Seeing through the haze: the discovery of chemical markers for smoke exposure

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Bushfires and controlled burns occur frequently throughout Australia. As a result, vineyards cannot avoid exposure to smoke drift. Unfortunately, wine produced from grapes exposed to smoke is often downgraded or unfit for sale, due to negative sensory characters. In order to manage the risk of smoke exposure, grapegrowers and winemakers rely on analytical tools. These tools identify grapes and wine affected, and influence decision-making as to whether grapes are suitable for harvest, purchase or in the production of wine. In the past, a single volatile phenol - guaiacol - in grapes has been successfully used as a first pass marker for smoke exposure. More recently, research at the AWRI has identified that, in many instances, much higher concentrations of numerous glycosidic metabolites are formed from smoke compounds and are present in grapes and wine. As a result, 40 markers have now been identified as indicators of smoke exposure. Some of these glycosides are present at relatively high concentrations. While they don't directly contribute to smoky aromas, they act as a 'silent' pool of precursors from which a smoky aroma can be released. Here, we summarise the status of our work to develop advanced diagnostic strategies that will allow industry to more reliably identify batches of grapes or wines that are at risk of developing smoke taint. We review management options in the winery and explore the mechanisms, as well as the complex interplay between a variety of free phenols and their glycosidic grape metabolites, that contribute to the development of smoke taint during winemaking and ageing.

INTRODUCTION

In 2003, following a series of bushfire events, it was obvious to many winemakers that smoke-affected vineyards were yielding grapes that made wine with undesirable sensory profiles. This wine was often characterised by objectionable 'smoky', 'burnt', 'ash', 'ashtray' and 'smoked salmon' aromas, and was described often as having 'an excessively drying' back-palate and a retronasal 'ash' character. It was clear that such wine would be difficult, if not impossible, to market. As seen from the 2006-07 bushfires in north-east Victoria alone, the financial loss to grape and wine producers was estimated to be $75-90 million[1]. Bushfire incidents in the vicinity of grapegrowing regions have significantly increased in the last decade with major fire events in 2003, 2007 and 2009. Unfortunately, we are most likely to experience even more bushfires in the future, as climate change forecasts indicate that south-east Australia will become hotter and drier, resulting in greater risk. The numbers of high and extreme fire danger days are expected to increase by up to 25% and 70% by 2020 and 2050 (relative to 1990), respectively[2].

RESEARCH FOCUS: DETECTION AND MITIGATION OF SMOKE EXPOSURE

In response to the increasing incidence of bushfires close to grapegrowing regions, The Australian Wine Research Institute (AWRI) has been investigating the effect of smoke exposure on grapes and wine[3,4,5]. Its key research objectives are to develop assays for the measurement of smoke exposure in grapes prior to winemaking. This will enable winemakers to characterise composition and sensory properties of wine made from smoke-affected grapes and to explore winemaking practices that might reduce or minimise undesirable smoke characters in wine.

Work undertaken at the AWRI and elsewhere has shown that winemaking and viticulture techniques that might help to limit the extraction or concentration of smoke-derived compounds in the final wine include hand harvesting and careful leaf removal, minimising skin contact, cold maceration [3,6,7], and reverse osmosis for the removal of smoke-related compounds[8]. The addition of oak chips or tannins during winemaking may also reduce the perception of smoke-affected characteristics due to the enhancement of wine complexity[7]. Such strategies might prove successful to reduce smoke taints in some cases, but might not provide a universal solution. More detailed information is available from the AWRI website under the Winemaking and Extension Services area (or by emailing winemakingservices@awri.com.au).

The options to manage smoke taint successfully in the winery are limited. Therefore, it is desirable to find ways to minimise or eliminate the uptake of smoke-related contaminants in the vineyard. While such research is currently under way, reliable solutions are not yet available to grapegrowers. So far, our efforts have been concentrated on the development of diagnostic assays to identify smoke exposure in grapes after a bushfire event. The aim is to assist grapegrowers and/or winemakers to make critical decisions with confidence and early: supporting their decisions concerning harvest, purchase of grapes or bulk wine, and winemaking following a smoke event.

HOW DO YOU KNOW IF YOUR SMOKE-AFFECTED GRAPES WILL MAKE SMOKE-AFFECTED WINE?

Initially, guaiacol and 4-methylguaiacol have been used in Australia and overseas as marker compounds to assess the degree of smoke exposure in grapes and wine. This has been for two reasons: because these compounds are found commonly in smoke from wood fires; and their concentrations in wine have been reported to correlate strongly with the overall sensory panel rating of the intensity of the smoke effect. However, after the 2009 Victorian bushfires, we found that almost 80% of grape samples submitted for guaiacol analysis had guaiacol concentrations below the detection limit (1µg/kg) and more than 95% of samples were found to be below 5µg/kg. Despite the presence of insignificant concentrations of guaiacol, the risk that these grapes from smoke-affected regions may produce tainted wine could not be ruled out because the concentration of guaiacol and 4-methylguaiacol have often been reported to increase during fermentation and after bottling[9,10,11]. In addition, guaiacol and 4-methylguaiacol can be found in grapes without smoke exposure as a natural component (up to 5µg/kg levels in some varieties, such as Shiraz). Additionally, these compounds are also extracted into wine from toasted oak (up to 100µg/L in oaked wine[12]). This complicates the interpretation of wine data and limits the diagnostic value of free guaiacol and 4-methylguaiacol at low concentrations. Clearly, these observations suggested that better markers to assess the extent of smoke exposure on grapes and wine were needed.

OTHER VOLATILE COMPOUNDS PRESENT IN SMOKE

As a starting point, we profiled the volatile phenols that are found commonly as major components of smoke. Using smoke generated from a prescribed burn-off, we identified numerous volatile phenols including syringol, methylsyringol, *o*-, *p*- and *m*-cresol, and phenol (Figure 1)[10]. Vinylguaiacol and vinylsyringol were also present as minor components. Being derived by the thermal oxidative decomposition of lignin, these volatile phenols have been found commonly as major components of smoke. Although the relative concentration profile of these volatile phenols can vary due to the variation of the fire conditions such as fuel source (e.g., straw, stubble, or forest), moisture content, combustion temperature and the availability of oxygen, as a group these volatile phenols are major components of smoke generated by wildfires and prescribed burnings. Some of these volatile phenols also have distinctive smoke-related sensory characters ('plastic', 'medicinal',' spicy', 'smoky') that might contribute to the overall smoke-taint in grapes and wine. Like guaiacol, these volatile phenols can also be formed by other variables, such as the toasting of oak barrels and through other oak treatment, and are sometimes found in trace amounts in some grape and wine samples despite the absence of known smoke exposure or oak treatment.

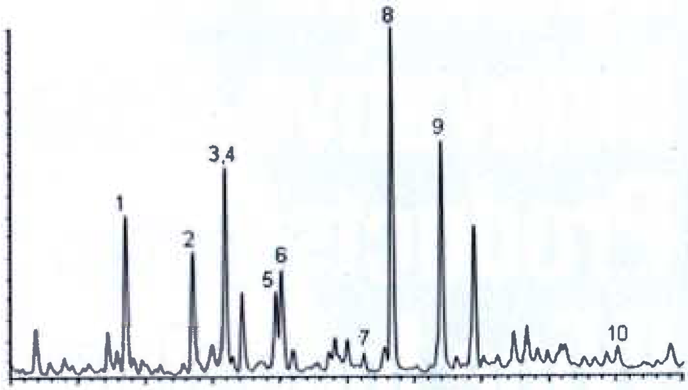

Figure 1. GC-MS analysis of smoke from a prescribed burn-off. Key: 1: guaiacol; 2: 4-methylguaiacol; 3: *o*-cresol; 4: phenol; 5: *p*-cresol; 6: *m*-cresol; 7: vinylguaiacol; 8: syringol; 9: 4-methylsyringol; 10: vinylsyringol.

Despite these limitations, which are similar to using guaiacol and 4-methylguaiacol, it has become clear that these volatile phenols have the potential to become additional smoke exposure markers and improve the diagnostic value of the GC-MS assay, especially in cases where only low guaiacol concentrations can be found in suspect grape samples.

NON-VOLATILE METABOLITES OF VOLATILE PHENOLS IN SMOKED GRAPES

In some instances, winemakers have observed that the smoke-related sensory characters increased after fermentation and continued intensifying in a wine over time, even after bottling. This anecdotal observation was experimentally confirmed by Kennison and colleagues[9] and again recently by Singh and colleagues[11], who found that guaiacol and 4-methylguaiacol can be released from smoke-affected grapes throughout fermentation, resulting in elevated concentrations in wine. This demonstrates the presence of precursors that are releasing free guaiacol and 4-methylguaiacol through hydrolysis with yeast-derived enzymes and/or acid. Subsequently, Hayasaka and colleagues[13] confirmed the presence of guaiacol glucoside as non-volatile smoke metabolite in grapes exposed to smoke from bushfires and model smoke experiments.

AWRI researchers then studied the formation of glycosidic metabolites in detail using a stable isotope tracer technique[14], directly applying a mixed d_0- and d_3-guaiacol solution to grape berries and leaves. As a result of this experiment, seven different glycoconjugates (glycosides) of guaiacol bearing the unique signature of the d_0- and d_3-guaiacol were found in grape berries and leaves that had been in direct contact with the guaiacol solution. Mass spectrometry helped us to tentatively identify these grape metabolites as the following guaiacol glycosides: glucosylglucoside (GG; glucose linked with glucose), monoglucoside (MG), glucosylpentoses (PGs; the glucoside linked with four different pentoses) and rutinoside (RG; glucose linked with rhamnose) (Figure 2). The study also indicated that minimal translocation of guaiacol glycosides occurred between grapevine leaves and berries (at least in the potted Cabernet Sauvignon vines used for the model experiments); the glycosides were present as low-level natural compounds in control leaves and berries; and the glycosides were present in significantly elevated amounts in leaves and berries following exposure of grapevines to smoke derived from actual bushfires. These guaiacol glycosides are considered to be the precursors that act as a masked pool of free guaiacol in wine.

Further study conducted by the AWRI[10] confirmed the presence of a broad range of glycosides of other volatile phenols including methylguaiacol, syringol, methylsyringol, *o*-, *p*- and *m*-cresol, and phenol which were found and identified in both smoked grapes and wine. As a consequence of grapevine exposure to smoke, these volatile phenols were taken up by grapes and subsequently converted to their non-volatile metabolites in a similar fashion to guaiacol. In smoke-affected grape samples, the phenolic glycosides were often present in concentrations hundreds or even thousands of times higher compared with the concentration of the free volatile phenols. This represents a large pool of metabolites with the potential to release significant concentrations of free aroma compounds during the winemaking and ageing process. Importantly, the phenolic glycosides were found to be present in grapes after exposure to smoke and in the corresponding wines at significantly greater concentrations compared with the non-smoked control grapes and wines which contained only trace levels.

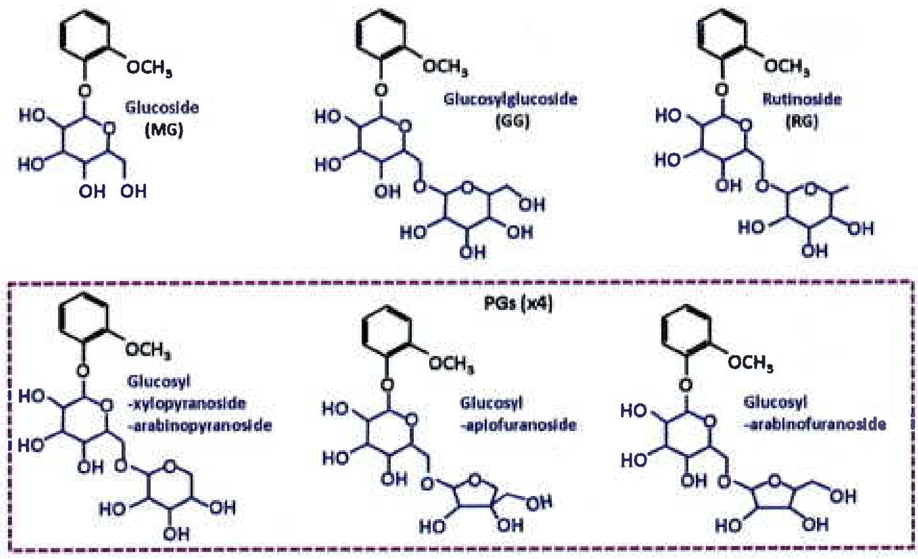

Figure 2. Guaiacol glycosides found in smoked grapes. Key: MG, guaiacol-monoglucoside; GG, glucosylglucoside (glucose linked with glucose); RG, rutinoside (glucose linked with rhamnose); PGs, glucosylpentoses (a group of four different disaccharides from glucose linked to four different pentoses).

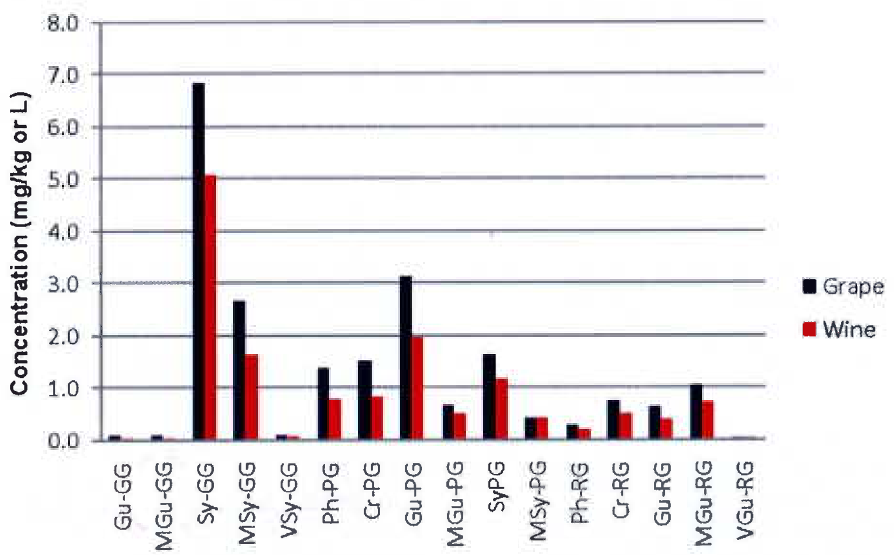

Figure 3. Profile of phenolic glycosides in smoke-affected Cabernet Sauvignon grapes and wine made from the smoke-affected grapes. Key: Ph, phenol; Cr, cresol; Gu, guaiacol; MGu, methylguaiacol; VGu, Vinylguaiacol; Sy, syringol; MSy, methylsyringol; VSy, vinylsyringol.

RELEASE OF SMOKE-RELATED PHENOLS BY WINEMAKING PROCESSES AND STORAGE

To establish the profiles of phenolic glycosides in grape and wine samples, we determined their concentration in bushfire-smoked Cabernet Sauvignon grapes, and in wine made from these grapes[10]. The results showed that the distinctive distribution pattern of the glycosides in the smoke-affected grape samples was similar to that in the resulting wines (Figure 3). At the same time, the concentration of total glycosides (sum of all glycosides measured) declined from around 21mg/kg in the grapes to 14mg/L in the wine (Figure 3). A large

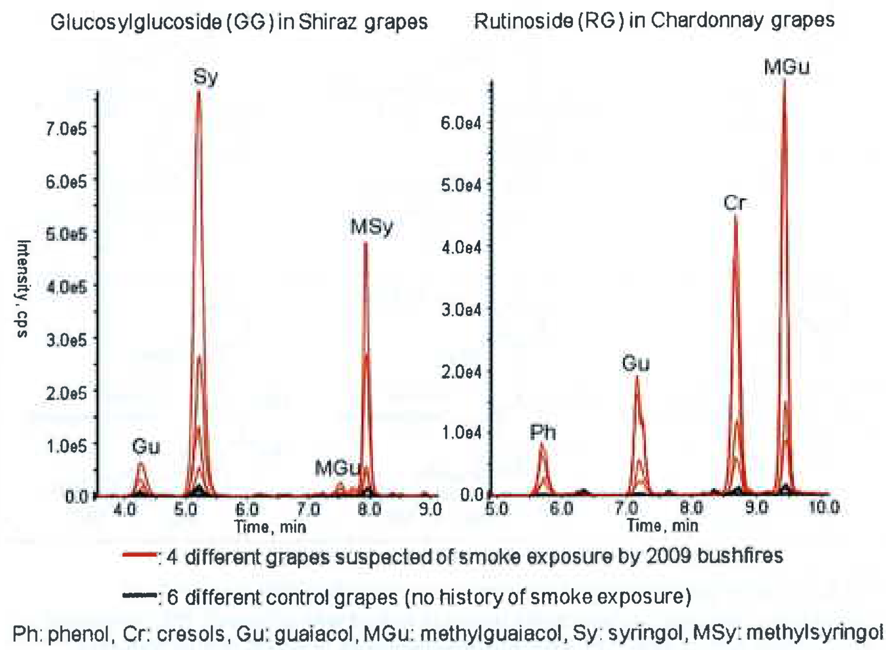

Figure 4. Comparison of HPLC-MS/MS chromatograms of smoked and control grapes.

portion (66% by concentration) of the glycosides from grapes was found in wine due to their high water-solubility. This means that the phenolic glycosides are readily extracted during crushing and fermentation. These glycosides are non-volatile and have no aroma, but likely act as precursors for the corresponding free volatile phenols, resulting in an increase in the concentration of volatile phenols during fermentation[9,10,11]. The ability of the volatile phenols to be chemically released from the glycosides was demonstrated by subjecting bushfire-smoked grapes (Chardonnay and Cabernet Sauvignon) and the corresponding wines to harsh acid hydrolysis conditions. After acid hydrolysis, a large portion of the total glycosides in the grape and wine samples of both varieties disappeared. This was accompanied by a concomitant increase in the free volatile phenols in the hydrolysates[10]. Therefore, the concentration of these phenolic glycosides in grapes can be used to identify grape exposure to smoke and is indicative of the potential for wine to develop smoke-related aroma compounds throughout winemaking and storage.

PHENOLIC GLYCOSIDES AS MARKERS FOR SMOKE EXPOSURE

To further corroborate the concept that the various phenolic glycosides can be used as diagnostic markers to detect smoke exposure, the AWRI has developed a robust and specific method for the quantification of phenolic glycosides in grape and wine samples using HPLC-MS/MS. Using this new analytical method, we analysed grapes suspected of smoke exposure from the 2009 bushfires and control grapes with no history of smoke exposure (Figure 4). Trace amounts of phenolic glycosides were present as natural compounds in the control grapes and their intensities were quite consistent[10,14]. The smoke-exposed grape samples exhibited a great range and much higher concentrations of phenolic glycosides compared with the control samples. On the other hand, low concentrations of free guaiacol (2-3μg/kg) and 4-methylguaiacol (<2μg/kg) were found in approximately 50% of the grape samples after smoke exposure, revealing the difficulty in making the decision on whether grapes were smoked or not, based on free guaiacol and 4-methylguaiacol alone. The much improved distinction between non-smoked (control) and smoked samples revealed by the HPLC-MS/MS measurement of the phenolic glycosides is now being used as a reliable diagnostic strategy to identify smoke exposure and to assess the impact of smoke exposure in grapes and wine.

ADVANTAGES OF PHENOLIC GLYCOSIDES AS SMOKE EXPOSURE MARKERS

In summary, the measurement of phenolic glycosides provides a versatile and reliable method to identify and manage smoke exposure:

- when a grapevine is exposed to smoke, the amount of the volatile phenols taken up by grapes can be related to the intensity and duration of smoke exposure[15]
- once taken up by grapes, the volatile phenols are metabolised rapidly into their more stable and non-volatile glycosidic forms[16]
- the smoke-induced glycosides persist and accumulate in grapes until the time of harvest[14,16]. Therefore, the amount of the grape glycosides can be correlated to the intensity of smoke exposure, and possibly also used to quantify extended or repeat smoke exposure
- the phenolic glycosides are not present in non-smoked grapes and are also not found (unlike free guaiacol) in oak in significant concentrations
- the phenolic glycosides are easily extracted into wine and act as a pool of precursors to release volatile phenols during alcoholic and/or malolatic fermentation, ageing, and storage[8,9,11]. This implies that measurement of phenolic glycosides can be used to estimate the potential of grapes to produce smoke-related taints after fermentation and at the time of consumption.

WHERE TO FROM HERE?

In collaboration with industry partners, a comprehensive survey of the baseline levels of volatile phenols and their glycosides in samples of un-smoked (control) grapes and un-oaked laboratory-scale wines is under way. We have sourced samples from 11 grapegrowing regions and five major varieties: Cabernet Sauvignon, Chardonnay, Pinot Noir, Riesling and Shiraz. Volatile phenols, including guaiacol, methylguaiacol, *o*-, *m*-, and *p*-cresol, syringol and methylsyringol, as well as multiple bound versions (glycosides) of the above phenols have been measured over two vintages. Statistical analysis will improve our ability to identify 'normal' clean grapes and aid the interpretation of analytical data from samples suspected of smoke exposure. We are also currently undertaking a major sensory study to quantify the impact in wine of combinations of free volatile phenols and their glycosides on smoky aroma and perceived flavour.

While bushfires continue to form part of the Australian natural environment and smoke drift into vineyards cannot be avoided, we are now in a much better position to assist grapegrowers and winemakers with their management of the consequences of real or potential smoke exposure. By combining analytical data about free and glycosidically-bound phenols from smoke events with a comprehensive understanding of the natural occurrence of smoke exposure markers, their stability and release, and their sensory properties, grape and wine producers are in a stronger position to take control of smoke taint.

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