A GC-MS based analytical method for detection of smoke taint associated phenols in smoke affected wines.

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ABSTRACT

Guaiacol and 4-methylguaiacol are routinely used as markers to determine extent of smoke impact on winegrapes and wines. However, smoke contains a complex group of compounds which may contribute to smoke taint in winegrapes and wine. In this study, a gas chromatography-mass spectrometry (GC-MS) based analytical method was developed and validated for the profiling of various smoke taint compounds in wines made from smoke affected fruit. A total of 22 analytes were separated and identified in the GC-MS chromatogram, all of which were selected to evaluate the samples and precision of the method. The GC-MS method showed good repeatability/reproducibility with intra- and inter-day relative standard deviation (RSD) of ±14%. The method was used to demonstrate that the smoked grapes and resultant wines, compared to unsmoked wines, contained significantly enhanced levels of guaiacol and 4-methylguaiacol along with other lignin derived phenols such as cresols and syringol. In smoke affected grapes and young wines, volatile phenols exist as glyco-conjugates (potential taint) which hydrolyse slowly leading to unacceptable levels of taint accumulation in wine during storage. The GC-MS method reported here, in conjunction with the optimised acid hydrolysis of phenol glyco-conjugates, was successfully used to determine potential levels of smoke taint compounds in wines. Thus, the method can be used for screening smoke exposed grapes for potential taint levels prior to wine making. The results presented here highlight the need to include an array of smoke derived phenols to develop a complete picture of smoke taint and associated aroma in affected grapes and wines.

KEY WORDS: acid hydrolysis, gas chromatography-mass spectrometry, glycosides of phenols, lignin, smoke taint, solid phase extraction, volatile phenols, wine.

INTRODUCTION

Research conducted in the last five years has found that smoke affected winegrapes and wines produced from these grapes have “smoke taint” aroma [1-5]. Common descriptors of smoke taint aroma in wines are smoky, dirty, earthy, burnt, smoked meat, bacon, damp fire, plastic, ashtray and band aid characters. These unpleasant characteristics in the wines, prepared from smoke affected fruit, have resulted in low consumer appeal and financial loss to the wine grape industry [3].

The vegetative biomass consumed in bushfires and fuel reduction burning is primarily composed of cellulose (40-45%), hemicelluloses (20-35%) and lignin (18-35%) compounds [6]. It is widely believed that the pyrolysis of lignin in a fuel releases phenols that give smoke its distinctive smell and these compounds are normally associated with the tastes and smells of smoke cured foods [7, 8]. However, production and concentration of these compounds in the smoke depend upon oxidative combustion conditions such as temperature, moisture content and fuel type [9-11].

Guaiacol and 4-methylguaiacol, which are thermal degradation products of lignin, have been widely used as indicator compounds in assessing smoke taint levels and the degree to which fruit and wines have been affected by smoke [2-4]. However, concentrations of guaiacol and 4-methylguaiacol are not always a reliable indicator of the extent of smoke exposure. In some cases these compounds were not detected, or detected at low levels, in the fruit while high levels were subsequently identified during or after winemaking or storage [12-14]. This discrepancy was attributed to the presence of glycosidic conjugates of volatile phenols in the grapes, which were thought to evolve into smoke taint during fermentation and wine making. Later research involving high pressure liquid chromatography mass spectrometry (HPLC-

77 MS/MS) and hydrolysis under acid or enzymatic conditions confirmed the presence of
78 glycosidic conjugates in grapes and wine [1, 5, 15].

79 Pyrolysis of smoke produced from the combustion of vegetative biomass
80 contains several other volatile and semi-volatile phenols [10, 11, 16], which can
81 contribute to smoke taint and hence, to the overall sensory properties of smoke
82 affected fruit and wine. Recently, elevated levels of free phenols and their glycosides
83 such as cresols, syringol and syringol derivatives have been reported in smoke
84 affected fruit and wine [1, 17] indicating that identification and quantification of
85 guaiacol and 4-methylguaiacol may not present the complete picture of smoke taint
86 and associated aroma in fruit and wine. Additionally, individual concentrations of
87 these phenols may be well below sensory thresholds but their combined
88 concentrations may result in a perceived sensory effect. Therefore, it is important to
89 investigate whether different phenols contribute to smoke taint.

90 The present paper describes the development (optimisation and validation) of
91 a gas chromatography-mass spectrometry (GC-MS) based analytical method to
92 identify and quantify the characteristic organic compounds (i.e. volatile phenols)
93 emitted during pyrolysis of wood (or lignin) in wines prepared from smoke affected
94 fruit. The method involved solvent extraction and a subsequent capillary GC-MS
95 detection and determination of volatile phenols in wine made from fruit exposed to
96 smoke. Glycoside bound phenols were extracted from the wine using solid-phase
97 extraction (SPE) before acid hydrolysis to generate aglycones followed by solvent
98 extraction and GC-MS analysis.
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MATERIALS AND METHODS

Chemicals

HPLC grade acetonitrile, methanol, ethanol, sulphuric acid, and sodium hydroxide were purchased from Merck and Co. Inc. (Darmstadt, Germany). Standards for phenol, *o*-, *m*- and *p*-cresol, 4-ethylphenol, 4*n*-propylphenol, 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4*n*-propyl-2-methoxyphenol (4*n*-propylguaiacol), 2-methoxy-4-vinylphenol (4-vinylguaiacol), 2,6-dimethoxyphenol (syringol), 2,6-dimethoxy-4-methylphenol (4-methylsyringol), 4-allyl-2,6-dimethoxyphenol (4-allylsyringol) and syringaldehyde were acquired from BioScientific Pty Ltd. (Gymea, NSW, Australia). Eugenol, isoeugenol, guaiacol, 4-methylguaiacol, vanillin, acetovanillone, acetosyringone standards, ethylacetate and *n*-hexane (GC grade) were obtained from Sigma-Aldrich (St. Louis MO, USA). 2-methoxy-d_3-phenol (d_3-G) was purchased from CDN isotopes (Pointe-Claire, QB, Canada). Purity of all standards was verified by GC-MS before preparation of stock solutions. Deionised water was obtained through a MilliQ system (Milli-RX Analytical-Grade Water Purification System, Millipore, Billerica MA, USA).

Wine Samples

Wines were made from *Vitis vinifera* L. cv. Chardonnay, Merlot, Shiraz, Sangiovese and Cabernet Sauvignon fruit collected from the King Valley wine region of north eastern Victoria (36°42’ South, 146°25’ East), Australia. Fruit was collected in March 2007 following bushfire events in December 2006 and January 2007 [5]. To meet quarantine regulations, fruit was frozen at -20 °C for at least seven days prior to shipping to a small scale winery for winemaking. Wines were made according to a standardised methodology [18]. For comparison, wines were made from smoke unexposed grapes of Chardonnay, Shiraz and Cabernet Sauvignon varieties (2006 and

2009 vintage) from the Mildura region (34°42’ South 142°28’ East) and analysed for both free and bound forms of volatile phenols. The Mildura region had no bushfire activity in 2005-06 and 2008-09.

Sample preparations

Free forms of phenols were measured by extracting 5 mL of the wine samples with 2 mL of ethylacetate:*n*-hexane (1:1, v/v) after spiking with 10 µL of d_3-G and adding 1.05 g NaCl. The samples were vortexed for 1 min followed by incubation at room temperature for 60 min. The incubation at the room temperature was continued for another 1-2 h after addition of 2 mL of ethylacetate:*n*-hexane (1:1, v/v) and vortexing for 1 min. A 1 mL portion of the organic phase, obtained after spinning at 2,469 x g for 5 min, was transferred to a 2 mL GC autosampler vial, capped and analysed for various phenols using the GC-MS method described below.

For analysis of glyco-conjugated phenols, the following sample preparation, extraction and acid hydrolysis procedures were performed prior to GC-MS analysis. A 20 mL aliquot of the wine to be analysed was frozen in liquid nitrogen and dried using a freeze dryer (Freezone, Labconco Corporation, Kansas City MO, USA) at -75 °C. The dried samples were redissolved in 10 mL of deionised water. 1.5 mL of 10 M NaOH added and the solution filtered through a 0.45 µm polypropylene syringe filter (Whatman, Kent, UK).

Solid-phase extraction (SPE) was utilised to extract bound forms of phenols from freeze dried wine samples and to remove non-phenolic substances (sugars, organic acids, proteins and pigments) which can interfere with the chromatographic separation. An Oasis® HLB Plate 96-well plate (Waters Corporation, Milford MA, USA) was used for SPE as reported previously [1, 5, 19-21]. Solid-phase plates were conditioned with 0.5 mL methanol followed by a rinse with 0.5 mL deionised water.

One mL of wine samples were loaded into 8 wells and the liquid was removed under vacuum. The wells were rinsed three times with 1 mL aliquots of deionised water.

The solid-phase plate columns were eluted under vacuum with 0.17 mL ethanol (99.9%) and rinsed with 0.33 mL deionised water into a clean 2 mL 96 well plate. One mL of each sample was then transferred in three replicates to 20 mL GC-MS head-space autosampler vials. To this was added 4 mL of 5 N H_2SO_4 (pH1.0). The sealed autosampler vials were incubated for 1 h at 100 °C. Samples were cooled on ice and transferred to Kimble tubes (PYREX® Corning, New York, USA) containing 1.05 g NaCl. These samples were spiked with 10 µL of internal standard (1 mg/L d_3-G in ethanol) and extracted with 2 mL of ethylacetate:*n*-hexane (1:1, v/v) as described above for the analysis of free forms of volatile phenols.

Gas chromatography mass spectral analysis of various phenols

Grape and wine samples were analysed for various phenols using an Agilent 7890A gas chromatograph and 5975 mass spectrometer (Agilent Technologies, Palo Alto CA, USA) equipped with a fused silica capillary column (AT-5MS, 0.25 mm I.D. x 30 m length and 0.25 µm film thickness, GRACE, Deerfield, USA). Helium (ultra purity grade, BOC Gases, Adelaide SA, Australia) was used as a carrier gas with an average linear velocity of 37 cm/s and a flow rate of 1 mL/min. Liquid sample (1 µL) was injected using a CTC-PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) into the GC inlet injector at 240 ºC fitted with a 4 mm id liner (Agilent Technologies, Palo Alto CA, USA). The GC injector (inlet 1) was operated in the pulsed/splitless mode with a pulsed pressure of 40 psi for 0.5 min followed by a split flow of 100 mL/min for 1 min. Oven temperature started at 50 ºC and was increased by 15 ºC/min until reaching 280 ºC and was held at 280 ºC for 1 min. Under this temperature program the elution order was phenol, cineole, *o*-cresol, *m*-cresol, *p*-

cresol, guaiacol, 2,4-dimethyphenol, 4-ethylphenol, 4-methylguaiacol, 4*n*propylphenol,4-ethyl-guaiacol, 4-vinylguaiacol, syringol, eugenol, 4*n*-propyl-guaiacol, vanillin, 4-methylsyringol, isoeugenol, acetovanillone, allylsyringol, syringaldehyde, acetosyringone, and d3-G Fig. (**1**).

The MS ion source temperature was 230 ºC and the GC-MS transfer line temperature was 220 ºC. A solvent delay of 3 min was set up and data acquisition mode was set to Selective Ion Monitoring (SIM) mode. The ions monitored are detailed in Table **1** (Source: National Institute of Standards and Technology virtual library).The selected ions were monitored for 50 ms each. Samples were analysed in triplicate. The detector showed good linear response for each of the 22 analytes ($r^2 \geq 0.99$).

Calibration standards and method validation

Solutions containing 1000, 500, 250, 100, 50, 25, 10, 5, 2.5 and 1.0 μg/L phenol, cineole, *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, 2,4-dimethyphenol, 4-ethylphenol, 4-methylguaiacol, 4*n*-propylphenol, 4-ethylguaiacol, 4-vinylguaiacol, syringol, eugenol, 4*n*-propylguaiacol, vanillin, 4-methylsyringol, isoeugenol, acetovanillone, allylsyringol, syringaldehyde and acetosyringone were prepared in ethylacetate:*n*-hexane (1:1, v/v). The calibration standard curves were prepared by transferring 1.0 mL of a solution containing all the 22 compounds to a 2 mL vial and adding 10 μL of d_3-G internal standard solution. A typical chromatogram from a standard solution containing the 22 analytes is shown in Fig. 1A.

The specificity, precision and validation of the analytical method were determined by spiking a series of standards to red wine (Shiraz). The wines were spiked in triplicate with 0, 10, 20, 40, 80, and 160 μg/L of mixed standards to determine analyte recoveries.

The limit of detection (LOD) and limit of quantification (LOQ) of all 22 phenols according the statistical procedures described previously [22].

RESULTS AND DISCUSSION

Calibration and performance characteristics

Lignins are primarily polymers of three monolignols i.e. para-coumaryl, coniferyl and sinapyl alcohols which differ in their degree of methoxylation [23]. A total of 22 compounds, covering different chemical families of lignin were studied (Table 1). Eight *p*-coumaryl alcohols (phenol, cineole, *o*-, *m*- and *p*-cresols, 4-ethylphenol, 2,4-dimethylphenol and 4*n*-propylphenol), nine coniferyl alcohols (guaiacol, 4-methylguaiacol, 4-ethylguaiacol, eugenol, isoeugenol, 4*n*-propylguaiacol, 4-vinylguaiacol, vanillin and acetovallinone) and five sinapyl alcohols (syringol, 4-methylsyringol, allylsyringol, syringaldehyde and acetosyringone) were selected for the method development and validation. The representative compounds for each monolignol class (Table 1) were chosen by considering published data on composition of smoke from lignin pyrolysis and liquid smoke flavourings [6-8, 24-27]. The presence of some of these compounds was also confirmed in the smoke from prescribed burns [1]. This further highlighted the risk of exposure of grape vines to various volatile phenols in smoke from bushfire or prescribed burns and development of smoke taint in grapes and wine.

Ethylacetate has been used to extract non-flavonoid phenols in wines [28] and maple products [29] for analyses by HPLC with very good reproducibility and without any chemical modifications. Previous researchers have observed that the mean percentage recovery for all phenolic and furfural compounds using different methods of extraction was, in decreasing order: ethyl acetate (87.6%) > Sep-Pak

(82.2%) > lyophilization (62.9%) > ether (44.3%) > Supelclean (41.8%). Recently, Hayasaka *et al.* [1] used ethylacetate:*n*-pentane (1:1, v/v) for extraction of volatile phenols from grape juice and wines without reporting any artefacts. Preliminary trials substituting *n*-pentane with *n*-hexane showed improved baseline on chromatographs (Figure1 and data not shown); thus in this study we used ethylacetate:*n*-hexane (1:1, v/v) for extraction of volatile phenols from unsmoked (control) and smoke affected wines. The parameters of the method were optimised by using standards of various phenols (Table 1) in ethylacetate:*n*-hexane (1:1, v/v). For each standard, ten concentrations (1-1000 μg/L) were tested in 5-10 replicates; these concentrations covered the concentration ranges expected for these compounds in wine. Total ion chromatogram and retention times of all the studied compounds are shown in Figures 1A, 1B and Table 1. Target ion and one or two qualifier ions were used to calculate the (volatile compound/internal standard) ion peak area ratio for each studied volatile compound (Table 1). For all the compounds examined, the relationships between ion peak area ratios and analyte concentration ratios were linear over the entire calibration range (1-1000 μg/L). The coefficients of determination (r^2) were ≥ 0.99.

The limits of detection and quantitation of the analytes were low enough (Table 2) to detect and/or quantitate these compounds in wine samples from frapes unaffected by smoke. The LOQ values determined in this work were close to the lowest concentration of the calibration range and are comparable to those published elsewhere [1].

Accuracy, recovery, repeatability and reproducibility

In order to calculate the accuracy of the method, a recovery study was carried out. Known concentrations of the volatile phenols were spiked in triplicate into a smoke unaffected wine and the concentrations before and after the addition were

251 determined. On the evidence of these concentrations, the percent recovery for each
252 studied compound was calculated (Table 2). The majority of the compounds had
253 reasonably high recovery (> 90%) except cineole (< 50%). Compounds such as
254 eugenol and isoeugenol showed intermediate levels of recovery (79-84%). The
255 recovery of some of the smoke taint compounds was better than 100% with relative
256 standard deviation (RSD) < 10% (Table 2). This could be due to hydrolysis of soluble
257 precursors at higher injector block temperatures as has also been reported previously
258 [5, 30]. Another reason for this discrepancy could be matrix enhancement of the GC
259 response; usually from the active sites in the liner and column being shielded by
260 compounds in the matrix resulting in a larger response for the target compounds.
261 Nevertheless, these results are similar to spiked recoveries observed in previous
262 studies, suggesting that wine components may affect the extraction of volatile phenols
263 [31].

264 The intra-day (repeatability) and inter-day (reproducibility) precision of the
265 method were calculated by means of eight samples extracted in triplicate at the same
266 time and another eight extractions performed on different days. No significant
267 differences were observed between the sets of data produced either intra- or inter-day.
268 As can be seen in Table 2, the precision were broadly comparable for the intra- (2.2-
269 12.2%) and inter-day (1.9-14.2%) runs indicating the robustness of the method.

270 The detection limits (LOD) determined for most of the chemicals analysed
271 was < 5 μg/L (Table 2). These values were close to the lowest concentration level of
272 the working range. It was verified that these analytes presented rates of recovery and
273 levels of detection compatible with their thresholds of perception and the
274 concentrations expected in non-smoked fruit and wine [32] (Table 3). In summary,
275 taking into account recovery, repeatability, reproducibility, LOD and LOQ, the

method developed here provides an acceptable level of accuracy for the determination of volatile phenols which may contribute to smoke taint in wines prepared from smoke affected fruit.

Determination of volatile compounds in wines

Previous research has established a strong link between smoke exposure and development of smoke aroma in winegrapes and the wine product. We used the GC-MS based analytical method developed here to examine the levels of free and glyco-conjugates of phenols in wine samples prepared from smoke exposed and unexposed grapes. Fig. 1B shows the total ion chromatogram of one of the smoke affected wine samples showing the presence of various smoke related phenols.

The analytical method was used successfully to show that bushfire smoke affected wines, compared to unaffected control wines, contained markedly elevated levels of a range of smoke taint compounds for all the varieties and hence different wine matrices examined (Table 3). Cineole, eugenol, and isoeugenol were not detected in the smoked or unsmoked control wines. This may be due to degradation of these analytes during analysis as suggested previously [1, 12] or these analytes were present at levels lower than the detection limits of the method described above.

Vanillin, the main phenolic aldehyde and its derivatives contribute to vanilla aromas [33], was detected at slightly elevated levels in wines prepared from smoke affected grapes compared to unsmoked control wines (Table 3). Free acetovallinone content was significantly higher in smoke affected wine for all varieties, but the levels in Cabernet Sauvignon, Merlot and Sangiovese wines were approximately twice those in Chardonnay and Shiraz wines. Syringol and acetosyringone were the most dominant sinapyl volatile phenols. This is consistent with a previous report where enhanced levels of syringol have been observed in smoke affected grapes [1]. Other

301 related compounds such as syringaldehyde which contribute to the enhancement of
302 aged wine's flavour were detected in free form in only two of the varieties: Cabernet
303 Sauvignon (122 µg/L) and Merlot (98 µg/L) (Table 3). The reason for this differential
304 result is not clear but may indicate a varietal difference in accumulation.

305 Wines produced from grapes not exposed to smoke had low levels of some of
306 the volatile phenols studied here in both the free as well as bound forms as evident
307 from only slightly elevated levels after acid hydrolysis (Table 3). These results
308 suggest that small amounts of the phenolic glycosides are naturally present in grapes
309 and are released during yeast fermentation or aging of bottled wines. Previous studies
310 have reported glycosides of guaiacol in the berries of Tempranillo, Grenache [34],
311 Shiraz [5, 13], Merlot [12] and vanillin as glycoside in grapes, cherry and strawberry
312 [35]. Recently glycosides of phenol, cresols, methylsyringol and syringol had been
313 detected in unsmoked Chardonnay berry juice [1, 17]. It is also possible that some of
314 these phenols derive at least partially from degradation of certain lignified zones of
315 the fruit for example, the seed [36]. Therefore, it will be interesting to process grapes
316 and yeast fermentation samples with or without seeds to shed further light on the
317 provenances of these chemicals.

318 Concentrations of hydrolytically released *p*-coumaryl alcohols from their
319 bound forms ranged from 52 µg/L (unsmoked Chardonnay, control wine) to 1260
320 µg/L (smoked Merlot wine). The glycoside-bound *p*-coumaryl pool of compounds
321 was generally dominated by cresols (Table 3). Among all the wines prepared from
322 smoke affected fruit, the highest concentrations of hydrolytically released *p*-coumaryl
323 alcohols were observed in Chardonnay, Cabernet Sauvignon and Merlot, while the
324 lowest concentrations were observed in wines from Sangiovese and Shiraz grapes.
325 Concentrations of the hydrolytically released phenols from the coniferyl alcohol

group ranged from 141 µg/L in smoke unaffected Shiraz wines to 1698 µg/L in smoke tainted Cabernet Sauvignon wines (Table 3). Concentrations of the hydrolysed sinapyl alcohol group of compounds showed a two order of magnitude range (69 µg/L in control Chardonnay wines to 6487 µg/L in smoke tainted Cabernet Sauvignon wines) (Table 3). Generally, the highest concentrations of hydrolytically released compounds were observed in red wines, while the lowest concentrations were observed in wines from Chardonnay grapes. This observation was consistent with previous reports that wines made from white grapes tended to have lower levels of guaiacol and 4-methylguaiacol and that this was due to the absence of skin contact during winemaking with the wines being made from free-run juice [14]. This suggests that smoke taint compounds accumulate differentially in different tissues of grapes. It would therefore be interesting to analyse these compounds in each of the berry tissues (skin, seeds and flesh) separately to localise their distribution in the berry.

Guaiacol and 4-methylguaiacol concentrations in wines, made from bushfire smoke exposed fruit, were considerably higher than those reported in wines prepared from fruit exposed to smoke under experimental conditions [2, 4, 14, 17]. This is likely to be a function of the density and duration of smoke exposure, which has been shown to influence guaiacol levels in smoke tainted wine [37]. However, for the samples analysed here the bushfire smoke density and duration of exposure were not known although these are likely to be denser and longer than the experimental smoke exposure conditions based on anecdotal reports. It is also possible that there are differences in varietal sensitivity and accumulation of smoke taint compounds, and it is worth verifying whether this indeed is the case. The results from this work collectively demonstrate that smoke unaffected wines contain high totals of background (constitutive) levels of lignin derived compounds ranging from 498 µg/L

in Chardonnay to 1549 μg/L in Cabernet Sauvignon (Table 3). The primary effect of smoke exposure is less a matter of generating new smoke taint compounds than of elevating the levels of lignin-derived compounds that are naturally found in grapes and wines. Thus, in this respect, in wines made from smoke exposed grapes, the background levels were increased by 5 -10 fold (Fig. 2).

CONCLUSIONS

We have optimised the conditions for the analysis of smoke derived volatile phenols that may possess smoky aromas in winegrapes and finished wines by GC-MS. Under the optimised conditions developed in this study, SPE can be considered an appropriate technique for the extraction of bound forms of smoke taint compounds from complex matrices such as wines. The detection and quantitation limits, and the accuracy obtained are adequate for the quantification of the studied phenols.

Several of these volatile phenols were detected in wines prepared from fruit exposed to smoke from 2006-07 bushfire event in the north eastern Victoria. In view of the results obtained here and the method's capability for analysing a wider range of smoke taint compounds than has been hitherto reported, this method will be a valuable tool in furthering smoke taint research. Furthermore, evaluation of additional smoke taint associated compounds with this method provides opportunities to explore the impact on predictive assays and additive or cumulative effects on sensory analyses.

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Table 1. Characteristics of the calibration curves generated by using GC-MS based analytical method (described in materials and methods section) to examine smoke taint related phenols.

Compound	Quantifying ions (*m/z*)	Retention time (min)	Studied range (μg/L)	r^2	mean slope	RSD (%)
phenol	94, 66, 65, 39	4.284	2.5-1000	0.9983	0.2106 ± 0.010	3.2
cineole	154, 139, 108, 111	4.856	2.5-1000	0.9975	0.2561 ± 0.008	3.2
***o*-cresol**	108, 107, 79, 77	5.022	2.5-1000	0.9994	0.1772 ± 0.010	2.9
***p*-cresol**	107, 108, 79, 77	5.198	2.5-1000	0.9996	0.3914 ± 0.010	2.9
***m*-cresol**	108, 107, 79, 77	5.23	2.5-1000	0.9991	0.3473 ± 0.010	3.1
guaiacol	109, 124, 81	5.409	1-1000	0.9998	0.1715 ± 0.003	1.9
2,4-dimethylphenol	122, 107,121, 77	5.962	1-1000	0.9996	0.3126 ± 0.010	2.8
4-ethylphenol	107, 122, 77	6.122	1-1000	0.9993	0.3846 ± 0.010	2.9
4-methylguaiacol	138, 123, 95	6.439	1-1000	0.9984	0.1465 ± 0.003	1.8
4*n*-propylphenol	107, 136, 77	7.009	1-1000	0.9981	0.3428 ± 0.010	3.2
4-ethylguaiacol	137, 152, 122	7.263	2.5-1000	0.9964	0.2823 ± 0.010	2.1
4-vinyl guaiacol	150, 134, 107	7.588	1-1000	0.9962	0.0891 ± 0.002	2.0
syringol	139, 154, 111	7.909	2.5-1000	0.991	0.0473 ± 0.003	7.3
eugenol	164, 149, 131, 103	7.985	2-1000	0.9983	0.1092 ± 0.007	6.7
4*n*-propylguaiacol	137, 166, 122, 94	8.027	1-1000	0.9914	0.3790 ± 0.004	1.1
vanillin	151, 152, 109, 123	8.371	5-1000	0.9977	0.0311 ± 0.003	9.7
4-methylsyringol	168, 153, 125, 151	8.746	5-1000	0.9873	0.0416 ± 0.004	9.1
isoeugenol	164, 77, 149, 91	8.795	2.5-1000	0.9957	0.0981 ± 0.007	7.1
acetovallinone	151, 166, 123	9.118	10-1000	0.9918	0.0088 ± 0.001	8.5
allysyringol	194, 179, 167	10.025	5-1000	0.9973	0.0298 ± 0.002	7.9
syringaldehyde	182, 181, 96, 111	10.488	5-1000	0.9981	0.0218 ± 0.001	4.0
acetosyringone	181, 196, 153	11.039	5-1000	0.997	0.0309 ± 0.002	7.1

Table 2. Performance characteristics of the analytical method developed to detect and measure various phenols potentially associated with smoke taint in wines.

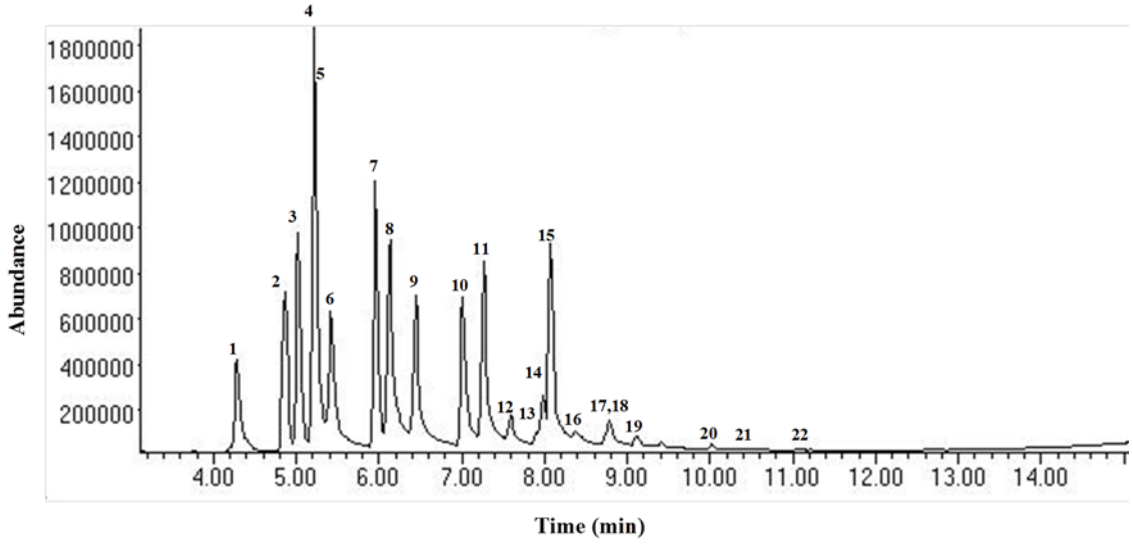
Compound	Detection limit (LOD, µg/L)	Quantitation limit (LOQ, µg/L)	Recovery (%)	Repeatability (RSD, %)	Reproducibility (RSD, %)
phenol	1.1	3.2	96.4	4.1	5.8
cineole	1.3	4.0	46.4	-	-
***o*-cresol**	0.4	1.3	117.5	4.0	3.2
***p*-cresol**	0.3	1.0	110.6	3.7	4.6
***m*-cresol**	0.3	0.9	110.7	4.4	5.8
guaiacol	0.5	1.4	121.9	5.9	5.3
2,4-dimethylphenol	1.1	3.4	-	-	-
4-ethylphenol	0.5	1.3	121.5	3.0	1.9
4-methylguaiacol	0.4	1.1	108.5	4.7	6.6
4*n*-propylphenol	0.4	1.0	106.0	9.6	4.2
4-ethylguaiacol	0.4	1.3	114.4	3.9	6.3
4-vinyl guaiacol	0.9	2.6	-	-	-
syringol	2.4	7.1	95.3	9.6	8.3
eugenol	2.1	6.1	79.0	2.5	-
4*n*-propylguaiacol	0.5	1.5	104.3	4.9	4.5
vanillin	2.1	6.4	98.6	12.2	14.2
4-methylsyringol	1.5	4.6	94.2	5.2	6.8
isoeugenol	2.1	6.4	83.7	12.8	6.4
acetovallinone	2.8	8.5	134.2	9.6	6.9
4-allysyringol	1.8	5.4	145.8	12.2	10.4
syringaldehyde	2.8	8.4	100.3	2.4	7.2
acetosyringone	1.2	3.7	107.3	2.2	4.3

Table 3. Concentrations (μg/L) of free and bound forms (determined after acid hydrolysis) of volatile phenols in wines made from smoke affected and unaffected (control) grapes. Wines were analysed by the method described here and values represent mean ± s.e. of analytical replicates (n=3).

		Location							
		Mildura (34°42' South 142°28' East)	**Mildura (34°42' South 142°28' East)**	**Mildura (34°42' South 142°28' East)**	**Whitfield (36°45' South 146°24' East)**	**Cheshunt Sth (36°55' South 146°23' East)**	**Whitfield (36°45' South 146°24' East)**	**Edi Upper (36°41' South 146°29' East)**	**Cheshunt (36°47' South 146°25' East)**
		Variety							
Free	**Volatile phenols**	**Chardonnay (Control)**	**Shiraz (Control)**	**Cabernet sauvignon (Control)**	**Chardonnay**	**Shiraz**	**Sangiovese**	**Cabernet sauvignon**	**Merlot**
p-coumaryl alcohol	phenol	21.32±1.4	9.3±1.1	12.5±0.8	60.9±0.7	107.1±5.6	125.3±3.2	249.8±13.9	59.0±3.4
	o-cresol	ND	ND	ND	15.8±0.4	60.6±3.7	106.1±2.3	108.4±5.5	25.4±1.3
	p-cresol	2.9±0.3	10.7±1.2	6.5±0.3	28.7±0.4	35.1±2.3	62.6±1.5	77.6±4.7	23.6±0.8
	m-cresol	3.7±0.4	9.6±0.9	3.6±0.3	30.3±0.3	37.9±2.6	69.2±2.1	83.7±5.2	25.5±1.3
	4-ethylphenol	ND	ND	ND	ND	ND	ND	ND	ND
coniferyl alcohol	guaiacol	1.7±0.2	21.8±1.4	8.4±0.6	86.5±1.5	283.6±14.5	487.0±9.0	306.8±15.7	100.7±4.3
	4-methylguaiacol	ND	ND	ND	61.3±1.7	68.4±3.6	123.8±2.5	180.5±10.9	45.6±2.3
	4-ethylguaiacol	ND	ND	ND	42.4±1.3	21.5±1.7	44.7±0.8	80.8±4.6	25.7±0.6
	eugenol	ND	ND	ND	ND	ND	ND	ND	ND
	4*n*-propylguaiacol	ND	1.7±0.2	ND	6.3±0.1	ND	4.1±0.1	9.0±0.4	2.6±0.1
	vanillin	27.7±2.8	32.7±0.4	35.6±1.8	48.0±1.0	49.3±1.5	46.3±1.3	47.5±1.3	29.8±0.8
	acetovallinone	40.4±2.1	73.4±1.0	165.3±1.9	303.5±8.5	305.5±11.9	779.0±27.0	707.8±27.8	617.2±23.0
sinapyl alcohol	syringol	20.3±1.5	ND	474.3±28.3	370.6±5.4	570.0±104.9	421.6±19.4	1649.8±114.1	831.4±21.5
	4-methylsyringol	ND	23.9±3.2	10.9±0.5	160.5±2.4	197.2±8.6	154.2±5.6	686.7±45.0	232.7±8.2
	allysyringol	ND	61.8±2.3	ND	90.3±3.6	118.3±9.0	41.0±0.8	127.0±6.4	49.8±3.6
	syringaldehyde	ND	ND	ND	ND	ND	ND	122.1±2.9	98.5±4.2

	acetosyringone	32.0±2.0	299.3±12.7	164.0±12.1	286.9±8.7	1054.3±17.5	966.3±28.0	1253.0±50.9	1755.5±32.1
Bound									
p-coumaryl alcohol	phenol	10.6±0.4	6.3±0.5	10.2±0.5	149.6±3.1	70.0±2.3	110.8±7.7	178.2±2.2	224.8±5.6
	o-cresol	ND	ND	ND	328.2±1.24	23.4±.8	19.6±3.3	49.9±.8.2	461.8±13.5
	p-cresol	4.2±0.6	6.7±0.2	22.5±3.5	105.9±3.7	68.9±3.3	62.7±2.6	246.0±8.6	245.4±12.1
	m-cresol	6.2±0.4	9.2±0.4	ND	138.8±9.6	52.4±2.6	72.7±9.4	314.7±15.0	328.7±19.0
	4-ethylphenol	32.1±1.0	ND	ND	31.6±0.6	ND	ND	ND	ND
coniferyl alcohol	guaiacol	3.5±0.2	17.7±0.3	7.3±0.4	130.0±3.5	209.9±7.1	253.6±9.9	235.6±2.5	377.3±11.4
	4-methylguaiacol	ND	ND	ND	63.4±2.4	57.8±3.8	114.6±9.3	132.1±3.7	210.3±7.2
	4-ethylguaiacol	8.9±0.3	ND	2.4±0.3	16.9±0.7	9.4±0.5	27.0±2.8	37.6±0.8	60.9±2.1
	eugenol	ND	ND	ND	ND	ND	ND	ND	ND
	4*n*-propylguaiacol	ND	ND	ND	1.1±0.1	ND	1.96±0.3	4.4±0.7	10.2±0.6
	vanillin	135.7±5.3	113.5±2.4	178.7±4.8	736.2±64.5	395.9±21.7	809.3±16.3	740.4±30.4	299.0±16.0
	acetovallinone	76.8±3.6	9.9±0.8	92.7±4.4	376.2±26.5	197.2±23.6	398.8±18.3	548.3±69.8	22.5±2.2
sinapyl alcohol	syringol	18.2±0.9	18.6±0.6	71.0±3.4	506.2±30.5	566.8±45.1	1433.8±162.7	2985.4±186.5	3201.2±167.5
	4-methylsyringol	ND	ND	ND	ND	7.2±0.5	9.0±1.5	49.2±1.5	11.7±1.1
	allysyringol	ND	ND	ND	ND	ND	ND	ND	ND
	syringaldehyde	ND	134.8±16.0	ND	ND	504.6±25.4	906.5±25.3	3040.7±208.5	1223.8±62.6
	acetosyringone	51.5±3.7	265.7±9.7	283.2±11.5	229.2±14.8	330.8±13.4	384.6±42.4	411.8±51.4	525.4±51.6

A.



B.

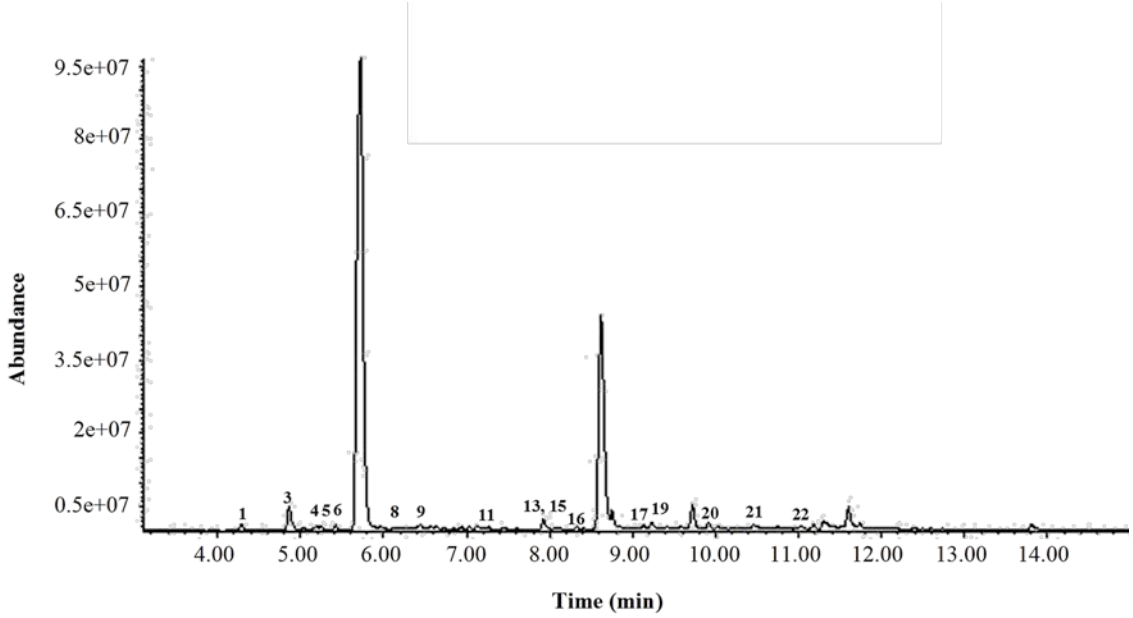


Fig. (1). SIM chromatograms showing retention times (min) of various phenolic standards (A) and ethyl acetate: n-hexane (1:1) extract of wine (B) on GC-MS AT-5MS silica capillary column (GRACE, Deerfield, IL; 30 m, 0.25 mm id and 0.25 μm film thickness). The retention times (min): 1. phenol (4.284); 2. cineole (4.856); 3. *o*-cresol (5.022); 4. *m*-cresol (5.198); 5. *p*-

cresol (5.23); 6. guaiacol (5.409); 7. 2,4-dimethyphenol (5.962); 8. 4-ethylphenol (6.122); 9. 4-methylguaiacol (6.439); 10. 4*n*-propylphenol (7.009); 11. 4-ethylguaiacol (7.263); 12. 4-vinylguaiacol (7.588); 13. syringol (7.909); 14. eugenol (7.985); 15. 4*n*-propylguaiacol (8.027); 16. vanillin (8.371); 17. 4-methylsyringol (8.746); 18. isoeugenol (8.795); 19. acetovanillone (9.118); 20. allylsyringol (10.025); 21. syringaldehyde (10.488); and 22. acetosyringone (11.039).

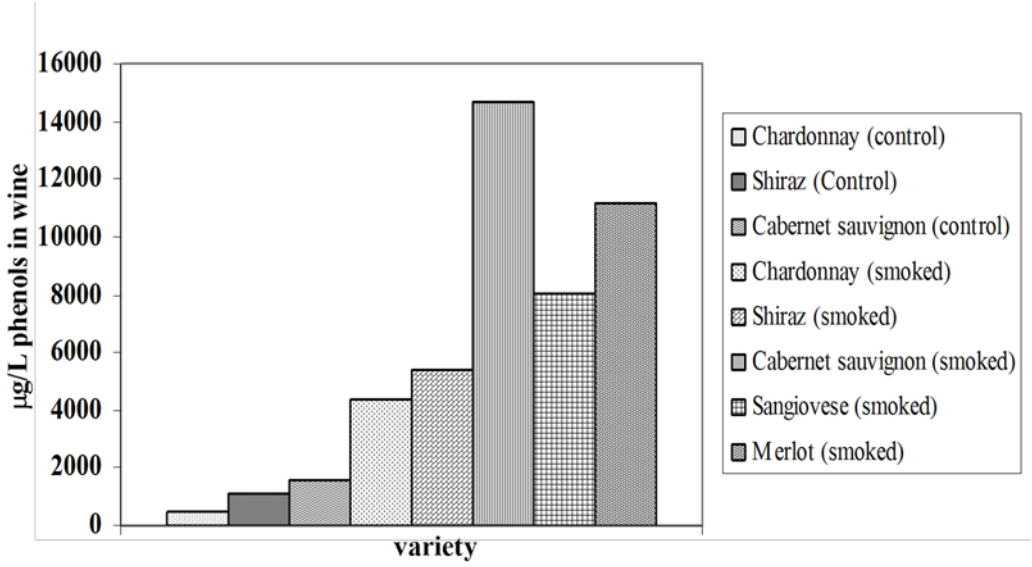


Fig. (2). Concentration of total phenols in wines prepared from grapes exposed to smoke as a result of bushfires in the 2006-07 season in north eastern Victoria Values represent mean of analytical replicates (n=3). Total represents the sum of free and bound forms of *p*-coumaryl, coniferyl and sinapyl alcohols investigated in this study.