AUSTRALIAN SOCIETY
OF VITICULTURE AND
OENOLOGY | ASVO

Research Article

Modelling Smoke Flavour in Wine from Chemical Composition of Smoke-Exposed Grapes and Wine

Mango Parker [ID],[1] **WenWen Maddy Jiang** [ID],[1] **Eleanor Bilogrevic** [ID],[1] **Desireé Likos** [ID],[1]
John Gledhill,[2] **Adrian D. Coulter** [ID],[1] **Geoff D. Cowey,**[1] **Con A. Simos,**[1] **I. Leigh Francis** [ID],[1]
and Markus J. Herderich [ID][1]

[1]*The Australian Wine Research Institute, Glen Osmond, Adelaide, SA 5064, Australia*
[2]*WIC Winemaking Services, Glen Osmond, Adelaide, SA 5064, Australia*

Correspondence should be addressed to Mango Parker; mango.parker@awri.com.au

Received 10 November 2022; Revised 23 January 2023; Accepted 24 March 2023; Published 21 April 2023

Academic Editor: Liz Waters

Wine grapes exposed to smoke and wine made from grapes exposed to smoke can robustly be identified through their elevated concentrations of volatile phenols and phenolic glycosides serving as smoke markers, compared to concentrations typically found in non-smoke-exposed samples. Smoke-affected wines with high concentrations of volatile phenols and glycosides can have smoky flavours, but the relationship between concentrations of specific smoke markers in grapes and the intensity of smoky sensory attributes in the resulting wine has not been established. This study sought to determine whether volatile phenols and glycoside concentration in grapes and wine are suited to predict smoke flavour, to identify the key drivers of smoke flavour in both matrices. The study aimed to determine what concentrations of volatiles and glycosides in grapes impart an unacceptable smoke flavour in the resulting wine, to provide a guide for producers assessing suitability of smoke-exposed grapes for wine production. During vintage 2020, a total of 65 grape samples were collected from vineyards exposed to bushfire smoke, as well as unaffected vineyards. Chardonnay, Pinot Noir, and Shiraz grapes were harvested from vineyards in New South Wales, South Australia, and Victoria. Unoaked wines (50 kg scale) were produced under controlled conditions. The wines had a wide range of smoke flavour intensities rated by a trained sensory panel. Statistical models based on guaiacol, *o*-cresol, *m*-cresol, *p*-cresol, and some glycosides gave good predictions of smoke flavour intensity, with a slightly different optimal model for each cultivar. Subsequently, critical concentrations for quality defects were estimated to provide a guide for producers. A subset of smoke exposure markers in wine grapes affected by smoke from bushfires can be used to predict the degree of smoke flavour in wine. This information provides a first guide for assessing the risk of producing smoke tainted wine from smoke-exposed grapes.

1. Introduction

Bushfire smoke has caused billions of dollars of losses to the global wine industry since it was first identified in 2003 as the source of unpleasant smoky flavours [1, 2]. Volatile phenols in smoke are taken up by berries and metabolized, forming phenolic glycosides which accumulate during the season, resulting in elevated concentrations of volatile phenols and/or phenolic glycosides at harvest [3–5]. Wines made from smoke-affected grapes can have elevated concentrations of volatile phenols and phenolic glycosides, particularly for wines made with skin contact [1, 6, 7]. These wines have

unpleasant smoky aromas, flavours, and aftertaste, which are considered undesirable quality defects by many in the wine industry [8].

"Smoky," "medicinal," and "cold ash" aromas, flavours and aftertaste have been attributed to volatile phenols and glycosides, particularly the potent odorants guaiacol, *o*-cresol, *m*-cresol, and *p*-cresol [9–11]. "Smoky" and "medicinal" flavours are due to combinations of volatile phenols and phenolic glycosides, that can contribute even when below their individual sensory thresholds, due to sub-threshold interactions and additive effects [9]. Phenolic glycosides contribute to smoke flavour and aftertaste by

hydrolysis in-mouth during tasting [9, 10]. Earlier studies have shown that consumers dislike smoky flavours in rosé style wines made from smoke-exposed grapes, and red wines with 50 μg/L of guaiacol added [12, 13]. While the volatile phenols and glycosides have been related to smoke flavour in highly smoke-affected wines of various cultivars [9, 14], there is little information about the minimum concentrations of volatile phenols and/or phenolic glycosides required to impart a perceptible smoke flavour when present below individual sensory threshold concentrations.

Notably, some smoke flavours or compounds can be desirable in certain wine styles. For example, oaked wines can have distinct toasty or smoky flavours due to guaiacol and other volatile phenols formed during toasting of oak [15, 16]. Also, in some white wines, a smoky/struck flint character can be found, attributed to phenylmethanethiol (syn. benzenemethanethiol), a potent odorant with an odour detection threshold of approximately 0.3 ng/L [17].

Analytical protocols for identifying smoke exposure are available to the wine sector [18]. A suite of seven volatile phenols and six phenolic glycosides are routinely analysed to identify smoke exposure of grapes; guaiacol, 4-methylguaiacol, o-cresol, m-cresol, p-cresol, syringol, 4-methylsyringol, syringol gentiobioside, methylsyringol gentiobioside, cresol rutinosides (including rutinosides of o-, m-, and p-cresol), guaiacol rutinoside, methylsyringol rutinoside, and phenol rutinoside [18, 19]. In addition to those routinely analysed, a large number of phenolic glycosides including pentose-glucosides and trisaccharides have been identified [19–21]. Smoke exposed grapes and wine can be identified by comparing concentrations of volatile phenol and glycoside smoke markers to typical background levels found in non-smoke-exposed samples [22]. If volatile phenols or phenolic glycosides are above concentrations typically found in non-smoke-exposed samples for that cultivar, the sample is considered to be smoke-affected. If analysis of grapes shows high levels of smoke markers, growers may choose not to harvest affected blocks or winemakers might choose to modify their winemaking protocols or not to proceed with winemaking.

There are a number of steps that can be taken in the vineyard and winery to minimise the sensory impacts of smoke exposure. These include hand harvesting instead of machine harvesting, excluding leaves and stems, keeping fruit cool prior to juice extraction, separating press fractions, reducing fermentation time on skins, and fining techniques and reverse osmosis treatment for removal of negative characters from juice or wine [7, 13, 23].

When wine is made from smoke-affected grapes, phenolic glycosides in grapes are readily transferred into wine and can release volatile phenols, in addition to volatile phenols transferred directly from grape to wine [8]. The sum of phenolic glycosides remaining in wine compared to the concentrations in smoke-exposed grapes has been reported to range between 17 to 78% in individual examples from seven cultivars [5, 14]. Monitoring individual glycosides during winemaking, most glycosides decrease but some increase (e.g. guaiacol rutinosides) [6, 21, 24]. Apart from a small number of heavily smoke-affected samples, there is

little published data comparing the concentrations of volatile phenols and phenolic glycosides in grapes, with their concentrations in the wines and the intensity of smoky sensory attributes in the resulting wine, and no data are available for mildly smoke-exposed grapes [8].

Given the increasing number of smoke events, wine producers seek to understand the likelihood of producing an unacceptable smoky flavoured wine from mildly smoke-affected grapes. This information is needed in order to make production decisions on whether to harvest fruit, based on evidence of smoke exposure, and whether it is possible to produce wine from fruit without product quality downgrade or if winemakers are best advised not to proceed with expensive winemaking processes. There is a significant gap in the knowledge about predicting smoke flavour from volatile phenols and phenolic glycoside concentrations in grapes. Currently, it is not known whether the volatile phenols and phenolic glycosides used to assess smoke exposure are suitable to predict smoke flavour with acceptable rigour or whether additional analytical targets are needed for modelling smoke taint in wine from grape composition. Critically, the concentrations required to render a wine unacceptably smoky have not been defined.

This study aimed to establish relationships between the concentration of smoke markers in grapes from wildfire affected vineyards and the corresponding wine made under controlled or standard winemaking conditions and the smoky sensory attributes in the resulting wine. We included three cultivars widely grown across Australia and other grape producing countries: Chardonnay, Pinot Noir, and Shiraz which together constitute almost half of Australia's winegrape production [25, 26]. The study sought to determine whether the concentration of volatile phenols and phenolic glycosides in grapes and wine can adequately predict smoke flavour, to identify the key drivers of smoke flavour in both sample types. The study also aimed to determine what concentrations of volatiles and glycosides in grapes would impart a discernible smoke flavour in the resulting wine, to provide a guide for producers assessing suitability of smoke-exposed grapes for wine production.

2. Materials and Methods

2.1. Chemicals

2.1.1. Winemaking Additives. Maurivin PDM yeast and Rohavin-L pectolytic enzyme were sourced from AB Biotek, North Ryde, NSW, Australia, GoFerm from Lallemand, Edwardstown, SA, Australia, tartaric acid, diammonium phosphate, bentonite and potassium metabisulfite from E. E Muirs, Australia, and hydrogen peroxide from Rowe Scientific.

2.2. Grapes

2.2.1. Study A: Grapes Exposed to Early Season Smoke. The Cudlee Creek fire of December 2019 produced a large amount of smoke which affected vineyards in the Adelaide Hills with small green berries at Eichhorn–Lorenz (E-L) stage 29, after which, negligible further smoke exposure

occurred [27]. *Vitis vinifera* L. cv. Chardonnay, Shiraz, and Pinot Noir grapes from Adelaide Hills wine region in South Australia were hand harvested during March 2020. Eight samples were collected for each cultivar including samples from vineyards that had no evidence of smoke exposure (control samples); that had mild smoke exposure based on grape maturity smoke compound analysis; and samples from vineyards with evidence of mild fire activity within the block, such as burnt grass undervine and in the midrow. All details of the grapes, wines, and sensory analysis are described elsewhere [28].

2.2.2. Study B: Grapes Exposed to Diverse Smoke Events. *Vitis vinifera* L. cv. Chardonnay, Shiraz, and Pinot Noir grapes from Victoria, New South Wales, Canberra District, and South Australia were hand harvested during February-March 2020. A total of 40 samples were collected, representing at least nine samples per cultivar that had been exposed to smoke from multiple bushfires that burnt across eastern Australia in the spring and summer of 2019-2020. In addition, control samples with no known smoke exposure were sourced from Langhorne Creek, McLaren Vale, and parts of the Adelaide Hills that were not affected by smoke, as shown in Table S1. The geographic indications included Alpine Valleys, Beechworth, and King Valley in Victoria; Adelaide Hills, Barossa Valley, Langhorne Creek, and McLaren Vale in South Australia; Cowra, Mudgee, Orange, and Riverina in New South Wales; and Canberra District. The grapes were hand harvested at commercial ripeness for table wine styles (approximately 20°Brix), approximately 50–80 kg each. The whole bunches were frozen due to logistical and biosecurity considerations and transported frozen to WIC Winemaking Services in Adelaide in April 2020 for winemaking commencing late April.

Frozen grapes were thawed at 4°C over the weekend prior to commencement of winemaking. Once thawed, prior to winemaking, a randomised subsample of 2 kg of bunches was taken for grape analysis. Approximately 50 kg of each sample was used for winemaking, with one 50 kg fermentation replicate per cultivar. The primary goal was to capture as much variation as possible in smoke exposure at vineyard level, with replicated winemaking for each sample not feasible with the limited resources available.

2.3. Winemaking. The thawed Chardonnay grapes were destemmed and pressed, pectolytic enzyme added at a rate equivalent to 40 mL/tonne, tartaric acid adjusted to target a pH of between 3.4-3.5 where practical, batches with soluble solids greater than 22°Brix for Chardonnay grapes, and 24°Brix for Pinot Noir and Shiraz grapes were adjusted using reverse osmosis purified water generated in the winery (see Table S2), settled, racked to a new fermenter vessel targeting turbidity of 200 NTU, and then inoculated with 250 mg/L PDM yeast and GoFerm. Shiraz and Pinot Noir grapes were thawed then destemmed prior to inoculation. Fermentation temperature was kept between 14.2°C and 18.3°C, and all fermentations were completed within 11–14 days. Additions of 100–250 mg/L diammonium phosphate were made to the ferments targeting 250 mg/L yeast available nitrogen at 8 ± 2° Baumé. Once fermentation was complete, 80 mg/L total sulfur dioxide (SO_2) was added as a 10% SO_2 solution made by dissolving potassium metabisulfite, and the wines were racked into 18 L stainless steel kegs for cold stabilisation at 0°C for 4 weeks, then racked off gross lees. The wines were filtered with a crossflow filter of nominal pore size 0.2 μm and bottled into 375 mL OI 30157 AG Punted Claret BVS bottles with screwcap closures (Vinpac International, Angaston, SA, Australia). Wines were stored at 15°C until analysis.

2.4. Wine Composition. Chemical analysis was performed on the wines by AWRI Commercial Services (now Affinity Labs) three weeks after bottling. Analysis included alcohol, glucose and fructose, malic acid, pH, titratable acidity (TA), free and total SO_2, and volatile acidity [29]. Affinity Labs also analysed the grapes and wines for smoke exposure markers: volatile phenols including guaiacol, 4-methylguaiacol, syringol, 4-methylsyringol, *o*-cresol, *m*-cresol, and *p*-cresol, and six glycosides of volatile phenols: syringol gentiobioside, methylsyringol gentiobioside, phenol rutinoside, guaiacol rutinoside, methylguaiacol rutinoside, and cresol rutinosides [19]. Note that the calibration range for the phenolic glycosides was 1–200 $\mu g/L$ or $\mu g/kg$ for grape analysis, and values above 200 were estimated by extrapolating the calibration function. This was deemed an acceptable approach because the method was found to be linear up to 1,000 $\mu g/L$ or $\mu g/kg$ [19].

2.5. Sensory Smoke Rating. Formal sensory analysis was performed on the wines six weeks after bottling. The sensory assessment for Study A has been described previously [28] and a closely similar procedure was followed for Study B. A panel of screened, qualified, and experienced assessors was convened to evaluate each of the wine sets. The assessors were selected from a pool of AWRI staff members, all of whom were chosen for their ability to perceive smoke flavour from phenolic glycosides and have previous experience in smoke sensory analysis. The panel of assessors (10 for the Chardonnay, Pinot Noir, and Shiraz sets in Study A, and 9 for the Chardonnay, 12 for the Pinot Noir, and 10 for the Shiraz sets in Study B) rated 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), smoke flavour (defined as including bacon, smoked meat, and ashy aftertaste), overall fruit aroma (defined as including red fruit, red berry, strawberry, raspberry, and cherry for the Pinot Noir rosé and Shiraz and defined as any type of citrus fruit, stone fruit, and tropical fruits including pineapple for the Chardonnay), and overall fruit flavour for each of the wine sets. An "other" term was available for both aroma and flavour to capture any additional noteworthy characteristics in the wines. 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.

All wines were assessed in duplicate and separated by study and cultivar. Study A was assessed over 3 days (different cultivar per day), while Study B was assessed over 5 days (one day for Chardonnay assessment, and two days for each of the Pinot Noir and Shiraz sets) due to a larger number of wines in the set. All wines were presented to panellists in 30 mL aliquots in 3-digit-coded and covered, ISO standard wine glasses at 22–24°C, in isolated booths under colour-masking lighting, with randomised presentation order using a modified Williams Latin Square design generated by Compusense20 sensory evaluation software (Compusense, Guelph, ON, Canada). A minimum 30-second delay was enforced before assessors could finalise the palate ratings to account for any lingering attributes and aftertastes and then a 2-minute rest between each sample and a 10-minute rest between sets of two and three samples, to minimise carryover [23, 30]. Water was provided for palate cleansing. Data were acquired using Compusense Cloud sensory evaluation software.

2.6. Data Analysis. Panellist performance was assessed using Compusense software and R with the SensomineR (senso-miner.free.fr/) and FactomineR (factominer.free.fr/) packages. The performance assessment included analysis of variance (ANOVA) for the effect of assessor, 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 assessor by attribute. All assessors were found to be performing to an acceptable standard.

For the sensory data, ANOVA was conducted using XLSTAT (Addinsoft, 2020, Paris, France). For each cultivar in the separate studies, the fixed effects of wine, presentation replicate, the random effect of judge, and their two-way interactions were assessed, followed by a Dunnett's means comparison test to determine whether the wines were rated significantly higher than a control. It was decided that the control that received the higher smoke flavour score would be the wine used as the specified control for all Dunnett's calculations for that set to account for variation commonly observed in wines without smoke exposure.

Partial least squares (PLS) regression was carried out using The Unscrambler 11.0 (CAMO Technologies Inc., Woodbridge, NJ). All PLSR analyses were carried out using standardized data with full cross-validation, with the y data set being the sensory smoke flavour scores, and the x data being the chemical compositional data. Where an analyte was reported as below the limit of quantification; the value of half of the limit of quantification was used for the PLS models. The correlation of predicted versus measured smoke flavour as indicated by R^2 of calibration, and standard error of cross-validation (SE) were used to compare the models.

3. Results and Discussion

3.1. Winemaking. Details for Study A have already been described [28]. Importantly, grapes in this study were exposed to smoke from a single wildfire, ca. 3 months prior to harvest and winemaking, and sampled at the same day for each variety. This resulted in some variability in maturity across the samples but maintained the time between smoke exposure and sampling a constant. Smoke exposure did not affect the progress of the fermentations, with all musts completing primary fermentation at similar rates, and all within 11–14 days.

The basic wine compositional measures are provided in Table S1 and for Study A have been previously reported [28]. Chardonnay wines ranged in alcohol concentration from 12.0–15.8% (v/v). There was also variation in glucose and fructose, pH, and titratable acidity. The two Chardonnay control wines both had relatively high alcohol content (14.9 and 15.8% v/v), one contained some residual sugar (2.9 g/L glucose and fructose) and had relatively high pH values.

Pinot Noir wines also varied in their basic wine composition. Alcohol varied from 11.1–15.1% (v/v), all the Pinot Noir wines had a pH between 3.45 and 3.55, and titratable acidity ranged from 5.0 to 6.7 g/L tartaric acid equivalents, and malic acid was less than 0.2 g/L. All wines had residual sugar at or below 1 g/L glucose and fructose. Two wines were removed from the study due to a dominating 'nail polish remover' aroma, associated with a high ethyl acetate concentration (data not shown).

The Shiraz wines varied in alcohol from 12.8-14.8% v/v, pH from 3.40-3.69, and titratable acidity ranged from 5.9 to 6.8 g/L. All wines had less than 1 g/L of glucose and fructose, volatile acidity below 0.6 g/L, and malic acid less than 0.2 g/L.

3.2. Volatile Phenols and Glycosides in Grapes and Wines. Compositional data for all control grape and control wine samples were consistent with data reported from non-smoke-exposed samples [22] for all phenolic smoke markers measured, with the exception of one Shiraz sample (SHI-03-Control) which had a slightly elevated concentration of guaiacol (Table S3 and [28]). These observations provide further confirmation of background levels of phenolic compounds typically found in grapes and wine without a history of known smoke exposure.

In Study A, vineyards had been exposed to a single smoke event preveraison when grapes were still, very small, hard, and unripe [28]. At harvest smoke-exposed grapes had elevated concentrations of many of the phenolic glycosides and volatile phenols compared to non-smoke-exposed samples in the study. Syringol gentiobioside was the most abundant phenolic glycoside, up to 150 μg/kg in the grapes at harvest (Table 1). The concentrations of volatile phenols in grapes (up to 32 μg/kg guaiacol and 16 μg/kg o-cresol) was surprising and is in contrast to other reports. For example, grapes exposed to smoke in 2009 in Australia had concentrations of guaiacol below 5 μg/kg and concentrations of syringol gentiobioside reaching 1623 μg/kg [19], as well as samples affected by Californian fires in 2008 that had concentrations of guaiacol below 2 μg/kg and high levels of guaiacol released by enzymes [31]. Syringol and 4-methylsyringol were not found above the limit of quantification in any of the grape samples.

TABLE 1: Summary of volatile phenol and phenolic glycoside abundance in smoke-exposed grapes.

Cultivar and study		4-Methyl-guaiacol (µg/kg)	Guaiacol (µg/kg)	o-Cresol (µg/kg)	m-Cresol (µg/kg)	p-Cresol (µg/kg)	GuRG (µg/kg)	MGuRG (µg/kg)	MSyGG (µg/kg)	PhRG (µg/kg)	CrRG (µg/kg)	SyGG (µg/kg)
Chardonnay Study A	Range	LoQ-5.7	1-16	2.3-11	1.7-11	1.3-8.0	1-14	3-31	LoQ-25	1.5-7.0	2.8-11	2.3-136
	Median	3	8.7	6.7	6.9	4.5	6.6	15	9.4	5.0	7.7	55
Chardonnay Study B	Range	LoQ-10	1-33	1-28	LoQ-9	LoQ-4	2-18	6-73	3-151	2-20	3-27	32-868
	Median	1.5	4.5	4.5	1.5	<LoQ	3.0	8.5	6.5	5.5	6.5	68
Pinot Noir Study A	Range	LoQ-5.0	1.7-32	1.7-16	1.0-11	LoQ-4.7	2.9-11	3.0-49	1.0-16	2.2-27	2.9-54	2.8-146
	Median	2.4	16	7.2	5.0	2.2	5.4	15	7.8	9.7	15	59
Pinot Noir Study B	Range	LoQ-3	2-20	3-15	LoQ-4	LoQ-2	2-19	4-48	2-35	5-47	4-49	21-456
	Median	<LoQ	6	5	1	<LoQ	5	11	5	10	12	56
Shiraz Study A	Range	LoQ-2.7	5-16	1.0-4	LoQ-2.7	LoQ-1.7	4.3-22	6-44	LoQ-13	1.3-5.0	4-15	4.4-71
	Median	2.4	8.7	6.7	5.0	2.2	5.4	15	7.8	5.5	7.7	59
Shiraz Study B	Range	LoQ-7	2-59	LoQ-17	LoQ-3	LoQ-1	6-74	5-93	3-189	2-25	3-27	24-977
	Median	<LoQ	5.0	2.0	0.5	<LoQ	18	21	17	14	12	164

LoQ, limit of quantitation = 1 µg/kg for all analytes apart from 4-methylsyringol and syringol with LoQ = 2 µg/kg; 4-methylsyringol and syringol were at or below LoQ for all samples so have been excluded from this table; GuRG, guaiacol rutinoside; MGuRG, methylguaiacol rutinoside; MSyGG, methylsyringol gentiobioside; PhRG, phenol rutinoside; CrRG, cresol rutinosides; SyGG, syringol gentiobioside.

In the smoke-exposed grape samples of Study B, the concentrations of phenolic glycosides were much higher than those observed in Study A, with syringol gentiobioside reaching approximately 980 μg/kg (Tables 1 and S3). The pattern of abundance was different to that observed in Study A, likely due to a number of factors such as smoke composition, the timing of smoke exposure and metabolism in the berries. In contrast, in Study B, the concentration of the volatile phenols relative to phenolic glycosides was generally lower (Table S3). Guaiacol again was the most abundant volatile phenol in the grapes, up to 59 μg/kg in Shiraz grapes. In contrast to the phenolic glycosides, guaiacol was above typical concentrations found in non-smoke-exposed grapes in only 20 of the 40 samples. This is in line with recent observations of volatile phenols and phenolic glycosides in smoke-exposed grapes from the 2020 vintage [32]. o-Cresol was elevated in 17 of the 40 samples and was particularly abundant in the Chardonnay and Pinot Noir samples (up to 28 μg/kg). Syringol and 4-methylsyringol were below or near to the limit of quantitation in all of the grape samples. It is interesting to note in both studies that general patterns of abundance of volatile phenols and phenolic glycosides differed by cultivar, which could be due to possible differences in the chemical composition of the smoke in different vineyards, and differences in uptake and metabolism by the different cultivars [14].

In the wines of Study A, the guaiacol was the most abundant volatile phenol (up to 78 μg/L), followed by syringol (up to 65 μg/L) (Tables 2 and S4). Syringol and 4-methylsyringol were found in the wines despite being absent from the grape samples, presumably due to release from glycosidic precursors during winemaking. Syringol gentiobioside was the most abundant phenolic glycoside in wine, up to 71 μg/L. Each of the three cresol isomers was observed up to 17 μg/L in the wines and were particularly abundant in the Pinot Noir wines.

In the wine samples of Study B, the most abundant smoke exposure marker was syringol gentiobioside (up to 690 μg/L) (Tables 2 and S4), and guaiacol was the most abundant volatile phenol in the wines (up to 125 μg/L), followed by syringol (61 μg/L) as was seen in Study A. Like Study A, cresols were particularly abundant in the Pinot Noir wines of Study B.

3.3. Comparing Volatile Phenols and Phenolic Glycosides in Grapes and Wine.

As is common practice, the Chardonnay wines were made with minimal skin contact, and the red wines were fermented on skins. This resulted in different relationships between grape and wine composition between the red and white cultivars. The red wines, which were made with skin contact, had higher concentrations of volatile phenols. The concentrations of guaiacol and cresols were higher in the red wines compared to grapes (example Study B 59 μg/kg guaiacol in grapes and 125 μg/L in wine), which is likely due to both the extraction of guaiacol and cresols from the grape skins and release of guaiacol and cresols from glycosides during the winemaking process. Syringol and 4-methylsyringol were rarely detected in the grapes yet were commonly found in both red and white wines (up to concentrations of 61 and 25 μg/L respectively), due to release from glycosides during wine production. Refer to Tables 1, 2, S3–S5 for details of volatile phenols and phenolic glycoside concentrations in grapes and wines. On the other hand, lower concentrations of volatile phenols were found in the Chardonnay wines (example Study B max 14 μg/L guaiacol in wine compared to max 33 μg/kg guaiacol in grapes), in line with previous studies on the effect of skin removal [1, 6, 7, 33].

In contrast, the summed concentrations of the glycosides in the red wines were similar to those found in the grapes. Close examination of individual glycosides shows a complex pattern, and a variable proportion of individual glycosides persisted in the wine compared to the concentration in the grapes: some glycosides were lower in concentration in the wines than in the grapes, and some were higher in the wine. Shiraz and Pinot Noir wines generally had lower concentrations of syringol gentiobioside and methylsyringol gentiobioside in the wines compared to grapes, and higher concentrations of rutinosides, with some individual samples showing other patterns. Guaiacol, cresols, guaiacol rutinoside, and cresol rutinosides in grapes were strongly related to the concentrations found in Chardonnay and Pinot Noir wine, and the associations were weaker in Shiraz but unrelated to water additions to the musts to reduce excessive sugar concentrations.

3.4. Predicting Smoke Flavour Intensity from Volatile Phenols and Phenolic Glycosides in Wine.

In both studies, smoke-affected red wines had a wide range of smoke aroma and flavour sensory rating values, whereas the smoke-affected Chardonnay wines had lower scores for smoke aroma and flavour (Table S6) [28]. Control wines for each cultivar generally had low scores for smoke aroma and flavour, showing the sensory panel was well trained and was able to differentiate non-smoke-exposed samples and smoke-affected samples. Not all wines made from smoke-exposed grapes had smoke ratings significantly higher than the control wines, and some smoke-exposed wines had lower ratings of smoke flavour than the controls. In addition, some wines exhibited strong "green," "eucalyptus," "reduced," and "tropical" notes which could have masked smoke flavour, and others were "reduced" with burnt rubber characters that could be confused with smoke. Some assessors noted "smoky/struck flint" characters in some Chardonnay wines that are easily confused with smoke-related characteristics. Across all the wines, three were removed from the smoke flavour models due to comments indicating competing strong characteristics: one Shiraz with strong green/eucalyptus characters (SHZ-I Study A) and two Shiraz with reduced rubber and cooked vegetable notes (Study B SHZ-13-Smoke and SHZ-15-Smoke). A total of 20 samples for Chardonnay, 19 for Pinot Noir, and 21 for Shiraz wines were used for further data analysis. Notably, the smoke flavour was apparently unaffected by the water additions made to the musts with excessively high soluble solids.

TABLE 2: Summary of volatile phenol and phenolic glycoside abundance in smoke-exposed wines.

Cultivar and study		4-Methyl-guaiacol (μg/L)	Guaiacol (μg/L)	o-Cresol (μg/L)	m-Cresol (μg/L)	p-Cresol (μg/L)	4-Methyl-syringol (μg/L)	Syringol (μg/L)	GuRG (μg/L)	MGuRG (μg/L)	MSyGG (μg/L)	PhRG (μg/L)	CrRG (μg/L)	SyGG (μg/L)
Chardonnay Study A	Range	LoQ	LoQ-2	LoQ	LoQ-1	LoQ-2	LoQ	LoQ	1.5–7.8	LoQ-12	LoQ-2	1.1–3.9	LoQ-5.4	2.8–41
	Median	LoQ	1.0	LoQ	LoQ	1.0	LoQ	LoQ	4.9	4.5	<LoQ	2.3	2.0	18
Chardonnay Study B	Range	LoQ-5	1–14	LoQ-10	LoQ-12	LoQ-2	LoQ-9	LoQ-13	3–35	8–87	LoQ-44	2–28	4–45	17–525
	Median	<LoQ	2.5	2	1.5	<LoQ	<LoQ	<LoQ	6.5	12	3	6	10	53
Pinot Noir Study A	Range	2–15	5–52	2–11	3–17	3–15	LoQ-19	4–65	1.7–30	2.4–29	LoQ-3.1	LoQ-6.8	3.3–19	2.1–63
	Median	7.0	26.0	4.0	8.0	6.0	7.0	12.0	4.6	5.5	LoQ	1.6	4.4	13
Pinot Noir Study B	Range	1–13	5–53	5–29	3–22	1–15	LoQ-4	3–16	5–40	7–83	1–28	7–50	8–68	18–411
	Median	3	16	11	9	4	<LoQ	6	12	23	3	12	18	57
Shiraz Study A	Range	2–13	21–78	2–8	2–7	2–6	LoQ-2	5–7	11–53	8.4–55	LoQ-5.3	1.1–6.6	4.2–15	9.6–71
	Median	6.5	54.5	5.5	4.5	4.0	2.0	6.5	28	41	3.1	2.7	7.5	42
Shiraz Study B	Range	LoQ-25	11–125	2–24	LoQ-18	LoQ-8	LoQ-25	5–61	12–85	6–109	1–72	2–22	4–27	21–689
	Median	1.0	23.0	3.0	2.0	2.0	3.0	14.0	29	28	9.0	16	16	123

LoQ, limit of quantitation = 1 μg/L for all analytes apart from 4-methylsyringol and syringol with LoQ = 2 μg/L; GuRG, guaiacol rutinoside; MGuRG, methylguaiacol rutinoside; MSyGG, methylsyringol gentiobioside; PhRG, phenol rutinoside; CrRG, cresol rutinosides; SyGG, syringol gentiobioside.

Smoke aroma was highly correlated with smoke flavour in the red wines ($r > 0.978$) and slightly less correlated in the Chardonnay wines ($r > 0.948$) (Table S6). Given the close correlation, with smoke flavour being more discriminating, it was considered the most reliable indicator of smoke taint.

The first step to modelling smoke flavour in wine from its chemical composition investigated whether volatile phenols and phenolic glycosides in wine could be used to predict smoke flavour intensity in wine. PLS models for smoke flavour were explored for each cultivar in each of the two studies (Table 3). Good models for predicting wine smoke flavour were generated from wine compositional data for each of the three cultivars, using all 13 smoke markers ($R^2 > 0.86$, SE < 1.3). The exception was Chardonnay wine set from Study A ($R^2 = 0.63$, SE = 0.62) where the model was not as predictive, likely as this set had overall low smoke flavour ratings (Table 3). Overall, the volatile phenols and phenolic glycosides could be used to predict smoke flavour in wine.

As a second step to identify the most important volatile phenols and phenolic glycosides for modelling smoke flavour, the PLS model coefficients were examined. Table 3 lists the PLS model coefficients of the independent variables (volatile phenols and phenolic glycosides) modelling smoke flavour. The jack-knife cross-validation method [34] was applied to identify significant variables which are highlighted in bold in the table, although variables can still be considered important to the model if they are nonsignificant in this particular statistical test. Generally, variables that have the largest regression coefficient values, greater than 0.1 are the most important to the model, and coefficients of less than 0.05 can be considered not important [34].

Guaiacol, which by itself is not necessarily a taint compound, was one of the most important predictors of smoke flavour for all the sample sets. The three cresol isomers were important to most of the sample sets, which is in line with previous observations that the volatile compounds guaiacol and the three cresols, in combination, are likely to drive the perception of smoke flavour in smoke-affected wines, due to their low sensory thresholds relative to the other volatile phenols (measured in red wine: guaiacol 23 μg/L, o-cresol 62 μg/L, m-cresol 20 μg/L, p-cresol 64 μg/L, and in water: 4-methylguaiacol 21 μg/L, syringol 570 μg/L, and 4-methylsyringol 10,000 μg/L) [9–11, 35, 36]. Syringol and 4-methylsyringol were found at concentrations much lower than the reported thresholds even in the most severely smoke-affected wines and were considered unlikely to contribute to the smoke flavour directly [9, 11]. 4-Methylguaiacol was generally found at much lower concentrations than guaiacol in smoke-affected wines and has a much higher threshold than guaiacol in water (21 μg/L compared to 0.84 μg/L, respectively), so is less likely to contribute to smoke flavour directly [37]. Phenolic glycosides were strongly associated with the smoke flavour in both Pinot Noir wine sets, but the pattern differed across studies for the Shiraz, with only some glycosides strongly contributing to the models, and these compounds were generally less important to the Chardonnay models.

For the Chardonnay wines of Study B, the p-cresol, m-cresol, and guaiacol volatiles were strong predictors of smoke flavour, and the glycosides were less important. The data from the Chardonnay wines of Study A generated a less strong model with lower regression coefficient values, with several glycosides contributing.

For the Pinot Noir wines, there was a particularly high degree of co-correlation among the smoke marker compounds. Almost all of the variables had similar strong regression coefficients for both Pinot Noir sets (one factor model) although syringol and 4-methylsyringol were indicated to have less importance in Study A.

For the Study B Shiraz wines (3 factor model), the compounds most associated with smoke flavour were guaiacol, o-cresol, and phenol rutinoside, whereas methylsyringol gentiobioside was strongly negatively associated. For the Study A Shiraz wine set 4-methylguaiacol, guaiacol and o-cresol were most important to the model and in contrast in Study B methylsyringol gentiobioside was positively associated as was syringol gentiobioside. 4-Methylguaiacol was a good predictor of smoke flavour for the red wines, but as the concentrations were below 30 μg/L in all wine samples, and the threshold in water is reportedly 25 times higher than guaiacol, it was considered unlikely to be directly contributing to the smoke flavour in most samples [37]. Overall, the volatile phenols and phenolic glycosides in wine were able to predict the smoke flavour.

Guaiacol and the cresols have been previously indicated to be important compounds contributing to the perception of smoky and medicinal characters in smoke-affected wines [10]. In addition, glucosides of guaiacol and m-cresol have been shown to impart smoke flavour and aftertaste [9, 10]. It is plausible that the guaiacol, cresols, guaiacol glycosides, and cresol glycosides together contribute to the smoke flavour. To test this hypothesis, PLS models for smoke flavour were explored using a subset of the smoke marker compounds deemed most likely to be sensory drivers; guaiacol, o-cresol, m-cresol, p-cresol, guaiacol rutinoside, and cresol rutinosides.

Smoke flavour was predicted well by PLS regression analysis based on a subset of smoke compounds in wine (guaiacol, o-, m-, p-cresol, guaiacol rutinoside, and cresol rutinoside) for all sample sets ($R^2 > 0.90$) apart from Study A Chardonnay which was less well modelled ($R^2 = 0.59$). Guaiacol and m-cresol had the highest loadings in most models. o-Cresol was important to the model for the Pinot Noir wines in Study B and the Shiraz in Study A to a lesser extent. p-Cresol, guaiacol rutinoside, and cresol rutinoside were important for some sets. The best models were obtained for Pinot Noir. The low sensory scores for Chardonnay wines again limited the model development for this white varietal.

For most sample sets, the smoke flavour models could not be not significantly improved by adding basic wine parameters such as alcohol, pH, TA, residual sugar, other volatile phenol, and phenolic glycoside smoke markers. The exceptions to this were the Study B Pinot Noir and Shiraz models which were improved by adding basic wine composition, indicating that the sensory results were influenced by basic wine composition, particularly volatile acidity in the Pinot Noir wines and pH, TA, and alcohol in the Shiraz

TABLE 3: Partial least squares regression coefficients of the predictive models for smoke flavour from volatile phenols and phenolic glycosides in wine for two studies, each comprising three cultivars. Variables identified as significant by the martens and martens jack-knife cross-validation method are highlighted in bold.

	Chardonnay Study A	Chardonnay Study B	Pinot Noir Study A	Pinot Noir Study B	Shiraz Study A	Shiraz Study B
Number of factors	1	4	1	1	2	3
SE	0.62	0.67	1.21	0.78	1.1	0.52
R^2	0.63	0.93	0.87	0.86	0.93	0.96
4-Methylguaiacol	NA	0.18	**0.20**	**0.12**	**0.33**	**0.24**
Guaiacol	0.08	**0.26**	**0.20**	**0.12**	0.23	**0.75**
o-Cresol	0.06	**0.27**	**0.20**	**0.12**	0.19	**0.30**
m-Cresol	NA	−0.22	0.15	**0.09**	0.21	**0.26**
p-Cresol	NA	0.07	**0.19**	**0.12**	0.21	**0.40**
4-Methylsyringol	0.08	**0.62**	**0.20**	**0.10**	0.17	**0.26**
Syringol	**0.07**	−0.13	0.15	**0.10**	−0.27	**0.18**
Sum of volatile phenols	0.07	**0.11**	**0.19**	**0.12**	0.22	**0.50**
GuRG	0.05	0.09	**0.18**	**0.12**	−0.01	−0.12
MGuRG	**0.05**	0.03	**0.18**	**0.12**	0.19	−0.13
MSyGG	0.00	0.02	0.15	**0.11**	0.31	−0.42
PhRG	0.06	0.00	**0.19**	**0.11**	−0.17	0.35
CrRG	0.04	0.06	**0.18**	**0.11**	−0.11	0.10
SyGG	**0.07**	0.04	0.16	**0.11**	0.33	−0.35
Sum of phenolic glycosides	**0.07**	0.04	0.19	**0.11**	0.20	−0.29

SE = standard error of cross-validation, GuRG, guaiacol rutinoside; MGuRG, methylguaiacol rutinoside; MSyGG, methylsyringol gentiobioside; PhRG, phenol rutinoside; CrRG, cresol rutinosides; SyGG, syringol gentiobioside. Where analytes were not detected in the sample set, they are absent from the PLS model, and denoted NA. Note, the sum of volatile phenols includes the seven volatile phenols listed in the table, and sum of phenolic glycosides includes the six phenolic glycosides listed in the table.

wines. The effect of the basic wine composition was of most concern with the Study B Chardonnay wines, because the two unsmoked control wines were both high in alcohol, and one also contained residual sugar; however, the PLS models for smoke flavour did not improve when these parameters were added to the model. When 4-methylguaiacol was added to the model, the models did not improve. Overall, the results were in line with previous observations on smoke-affected wines from various regions, vintages, and cultivars which indicated that a range of volatile phenols and phenolic glycosides are important to model smoke flavour [9]. In summary, a subset of the smoke markers, namely guaiacol, o-, m-, p-cresol, guaiacol rutinoside, and cresol rutinosides, could predict wine flavour, using PLS regression models.

Many wine producers may not have access to specialised statistical software packages that would allow use of a multifactor model, and a simple, practical way to interpret the analysis results is preferred. In an attempt to streamline data analysis, we noted that the simple sum of [guaiacol + o-cresol + m-cresol + p-cresol concentrations] in wine enabled very good prediction of smoke flavour intensity in these sample sets (Figure 1). Also, it was evident that the models for the different cultivars had different slopes, likely reflecting variety-specific matrix effects as well as differences in chemical composition between the cultivars. For example, the Pinot Noir wines had particularly high concentrations of cresols, whereas Shiraz wines had high concentration of guaiacol, and Chardonnay had much lower volatile phenols. This simplified sum of concentrations parameter should be used with caution, bearing in mind that it does not take into account the contribution of the glycosides. There was a high degree of correlation observed among the volatile phenols

and phenolic glycosides in the samples, but this may not always be the case if treatments have been applied to selectively remove volatile phenols or phenolic glycosides, and the model may not be applicable to those wines.

Overall, smoke flavour in wine made from grapes with a varying degree of smoke exposure could be predicted by quantifying volatile phenols and phenolic glycosides in wine. The compounds key to predict smoke flavour were guaiacol, o-, m-, p-cresol, guaiacol rutinoside, and cresol rutinoside, although additional phenols and other compounds not measured in this study may be important too.

3.5. Predicting Smoke Flavour from Volatile Phenols and Phenolic Glycosides in Grapes. Preharvest chemical analysis data from grape samples are critical for making appropriate harvest or processing decisions for vineyards suspected of smoke exposure. The key question is if smoke exposure markers in *grape berries* can be reliably used to identify whether smoke flavour will be evident in *wine produced from smoke-affected grapes*? Encouraged by our ability to predict smoke flavour in wine from wine compositional data, PLS models to predict smoke flavour in wine from volatile phenols and phenolic glycosides in grapes were explored for each cultivar for both studies. The volatile phenols and phenolic glycosides in grapes predicted smoke flavour well in each cultivar and in each study ($R^2 > 0.86$, SE < 1.2 for Pinot Noir and Shiraz), although like the wine models, the models were not as strong for Chardonnay ($R^2 > 0.71$, SE 0.68) (Table 4). Table 4 lists the PLS model coefficients of the independent variables in grapes (volatile phenols and phenolic glycosides) predicting smoke flavour.
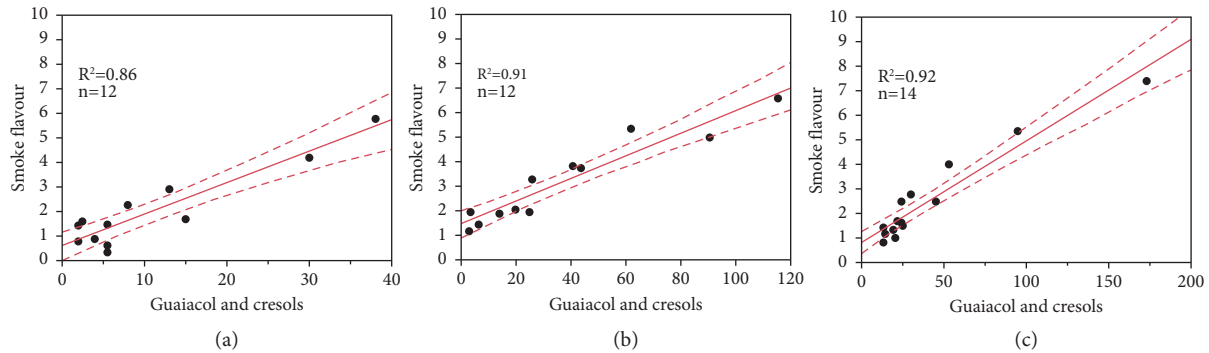
Figure 1: Correlation between the sum of guaiacol and cresols (*m*-cresol, *o*-cresol, and *p*-cresol) in wine and smoke flavour in each of the three cultivars: (a) Chardonnay (b) Pinot Noir, and (c) Shiraz shown here for Study B. Linear fit is shown by the solid line and confidence of the fit is shown by dotted lines.

Table 4: Partial least squares regression coefficients of the predictive model for smoke flavour in wine from volatile phenols and phenolic glycosides in grapes for two studies, each comprising three cultivars. Variables identified as significant by the Martens and Martens jack-knife cross-validation method are highlighted in bold.

	Chardonnay Study A	Chardonnay Study B	Pinot Noir Study A	Pinot Noir Study B	Shiraz Study A	Shiraz Study B
Number of factors	2	2	3	7	1	1
SE	0.68	0.87	0.83	0.58	1.1	0.75
R^2	0.71	0.86	0.99	0.99	0.88	0.88
4-Methylguaiacol	0.07	**0.26**	0.08	0.18	**0.09**	**0.08**
Guaiacol	0.22	**0.25**	**0.33**	**1.48**	**0.09**	**0.08**
o-Cresol	0.37	0.19	0.09	−0.60	**0.10**	**0.08**
m-Cresol	0.39	0.28	**0.58**	−0.25	**0.10**	**0.08**
p-Cresol	0.39	0.19	0.46	−0.54	0.08	0.06
Syringol	NA	NA	NA	NA	NA	0.04
Sum of volatile phenols	0.29	0.23	**0.29**	**0.47**	**0.09**	**0.08**
GuRG	−0.26	0.05	−0.39	0.41	**0.07**	**0.08**
MGuRG	−0.06	0.02	−0.19	0.44	**0.08**	**0.08**
MSyGG	−0.32	−0.04	0.03	−0.21	**0.09**	**0.08**
PhRG	−0.04	0.02	−0.16	0.01	**0.04**	0.07
CrRG	−0.04	0.04	−0.03	−0.11	0.05	0.06
SyGG	−0.17	0.01	−0.05	−0.28	**0.09**	**0.08**
Sum of phenolic glycosides	−0.17	0.01	−0.08	−0.13	**0.09**	**0.08**

SE = standard error of cross-validation, GuRG, guaiacol rutinoside; MGuRG, methylguaiacol rutinoside; MSyGG, methylsyringol gentiobioside; PhRG, phenol rutinoside; CrRG, cresol rutinosides; SyGG, syringol gentiobioside. Where analytes were not detected in the sample set, they are absent from the PLS model, and denoted NA. 4-methylsyringol was not listed in the table due to no detection. Note, the sum of volatile phenols includes the seven volatile phenols listed in the table and sum of phenolic glycosides includes the six phenolic glycosides listed in the table.

As found for the wine composition models, grape guaiacol concentration was one of the most important predictors across all sample sets. 4-Methylguaiacol and *m*-cresol were also strong predictors of smoke flavour in the Chardonnay wines of Study B, and surprisingly the glycosides were less important than the volatile phenols in the model prediction from both studies for this cultivar.

Guaiacol rutinoside and methylguaiacol rutinoside were strongly positively associated with wine smoky flavour in the Pinot Noir Study B set, and the cresols were negatively related. In contrast, *m*-cresol and *p*-cresol had high positive regression coefficients in the Study A set. The cresols were much higher in concentration in the grapes of Study A compared to Study B, possibly reflecting differences in the smoke composition and timing of exposure.

Almost all variables had equal high regression coefficients for the Shiraz grapes in both studies, with indications that cresol rutinoside, phenol rutinoside, *p*-cresol, and syringol were slightly less important. Overall, the volatile phenols and phenolic glycosides in grapes were able to predict the smoke flavour well in the corresponding wines for this cultivar.

In the models for smoke flavour from wine composition discussed above, the key drivers of predicting smoke flavour in wine from grape phenol analysis were identified as guaiacol, cresols, guaiacol rutinoside, and cresol rutinoside. Guaiacol and cresols in grapes can be transferred directly into the juice or must. Guaiacol glycosides and cresol glycosides in grapes can also hydrolyse to release guaiacol and cresols during wine production and ageing. Logically, guaiacol, cresols, and glycosides of guaiacol and cresols in

TABLE 5: Concentrations of volatile phenols and phenolic glycosides (μg/kg) in grapes that resulted in wines with significant smoke flavour (high risk) compared to controls, and concentrations above which only some wines were significantly smoky and some wines were not (moderate risk).

Analyte in grapes	Chardonnay		Pinot Noir		Shiraz	
	Moderate risk	High risk	Moderate risk	High risk	Moderate risk	High risk
4-Methylguaiacol	4.0	5.7	n.d.	n.d.	1.0	n.d.
Guaiacol	14.3	16.0	4.0	4.0	7.0	12.0
o-Cresol	10.3	10.3	3.0	5.0	2.0	3.0
m-Cresol	6.0	10.0	n.d.	n.d.	n.d.	n.d.
p-Cresol	2.0	7.3	n.d.	n.d.	n.d.	n.d.
4-Methylsyringol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Syringol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
GuRG	9.2	13.7	3.5	3.8	9.2	23.0
MGuRG	25.0	30.6	6.1	10.0	22.3	23.0
MSyGG	15.9	25.4	2.0	5.0	5.3	18.0
PhRG	5.0	7.0	4.4	10.0	1.8	16.0
CrRG	11.1	11.0	5.9	13.0	5.4	14.0
SyGG	101.2	135.7	22.2	53.0	28.6	176.0

GuRG, guaiacol rutinoside; MGuRG, methylguaiacol rutinoside; MSyGG, methylsyringol gentiobioside; PhRG, phenol rutinoside; CrRG, cresol rutinosides; SyGG, syringol gentiobioside; n.d. not determined as concentrations were below LoQ.

grapes could contribute to smoke flavour in wine. A diverse range of glycoconjugates of guaiacol and cresol have been identified in smoke-exposed grapes and wine, including monoglucosides, rutinosides, gentiobiosides, pentosylglucosides, trisaccharides, and more. While there is a lack of quantitative data about many of these compounds, the limited quantitative data available suggest they are highly correlated [19, 21]. The routinely analysed glycosides, guaiacol rutinoside, and cresol rutinoside can be considered representatives of the broader diversity of glycoconjugates present [19]. Therefore, the subset of markers *in grapes* most likely to cause smoke flavour in wine was identified as guaiacol, o-cresol, m-cresol, p-cresol, guaiacol rutinoside, and cresol rutinoside.

Smoke flavour in wine was modelled surprisingly well by PLS regression analysis based on a subset of grape compounds (guaiacol, o-, m-, p-cresol, guaiacol rutinoside, and cresol rutinoside), for all sample sets ($R^2 > 0.86$) apart from Study A Chardonnay which was less well modelled ($R^2 = 0.77$). In fact, the models using a subset of the markers were as good or better than the models using all 13 volatile phenols and phenolic glycosides. Guaiacol and m-cresol had the highest loadings in most models. o-Cresol and p-cresol were most important to the model for the Shiraz wines, especially in Study A and in Study B to a lesser extent. p-Cresol and guaiacol rutinoside were notably important for some sets. The best models were obtained for Pinot Noir ($R^2 > 0.99$). The narrow range of smoke flavour intensity ratings for Chardonnay wines from Study B limited the model development, and the Chardonnay wines in Study A were considered not suitable for developing variable subset models (Table S6).

The PLS regression approaches discussed above suggest that volatile phenols in the grapes are the most important determinants of smoke flavour intensity in the resulting wine. It is worth reiterating that in this sample set, volatile phenols and glycosides were highly correlated in the grapes and wines. In other words, the most severely smoke-affected

grape samples had high concentrations of both volatile phenols and glycosides. Grape samples from different smoke events in different vintages have shown other patterns, such as high concentration of glycosides and low levels of volatile phenols [19, 31, 32], and anecdotal reports suggest that grapes with this pattern can also produce smoky wines. Further research is required to establish whether the relationships and observations from this study can be applied more broadly to other smoke events and cultivars. On the other hand, smoke flavour in wine can be minimised by tailoring wine production, for example minimising skin contact, and carbon fining juice prior to fermentation [7, 33]. If these treatments are applied, the relationships described above would not be expected to apply, and phenolic glycosides may play a more important role to drive and predict smoke flavour.

3.6. Critical Concentrations of Volatile Phenols and Phenolic Glycosides in Grapes Likely to Produce Smoky Wines.

One of the aims of the study was to assess the concentrations of grape compounds that resulted in wines with perceptible smoke flavour and to provide practical guidance for producers assessing grape samples from smoke-exposed vineyards. Importantly, only some of the wines made from smoke-exposed grapes were rated as significantly more smoky by sensory analysis than the wines made from control grapes. In other words, a significant proportion of grape samples may have elevated concentrations of smoke exposure markers demonstrating smoke exposure of vineyards, yet not give rise to obviously smoke tainted wine after fermentation.

As a new approach in this study, wines were categorised as "smoky" if smoke flavour was significantly higher than the controls, using Dunnett's means comparison test. Table 5 summarises the critical concentration of volatile phenols and phenolic glycosides and the risk, as defined here as moderate or high, of producing smoky

wines. All grape samples with a concentration at or above the high-risk value produced wines that were significantly smoky. For grapes with concentrations between the moderate risk and high-risk concentrations, some wines were significantly smoky and some wines were not. No significantly smoky wines were observed in this study below the moderate risk concentrations described in Table 5.

In summary, this study is the most comprehensive to date on the relationships between volatile phenols and phenolic glycosides in smoke-exposed grapes and smoke flavour in wine, using two separate sets of diverse grapes and wines sourced after various wildfire events, with both studies giving similar results. Still, it is limited to the one vintage in Australia (2019-2020), and we cannot fully rule out that different smoke-exposure events or cultivars may lead to different patterns of abundance in grapes and wines, such as low concentrations of volatile phenols concomitant with elevated concentrations of glycosides [31, 32]. The most important volatile phenols and phenolic glycosides to assess risk have been identified in this study, yet it would be prudent to consider assessing the profiles of additional compounds in future events. Finally, our data are based on sensory assessment of young wines at 6 weeks after bottling. While there is evidence that volatile phenols increase and glycosides are quite stable over 5-6 years ageing in bottle [38], there is a need to include more samples and monitor wines over time.

Notwithstanding the limitations of the current study, the results enable better decisions to be made when assessing smoke-exposed grapes and wines, a decision with major financial and business implications that has been lacking data until now. Winemakers will be less likely to produce undesirably smoky wines, by identifying the risk early and applying suitable production techniques. Grape growers may be able to seek alternative uses for smoke-affected grapes. Producers will be able to compare results from their grape and wine analysis to the data presented herein and better understand the risk of producing unacceptably smoky wine. This will enable the wine sector to be better prepared to manage smoke events of the future.

Abbreviations

GC-MS: Gas chromatography-mass spectrometry
LC-MS: Liquid chromatography-mass spectrometry.

Data Availability

All the data from the study are available in the supplementary information attached.

Conflicts of Interest

The authors' employer, The Australian Wine Research Institute, is a not-for-profit organisation and provides smoke marker analysis as a commercial service where clients cover the cost to third parties through Affinity Labs. The analytical method has been published, relevant standards are commercially available, and other commercial laboratories within Australia and internationally also offer this service.

Supplementary Materials

The following data are provided in the supplementary materials: Table S1: Study B Region and basic wine composition. Table S2: Water additions made to musts of Study B to reduce high total soluble solids. Table S3: Study B Concentration of volatile phenols and phenolic glycosides in grapes. Table S4: Study B Concentration of volatile phenols and phenolic glycosides in wines. Table S5: Correlation coefficients for volatile phenols and phenolic glycosides comparing the concentrations in grapes to the concentrations in wine, for each cultivar and both studies. Table S6: Mean values of the smoke aroma and flavour ratings (0–10) for the wines from Study B. (*Supplementary Materials*)

References

[1] P. Høj, I. Pretorius, and R. J. Blair, *The Australian Wine Research Institute Annual Report*, Urrbrae, South Australia, Australia, 2003.

[2] M. Krstic, J. Culbert, M. Parker, and M. Herderich, "Managing wine quality," *Smoke Taint and Climate Change*, pp. 763–778, Elsevier, Amsterdam, Netherlands, 2021.

[3] Y. Hayasaka, K. A. Dungey, G. A. Baldock, K. R. Kennison, and K. L. Wilkinson, "Identification of a beta-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke," *Analytica Chimica Acta*, vol. 660, no. 1-2, pp. 143–148, 2010.

[4] K. R. Kennison, K. L. Wilkinson, A. P. Pollnitz, H. G. Williams, and M. R. Gibberd, "Effect of timing and duration of grapevine exposure to smoke on the composition

and sensory properties of wine," *Australian Journal of Grape and Wine Research*, vol. 15, no. 3, pp. 228–237, 2009.

[5] Y. Hayasaka, G. A. Baldock, M. Parker et al., "Glycosylation of smoke-derived volatile phenols in grapes as a consequence of grapevine exposure to bushfire smoke," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 20, pp. 10989–10998, 2010.

[6] R. Ristic, P. Osidacz, K. A. Pinchbeck, Y. Hayasaka, A. L. Fudge, and K. L. Wilkinson, "The effect of winemaking techniques on the intensity of smoke taint in wine," *Australian Journal of Grape and Wine Research*, vol. 17, no. 2, pp. S29–S40, 2011.

[7] Y. A. Mirabelli-Montan, M. Marangon, A. Graça, C. M. Mayr Marangon, and K. L. Wilkinson, "Techniques for mitigating the effects of smoke taint while maintaining quality in wine production: a review," *Molecules*, vol. 26, no. 6, p. 1672, 2021.

[8] M. P. Krstic, D. L. Johnson, and M. J. Herderich, "Review of smoke taint in wine: smoke-derived volatile phenols and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint," *Australian Journal of Grape and Wine Research*, vol. 21, pp. 537–553, 2015.

[9] C. M. Mayr, M. Parker, G. A. Baldock et al., "Determination of the importance of in-mouth release of volatile phenol glycoconjugates to the flavor of smoke-tainted wines," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 11, pp. 2327–2336, 2014.

[10] M. Parker, P. Osidacz, G. A. Baldock et al., "Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 10, pp. 2629–2637, 2012.

[11] D. Kelly and A. Zerihun, "The effect of phenol composition on the sensory profile of smoke affected wines," *Molecules*, vol. 20, no. 6, pp. 9536–9549, 2015.

[12] M. J. Herderich, T. E. Siebert, M. Parker et al., "Spice up your life: analysis of key aroma compounds in Shiraz," in *Flavor Chemistry of Wine and Other Alcoholic Beverages*, M. Qian, Ed., pp. 3–13, ACS, Washington, DC, United States, 2012.

[13] M. Herderich and M. Krstic, *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*, The Australian Wine Research Institute, Urrbrae, South Australia, 2020.

[14] R. Ristic, A. L. Fudge, K. A. Pinchbeck et al., "Impact of grapevine exposure to smoke on vine physiology and the composition and sensory properties of wine," *Theoretical and Experimental Plant Physiology*, vol. 28, no. 1, pp. 67–83, 2016.

[15] R. Wittkowski, J. Ruther, H. Drinda, and F. Rafiei-Taghanaki, "Formation of smoke flavor compounds by thermal lignin degradation," In *Flavour precursors American Chemical Society*, vol. 490, pp. 232–243, 1992.

[16] P. J. Spillman, M. A. Sefton, and R. Gawel, "The effect of oak wood source, location of seasoning and coopering on the composition of volatile compounds in oak-matured wines," *Australian Journal of Grape and Wine Research*, vol. 10, no. 3, pp. 216–226, 2004.

[17] T. Tominaga, G. Guimbertau, and D. Dubourdieu, "Contribution of benzenemethanethiol to smoky aroma of certain *Vitis vinifera* L. Wines," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 5, pp. 1373–1376, 2003.

[18] E. Wilkes, "Smoke analysis at the AWRI: a testing year," *Australia and New Zealand Grapegrower and Winemaker*, vol. 684, pp. 58–61, 2021.

[19] Y. Hayasaka, M. Parker, G. A. Baldock et al., "Assessing the impact of smoke exposure in grapes: development and validation of a HPLC-MS/MS method for the quantitative analysis of smoke-derived phenolic glycosides in grapes and wine," *Journal of Agricultural and Food Chemistry*, vol. 61, no. 1, pp. 25–33, 2013.

[20] M. Noestheden, E. G. Dennis, E. Romero-Montalvo, G. A. DiLabio, and W. F. Zandberg, "Detailed characterization of glycosylated sensory-active volatile phenols in smoke-exposed grapes and wine," *Food Chemistry*, vol. 259, pp. 147–156, 2018.

[21] A. Caffrey, L. Lerno, A. Rumbaugh et al., "Changes in smoke-taintvolatile-phenol glycosides in wildfire smoke-exposed Cabernet Sauvignon grapes throughout winemaking," *American Journal of Enology and Viticulture*, vol. 70, no. 4, pp. 373–381, 2019.

[22] A. Coulter, G. A. Baldock, M. Parker, Y. Hayasaka, I. L. Francis, and M. Herderich, "Concentration of smoke marker compounds in non-smoke-exposed grapes and wine in Australia," *Australian Journal of Grape and Wine Research*, vol. 28, no. 3, pp. 459–474, 2022.

[23] A. Oberholster, Y. Wen, S. Dominguez Suarez et al., "Investigation of different winemaking protocols to mitigate smoke taint character in wine," *Molecules*, vol. 27, no. 5, p. 1732, 2022.

[24] C. Szeto, R. Ristic, D. Capone et al., "Uptake and glycosylation of smoke-derived volatile phenols by Cabernet Sauvignon grapes and their subsequent fate during winemaking," *Molecules*, vol. 25, no. 16, p. 3720, 2020.

[25] Wine Australia, "Vintage 2020: Quantity constrained but value continues to grow," in *Market Bulletin*Wine Australia Vintage, Adelaide, South Australia, 2020.

[26] Wine Australia, "Vintage Survey Dashboard," 2022, https://marketexplorer.wineaustralia.com/vintage-survey.

[27] B. G. Coombe, "Growth stages of the grapevine: adoption of a system for identifying grapevine growth stages," *Australian Journal of Grape and Wine Research*, vol. 1, no. 2, pp. 100–110, 1995.

[28] W. Jiang, E. Bilogrevic, M. Parker et al., "The effect of pre-veraison smoke exposure of grapes on phenolic compounds and smoky flavour in wine," *Australian Journal of Grape and Wine Research*, vol. 2022, Article ID 9820204, 15 pages, 2022.

[29] P. Iland, N. Bruer, G. Edwards, S. Caloghiris, and E. Wilkes, *Chemical Analysis of Grapes and Wine: Techniques and Concepts*, Patrick Iland Wine Promotions Pty Ltd, , Patrick Iland Wine Promotions Pty Ltd, Athelstone SA 5076, Australia, 2 edition.

[30] J. A. Fryer and E. Tomasino, "Analysis of retronasal flavor alterations in smoke-affected wines and the efficacy of various inter-stimulus rinse protocols in clearing smoke-related attributes," *Beverages*, vol. 8, no. 2, p. 23, 2022.

[31] H. H. Chong and M. T. Cleary, "Smoke taint aroma assessment in 2008 California grape harvest," *Flavor chemistry of wine and other alcoholic beverages*, American Chemical Society, vol. 1104 , pp. 67–79, 2012.

[32] K. Wilkinson and R. Ristic, *Comparing the Chemical and Sensory Consequences of grapevine Smoke Exposure in Grapes and Wine from Different Cultivars and Different Wine Regions in Australia*" 13th International Terroir Congress, Adelaide, Australia, 2020.

[33] J. A. Culbert, W. Jiang, E. Bilogrevic et al., "Compositional changes in smoke-affected grape juice as a consequence of activated carbon treatment and the impact on phenolic

compounds and smoke flavor in wine," *Journal of Agricultural and Food Chemistry*, vol. 69, no. 35, pp. 10246–10259, 2021.

[34] H. Martens and M. Martens, "Modified jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (plsr)," *Food Quality and Preference*, vol. 11, no. 1-2, pp. 5–16, 2000.

[35] G. A. Burdock, *Fenaroli's Handbook of Flavor Ingredients*, CRC Press, Boca Raton, FL, USA, 4 edition, 2002.

[36] R. Lopez, M. Aznar, J. Cacho, and V. Ferreira, "Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection," *Journal of Chromatography A*, vol. 966, no. 1-2, pp. 167–177, 2002.

[37] M. Czerny, M. Christlbauer, M. Christlbauer et al., "Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions," *European Food Research and Technology*, vol. 228, no. 2, pp. 265–273, 2008.

[38] R. Ristic, L. van der Hulst, D. L. Capone, and K. L. Wilkinson, "Impact of bottle aging on smoke-tainted wines from different grape cultivars," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 20, pp. 4146–4152, 2017.