Winemaking practice affects the extraction of smoke-borne phenols from grapes into wines

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Running title: Uptake of smoke-borne phenols in grapes and wines

Abstract

Background and Aims: Exposure to smoke and uptake of taint imparting phenols in grapes and wines is a significant problem in bushfire-prone regions of Australia and other countries. The effects of smoke exposure on taint occurrence in wines, however, can be variable. This study assessed the influence of cultivar on uptake and accumulation of smoke-borne phenols in grapes and of subsequent processing and winemaking methods on extraction of phenols into wines.

Methods and Results: Smoke exposure experiments were conducted in commercial vineyards of Chardonnay, Merlot and Sauvignon Blanc 14 days after the onset of veraison. At maturity, grapes were harvested for winemaking, which included malolactic fermentation (MLF) for Merlot. Volatile and glycoconjugated phenols were determined in grapes and the resultant wines. All cultivars had a similar concentration of smoke-derived total phenols in their grapes. The apparent extraction of total phenols from grapes into wines, however, differed markedly among the three traditional winemaking methods. Red winemaking (Merlot) with skin contact extracted 88% of total grape phenols, whereas white winemaking either by crushing before pressing (Sauvignon Blanc) or by whole-bunch pressing without crushing (Chardonnay), respectively, released 39 and 18% of total phenols. For Merlot wines, MLF did not affect the extraction of total smoke-derived phenols.

Conclusions: Under standardised exposure conditions (duration, intensity and phenology), the three cultivars studied accumulated a similar amount of total phenols in grapes. The grape processing and winemaking methods, however, can bring about fourfold difference in the concentration of total phenols of wines. The smoke-derived phenols extracted from grapes into wine and the distribution of these phenols between the volatile and conjugated pools was not affected by MLF.

Significance of the Study: The key findings reported here have the potential to improve decision making by grapegrowers and winemakers on the effect of cultivar and winemaking on potential smoke taint in wine.

Keywords: *Chardonnay, cresols, guaiacol, glycoconjugated phenols, Merlot, phenol, Sauvignon Blanc, smoke exposure, syringol,* Vitis vinifera *L., volatile phenols*

Introduction

Wine grapes exposed to smoke from wildfires and controlled burns produce wines with an elevated concentration of volatile and glycoconjugated phenols (Kennison et al. 2007, Hayasaka et al. 2010, 2013, Kelly et al. 2012, Singh et al. 2012). At such an elevated concentration, volatile phenols and glycoconjugated phenols impart unpleasant characters to wines, including burnt, smoky, medicinal and dirty aromas and flavour attributes (Kennison et al. 2009, Ristic et al. 2011, Parker et al. 2012). Such wines have low consumer acceptance, and thus smoke exposure can cause significant negative economic impact on grapegrowers and winemakers (Whiting and Krstic 2007). Consequently, when a vineyard is subject to smoke, grapegrowers and winemakers endeavour to understand the likely level of smoke taint in grapes and in wines from a smoke-exposed vineyard. Several factors, however, are likely to influence the level of accumulation of smoke-borne putative taint compounds in grapes and into wines. First, from the work reported to date, it is unclear whether different winegrape cultivars accumulate a similar concentration of smoke-borne phenols under comparable smoke exposure conditions. Differences among cultivars in the concentration of phenols from wildfire smoke exposed fruit have been reported (Hayasaka et al. 2010, Dungey et al. 2011, Singh et al. 2012), but these differences may be due to variation in the intensity and duration of smoke exposure as well as the timing of smoke exposure in relation to vine phenology (Kennison et al. 2009).

Second, the transformations and estimates of the expected concentration of phenols in finished wines under different winemaking practices are not well understood. Recently, there have been advances in the analysis of phenols in smoke-affected fruit and resultant wines (Hayasaka et al. 2010). However, estimates of the expected proportion of volatile phenols and glycoconjugated phenols extractable from grapes into wines (that can be used as industry guidelines) may not be fully inferred from these reports either due in part to the use of non-traditional winemaking (Hayasaka et al. 2010) or to the limited range of glycoconjugated phenols reported (Ristic et al. 2011). In smoke-affected grapes, a high proportion of glycoconjugated phenols is sequestered in the skin (Dungey et al. 2011), and skin maceration and contact during winemaking may affect the extraction of these compounds into wine. Thus, it is imperative to understand the likely extent of extraction of a comprehensive range of glycoconjugated phenols from smoke-impacted grapes under commonly used white and red winemaking practices.

Third, the sensory impact of smoke taint in wine is influenced by the distribution of volatile and glycoconjugated phenols (Parker et al. 2012). The production of red wines often includes malolactic fermentation (MLF) by inoculation with lactic acid bacteria. The metabolic activity of lactic acid bacteria can influence wine aroma complexity by hydrolysing wine aroma glycosides (Ugliano et al. 2003, D’Incecco et al. 2004). The effect of MLF, however, on the distribution of glycoconjugated phenols is yet to be reported.

The objectives of this study were threefold:

- to determine whether the cultivar influences the uptake of smoke-borne phenols and accumulation in grapes of the three cultivars Chardonnay, Sauvignon Blanc and Merlot . To minimise potential confounding factors, the smoke-exposure experiments were replicated both with respect to the panels of vines exposed and to the exposure conditions (i.e., fuel composition, mass, pyrolysis of fuel), and the vines were exposed to smoke at the same phenological stage, 14 days post-veraison, in commercial vineyards.

- to determine the likely proportion of glycoconjugated phenols that are extracted from grapes into wines under commercial red and white winemaking techniques, including when fruit is crushed and de-stemmed and when fruit is whole-bunch pressed. These results may provide guidelines for expected smoke-derived phenols in wines based on the concentration determined in affected grapes under different wine making practices.
- to assess whether the glycosidase activity of lactic acid bacteria contributes significantly to hydrolysis of glycoconjugated phenols to volatile phenols, by comparing MLF and no MLF Merlot wines.

Materials and methods

Fuel types and fuel compilation

The experiments were conducted in ten-year-old commercial Sauvignon Blanc, Chardonnay and Merlot vineyards located in the Margaret River wine region (33°57′S, 115º01′E) in the southwest of Western Australia. These vineyards are typically located in close proximity to forests and agricultural areas, and bushfire emissions that may contribute to the accumulation of smoke compounds in wine grapes can arise from remnant native forest, plantations and farmland vegetation. Two fuels, the softwood species radiata pine (*Pinus radiata* D. Don) and a pasture grass, wild oats (*Avena fatua* L.) were collected and prepared as described in Kelly et al. (2012) to compare uptake of smoke-borne phenols between cultivars.

Grapevine smoke exposure

The design and conduct of the smoke exposure experiments followed that of Kelly et al. (2012). Briefly, the experiments were established as randomised block designs. To minimise variability within an experimental block, each block had vines with uniform canopy size and yield. The treatments and controls within each block were randomly allocated to experimental units where the smoke generation and exposure consisted of the fuels described above plus a control (not exposed to smoke). The smoke exposure treatments and controls were replicated three times for Chardonnay and Sauvignon Blanc and five times for Merlot. Each experimental unit consisted of a panel of five vines separated by at least two panels of vines to avoid cross contamination. Smoke exposure of the experimental vines occurred 14 days post-veraison as per Kelly et al. (2012), with smoke events lasting 30 min. For Chardonnay, due to logistical constraints, the smoke-exposure treatment involved one fuel type only, namely wild oats.

Winemaking

Fruit was harvested at commercial maturity, ~23°Brix total soluble solids, approximately 6 weeks after smoke exposure. The fruit from each replicate panel was harvested, processed and fermented separately. The Chardonnay and Sauvignon Blanc wines were made by conventional white winemaking methods where there was minimal skin contact before commencement of fermentation. The Sauvignon Blanc replicates were separately de-stemmed, crushed and pressed while the Chardonnay replicates were whole bunch-pressed, each with addition of 100 mg/L potassium metabisulfite (PMS) (Chem Supply AR grade, Gillman, SA Australia). For both cultivars the must was inoculated with *Saccharomyces cerevisiae* EC1118 (Lallemand Inc., Montreal, QC, Canada) at 300 mg/L and supplemented with 100 mg/L diammonium phosphate (Sigma- Aldrich, Sydney, NSW, Australia). Each replicate was fermented in 25-L glass demijohns to dryness (<1 g/L residual sugars), racked from gross lees with the addition of 60 mg/L PMS and cold stabilised at -4°C for 21 days.

The wines were filtered through a 0.2 μm pore size cartridge (Sartorius Sartopure 2 Maxicap, Sartorius, Gottingen, Germany) and bottled under food grade nitrogen with Stelvin closures.

The Merlot wines were made by traditional red winemaking methods as described in Kelly et al. (2012). After the ferments reached dryness (<1g/L residual sugars), the wines were racked from gross lees, and divided into two equal portions by volume. The first half (no MLF wines) were cold stabilised at -4°C for 21 days with the addition of 60 mg/L PMS and the second half (MLF wines) was inoculated with *Oenococcus oeni* (Viniflora CH 16, CHR Hansen, Hørsholm, Denmark) at 10 mg/L to initiate malolactic conversion (hereafter MLF wines). The MLF replicates were kept at 23°C until the malic acid concentration dropped to <0.1 g/L (19–60 days) and subsequently cold stabilised at -4°C for 21 days with the addition of 60 mg/L PMS. Both the no MLF and MLF wines were filtered as described above.

Chemical analyses

Grape and wine samples were analysed for seven volatile phenols and 14 glycoconjugated phenols at The Australian Wine Research Institute's commercial services using the methods described by Hayasaka et al. (2013). Volatile and glycoconjugated phenols were analysed on five and three replicate samples, respectively. Wines were analysed at 7 months (Chardonnay), 30 months (Sauvignon Blanc) and at 40 months (Merlot), post-bottling.

Statistical analysis

Analysis of variance was carried out using the general linear model procedure using SPSS 20 (IBM SPSS Statistics, Chicago, IL, USA). Reported treatments effects are significant at $P < 0.05$.

Results and discussion

Accumulation of smoke-borne phenols in grapes

Volatile phenols. Volatile phenols are expected to occur, although at low concentration, constitutively in lignin-bearing plants or parts thereof. Accordingly, for all three cultivars, the concentration of volatile phenols in grapes from the unsmoked, control vines were either below the limit of detection of the analytical method used (< 2.5 nmol/kg) or present at trace concentration. Smoke exposure early in the grape-ripening phase significantly increased the concentration of many of the volatile phenols, particularly in Sauvignon Blanc grapes (Table 1). Nonetheless, the concentration of total volatile phenols in smoke-exposed grapes were still low, ≤ 331 nmol/kg. Volatile phenols are toxic and reactive (Whetten and Sederoff 1995) and the low overall concentration in grapes, therefore, indicates that following uptake of volatile phenols, they are converted to and stored as physiologically compatible complexes by binding with sugars (Hayasaka et al. 2010). In Chardonnay, only *o*-cresol was present at measurable concentration. Generally, cultivar responses to smoke exposure in terms of the levels of individual volatile phenols and/or their total pools in grapes were of the order: Sauvignon Blanc >> Merlot > Chardonnay. Where smoke exposure increased the total pool of volatile phenols, the major contributors were the cresol isomers, guaiacol and syringol. While smoke exposure affected the concentration of volatile phenols in grapes, there was no consistent effect of fuel type (smoke source) across cultivars or phenol types.

Glycoconjugated phenols. Depending on cultivar, grapes from the unsmoked vines contained up to 175 nmol/kg endogenous total glycoconjugated phenols, including glucosylglucosides (GG), pentosylglucosides (PG) and rhamnosylglucosides (RG). Smoke exposure, averaged across fuel types and cultivars, increased the total pool of grape glycoconjugated phenols by >14-fold (96 vs 1392 nmol/kg, Table 2). The source of smoke (fuel type), however, had no significant effect. Similarly, there were no significant effects of cultivar on the total glycoconjugated phenols of grapes at commercial harvest, nor were there significant cultivar by fuel type interactions. Earlier work suggested cultivar sensitivity in the accumulation of smoke-borne phenols (Whiting and Krstic 2007), although it was not clear whether the cultivars were at similar stage of berry development when the smoke exposure event occurred. This is an important consideration in determining the effect of the cultivar, since the uptake of smoke-borne phenols changes markedly throughout berry development (Kennison et al. 2009). Our results indicate that when smoke exposure events occur at comparable stage of berry development (in this case, 14 days post-veraison), the cultivar has no effect on the accumulation of total glycoconjugated phenols. These observations underscore the importance of standardising smoke exposure conditions in experiments, such as duration, intensity and as well as timing in relation to grape development, and further suggest that the apparent variation in cultivar sensitivity of earlier reports may relate more to phenology at the time of exposure and exposure conditions than to cultivar differences. Furthermore, to compare cultivar responses properly it is necessary to standardise environmental conditions and management practices that affect leaf conductance, e.g. temperature, leaf-to-air vapour pressure deficit, soil moisture, canopy management and leaf area to fruit weight ratio. In this study although smoke was applied at the same phenological stage, possible difference in environmental conditions during smoke exposure and in grapevine management among cultivars might have masked differences in cultivar sensitivity.

Although cultivar and fuel type had little influence on the concentration of total glycoconjugated phenols in grapes, both treatments affected the composition of the phenols. For example, while the white cultivars accumulated an equivalent concentration of total phenols and total cresols, the concentration of these phenols in the red cultivar, Merlot, was significantly lower (i.e. SB = CH > M). This apparent red vs white cultivar dichotomy in phenol uptake does not apply to all cultivars since accumulation of total guaiacol was similar between Sauvignon Blanc and Merlot (~445 nmol/kg), which was higher than the ~≤ 166 nmol/kg observed in Chardonnay. Further quantitative differences between cultivars were also evident when composition was considered by the glycone moieties of glycoconjugated phenols. While a clear cultivar pattern was not apparent across all the glycoconjugate types and all phenols, for all the rhamnosylglucoside conjugates (RG), the following cultivar ranking was evident: Sauvignon Blanc > Chardonnay = Merlot. Clearly, in glycosylated form, these glycoconjugated phenols are aroma and, perhaps, flavour inactive. Whether such a difference in the concentration of diglycosides, which require sugar-specific exoglycosidases for cleavage of the sugar-sugar bonds that makes the resultant phenolic monoglucoside amenable to attack by a glucosidase and release of sensorially potent volatile phenols (Sarry and Günata 2004), influences the extent to which smoke taint can develop is not known.

The glycoconjugated phenols that reflected the fuel source of smoke were methylguaiacol–PG, and –RG and syringol–GG and –PG, which in part reflected the lignin composition of the fuels. Thus, for example, grapes exposed to the smoke of the pine fuel, whose lignin contains a relatively high concentration of methylguaiacol compared to that of the oat fuel (Kelly et al. 2012), accumulated a concentration of methylguaiacol–PG and –RG significantly higher than that of grapes exposed to oat smoke. The reverse occurred for the syringol diglycosides. Grapes exposed to smoke of oat fuel, which has a high concentration of syringols in its lignin compared to that of pine fuel (Kelly et al. 2012), contained a concentration of syringol–GG and –PG significantly higher than that of grapes exposed to pine smoke (Table 2). Notwithstanding these observations, the detection of an elevated concentration [~seven times the background concentration, Table 2, also see Kelly et al.

(2012)] of syringol diglycosides in grapes exposed to pine smoke, which has negligible syringols in its lignin, also shows that the accumulation of phenols into grapes does not match the phenol composition of the smoke's fuel source. The source of syringol in grapes exposed to pine fuel smoke remains unclear; however, in planta transformation (methoxylation) of the xenobiotically acquired hydroxy- and methoxy-phenols can be ruled out (Mr David Kelly and Dr Ayalsew Zerihun, unpubl. data, 2013).

Across cultivars and fuel types, the dominant (70–85%) contributors to the total glycoconjugated phenol pool were the diglycosides of phenol, cresol and guaiacol (Table 2). Interestingly, glycosides of syringol and methylsyringol made up ≤ 20% of the total smoke-derived glycoconjugated phenol in grapes. These results contrast with those reported in Hayasaka et al. (2010, 2013) in which syringol-GG was the single most dominant contributor to the total glycoconjugated phenols in grapes of a range of cultivars exposed to bushfire smoke. The source of this variance for the relative contribution is not clear apart from methodological differences in smoke generation (experimental vs wildfire smoke) as well as the intensity and duration of exposure.

Influence of wine making techniques on wine phenols

Volatile phenols. The winemaking practices varied for the three cultivars examined. Thus, cultivar effects on volatile and glycoconjugated phenols are necessarily subsumed in the effect of winemaking practices, which influenced the wine volatile phenol levels (Table 3). For both control and smoke exposure treatments, Chardonnay wines made from whole bunch-pressed juice had no measurable concentration (<2.5 nmol/kg) of volatile phenols, as was generally the case in the grapes (Table 3). Although smoke-exposed grapes generally contained no volatile phenols, the apparent absence of volatile phenols in the resultant wines was unexpected. This is indicative of a low overall extraction of phenols into whole bunch-pressed juice and subsequent negligible hydrolysis of the diglycoside bound phenols during and/or post-fermentation. These findings contrast with the high concentration of volatile phenols observed in Chardonnay fruit exposed to bushfire smoke and fermented on skins (Hayasaka et al. 2010) or in wines made after crushing and pressing Chardonnay juice (Singh et al. 2012). These examples highlight the effect of processing and/or winemaking practice on extraction of phenols.

Wines from the control treatments of Sauvignon Blanc and Merlot grapes had no measurable (<2.5 nmol/kg) volatile phenols, except guaiacol and syringol in the Merlot wines which were fermented on skins (Table 3). In contrast to that of the Chardonnay wines from whole bunch-pressed juice, Sauvignon Blanc and Merlot wines from the smoke-exposed, de-stemmed and crushed grapes had an elevated concentration of six volatile phenols (Table 3). Between these two latter groups, however, a significantly higher concentration of volatile phenols was present in Merlot wines that were fermented on skins than in Sauvignon Blanc wines made from de-stemmed, crushed and pressed juice (Table 3). Interestingly, the volatile phenol concentration was comparable in smoke-affected Sauvignon Blanc grapes and the resultant wines, whereas in Merlot the concentration in wine was higher than that in grapes (Tables 1, 3) suggesting the extended skin contact may have facilitated hydrolysis of glycoside-bound phenols as observed for example by Kennison et al. (2008).

Glycoconjugated phenols. The concentration of total glycoconjugated phenols varied significantly between Chardonnay (whole bunch-pressed without crushing), Sauvignon Blanc (de-stemmed, crushed and pressed) and Merlot (de-stemmed, crushed and fermented on skins) wines in the ratio of approximately 2:5:10, respectively (Table 4). Since the concentration of the total glycoconjugated phenols in grapes was comparable across cultivars and fuel types, the difference between wines primarily reflected the effect of fruit processing and/or winemaking practices that are applied to these cultivars. These results highlight that extraction of glycoconjugated phenols not only differs between the red wine (made with skin contact) and white wine (made without skin contact) practices, but also between white grape processing and/or winemaking practices, with grape crushing before pressing releasing considerably more (~2.5-fold) glycoconjugated phenols than whole-bunch pressing without crushing.

Fruit processing and winemaking methods also had significant influence on the concentration of all 14 glycoconjugated phenols (Table 4). With the exceptions of syringol-PG and methylsyringol-PG, the ranking of the concentration of the remaining 12 glycoconjugated phenols among the wines of the three cultivars was the same as that for the total glycoconjugated phenols, i.e. Merlot > Sauvignon Blanc > Chardonnay. Of the total pool, the diglycosides of cresol and phenol contributed the largest proportion (ranging from 44% in Merlot wines to 70% in Chardonnay). The second largest class of phenols was the diglycosides of guaiacol accounting for between 20% (Chardonnay wines) and 33% (Merlot wines). Collectively, the phenol, cresol and guaiacol diglycosides made up 78% (Merlot) and 89% (Chardonnay and Sauvignon Blanc) of the total glycoconjugated phenols in these wines. The contribution of the syringol and methylsyringol glycosides to the total pool was only about 10% or less. The relative abundance of the different phenol classes in wines is broadly comparable to the respective proportion observed in grapes (data not shown but compare Tables 2 and 4). The low contribution of syringol glycosides (<10% of total) observed in this study, while similar to the results from Kelly et al. (2012), contrasts to results for wines from bushfire smoke-affected fruit in which syringol glycosides were the single largest component (Hayasaka et al. 2010, 2013, Singh et al. 2012). While the exact reason for this variance is unclear, given the similarity of the relative proportion in fruit and wine in the current study, the low values here suggest differences are probably related to conditions, such as exposure intensity and duration, during accumulation in grapes.

Averaged across cultivars, wines from smoke exposed grapes had more than 16 times the concentration of total glycoconjugated phenols than the control wines (Table 4). The fuel source of smoke had no influence on the total glycoconjugated phenols. Of the individual glycoconjugated phenols, however, exposure to pine smoke generally tended to produce a higher concentration of phenols of the *p*-hydroxyphenly– and guaiacyl–lignin origin, although the fuel effects were significant only for methylguaiacol–PG and –RG. In contrast, wines made from fruit exposed to grass smoke had a concentration of phenols of the syringyl-lignin provenance (particularly, syringol–GG and –PG) significantly higher than that of the wines from the pine smoke treatment (Table 4). Such differences mirror broadly the concentration of these glycoconjugated phenols in fruit.

Effect of winemaking practice on extraction of grape glycoconjugated phenols into wines

The extraction of total glycoconjugated phenols from grapes into wines varied significantly among the three wines (Figure 1). Merlot wines, which were made according to the standard red winemaking practice of fermenting on skins until dryness, extracted about 85% of the grape glycoconjugated phenols, which is comparable to results for skin-fermented Cabernet Sauvignon and Chardonnay (Hayasaka et al. 2010). The extraction rate for the white wines, which did not involve skin contact, was considerably lower, averaging about 25% of the grape total glycoconjugated phenols. This average, however, masks effects of different fruit processing/handling practices that are customarily used in white winemaking. Sauvignon Blanc wines, made following crushing of fruit prior to pressing, extracted 39% of fruit total glycoconjugated phenols, approximately twice the extraction rate of wines from whole bunch-pressed must without crushing , i.e. Chardonnay, ~18% (Figure 1). It appears thus that white and red grape cultivars exposed to an identical bushfire smoke will have a markedly different concentration of putative smoke-taint compounds in wines under typical winemaking conditions. Whether these differences, however, translate into sensory differences (i.e., less negative impact in white wines than in red wines) is not clear, since sensory impacts may be modulated by the red vs white wine matrix effects (Boidron et al. 1988).

The extraction rates of the grape glycoconjugated phenols into wines varied between the different phenol classes and wines. In whole-bunch pressed Chardonnay, the extraction of glycosides of phenols of the *p*-hydroxyphenyl, guaiacyl and syringyl classes were 14, 19 and 11%. In Sauvignon Blanc, the corresponding extraction rate was 39, 27 and 11%, and by comparison 90, 75 and 74% for Merlot wines. Such differences in extraction rate between winemaking practices reflect the localisation of a high proportion of the total grape glycoconjugated phenols in skins (Dungey et al. 2011).

Differential extraction rates also occurred between glycoside type and conjugated phenol type. For example, guaiacol glucosylglucoside had a low extraction rate (≤ 7%) regardless of winemaking style (cultivar) as also reported in Ristic et al. (2011) for Shiraz and Grenache wines. Similarly, low extraction (~ 10%) was observed for syringol glucosylglucoside in both Sauvignon Blanc and Chardonnay wines. These low apparent extraction rates, however, are not generalisable for all glucosylglucoside phenols, since high extraction of syringol glucosylglucoside [Merlot, 63%, this study; as well as >70% in Cabernet Sauvignon and Chardonnay wines fermented on skins, Hayasaka et al. (2010)] can also occur. While the low apparent extraction rate for the white cultivars can be attributed to winemaking practices, the low extraction rate of guaiacol-GG compared to that of syringol-GG in wines fermented on skins is unclear.

Effect of MLF on extraction and hydrolysis of glycoconjugated phenols in Merlot wines

Red wines normally undergo malolactic fermentation (MLF) by inoculation with lactic acid bacteria (LAB), often after the completion of alcoholic fermentation. Although MLF is primarily used for de-carboxylating malate to lactate, the metabolic activity of LAB can also modify wine aroma complexity by transforming many compounds, including hydrolysis of glycosides (Ugliano et al. 2003, D'Incecco et al. 2004) and thus potentially releasing volatile phenols. The odour and flavour sensory profile of smoke-affected wines is largely dependent on the concentration of volatile phenols in wines, although some deconjugation of glycoconjugated phenols (at least of monoglucosides) can occur in the mouth (Parker et al. 2012). Therefore, it can be expected, that LAB-mediated hydrolysis of glycosides that alters the distribution of volatile and glycoconjugates of phenols, can also alter the sensory profile of smoke-affected wines. It is not clear whether LAB are capable of significant hydrolysis of glycoconjugated phenols. Our results showed no significant change in the concentration of total volatile phenols, of the total glycoconjugated phenols or of the phenol components between no MLF and MLF Merlot wines (Figure 2). The glycoconjugated phenols in smoke-affected wines were mostly present as diglycosides. It is unclear whether the nature/form of the glycosides present in wines contributed to the apparent lack of hydrolysis of glycoconjugates in the LAB- inoculated wines. The hydrolysis and release of aglycones from diglycosides can occur either at once by actions of diglycosidases or sequentially by cleavage of the sugar–sugar link by sugar-specific exoglycosidases followed by release of volatile phenols by glucosidases (Sarry and Günata 2004). While LAB contain the complementary suite of enzymes (exoglycosidase and glucosidase) that may make sequential hydrolyses possible (Boido et al. 2002, D'Inecco et al. 2004), the presence of a diglycosidase in LAB is yet to be shown (Sarry and Günata 2004). If LAB are capable of releasing aglycone moieties from their diglycoside conjugates though sequential hydroylases, then the lack of response here may be a strain- specific response (Ugliano et al. 2003), and further evaluation of other LAB strains is warranted to gain a more complete picture of LAB capacity on release of sensorially potent aglycones from their glycoconjugated phenols.

Conclusion

This study investigated three issues: (i) cultivar sensitivity to uptake and accumulation in grapes of smoke-borne phenols; (ii) influence of fruit processing/winemaking practices on release of grape phenols into wines; and (iii) hydrolysis of glycoconjugated phenols and release of volatile phenols during MLF of red wines. For the three cultivars evaluated, when exposure to smoke occurred at the same stage of berry development (early in the berry ripening phase), no significant cultivar sensitivity was observed in the accumulation of total phenols in grapes, although the phenol composition varied. This finding has practical implications. For example, for a grapegrower with a property that adjoins bushland, the criteria for choosing planting material, for expansion or redevelopment, may not need to factor in cultivar sensitivity to smoke phenol uptake.

Fruit processing and winemaking practices markedly influence the amount and/or proportion of grape phenols that are released into wines. While red winemaking practices that involve skin contact release a proportion (≥ 80%) of grape phenols considerably higher than that for white winemaking practices (no skin contact, average 25%), there is also significant difference in phenol extraction between different grape processing methods for white winemaking: crushing before pressing releases ~40% of grape phenols compared to ~18% for whole-bunch pressing without crushing. Understanding how extraction of phenols from grapes changes as a function of fruit processing and winemaking practices may aid in mitigating and managing smoke taint in smoke-exposed grapes. These results provide practical guidelines on the likely proportion of grape phenols to be expected in the wines for the three traditional winemaking methods studied.

The results from this work found no evidence that MLF in red winemaking increases extraction and hydrolysis of glycoconjugated phenols. Thus, at least for *Oenococcus oeni* (Viniflora CH 16), in wines containing an elevated concentration of glycoconjugated phenols, MLF should not significantly alter the concentration or distribution of volatile and glycoconjugated phenols of the resultant wines.

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Table 1. Effect of fuel type and smoke exposure on the concentration of volatile phenols in the fruit of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

	Concentration of volatile phenols (nmol/kg)							
	Sauvignon Blanc			**Chardonnay**		**Merlot**		
Volatile phenols	**Control**	**Pine†**	**Grass‡**	**Control**	**Grass‡**	**Control**	**Pine†**	**Grass‡**
o-Cresol	nd	136.9±15.9	175.7±14.1	nd	20.0±1.5	nd	25.9±8.0	33.3±6.3
m-Cresol	nd	24.0±2.3	24.7±3.1	nd	nd	nd	nd	nd
p-Cresol	nd	20.3±1.8	nd	nd	nd	nd	nd	nd
Subtotal	nd	181.2±19.1	200.4±17.2	nd	20.0±1.5	nd	25.9±8.0	33.3±6.3
Guaiacol	nd	59.6±14.3	83.2±11.7	nd	nd	nd	17.7±5.3	27.4±5.5
4-Methylguaiacol	nd	31.8±5.4	14.5±0.1	nd	nd	nd	4.3±2.9	nd
Subtotal	nd	91.4±19.7	97.7±11.7	nd	nd	nd	22.0±7.5	27.4±5.5
Syringol	nd	40.2±11.5	32.4±23.4	nd	nd	25.9±8.5	20.8±2.4	16.9±7.3
4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	nd
Subtotal	nd	40.2±11.5	32.4±23.4	nd	nd	25.9±8.5	20.8±2.4	16.9±7.3
Total	nd	312.8±36.2	330.5±19.8	nd	20.0±1.5	25.9±8.5	68.7±14.2	77.6±14.9

Data are the mean ±1 SD (n=5, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where n=3). nd, not detected. †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡ from a pasture grass, wild oats (*Avena fatua* L.).

Table 2. Effect of fuel type and smoke exposure on the concentration of glycoconjugated phenols in the fruit of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

	Concentration of glycoconjugated phenols (nmol/kg)							
	Sauvignon Blanc			**Chardonnay**		**Merlot**		
Glycoconjugated phenols	**Control**	**Pine†**	**Grass‡**	**Control**	**Grass‡**	**Control**	**Pine†**	**Grass‡**
Phenol-PG	6.1±0.3	178.2±20.2	167.1±29.0	25.3±0.2	302.1±20.2	3.3±0.4	75.5±10.7	84.8±11.9
Phenol-RG	3.4±0.5	135.8±16.2	203.9±44.4	1.7±0.1	57.7±3.7	0.9±0.1	45.4±7.3	59.2±10.9
Cresol-PG	12.8±0.2	273.4±42.1	301.6±52.9	53.7±1.2	443.6±25.2	21.2±2.3	294.6±63.3	287.4±47.0
Cresol-RG	5.4±0.3	183.9±28.3	180.2±19.9	6.1±0.3	118.8±8.5	1.9±0.3	84.1±15.3	79.7±13.0
Guaiacol-GG	0.7±0.01	55.6±11.2	95.8±8.7	2.7±0.1	5.6±0.3	1.8±0.3	68.4±13.7	101.0±20.8
Guaiacol-PG	8.7±0.5	211.7±38.4	276.4±31.9	39.1±0.5	140.2±7.8	12.6±0.7	276.1±64.2	350.0±73.0
Guaiacol-RG	1.9±0.1	100.5±19.7	158.9±6.7	1.7±0.2	20.0±1.9	0.9±0.1	36.3±7.3	50.1±9.3
4-Methylguaiacol-GG	0.2±0.01	33.3±7.5	19.1±2.3	1.4±0. 1	1.3±0.2	0.3±0.01	28.2±9.4	15.0±4.2
4-Methylguaiacol-PG	1.5±0.1	83.5±15.1	34.4±1.3	13.0±0.2	39.3±2.6	2.5±0.3	79.4±28.1	43.3±9.6
4-Methylguaiacol-RG	2.0±0.2	106.3±19.4	52.9±2.4	6.2±0.2	22.9±1.1	1.2±0.1	73.6±13.1	41.6±6.0
Syringol-GG	4.3±0.3	51.9±14.7	168.6±19.9	6.5±0.1	55.5±6.2	4.3±0.5	71.0±23.9	209.2±59.5
Syringol-PG	5.1±0.2	15.5±3.0	35.9±3.7	11.5±0.2	31.0±1.8	6.1±0.7	15.2±4.1	31.1±7.8
4-Methylsyringol-GG	0.6±0.01	7.6±1.8	14.9±3.0	2.8±0.1	13.3±1.3	0.7±0.1	13.9±6.2	22.7±6.8
4-Methylsyringol-PG	1.3±0.1	3.0±0.5	4.3±0.9	2.6±0.1	6.7±0.4	1.4±0.1	4.2±1.1	4.8±0.9
Total	54.0±2.2	1440.2±206.0	1714.0±174.5	174.3±1.9	1258.0±74.4	59.1±4.0	1165.9±258.1	1379.9±260.7

Data are mean ±1 standard error (n=5, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where n=3). nd, not detected; PG, pentosylglucoside; RG, rhamnosylglucoside; GG, glucosylglucoside. †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡ from a pasture grass, wild oats (*Avena fatua* L.).

Table 3. Effect of fuel type and smoke exposure on the concentration of volatile phenols in wine of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

	Concentration of volatile phenols (nmol/kg)							
	Sauvignon Blanc			**Chardonnay**		**Merlot**		
Volatile phenols	**Control**	**Pine†**	**Grass‡**	**Control**	**Grass‡**	**Control**	**Pine†**	**Grass‡**
o-Cresol	nd	11.1±3.5	12.3±8.2	nd	nd	nd	77.7±18.2	88.8±11.9
m-Cresol	nd	27.7±9.2	27.7±16.0	nd	nd	nd	33.3±8.6	33.3±4.7
p-Cresol	nd	9.2±4.1	nd	nd	nd	nd	27.7±7.2	20.3±1.8
Subtotal	nd	48.0±14.1	40.0±22.2	nd	nd	nd	138.7±33.7	142.4±17.2
Guaiacol	nd	43.5±9.7	64.4±20.3	nd	nd	40.3±2.5	178.8±30.9	207.8±25.1
4-Methylguaiacol	nd	13.0±4.2	nd	nd	nd	nd	60.8±15.6	34.7±4.8
Subtotal	nd	56.5±13.8	64.4±20.3	nd	nd	40.3±2.5	239.6±46.2	242.5±29.8
Syringol	nd	15.6±4.4	45.4±9.9	nd	nd	122.0±8.0	131.0±6.9	205.0±25.2
4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	nd
Subtotal	nd	15.6±4.4	45.4±9.9	nd	nd	122.0±8.0	131.0±6.9	205.0±25.2
Total	nd	120.1±28.0	149.8±51.7	nd	nd	162.3±9.0	509.3±81.4	589.9±58.6

Data are mean ±1 standard error (n=5, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where n=3); nd, not detected.

†Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡ from a pasture grass, wild oats (*Avena fatua* L.).

Table 4. Effect of fuel type and smoke exposure on the concentration of glycoconjugated phenols in wine of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

	Concentration of glycoconjugated phenols (nmol/kg)							
	Sauvignon Blanc			**Chardonnay**		**Merlot**		
Glycoconjugated phenols	**Control**	**Pine†**	**Grass‡**	**Control**	**Grass‡**	**Control**	**Pine†**	**Grass‡**
Phenol-PG	6.0±0.6	141.1±21.5	114.5±24.4	10.8±0.4	41.3±1.3	4.9±0.5	164.6±24.6	147.1±15.7
Phenol-RG	1.4±0.2	59.7±3.6	60.8±16.7	0.6±0.2	19.0±1.2	1.1±0.1	74.9±7.3	90.1±8.7
Cresol-PG	9.7±0.6	170.8±28.4	148.3±24.4	16.6±1.1	94.5±5.3	22.3±2.3	265.4±36.1	266.0±23.5
Cresol-RG	3.1±0.6	76.8±9.3	55.1±7.0	0.8±0.1	19.8±1.3	2.7±0.3	105.7±9.9	121.0±12.5
GuaiacoliGG	0.3±0.01	0.6±0.2	1.6±0.7	0.2±0.01	0.2±0.01	2.0±0.5	7.0±0.8	8.2±1.4
Guaiacol-PG	6.2±0.5	113.0±17.3	128.5±8.7	11.8±0.7	42.8±2.2	14.2±1.2	319.2±52.7	387.8±42.8
Guaiacol-RG	1.0±0.1	35.3±8.3	38.0±2.9	0.4±0.01	5.7±0.2	1.1±0.1	46.9±5.7	76.1±9.4
4-Methylguaiacol-GG	nd	0.2±0.01	0.1±0.01	nd	nd	0.2±0.01	2.7±0.8	2.2±0.3
4-Methylguaiacol-PG	0.9±0.1	52.2±8.2	21.1±1.1	2.6±0.2	7.1±0.6	3.2±0.5	108.5±19.4	66.6±9.9
4-Methylguaiacol-RG	0.8±0.1	25.4±3.2	11.0±0.8	0.6±0.1	3.8±0.5	1.6±0.2	70.5±6.0	54.1±5.9
Syringol-GG	1.1±0.1	6.0±1.4	19.9±3.7	1.8±0.3	9.4±0.6	2.7±0.4	49.3±13.6	129.6±25.9
Syringol-PG	1.8±0.2	4.2±0.8	6.8±0.6	2.7±0.1	5.3±0.4	8.6±0.6	20.0±2.9	38.8±6.0
4-Methylsyringol-GG	0.1±0.01	0.6±0.1	1.2±0.2	0.1±0.01	0.6±0.01	0.2±0.01	4.8±1.4	10.3±2.6
4-Methylsyringol-PG	0.3±0.01	0.5±0.1	0.6±0.1	0.3±0.01	0.7±0.01	10.4±0.6	16.6±2.1	15.9±0.9
Total	32.7±2.2	686.4±76.6	607.5±85.5	49.3±0.8	250.2±10.9	75.2±3.9	1256.1±173.9	1413.8±139.1

Data are mean ±1 standard error (n=5, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where n=3). nd, not detected; PG, pentosylglucoside; RG, rhamnosylglucoside; GG, glucosylglucoside. †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡ from a pasture grass, wild oats (*Avena fatua* L.).

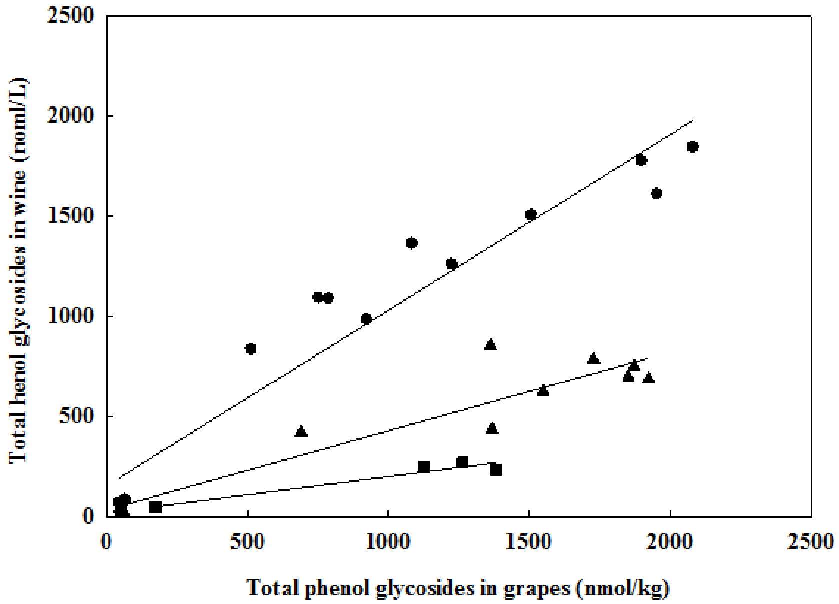

Figure 1. Apparent rate of extraction of total glycoconjugated phenols from grapes into wine as a result of whole-bunch pressing [Chardonnay (■), y = 20.0 + 0.18x, R^2_adj = 0.95], crushing, de-stemming and pressing [Sauvignon Blanc (▲) y = 35.3 + 0.39x, R^2_adj = 0.95] and fermentation on skins [Merlot (●) y =154.8 + 0.88x, R^2_adj = 0.93].

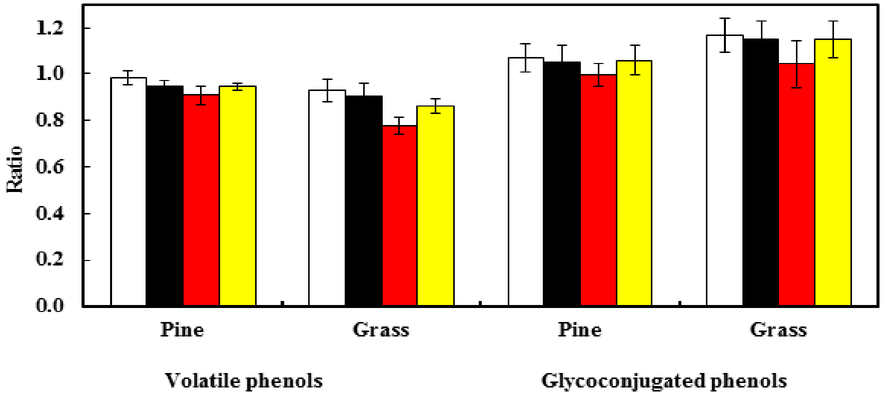

Figure 2. The effect of no malolactic fermentation (MLF) and MLF on the ratio of volatile phenols and glycoconjugated phenols in Merlot wines made from grapes exposed to smoke from pine and oat grass. Cresols and phenols (phenol not quantified in volatile form) (□), guaiacols (■), syringols (🟥) and totals (🟨). Data are means ±1 standard error. For a reference, the data for the MLF wines are shown in Tables 3 and 4.