JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Smoke-Derived Taint in Wine: The Release of Smoke-Derived Volatile Phenols during Fermentation of Merlot Juice following Grapevine Exposure to Smoke

KRISTEN R. KENNISON,[†,‡] MARK R. GIBBERD,[‡] ALAN P. POLLNITZ,[§] AND KERRY L. WILKINSON*[,‡,||]

Department of Agriculture and Food Western Australia, P. O. Box 1231, Bunbury, Western Australia 6230, Australia, Muresk Institute, Curtin University of Technology, PMB 1, Margaret River, Western Australia 6285, Australia, The Australian Wine Research Institute, P. O. Box 197, Glen Osmond, South Australia 5064, Australia, and School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia

The release of smoke-derived volatile phenols during the fermentation of Merlot grapes, following grapevine exposure to smoke, has been investigated. The concentrations of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, and eugenol were determined by gas chromatography−mass spectrometry and found to increase throughout the winemaking process. Only trace levels (≤1 μg/L) of guaiacol and 4-methylguaiacol could be detected in free run juice derived from the fruit of smoked vines; the highest levels, 388 μg/L and 93 μg/L, respectively, were observed in the finished wine. Control wine (derived from fruit of unsmoked vines) contained 4 μg/L guaiacol, with the volatile phenols either not detected or detected at only trace levels (≤1 μg/L) throughout fermentation. The role of enzyme and acid catalyzed hydrolysis reactions in releasing smoke-derived volatile compounds was also investigated. The volatile phenols were released from smoked free run juice by strong acid hydrolysis (pH 1.0) and enzyme (β-glucosidase) hydrolysis, but not mild acid hydrolysis (juice pH 3.2−3.7). Guaiacol was again the most abundant smoke-derived phenol, present at 431 μg/L and 325 μg/L in strong acid and enzyme hydrolysates, respectively. Only trace levels of each phenol could be detected in each control hydrolysate. This study demonstrates the potential for underestimation of smoke taint in fruit and juice samples; the implications for the assessment of smoke taint and quantification of volatile phenols are discussed.

KEYWORDS: β-Glucosidase; fermentation; grapes; grapevines; guaiacol; hydrolysis; 4-methylguaiacol; smoke exposure; smoke taint; volatile phenols; wine

INTRODUCTION

In recent years, significant forest fires have occurred in Asia, Africa, Europe, North America, South America, and Australia, and the incidence of such fires is expected to escalate as a result of climate-induced changes to weather, particularly increased temperature, drought, wind and natural ignition sources (*1*). In some cases, fires have occurred in close proximity to wine regions resulting in vineyard smoke exposure and smoke tainted wines. The taint, characterized by objectionable 'smoky', 'dirty' and 'burnt' aromas and a lingering retro-nasal 'ash' character on the palate (*2*), has caused significant financial loss for grape and wine producers and is therefore an issue of increasing concern.

Grape and grapevine exposure to smoke has been shown to affect the chemical composition and sensory properties of wine (*2, 3*). A number of volatile phenols including guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethlyphenol and eugenol (4-allylguaiacol) were detected in wines made from grapes which had received a postharvest exposure to smoke. Since these compounds were not present in wines made from unsmoked grapes, their origin was attributed to smoke exposure (*3*).

In wine, guaiacol, 4-methylguaiacol and eugenol are typically associated with oak barrel maturation (*4–6*), derived predominantly from the thermal degradation of oak lignin during the

* Corresponding author. School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia. Tel: +61 8 8303 7360. Fax: +61 8 8303 7116. E-mail: kerry.wilkinson@adelaide.edu.au.

[†] Department of Agriculture and Food Western Australia.

[‡] Curtin University of Technology.

[§] The Australian Wine Research Institute.

[||] The University of Adelaide.

10.1021/jf800927e CCC: $40.75
Published on Web 08/05/2008

toasting process of cooperage (*7*), although there are significant levels of eugenol present in untoasted oak (*5*). In contrast, 4-ethylguaiacol and 4-ethylphenol are bacterial in origin, arising from the action of *Brettanomyces*/*Dekkera* yeast on grape-derived *p*-coumaric acid and *p*-ferulic acid (*6, 8*). Numerous studies have identified these phenols as components of smoke and liquid smoke preparations (e.g, refs *9–12*), with guaiacol and 4-methylguaiacol reported as two of the most abundant phenols occurring in wood smoke, in both the vapor phase and aqueous extracts (*13*). Additionally, these volatile phenols are associated with smoke-like aromas (**Table 1**); guaiacol and 4-methylguaiacol in particular, which impart 'smoky', 'phenolish', 'aromatic', 'sharp' and 'sweet' aroma characters (*6, 10, 11*). Although guaiacol and 4-methylguaiacol are not considered solely responsible for smoke taint (*3*), they nevertheless represent useful marker compounds, with levels of guaiacol and 4-methylguaiacol indicative of levels of smoke taint.

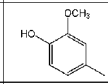
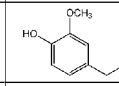
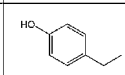
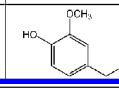
In previous studies in this laboratory, the intensity of smoke taint has been observed to increase during the fermentation of smoke affected grapes. This is consistent with anecdotal evidence from industry that smoky characters either appeared during fermentation of grapes which had not previously exhibited smoke taint, or increased throughout the winemaking process. The release of volatile secondary metabolites from grape and oak-derived flavor precursors via enzyme and acid catalyzed hydrolysis has been previously demonstrated (e.g., refs *14–17*). Accordingly, the hydrolytic release of smoke-derived volatiles from involatile precursors, such as glycoconjugates, could be responsible for the intensification of smoke aroma during fermentation. Indeed guaiacol has been previously reported as a component of acid and enzyme hydrolysates prepared from Merlot and Shiraz juices (*14, 16*), presumably deriving from glycoconjugate precursor forms. To date, the assessment of smoke taint relies on either sensory evaluation or quantification of guaiacol and 4-methylguaiacol. The presence of conjugated precursors is therefore problematic for both sensory and chemical analysis. This study was undertaken to investigate (i) the evolution of smoke-derived volatile phenols during fermentation, following grapevine exposure to smoke; (ii) the release of volatile phenols under acid and enzyme catalyzed reaction conditions; and (iii) the implications of the results for carrying out analysis of smoke affected grapes and juice.

MATERIALS AND METHODS

Field Application of Smoke to Grapevines. Merlot grapevines within a vineyard located in Capel, Western Australia were exposed to eight successive smoke applications (30 min each) between veraison and harvest, i.e., at 0, 3, 7, 10, 15, 18, 21 and 24 days post-veraison. Smoke applications were performed (in triplicate) using a purpose built smoke tent similar to that described in seed germination experiments by Dixon et al. (*18*), constructed from galvanized steel and greenhouse film (Solarweave). Smoke was generated in a metal drum (50 L) by combustion of dry straw and pumped into the smoke tent with the grapevines (3 per replicate) enclosed. Dry barley straw was selected as a fuel source to minimize variation in combustion conditions, enabling reproducible smoke application. Control grapevines were similarly enclosed in identical (smoke-free) tents for the duration of each smoke treatment to minimize differences in environmental conditions (such as humidity, temperature and light exposure). Tents were removed following each experimental treatment.

Winemaking. Grapes (three fruit replicates of approximately 16 kg each) were harvested from control (unsmoked) and smoked grapevines on the same day, corresponding to total soluble solids (TSS) contents of 22 °Brix and 19 °Brix, respectively. For each treatment, the fruit was processed to produce three replicate wines, according to standard small-lot winemaking procedures. The fruit was crushed, destemmed and fermented in 15 L fermentation vessels with EC1118 *Saccharomyces cerevisiae* yeast (Lallemand Inc., Montreal, Canada). The fermenting musts were plunged twice per day and the wine was pressed from the skins at a TSS level of 3.6 °Brix. Wines were transferred to 15 L demijohns and held at 15 °C until the residual sugar approached 0 g/L. The wines were then racked from gross lees and inoculated with *Leuconostoc oenos* (Vinaflora Oenos, Chr. Hansen, Denmark). On completion of malolactic fermentation, wines were again racked and free SO_2 adjusted (to 30 ppm) before being cold stabilized (2 °C for 28 days), filtered and bottled.

Table 1. Structures and Aroma Descriptors of Smoke-Derived Volatile Phenols

compound	structure	aroma descriptors
guaiacol		smoky, phenolish, aromatic, sharp, sweet (*6,10,11*)
4-methylguaiacol		smoky, toasted, ash, vanilla-like, sweet, phenolish, fruity, sharp (*6,10,11*)
4-ethylguaiacol		smoky, sweet, spicy, clove-like (*6,8,10*)
4-ethylphenol		horsy, leather, medicinal, smoky, barnyard, animal, stable, sweaty saddle (*6,8*)
eugenol		clove, vanilla-like, phenolish (*5,11*)

Sampling. Samples (approximately 50 mL aliquots) were collected from each smoked and control fermentation replicate, throughout the winemaking process. For smoked ferments, the sampling times were: after crushing (i.e., free run juice), after 1, 3, 5 and 7 days of maceration, at the end of alcoholic fermentation and after bottling (i.e., finished wine). The same sampling times were employed for control ferments, but with the inclusion of a sampling point after 10 days of maceration. The additional control sampling point was necessitated by differences in fermentation rates between smoked and control ferments. As in previous studies (*3*), smoke exposure increased fermentation rates, with smoked ferments completing alcoholic fermentation 3 days earlier than control ferments. Each ferment was also sampled immediately before and after pressing (i.e., at 7 and 10 days of maceration for smoked and control ferments, respectively); grape marc samples (approximately 50 g) were also collected after pressing. Finished wines were reanalyzed approximately 12 months post-bottling. Prior to analysis, must and wine samples were clarified by centrifugation and grape marc samples were crushed in liquid nitrogen.

Preparation of Acid and Enzyme Hydrolysates. Acid and enzyme hydrolysis experiments were conducted (in duplicate) using control and smoked free run juice, based on methodology described elsewhere (*17, 19*). Chemicals and enzymes were purchased from Sigma-Aldrich. Mild acid hydrolysates (i.e., juice pH: 3.2 for control juice and 3.7 for smoked juice) were prepared by heating grape juice (10 mL) for 1 h at 100 °C. Strong acid hydrolysates (i.e., pH 1.0, achieved by addition of concentrated sulfuric acid) were prepared by heating grape juice (10 mL) for 1 h at 100 °C. Enzyme hydrolysates were prepared by treating grape juice (10 mL) with almond emulsion β-glucosidase enzyme (25 mg) for 24 h at 30 °C.

Quantitative Gas Chromatography−Mass Spectrometry Analysis. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethyphenol, eugenol, furfural and 5-methylfurfural were quantified by the stable isotope dilution assay methods reported previously (*20–23*). These publications include details of the syntheses of the internal standards used herein. For all analytes: the linear dynamic range was 0, and 1−1000 μg/L; the limit of detection was 1 μg/L; and the precision was <5% relative standard deviation. The purity of all standards was verified by GC-MS.

Preparation of Must and Wine Samples for Analysis. A deuterated internal standards (I.S.) solution of d_4-furfural (1.06 μg), d_3-guaiacol

(1.13 μg), d_3-4-methylguaiacol (0.840 μg) and d_4-4-ethylphenol (0.721 μg), in ethanol (100 μL) was added to the sample (5 mL) in a screw cap vial using a glass syringe (100 μL Hamilton). The organic solvent (diethyl ether/n-pentane 1:2 (v/v) ca. 3 mL) was added, and the mixture was shaken briefly. A portion of the organic layer (ca. 2 mL) was then placed in a vial ready for instrumental analysis.

Preparation of Marc Samples for Analysis. For each marc sample, a 4.0 g subsample was accurately weighed into a screw cap vial. The I.S. solution as above (100 μL) and the organic solvent as above (10 mL) were added (to immerse the marc sample) and the lid screwed on. After 24 h at room temperature the vial was swirled briefly and then a portion of the organic layer (2 mL) was then placed in a vial ready for instrumental analysis.

Reference Standards. Reference standards containing 100 μL of deuterated internal standards ethanolic solution (as described above) and 100 μL of normal unlabeled analytes ethanolic solution (furfural (1.674 μg), 5-methylfurfural (2.073 μg), guaiacol (4.646 μg), 4-methylguaiacol (1.536 μg), 4-ethylguaiacol (2.025 μg), eugenol (2.108 μg) and 4-ethylphenol (1.798 μg)) in diethyl ether/*n*-pentane (1:2 (v/v), approximately 2 mL) were used.

Gas Chromatography−Mass Spectrometry Analysis. An Agilent Technologies 6890 gas chromatograph (GC) was equipped with a Gerstel MPS2 multipurpose sampler and coupled to an Agilent 5973N mass selective detector. The gas chromatograph was fitted with an approximately 30 m × 0.25 mm, 0.25 μm J&W DB-Wax fused silica capillary column. The carrier gas was helium (BOC Gases, high purity), linear velocity 50 cm/sec; flow rate 1.2 mL/min. vacuum compensated at the mass spectrometer interface. The oven temperature was started at 50 °C, held at this temperature for 1 min, increased to 240 at 10 °C/min, and held at this temperature for 20 min. The injector temperature was 200 °C and the transfer line was held at 240 °C. The sample volume injected was 2 μL. The splitter, at 30:1, was opened after 36 s, and the liner used was resilanized borosilicate glass, tapered, with a plug (2−4 mm) of resilanized glass wool at the column interface. The instrument was controlled with Agilent G1701CA ChemStation software in conjunction with the Gerstel MASter software (version 1.81). For quantification of the smoke volatiles, positive ion electron impact mass spectra at 70 eV were recorded in Selective Ion Monitoring (SIM) mode. The ions monitored were *m/z* 98, *100* for d_4-furfural (dwell 50 ms); *m/z* 95, *96* for furfural (dwell 50 ms); *m/z* 95, 97, *112* for 5-methylfurfural (dwell 50 ms); and *m/z* 77, 131, 149, *164* for eugenol (dwell 25 ms). The italicized ions were the ones used for quantitation (by peak area). 5-Methylfurfural was quantified versus d_4-furfural as internal standard (IS). Eugenol was quantified versus d_4-4-ethylphenol as IS. Other SIM conditions have been published previously (*20, 21*). The data was analyzed with Agilent MSD ChemStation software (Build 75).

Statistical Methods. Data were analyzed by two-way analysis of variance (ANOVA) using GenStat (8th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$.

RESULTS AND DISCUSSION

The volatile phenols, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol, were either not detected or detected at only trace levels (≤1 μg/L) in free run juice derived from fruit of smoke-exposed grapevines. However, the concentration of each compound increased dramatically and progressively throughout fermentation, with the highest levels observed in finished wine (**Table 2**). The corresponding finished control wine (derived from fruit of unsmoked grapevines) contained 4 μg/L guaiacol, but 1 μg/L or less of the other phenols of interest. As in previous studies (*3*), the absence of these compounds (at significant concentrations) in control wines indicates that in smoked samples they derive almost exclusively from the application of smoke to grapevines. Of the smoke-derived volatile phenols measured, guaiacol and 4-methylguaiacol were the most abundant, present in the finished wine at 388 μg/L and 93 μg/L, respectively. This is consistent with previous studies which reported guaiacol and 4-methylguaiacol as the most abundant phenolic components occurring in smoke (*13*). Eugenol was the least abundant phenol measured, with just 3 μg/L detected in the finished wine.

Table 2. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, and Eugenol in Ferments Derived from the Fruit of Smoked and Unsmoked Grapevines Throughout the Winemaking Process

	concentration[a] (μg/L)				
sample	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol	eugenol
		unsmoked			
free run juice	n.d.	n.d.	n.d.	n.d.	n.d.
after 1 day maceration	tr.	tr.	n.d.	n.d.	tr.
after 3 days maceration	tr.	tr.	n.d.	n.d.	tr.
after 5 days maceration	tr.	tr.	n.d.	n.d.	tr.
after 7 days maceration	tr.	tr.	n.d.	n.d.	tr.
after 10 days maceration	1	tr.	n.d.	n.d.	tr.
after alcoholic fermentation	1	tr.	n.d.	n.d.	tr.
finished wine	4	n.d.	tr.	tr.	tr.
12 months post-bottling	3	tr.	tr.	tr.	n.d.
		smoked			
free run juice	1 a	tr.	n.d.	n.d.	n.d.
after 1 days maceration	68 b	11 a	10 a	5 a	2 ab
after 3 days maceration	168 c	26 b	8 a	5 a	1 a
after 5 days maceration	203 cd	32 bc	9 a	15 b	2 a
after 7 days maceration	249 d	42 c	9 a	17 b	2 a
after alcoholic fermentation	249 d	43 c	8 a	23 c	1 a
finished wine	388 e	93 d	16 b	58 d	3 b
12 months post-bottling	371 e	124 e	29 c	94 e	4 c

[a] Values are the means from three replicates and were in agreement to ca. 10%. Values followed by a different letter within columns are significantly different ($P < 0.05$). n.d. = not detected; tr. = trace (i.e., positive identification but <1 μg/L).

Preliminary studies conducted by the Australian Wine Research Institute showed guaiacol and 4-methylguaiacol accumulated in skins, rather than pulp, of smoke affected grapes (*2*). As such, the increase in volatile phenol concentrations throughout winemaking could be attributed to ongoing extraction from skin tissues; except that phenol concentrations continued to increase during malolactic fermentation (i.e., after skins were pressed from the wine). The increased phenol content following pressing instead implies the presence of one or more precursor compounds.

Pressing itself had no apparent effect on the composition of wine, with very similar phenol concentrations observed in wine immediately before and after pressing (**Table 3**). Comparable levels of guaiacol and 4-methylguaiacol were observed in both marc and wine derived from smoke-exposed vines; but the marc retained approximately 2.5 times as much 4-ethylguaiacol and 4-ethylphenol than found in the wine. Small amounts of guaiacol and 4-methylguaiacol (6 μg/L and 2 μg/L, respectively) were observed in control grape marc, but only traces were detected in control wine, immediately before or after pressing.

Hydrolytic studies confirmed the release of smoked-derived volatile phenols under acid and enzyme catalyzed reaction conditions (**Table 4**), further supporting their accumulation in smoke affected grapes in conjugated precursor forms. The evolution of phenols through β-glucosidase activity alludes to glycoconjugate precursors, such as β-D-glucopyranosides. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, and 4-ethylphenol were identified as components of both strong acid and enzyme hydrolysates of smoked free run juice. These hydrolysates smelled strongly of 'smoke' and 'smoked meat', respectively, by informal sensory evaluation. In contrast, the mild acid hydrolysates and each of the control hydrolysates exhibited

Table 3. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, and Eugenol in Ferments (Pre- and Post-Pressing) and Grape Marc Derived from the Fruit of Smoked and Unsmoked Grapevines

	concentration[1] (μg/L or μg/kg)				
sample	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol	eugenol
		unsmoked			
wine (pre-pressing)	1	tr.	n.d.	n.d.	tr.
wine (post-pressing)	tr.	tr.	n.d.	n.d.	tr.
grape marc	6	2	n.d.	n.d.	n.d.
		smoked			
wine (pre-pressing)	249 a	42 a	9 a	17 a	2 a
wine (post-pressing)	246 a	41 a	9 a	15 a	1 a
grape marc	251 a	38 a	22 b	52 b	6 b

[1] Values are the means from three replicates and were in agreement to ca. 10%. Values followed by a different letter within columns are significantly different ($P < 0.05$). n.d. = not detected; tr. = trace (i.e., positive identification but <1 μg/L).

'berry', 'fruit' and 'jammy' aromas, with the volatile phenols detectable at only trace levels (<1 μg/L). Guaiacol and 4-methylguaiacol were again the most abundant smoke-derived phenols; present in the strong acid hydrolysate at 431 μg/L and 162 μg/L, respectively, and in the enzyme hydrolysate at 325 μg/L and 82 μg/L, respectively. Eugenol was again the least abundant phenol measured, with small quantities (5 μg/L or less) detected in strong acid hydrolysates of both smoked and control free run juice, respectively.

Guaiacyl β-D-glucopyranoside has been previously isolated from the fruit of anise (*Pimpinella anisum* L.) (*24*) and guaiacol has been identified in enzyme hydrolysates of several fruits, including tomato, mango and badea (*25–27*). This further supports our hypothesis of naturally occurring guaiacyl β-D-glucopyranoside. It is possible that plants (including grapevines) may glycosylate some volatile compounds in order to minimize toxic effects to cells, or to increase their solubility to facilitate cellular transportation. Certainly, there is literature precedence for the glycosylation of phenol in cultured plant cells (*28*). The provenance of glycosylated volatile phenols in smoke affected grapes and wine is therefore the subject of ongoing research.

Higher levels of guaiacol, 4-methylguaiacol and 4-ethylguaiacol were observed in the (smoked) strong acid hydrolysate compared to the (smoked) finished wine, suggesting incomplete hydrolysis of putative precursor compounds during fermentation. When the smoked wine was reanalyzed 12 months post-bottling, similar guaiacol levels were observed, but 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol levels increased (**Table 2**), suggesting further hydrolysis of soluble precursors with bottle age. This is akin to the accumulation of (toasted) oak derived volatile compounds in wine to different extents during aging, even with no further contact with the oak (*29*). The nature and concentration of smoke taint precursor compounds may influence the release of their free volatile aglycones under various conditions; certainly, the evolution of phenol derivatives post-bottling supports the presence of precursors in addition to simple β-D-glucopyranosides.

At juice pH and in the absence of glycosidase activity, glycoconjugates are relatively stable toward chemical hydrolysis (*30*), except where the carbohydrate unit is bonded to an activated hydroxyl group (*15*), which could explain the absence of smoke-derived phenols in the mild acid hydrolysate. Furfural and 5-methylfurfural were identified as acid hydrolysate components in both smoked and control samples, but their origin is likely attributable to acid catalyzed thermal degradation of carbohydrates, and not grapevine smoke exposure. The higher levels observed in the strong acid hydrolysates simply reflect the more aggressive hydrolysis conditions.

Significant quantities of guaiacol or 4-methylguaiacol would not be expected to form through hydrolysis of glycoconjugate precursors at juice pH. However, micro-organisms with β-glucosidase activity could certainly liberate these compounds during fermentation. The enzymatic release of smoked-derived volatile phenols therefore provides a plausible explanation for the observed intensification of smoke taint during fermentation. Most importantly, it should be recognized that if smoke-derived volatile compounds do indeed accumulate in grapes as odorless glycoconjugates following grapevine exposure to smoke, there may well be no apparent smoke taint at the time of harvest. However, the hydrolytic release of such volatiles could lead to the development of smoke aromas during fermentation, and subsequently smoke tainted wine.

For assessment of smoke taint contingent on guaiacol and 4-methylguaiacol determination, we recommend sample preparation be taken into consideration to ensure hydrolysis of any glycoconjugate precursors which might be present. In the current trial, where grapevines were deliberately exposed to repeated and relatively high intensity smoke applications, strong acid hydrolysis yielded higher levels of smoke-derived volatile phenols than enzyme hydrolysis. The strong acid hydrolysis conditions used in this study, i.e., pH 1.0 for 1 h at 100 °C, are those employed in the glycosyl-glucose assay for the quantification of glycosides in

Table 4. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, Eugenol, Furfural and 5-Methylfurfural in Free Run Juice, and Acid and Enzyme Hydrolysates of Juice Derived from Fruit of Smoked and Unsmoked Grapevines

	concentration[a] (μg/L)						
sample	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol	eugenol	furfural	5-methyl furfural
			unsmoked				
free run juice	n.d.	n.d.	n.d.	n.d.	n.d.	2	tr.
mild acid hydrolysate	tr.	tr.	tr.	tr.	n.d.	76	2
strong acid hydrolysate	tr.	tr.	tr.	tr.	2	15150	640
enzyme hydrolysate	tr.	tr.	tr.	tr.	n.d.	7	2
			smoked				
free run juice	1	tr.	n.d.	n.d.	n.d.	2	tr.
mild acid hydrolysate	tr.	tr.	tr.	tr.	n.d.	40	2
strong acid hydrolysate	431	162	31	48	5	12800	860
enzyme hydrolysate	325	82	13	27	n.d.	8	2

[a] Values are the means from three replicates for juice samples and two replicates for hydrolysate samples. Values were in agreement to ca. 10%. n.d. = not detected; tr. = trace (i.e., positive identification but <1 μg/L).

grapes, juice and wine (*19*). However, since these reaction conditions could also catalyze various aglycone side reactions (for example aglycone degradation), enzyme hydrolysis may be more appropriate for commercial samples, where less intense smoke exposure would likely give lower volatile phenol levels. Accordingly, the potential under-estimation of smoke taint can be reduced.

ACKNOWLEDGMENT

We gratefully acknowledge the vineyard owner and manager for their participation in this project, Bob Frayne, Eric Wootton, and Sue Wills (Department of Agriculture and Food, Western Australia) for invaluable technical assistance, and David Wollan (Memstar Pty. Ltd.), and Dr. Mark Sefton, Randell Taylor, Dr. Markus Herderich, and Con Simos (The Australian Wine Research Institute) for helpful discussions and suggestions.

LITERATURE CITED

(1) Overpeck, J. T.; Rind, D.; Goldberg, R. Climate-induced changes in forest disturbance and vegetation. *Nature* **1990**, *343*, 51–53.

(2) *The Australian Wine Research Institute, Annual Report*; Høj, P., Pretorius, I., Blair, R., Eds; The Australian Wine Research Institute: Adelaide, Australia, 2003; Vol. 3, pp 7−38.

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

(4) Chatonnet, P.; Boidron, J. N. Dosages de phénols volatils dans les vins par chromatographie en phase gazeuse. *Sci. Aliments* **1992**, *8*, 479–488.

(5) Chatonnet, P.; Dubourdieu, D. Comparative study of the characteristics of American white oak (*Quercus alba*) and European oak (*Quercus petraea* and *Q. robur*) for production of barrels used in barrel ageing of wines. *Am. J. Enol. Vitic.* **1998**, *49*, 79–85.

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

(7) Wittkowski, R.; Ruther, J.; Drinda, H.; Rafiei-Taghanaki, F. Formation of Smoke Flavor Compounds by Thermal Lignin Degradation. In *Flavour Precursors*; Teranashi, R., Takeoka, G. R., Günert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 232−243.

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

(9) Fiddler, W.; Doerr, R. C.; Wasserman, A. E. Composition of an ether-soluble fraction of a liquid smoke solution. *J. Agric. Food Chem.* **1970**, *18*, 310–312.

(10) Kim, K.; Kurata, T.; Fujimaki, M. Identification of flavor constituents in carbonyl, non-carbonyl neutral and basic fractions of aqueous smoke condensates. *Agric. Biol. Chem.* **1974**, *38*, 53–63.

(11) Baltes, W.; Wittkowski, R.; Söchtig, I.; Block, H.; Toth, L. Ingredients of Smoke and Smoke Flavour Preparations. In *The Quality of Food and Beverages*; Charalambous, G., Inglett, G., Eds.; Academic Press: New York, 1981; Vol. 2, pp 1−19.

(12) Maga, J. A. *Smoke in Food Processing*; CRC Press: Boca Raton, FL, 1988.

(13) Kornreich, M. R.; Issenberg, P. Determination of phenolic wood smoke components as trimethylsilyl ethers. *J. Agric. Food Chem.* **1972**, *20*, 1109–1113.

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

(15) Skouroumonis, G. K.; Sefton, M. A. Acid-catalysed hydrolysis of alcohols and their β-D-glucopyranosides. *J. Agric. Food Chem.* **2000**, *48*, 2033–2039.

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

(17) Wilkinson, K. L.; Elsey, G. M.; Prager, R. H.; Tanaka, T.; Sefton, M. A. Precursors to oak lactone. Part 2: Synthesis, separation and cleavage of several β-D-glucopyranosides of 3-methyl-4-hydroxyoctanoic acid. *Tetrahedron* **2004**, *60*, 6091–6100.

(18) Dixon, K. W.; Roche, S.; Pate, J. S. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **1995**, *101*, 185–192.

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

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

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

(22) Pollnitz, A. P. The Analysis of Volatile Wine Components Derived from Oak Products during Winemaking and Storage. Ph.D. Thesis, The University of Adelaide, Australia, 2000.

(23) Wilkinson, K. L. Oak Derived Flavour Compounds and Their Contribution to Wine and Spirits. Ph.D. Thesis, Flinders University, Australia, 2004.

(24) Fujimatu, E.; Ishikawa, T.; Kitajima, J. Aromatic compound glucosides, alkyl glucoside and glucide from the fruit of anise. *Phytochem.* **2003**, *63*, 609–616.

(25) Ortiz-Serrano, P.; Gil, J. V. Quantitation of free and glycosidically bound volatiles in and effect of glycosidase addition on three volatile tomato varieties (*Solanum lycopersicum* L.). *J. Agric. Food Chem.* **2007**, *55*, 9170–9176.

(26) Ollé, D.; Baumes, R. L.; Bayonove, C. L.; Lozano, Y. F.; Sznaper, C.; Brillouet, J.-M. Comparison of free and glycosidically linked volatile components from polyembryonic and monoembryonic mango (*Mangifera indica* L.) cultivars. *J. Agric. Food Chem.* **1998**, *46*, 1094–1100.

(27) Osorio, C.; Duque, C.; Suárez, M.; Salamanca, L. E.; Urueña, F. Free, glycosidically bound, and phosphate bound flavor constituents of badea (*Passiflora quadrangularis*) fruit pulp. *J. Sep. Sci.* **2002**, *25*, 147–154.

(28) Shimoda, K.; Sato, N.; Nakajima, N.; Hamada, H.; Ishihara, K.; Hamada, H. Glucosylation of phenols and phenylalkyl alcohols using cultured plant cells. *Biotechnol. J.* **2007**, *2*, 1294–1296.

(29) Campbell, J. I.; Pollnitz, A. P.; Sefton, M. A.; Herderich, M. J.; Pretorius, I. S. Factors affecting the influence of oak chips on wine flavour. *Aust. New Zeal. Wine Ind. J.* **2006**, *21* (4), 38–42.

(30) Skouroumounis, G. K. β-Damascenone precursors in grapes and wines. Ph.D. Thesis, The University of Adelaide, Australia, 1991.

Received for review March 26, 2008. Revised manuscript received June 22, 2008. Accepted June 28, 2008. This research was supported by Australia's grape growers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Federal Government.

JF800927E