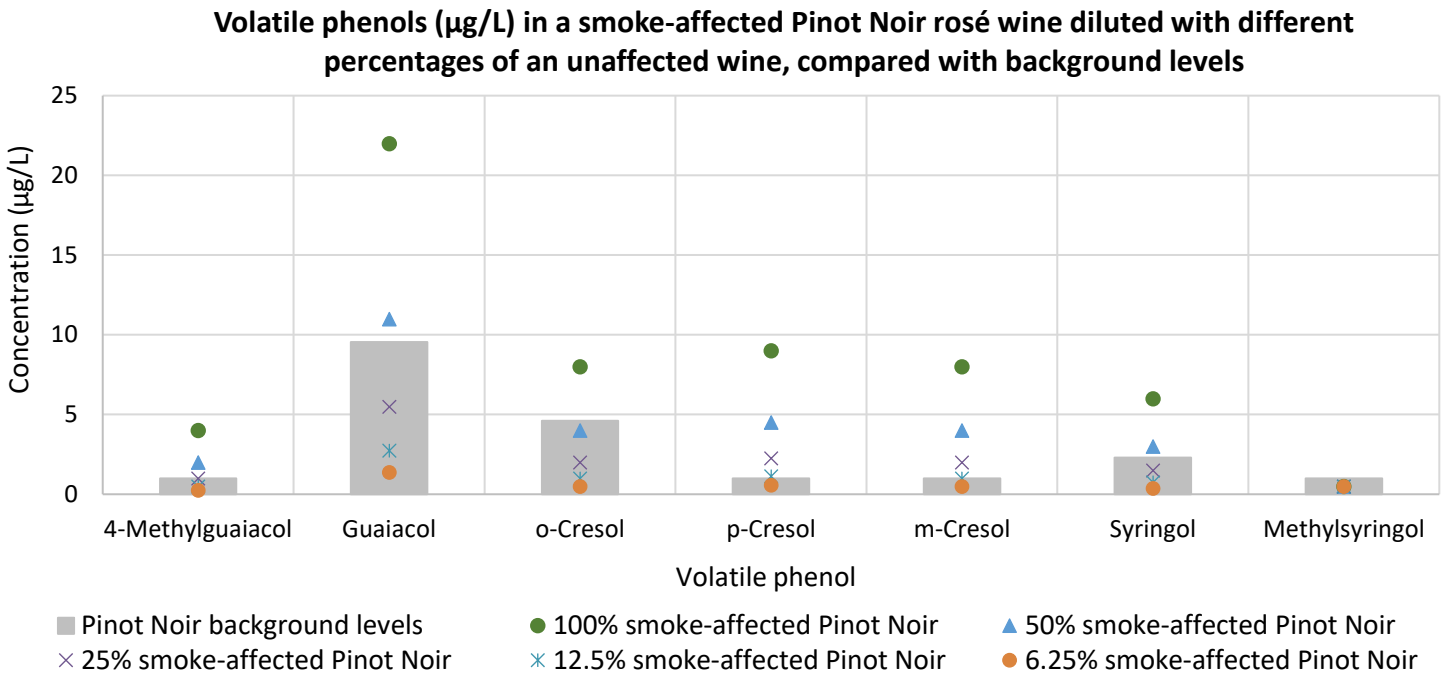


Figure 2. Smoke volatile phenol results for the diluted rosé wines (coloured symbols) compared to the 99th percentile values for Pinot Noir red wine (grey bars) from the AWRI's background levels database

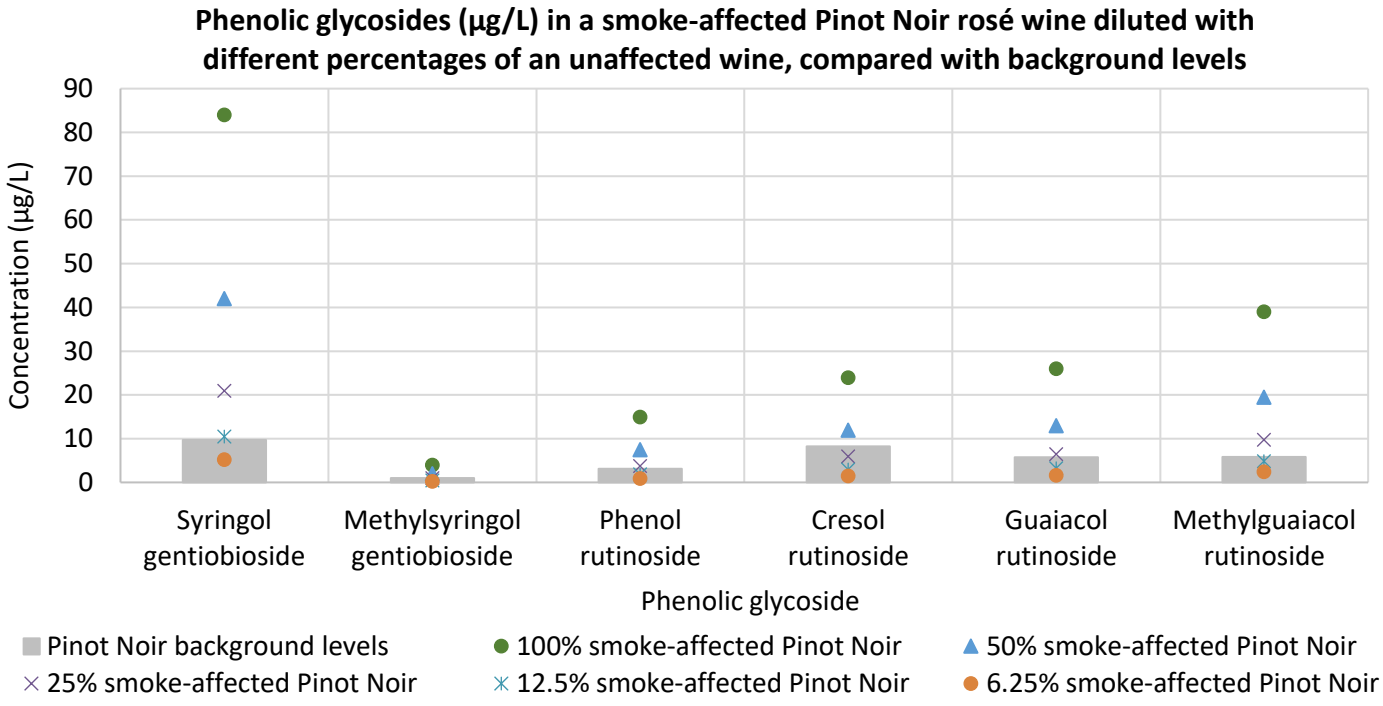


Figure 3. Smoke phenolic glycoside results for the diluted rosé wines (coloured symbols) compared to the 99th percentile values for Pinot Noir red wine (the grey bars) obtained from the AWRI's background levels database

Acknowledgement

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ECONOMIC ANALYSIS OF WINE REMEDIATION OPTIONS

Grape and wine value lost estimates, based on grape price; volumes of wine downgraded or lost ($0 value)

volumes & prices ex2019 Wine Australia National Vintage Report

	tonnes	price	grape value at 2% smoke exposed & tainted	L wine at 0.6 extraction rate		grape value at 10% smoke exposed & tainted	L wine at 0.6 extraction rate
grapes red 2019	941583	$ 845	$ 15,912,753	11,298,996		$ 79,563,764	56,494,980
grapes white 2019	786871	$ 462	$ 7,270,688	9,442,452		$ 36,353,440	47,212,260
totals	1728454		**$ 23,183,441** grape value lost at 2% of crush	**20,741,448** litres wine lost	(can't be sold)	$ 115,917,204	103,707,240

only attributes losses to losses in tainted grapes/wine, does not include additional winemaking cost and not including analytical testing of grapes or wine
0.6L/kg extraction rate based on typical range 500L - 750L (free run only - free run plus pressings)

For this evaluation it is estimated that exposure of vineyards to smoke could result in recognisable contamination of grapes and/or tainted wine that is unfit for sale and has an impact on approximately 2% to 10% of the national harvest in each year. This equates to approximately 34,570 to 172,845 tonnes of grapes which might not be harvested, cannot be sold or result in tainted wine (11.3 ML white wine and 9.4 ML red wine under the 2% exposure scenario). Based on average 2019 grape prices ($845/tonne red grapes, $462/tonne white grapes) economic losses from value of grapes that cannot be realised would range from $23.2 million pa to $115.9 million pa. These estimates exclude additional costs associated with analytical testing, winemaking steps needed when dealing with at risk grapes, damage to brand image etc. As such, total losses will very likely exceed the above estimates based on average grape prices.

Potential wine upgrade, based on minimisation of losses

Amelioration is not viable if value generated < cost of treatment

potential wine upgrades and associated value gains
bulk wine prices ex 2018 Austwine market update

red wine bulk	*value before/L*	*value after*		**value gain/L**	**value gain/1000L**	***value gain***	***notes***
carbon treat and sell into the lowest quality category	0	0.77	2018 dry red/rose bulk 1.10/L with 30% discount	**$ 0.77**	**$ 770**	***$ 754.60***	*corrected for volume losses during carbon treatment*
more optimistic, can sell into a low category	0	1.05	to bulk Shiraz/Rose with 30% discount	**$ 1.05**	**$ 1,050**	***$ 1,029.00***	
carbon treat and use as a blending wine	0	1.1	fining & blending cleaned up wine with bulk dry red/rose 1.1/L	**$ 1.10**	**$ 1,100**	***$ 1,078.00***	*
carbon treat and trade up from dry bulk to Shiraz	0.77	1.05	trade up from 2018 dry red bulk 1.10/L with 30% discount to bulk Shiraz/Rose with 30% discount	**$ 0.28**	**$ 280**	***$ 259.00***	#
carbon treat and dilute with clean Shiraz	1.05	1.5	fining & blending cleaned up Shiraz with bulk Shiraz 1.5/L	**$ 0.45**	**$ 450**	***$ 420.00***	*, #

** blending requires availability of 20-50x excess of clean base wine*
 # volume loss from carbon removal has a small impact, based on value of wine prior to fining

white wine bulk	*value before/L*	*value after*		**value gain/L**	**value gain/1000L**	***value gain***	*corrected for volume losses*
	0	0.63	2018 dry white bulk 0.90/L with 30% discount	**$ 0.63**	**$ 630**	***$ 617.40***	
	0	0.77	to bulk Chardonnay with 30% discount	**$ 0.77**	**$ 770**	***$ 754.60***	
	0	0.9	fining & blending cleaned up wine with bulk dry white 0.9/L	**$ 0.90**	**$ 900**	***$ 882.00***	*
	0.63	0.77	trade up from 2018 dry white bulk 0.9/L with 30% discount to bulk Chardonnay with 30% discount	**$ 0.14**	**$ 140**	***$ 124.60***	#
	0.77	1.1	fining & blending cleaned up Chardonnay with bulk Chardonnay 1.1/L	**$ 0.33**	**$ 330**	***$ 308.00***	*, #

Cost of carbon fining

	carbon/kg	cost @0.1g/1000l	cost 1g/1000l	cost additional bentonite 1kg/1000L
cheap cart	$7	$0.70	$7.00	$ 3
expensive	$14	$1.40	$14.00	$ 3

cost fining materials/1000L

low dose	***high dose***	
$3.70	$10.00	***cheap carbon***
$4.40	**$17.00**	***expensive carbon***

cost is excluding extra labor, analytical testing
wine losses after fining 2%; cost impact depends on value of starting wine

Notes:

The cost of the materials required for fining is very low ($3.70 to $17.00/1,000L wine, depending on dose rate and choice of product) and the impact of wine volume losses (typically 2%) is also marginal, given the low value of the wine prior to carbon fining. Addition of fining agents and their removal are standard operations in winery, do not require specialised equipment or significant extra labour inputs.

Summary of cost/benefit analysis for wine remediation of smoke taint

It's about minimising losses.

Amelioration is not viable, if value generated < cost of treatment

Potential wine upgrades and associated value gains

bulk wine prices ex 2018 Austwine market update

					value generated in fined wine corrected for volume losses	***value generated minus cost carbon high ($17)***	***grape to wine losses* per 1000L extraction rate 0.6*****	*doesn't include cost of winemaking ** conservative estimate; typical range 500L - 750L (free run only - free run plus pressings)	***benefit***	***% reduction in losses***	
red wine bulk	*value before/L*	*value after*		**value gain/L**	**value gain/1000L**							
	0	0.77	2018 dry red bulk 1.10/L with 30% discount	**$ 0.77**	**$ 770**	***$ 754.60***	*$ 737.60*	$ 1,408		***-$ 670.73***	**52%**	
	0	1.05	to bulk Shiraz with 30% discount	**$ 1.05**	**$ 1,050**	***$ 1,029.00***	*$ 1,012.00*	$ 1,408		***-$ 396.33***	**72%**	
	0	1.1	fining & blending cleaned up wine with bulk dry red 1.1/L	**$ 1.10**	**$ 1,100**	***$ 1,078.00***	*$ 1,061.00*	$ 1,408		***-$ 347.33***		blending with bulk dry red/rose
	0.77	1.05	trade up from 2018 dry red bulk 1.10/L with 30% discount to bulk Shiraz with 30% discount	**$ 0.28**	**$ 280**	***$ 259.00***	*$ 242.00*	$ 638	cost of grapes to make 1000L wine	***-$ 396.33***	**38%**	
	1.05	1.5	fining & blending cleaned up Shiraz with bulk Shiraz 1.5/L	**$ 0.45**	**$ 450**	***$ 420.00***	*$ 403.00*	$ 358	cost of grapes to make 1000L wine	***$ 44.67***		blending w bulk Shiraz[1]
white wine bulk	*value before/L*	*value after*		**value gain/L**	**value gain/1000L**							
	0	0.63	2018 dry white bulk 0.90/L with 30% discount	**$ 0.63**	**$ 630**	***$ 617.40***	*$ 600.40*	$ 770		***-$ 169.60***	**78%**	
	0	0.77	to bulk Chardonnay with 30% discount	**$ 0.77**	**$ 770**	***$ 754.60***	*$ 737.60*	$ 770		***-$ 32.40***	**96%**	
	0	0.9	fining & blending cleaned up wine with bulk dry white 0.9/L	**$ 0.90**	**$ 900**	***$ 882.00***	*$ 865.00*	$ 770		***$ 95.00***		blending w dry white[1]
	0.63	0.77	trade up from 2018 dry white bulk 0.9/L with 30% discount to bulk Chardonnay with 30% discount	**$ 0.14**	**$ 140**	***$ 124.60***	*$ 107.60*	$ 140	cost of grapes to make 1000L wine	***-$ 32.40***	**77%**	
	0.77	1.1	fining & blending cleaned up Chardonnay with bulk Chardonnay 1.1/L	**$ 0.33**	**$ 330**	***$ 308.00***	*$ 291.00*	$ -	cost of grapes to make 1000L wine	***$ 291.00***		blending w bulk Chardonnay[1]

cost of grapes

(they could be worth nothing but we make something by treating the wine)

[1] this is the best scenario: break even depending on costs

Definitions:

dry' is generic (non-varietal) commercial bulk wine

varietal' bulk is bulk wine with the variety stated

Notes:

Another key variable that impacts on value recovered is the extraction rate, or in other words the volume of wine produced from 1 kg of grapes. For this analysis the extraction rate was assumed to be 0.6 L wine/kg grapes, which is a conservative assumption for achievable extraction rates for smoke-tainted grapes and is positioned between extraction rates of 0.5 L wine/kg grapes (where free run juice only is used which may be lower in some taint compounds) and common extraction rates of 0.75 L wine/kg grapes (free run juice plus pressings which are likely to be higher in taint compounds and more difficult to improve).

Key risks to producers after carbon fining (as for any other mitigation option) include that the resulting wine cannot be sold - despite suitable quality attributes - because of damage to brand image and/or negative perception associate with smoke exposure, and a lack of market demand or pre-existing oversupply in the bulk market. There is therefore a risk that producers might incur additional costs which cannot be recovered and would further increase their losses.

Where there is a sufficient market for bulk wine, wine made after carbon fining of grape juice will contribute to cashflow and assist in regaining some of the value otherwise lost. The relative reduction in losses (or value recovered) can be in the range of 38 to 96% when based on the cost of wine which is otherwise not fit for sale.

Finally, the overall increase in value, reduction in losses and improvements to profitability that are achievable will also be a function of prize paid for grapes (or cost of producing grapes) which varies widely between < $300 to $2000+ for grapes from different varietals, for different quality brackets, and between warm and cool/temperate regions (Wine Australia, National Vintage Report 2019). Not surprisingly, the biggest gains can be anticipated from carbon fining of juice from white varietals, given that 71% of fruit from white varieties sold in 2019 was in the $300 to $600 category. Considering the overall higher grape prices for red varietals (62% in the $600 -$1500 category; 12% in the $1500-$2000+ category), together with the detrimental impact carbon fining has on red wine colour, it is estimated that the aggregate value recoverable from ameliorating smoke-tainted red wine would be similar to value recovered from smoke-exposed white grapes and smoke tainted white wine, but the relative reduction in losses is likely significantly smaller for most red wine compared to white wine.