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Smoke-derived Taint in Wine: Effect of Postharvest Smoke Exposure of Grapes on the Chemical Composition and Sensory Characteristics of Wine

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Although smoke exposure has been associated with the development of smoke taint in grapes and subsequently in wine, to date there have been no studies that have demonstrated a direct link. In this study, postharvest smoke exposure of grapes was utilized to demonstrate that smoke significantly influences the chemical composition and sensory characteristics of wine and causes an apparent ‘smoke taint’. Verdelho grapes were exposed to straw-derived smoke for 1 h and then fermented according to two different winemaking treatments. Control wines were made by fermenting unsmoked grapes. Sensory studies established a perceivable difference between smoked and unsmoked wines; smoked wines were described as exhibiting ‘smoky’, ‘dirty’, ‘earthy’, ‘burnt’ and ‘smoked meat’ characters. Quantitative analysis, by means of gas chromatography–mass spectrometry, identified guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, and furfural in each of the wines made from smoked grapes. However, these compounds were not detected in the unsmoked wines, and their origin is therefore attributed to the application of smoke. Increased ethanol concentrations and browning were also observed in wines made from grapes exposed to smoke.

KEYWORDS: Gas chromatography–mass spectrometry; grapes; guaiacol; smoke; taint; *Vitis vinifera*; wine

INTRODUCTION

Taint in grapes and wine as a consequence of grapevine exposure to smoke has resulted in a decline in product quality and significant financial losses for wine producers throughout the world. To date, the effects of smoke on either grapevine physiology or the organoleptic properties of grapes and wine have not been reported in the literature. However, some preliminary investigations have been carried out by the Australian Wine Research Institute (*1*). The role of smoke in stimulating the germination of dormant seeds of some native plant species has been well documented (for example refs *2*−*4*), and the effect of smoke on the photosynthetic gas exchange of *Chrysanthemoides monilifera* has been reported (*5*), but largely, research relating the effect of smoke on plant physiology and on the composition of fruit and fruit-derived products is limited.

Smoke and liquid smoke flavoring preparations have long been employed by the food industry to enhance the aroma, flavor, and color characteristics of foodstuffs, in particular, meat, fish, and cheese (*6*–*8*). Consequently, considerable research has been undertaken to establish the chemical composition of such preparations. Smoke is generated during the pyrolysis (combustion) of wood, with the composition dependent upon the fuel composition, particle size, moisture content, combustion temperature, and availability of oxygen (*9*, *10*). Wood is primarily composed of cellulose, hemicellulose, and lignin, contributing 40−45%, 20−35%, and 18−35% of total dry weight, respectively (*11*). During the pyrolytic process, thermal degradation of wood components generates a complex mix of volatile organic compounds. Numerous volatile compounds have been reported in smoke, smoke flavoring preparations, and smoked food products, including phenol derivatives, carbonyls, organic acids and their esters, lactones, pyrazines, and furan and pyran derivatives (*6*, *10*, *12*, *13*). Of these, smoke aroma has primarily been attributed to the phenol derivatives (*7*, *10*); in particular, guaiacol (2-methoxyphenol) and 4-methylguaiacol, which exhibit ‘smoky’, ‘phenolish’, ‘burning wood’, ‘ash’, ‘sharp’, ‘sweet’, ‘burnt’ and ‘smoked bacon’ aroma characters (*7*, *14*, *15*).

Guaiacol and 4-methylguaiacol are routinely identified in wines matured in oak barrels, at concentrations between 10 and

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Table 1. Aroma Detection Thresholds for Guaiacol and 4-Methylguaiacol in Water, Model Wine, White Wine, and Red Wine[a]

	aroma detection threshold (μg/L) in			
	water	model wine	white wine	red wine
guaiacol	5.5	20	95	75
4-methylguaiacol	10	30	65	65

[a] Reference *15*.

100 μg/L for guaiacol and between 1 and 20 μg/L for 4-methylguaiacol (*16*), both of which are derived from lignin degradation (*7*) during the toasting process of cooperage. The aroma detection thresholds of guaiacol and 4-methylguaiacol in water and wine are given in **Table 1** (*15*).

The contribution of oak-derived guaiacol to wine aroma has been previously reported. In an oak-aged Chardonnay, the concentration of guaiacol has been shown to positively correlate to the perceived intensity of the smoky aroma (*17*), whereas Rapp and Versini (*18*) found guaiacol to have a negative effect on wine aroma at concentrations exceeding 80 μg/L. Simpson et al. (*19*) found guaiacol to be responsible for an off-flavor in wine; the taint, originating from contaminated corks, was attributed to guaiacol levels ranging from 0.07 to 2.63 mg/L; a detection threshold of 20 μg/L was also reported in this study.

It is therefore conceivable that guaiacol and other phenol derivatives could accumulate in grapes as a result of smoke exposure, and at elevated concentrations, they could lead to an apparent taint. This study was undertaken to test this hypothesis and to demonstrate that smoke exposure of grapes can influence the chemical composition and sensory characteristics of wine.

MATERIALS AND METHODS

Smoke Treatment. Smoke treatments were performed in a purpose-built smoke house (3 × 3 × 3 m) located at the Kings Park and Botanic Gardens (Perth, Western Australia), similar to that described by Dixon et al. (*20*). Whole bunches of grapes were placed on wire racks within the smoke house and exposed to smoke generated by the combustion of dry straw in a metal drum (50 L), for one hour at ambient temperature (25 °C). Following smoke exposure, bunches were randomly mixed to reduce variation in smoke exposure.

Winemaking. Verdelho grapes (350 kg) were harvested when the total soluble solids (TSS) of the grapes reached 24 ± 0.5 °Brix, and a portion (130 kg) of the fruit was separated and exposed to smoke, as described above. The fruit was divided into parcels (approximately 60 kg each), two smoked fruit parcels and two unsmoked fruit parcels. Each fruit parcel was (separately) stored overnight in cool rooms (5 °C) and allowed to warm to ambient temperature (18 °C) before being crushed and destemmed. The fruit parcels were then processed and fermented to produce four experimental wines: (i) a wine made from free run juice of unsmoked grapes, the ‘unsmoked free run’ treatment; (ii) a wine made from free run juice of smoked grapes, the ‘smoked free run’ treatment; (iii) a wine made from free run juice fermented on skins from unsmoked grapes, the ‘unsmoked free run on skins’ treatment; and (iv) a wine made from free run juice fermented on skins of smoked grapes, the ‘smoked free run on skins’ treatment. These winemaking methods were specifically chosen to reflect commercial white and red wine production, that is, clarification and primary fermentation only for white wine production, and oxidative primary fermentation with skin contact, followed by malolactic fermentation, for red wine production. For free run wines, must was pressed immediately, the juice and pressings were combined, and tartaric acid was added to adjust the pH to 3.4 prior to settling (3 days at 5 °C). The clarified juice was then separated into 15 L demijohns (three replicates per treatment) and inoculated with EC1118 yeast (Lallemand Inc., Montreal, Canada). Following primary fermentation, the wines were racked and free SO_2 adjusted (to 30 ppm) before being cold stabilized (−2 °C for 7 days), filtered, and bottled. For free run on skins wines, must was separated into 30 L fermentation vessels (three replicates per treatment), tartaric acid added to adjust the pH to 3.4, and the samples were inoculated with EC1118 yeast. The fermenting musts were plunged twice per day, and the wine was pressed at a total soluble solids level of 3.6 °Brix. The wines were transferred to 15 L demijohns and held at 25 °C until the residual sugar approached 0 g/L. The wines were then racked from gross lees and inoculated with OENOS culture (Chr. Hansen, Hoersholm, Denmark). Following malolactic fermentation, the wines were again racked and free SO_2 adjusted (to 30 ppm) before being cold stabilized (−2 °C for 7 days), filtered, and bottled. The remaining unsmoked fruit (100 kg) was fermented as above to produce a base free run wine and a base free run on skins wine, for the purpose of blending for sensory trials. Ethanol concentrations were determined by distillation, alcohol hydrometry, and spectral measurements according to the method described by Iland et al. (*21*).

Gas Chromatography–Mass Spectrometry Analysis. Quantitative analyses were performed by the Australian Wine Research Institute’s Analytical Services Laboratory (Adelaide, Australia), using an Agilent 6890N gas chromatograph coupled to a 5975 inert source mass spectrometer. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, and furfural were quantified by the stable isotope dilution assay methods reported previously (*16, 22–24*).

Difference Testing of Smoked and Unsmoked Wines. Difference tests were conducted using the triangle test method described by Meilgaard et al. (*25*), using a panel of 24 members. Panelists were of European origin, aged between 18 and 55, with similar numbers of males and females. Wines were presented to the panel using a balanced, randomized presentation order where all possible configurations (ABB, BAA, AAB, BBA, ABA, BAB, where A denotes unsmoked wine and B denotes smoked wine) were presented an equal number of times. Panelists assessed two sets of wine; one set comprised of wines made from free run and one set comprised of wines made from free run on skins. Panellists smelt, but did not taste the samples, and were asked to identify the sample within each set that was different.

Aroma Detection Thresholds of Taint in Smoked Wines. The detection threshold of smoke taint in free run wine was determined according to the American Society for Testing and Materials (ASTM) method 679E, using 33 judges. Judges were of European origin, aged between 18 and 55, with similar numbers of males and females. Wines were presented (as part of a triangle test) in ascending order of concentration spaced by a factor of 3, with the smoked free run wine (0.11, 0.33, 1.0, 3.0, 9.0, 27.0, and 81.0 mL) diluted with base free run wine to 250 mL. Panellists smelt, but did not taste the samples. Those panellists who could detect the spike at all of these concentrations were then tested at lower concentrations; conversely, those who could not detect the spike at any of the concentrations were tested at higher concentrations. The detection threshold of smoke taint in free run on skins wine was determined in the same manner.

Statistical Methods. Data were analyzed by two-way analysis of variance (ANOVA) using GenStat (9th 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

Postharvest smoke exposure by grapes resulted in detectable differences in the chemical composition and sensory characteristics of wine. Difference tests (*25*) established a clearly perceivable difference in the aroma profile of smoked and unsmoked wines. The sensory panel, comprising 24 judges, scored 22 correct responses for the free run wine set and 24 correct responses for the free run on skins wine set. These results indicate smoked wines and unsmoked wines are significantly different at the 99.9% confidence level; hence, smoke exposure of grapes prior to vinification alters wine quality.

The detection thresholds of smoke taint were then determined to evaluate the intensity of the taint and the potential for its reduction through blending. Thresholds (*25*) are reported as the volume of smoked wine (free run or free run on skins) diluted

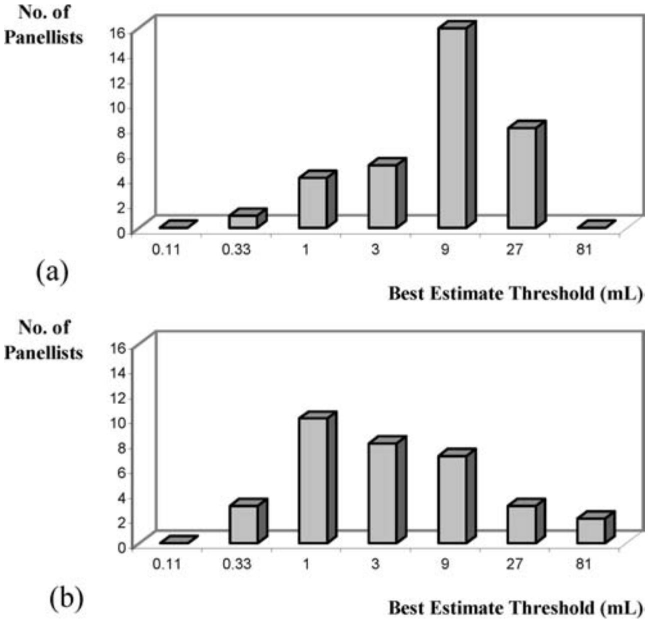

Figure 1. Histograms showing best-estimate threshold distributions for (**a**) smoke taint in smoked free run wine, and (**b**) smoke taint in smoked free run on skins wine.

with base wine (to 250 mL). For each smoked wine, a group threshold was calculated as the geometric mean of each panelist's individual best-estimate threshold, which was the geometric mean of the highest concentration missed and the next highest concentration tested. The aroma thresholds were calculated to be 3.9 mL for the smoked free run wine and 1.9 mL for the smoked free run on skins wine, corresponding to dilutions of 1.6 and 0.8% of original concentrations, respectively. The distributions of best-estimate thresholds for individual panelists are shown in **Figure 1**. The difference and detection threshold tests indicate that smoke exposure has a significant effect on the sensory characteristics of wine. Furthermore, in this study, the taint persisted even with high levels of dilution (by more than 98%), thus limiting options for blending.

The process by which smoke is generated involves the pyrolysis of wood (or other plant material) and is reminiscent of the toasting process of barrel cooperage. Both involve the thermal degradation of structural components, cellulose, hemicellulose, and lignin, resulting in the generation of volatile organic compounds. Stable isotope dilution assays have been developed to quantify oak-derived flavor compounds of organoleptic significance (including guaiacol and 4-methylguaiacol) in oak extracts and barrel-aged wines (*16, 22–24*). These assays were employed to ascertain the composition of smoked and unsmoked wines, and the results obtained indicate a significant treatment effect due to smoke exposure. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, and furfural were detected in wines made from smoked grapes, but not in wines made from unsmoked grapes, irrespective of the winemaking methods employed (**Table 2**).

The smoked wines, both free run and free run on skins, contained unusually high levels of guaiacol (1470 and 969 μg/L, respectively) and 4-methylguaiacol (326 and 250 μg/L, respectively). Typically, wines aged in oak (and not affected by smoke exposure) contain guaiacol and 4-methylguaiacol at concentrations of between 10 and 100 μg/L and between 1 and 20 μg/L, respectively (*16*). It should be noted that guaiacol has also been identified as a component of acid and enzyme hydrolysates prepared from Merlot and Shiraz juice, at concentrations up to 50 μg/L, apparently deriving from grape shikimic acids (*26*, *27*). However, in the present study, guaiacol could not be detected in unsmoked wines, and its origin is therefore attributed to the application of smoke. Previous studies (*1*) have indicated that smoke-derived guaiacol and 4-methylguaiacol preferentially accumulate in the skins of grapes, so in our study, they were expected to occur at higher concentrations in the wines made from smoked grapes fermented on skins. That higher concentrations were instead observed in the smoked free run wines suggests permeation of smoke into the grape berry. The lower levels of guaiacol derivatives in smoked free run on skins wines might also be attributed to winemaking conditions, that is, the loss of volatile compounds through either volatilization due to the higher fermentation temperatures and oxidative conditions or adsorption by grape skins. It is important to note that, in this study, permeation may reflect a relatively high intensity of smoke exposure and the fact that smoke was applied postharvest to bunches, whereas previous studies were based on field applications. Indeed, postharvest application was selected as a treatment to minimize potential confounding effects of field exposure (such as time, intensity, and smoke type). Regardless, the guaiacol and 4-methylguaiacol concentrations measured far exceed both detection threshold concentrations and concentrations typically reported in barrel-aged wines. Consequently, at these levels, both compounds would undoubtedly contribute to the intense smoky character evident in the smoked wines.

Table 2. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, Eugenol, Furfural, 5-Methylfurfural, and Vanillin Present in Smoked and Unsmoked Wines

	concentration[a] (μg/L) in			
	smoked free run	unsmoked free run	smoked free run on skins	unsmoked free run on skins
guaiacol	1470 a	n.d.	969 b	n.d.
4-methylguaiacol	326 a	n.d.	250 b	n.d.
4-ethylguaiacol	128 a	n.d.	111 b	n.d.
4-ethylphenol	59 a	n.d.	67 b	n.d.
eugenol	20 a	n.d.	26 b	n.d.
furfural	16 a	n.d.	13 b	n.d.
5-methylfurfural	n.d.	n.d.	n.d.	n.d.
vanillin	n.d.	n.d.	n.d.	n.d.

[a] Values followed by a different letter within rows are significantly different; n.d. = not detected. Mean values from three replicates. Values were in agreement to ca. 5%.

4-Ethylguaiacol, 4-ethylphenol, eugenol, and furfural were also detected in the smoked wines, albeit within concentration ranges previously reported in wine (*15*, *22*). Therefore, although the presence of these compounds is attributed to postharvest smoke exposure, they are unlikely to be key contributors to smoke taint. Interestingly, in wine, 4-ethylguaiacol and 4-ethylphenol are typically formed from grape-derived *p*-coumaric acid and ferulic acid (respectively) through the action of *Brettanomyces*/*Dekkera* yeast (*15, 28*). In this study, the absence of these compounds in the unsmoked wines instead supports a formation pathway involving the thermal degradation of lignin, such as proposed by Fiddler et al. (*29*). 5-Methylfurfural and vanillin were not detected in any of the wines made from smoked grapes. These compounds were either not formed at detectable levels under the conditions employed in this experiment or they experienced further degradation. Vanillin has been reported as an intermediate in the thermal degradation of lignin, with its decomposition yielding vanillic acid and guaiacol (*29*).

Quantitative GC-MS analysis established that the detection thresholds of smoke taint correspond to guaiacol and 4-methylguaiacol concentrations of 23 and 5 μg/L, respectively, for the

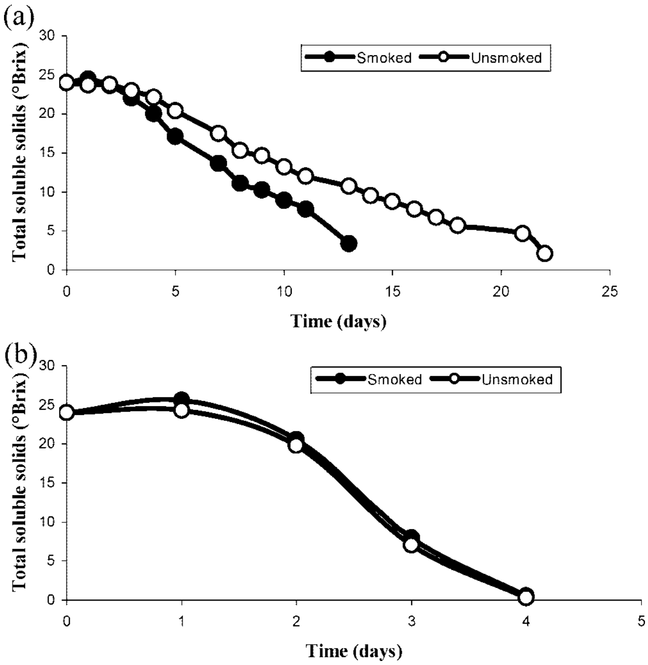

Figure 2. Fermentation curves for (**a**) smoked and unsmoked free run wines, and (**b**) smoked and unsmoked free run on skins wines. Mean values from three replicates; standard errors are obscured by symbols, so they are not shown, but they were <0.5 in all cases.

smoked free run wine and 7 and 2 μg/L, respectively, for the smoked free run on skins wine. Because these concentrations are near or below the detection thresholds reported for guaiacol and 4-methylguaiacol (*15, 19*), we therefore conclude that neither is solely responsible for the perception of smoke taint. The smoke taint threshold concentrations are also strongly supportive of this, with an increased threshold observed for the smoked free run wine relative to the smoked free run on skins wine; that is, threshold concentrations did not correlate with guaiacol and 4-methylguaiacol concentrations. It is quite likely that additional smoke-derived volatile compounds contribute to the taint observed in wines made from smoked grapes, and identification of these compounds is the subject of ongoing research. Nevertheless, guaiacol and 4-methylguaiacol are useful as indicators of smoke taint.

To investigate the development of smoke taint during the winemaking process, grapes were vinified according to two different winemaking methods, reflecting commercial white and red wine production. Free run wines were clarified (3 days at 5 °C) prior to fermentation, and free run on skins wines were fermented in open vessels with skin contact followed by malolactic fermentation. In the case of free run wines, postharvest smoke exposure resulted in an increased fermentation rate (completing fermentation 9 days earlier), but showed no effect on the fermentation rate of free run on skins wines (**Figure 2**).

Significant differences in ethanol concentrations and wine color were also observed between smoked and unsmoked wines (**Table 3**). Smoked wines had higher alcohol contents than their corresponding unsmoked wines, indicating a higher attenuation of sugars to ethanol during the fermentation process. Wines fermented on skins showed increased levels of brown pigments as compared with free run wines; this is not unexpected, because of the oxidative nature of this winemaking method. However, smoked wines also exhibited increased browning as compared with their corresponding unsmoked wines, irrespective of the winemaking methods employed. The effect of smoke exposure on both fermentation rate and development of brown pigments in white wine is the subject of ongoing further study. We anticipate that these observations may be explained by the effect of smoke compounds on membrane integrity within the grape berries and skins. Smoke exposure is likely to damage membrane-bound processes and, as such, may possibly lead to the release of proteases and other cellular enzymes associated with injury response. This response to smoke may then have the potential to considerably alter berry chemistry prior to fermentation, an effect which may have been exacerbated by our postharvest treatment.

Table 3. Ethanol Content and Color Analysis of Smoked and Unsmoked Wines[a]

	smoked free run	unsmoked free run	smoked free run on skins	unsmoked free run on skins
ethanol content (% v/v)[b]	14.3 a	14.1 b	14.7 c	13.8 d
estimated brown pigments (au)[c]	0.097 a	0.060 b	0.203 c	0.142 d

[a] Values followed by a different letter within rows are significantly different. [b] Mean values from three replicates; values were in agreement to ca. 0.5%. [c] Mean values from three replicates; values were in agreement to ca. 10%.

In this trial, dry straw was chosen as a model fuel for the application of a cold smoke treatment. Like wood, straw comprises cellulose, hemicellulose, and lignin, and its pyrolysis was therefore expected to generate smoke of similar composition to wood-derived smoke. The use of dry straw also enables the reproducible generation of smoke, as employed in current field trials involving the application of smoke to grapevines. Although it is recognized that forest fuels may contribute a broader range of potential smoke taint compounds the complexity of fuel composition, burn rates, combustion temperatures, and environmental conditions are confounding influences and are the subject of further studies.

This study has demonstrated a direct link between the smoke exposure of grapes and the development of smoke taint in subsequent wines. Smoke taint was readily perceived by sensory analysis, with the sensory panel able to detect the taint at dilutions of less than 2% of the original concentration. Further studies involving field exposure of grapevines to smoke are currently underway.

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