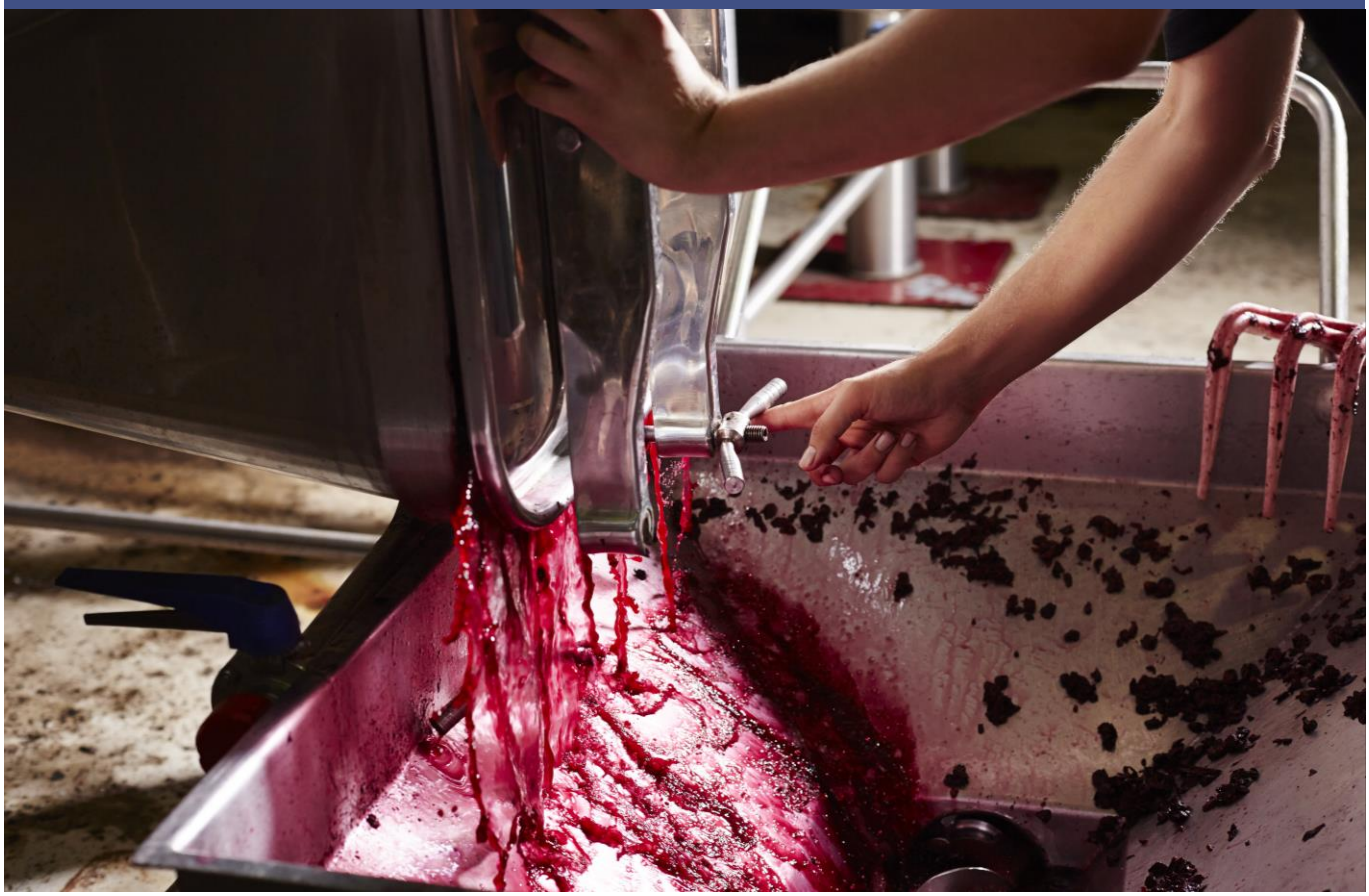
AWRI Australian Wine Research Institute

Fact Sheet
WINEMAKING

Treating smoke-affected wine with glycosidase enzymes



Background

When grapes are exposed to smoke, they can absorb volatile phenols, which bind to sugars in the grapes forming non-volatile phenolic glycosides (Figure 1). In juice and wine, both volatile phenols and their glycosides can cause unpleasant 'ashy' and 'smoky' sensory sensations and a lingering aftertaste, commonly described as 'smoke taint'.

What are glycosidases and how can they be used to mitigate smoke taint?

Glycosidases are enzymes that catalyse the hydrolysis of glycosidic bonds in complex sugars. In grapes, phenolic glycosides are formed when volatile phenols from smoke are absorbed into the grape berry. During fermentation, a portion of these glycosides can be broken down, due to glycosidase activity of added enzymes, yeast and bacteria. This leads to the release of volatile phenols into the wine and may increase its 'smoky' sensory characteristics. However, not all

phenolic glycosides are degraded during fermentation and as such they can remain in finished wine. Their presence in wine can be problematic as they can gradually hydrolyse during ageing and storage to release volatile phenols. For example, they could also pose a problem if present in sparkling base wines as the secondary fermentation might further degrade the phenolic glycosides and release additional volatile phenols into the sparkling wine. Furthermore, there is potential for phenolic glycosides to undergo in-mouth release during consumption due to the presence of glycosidases in human saliva (Parker et al. 2012, Mayr et al. 2014, Parker et al. 2020).

Consequently, the motivation for using glycosidase enzymes to combat smoke taint in wine is to reduce the concentrations of phenolic glycosides by cleavage of their sugar units releasing volatile phenols at early stages of winemaking. The volatile phenols can then be removed by treating with activated carbon or nanofiltration. Trials were conducted to investigate the viability of using commercially available glycosidases to treat smoke-affected wines.

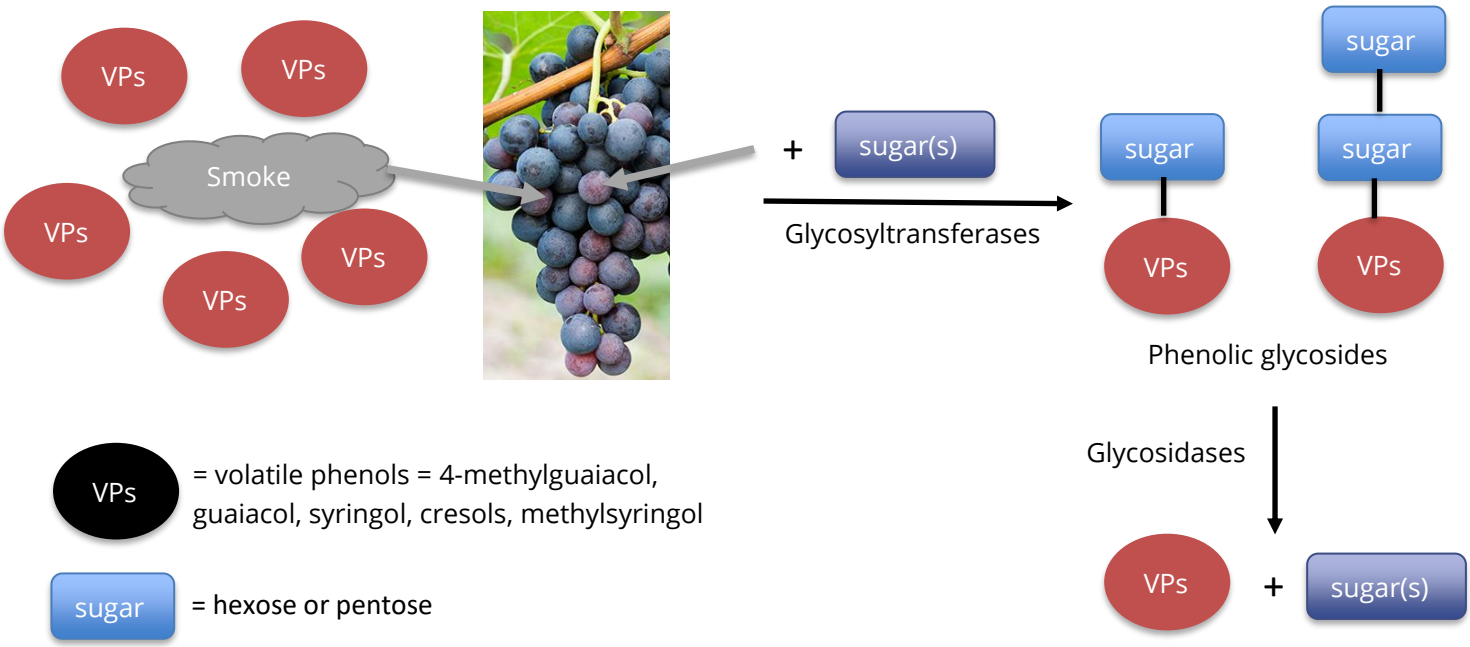


Figure 1. Schematic describing the formation of phenolic glycosides from volatile phenols (VPs) contained in smoke and sugars present in grapes and their degradation back to volatile phenols via glycosidase activity

Assessing the performance of glycosidases in treating smoke-affected wine

Glycosidases and wine types evaluated

Smoke-affected Chardonnay, Pinot Noir, Tempranillo, Durif, Shiraz and Muscat wines were treated with commercially available glycosidases in a commercial winery. The list of glycosidases evaluated, their manufacturer/supplier and the dose rates applied are listed in Table 1. Wines were typically treated at 15-17°C and glycosidase contact time varied from 2 to 8 weeks (typically a minimum of 3 weeks).

Table 1. *Glycosidases evaluated in smoke taint remediation trials and dose rates applied*

Glycosidase	Manufacturer (supplier)	Product name	Dose rate
1	Erbslöh (Victus International)	Trenolin® Bouquet PLUS	15 mL/hL
2	DSM	Rapidase Revelation Aroma	3 g/hL
3	Vason	Zimarom	3 g/hL
4	Martin Vialatte (Grapeworks)	Viazym Aroma	5 g/hL

Impact on smoke compounds and wine sensory characteristics

The sum of the volatile phenols (n=7) and sum of the phenolic glycosides (n=6), along with the mean ratings for smoke aroma and smoke flavour for all wines treated with glycosidases are provided in Table 2.

The concentrations of the six phenolic glycosides analysed were reduced by 30-100% (average 67%) with glycosidase treatment. The variation observed was influenced by the glycosidase used as well as treatment temperature, glycosidase contact time and wine type.

The Muscat wine (18.4 v/v% alcohol) had the lowest reduction, probably a consequence of the low wine storage temperature (only 12°C) and elevated sugar levels (i.e. 321 g/L) of this style, which likely inhibits glycosidase activity.

Of the four glycosidases evaluated, glycosidase 1 was the most effective at reducing the concentrations of the phenolic glycosides as it could degrade the rutinosides (RGs) in addition to the gentiobiosides (GGs, glucose-glucose). The other three glycosidases were only able to degrade the gentiobiosides. These results support previous research findings (Culbert et al. 2020). Glycosidase 1 reduced the concentrations of the six phenolic glycosides to below 1 in Chardonnay, Pinot Noir (wine 1) and Pinot Noir/Chardonnay wines after a contact time of 8 weeks at 17°C. Additional time course studies of these wines indicated that >90% of these phenolic glycosides were already broken down after a contact time of 3 weeks at 17°C.

Glycosidases 2, 3 and 4 behaved very similarly (both chemically and sensorially), and wines treated with those three glycosidases were blended prior to bottling and formal sensory evaluation.

Table 2. *Sum of volatile phenols, sum of phenolic glycosides and mean ratings for smoke aroma and smoke flavour for each of the control wines and wines treated with glycosidases*

Treatment	Sum of volatile phenols, µg/L (n=7)	Sum of phenolic glycosides, µg/L (n=6)	Smoke aroma	Smoke flavour	Significantly different from the control*?
Chardonnay wine					
Control	6	70	0.80	1.46	-
Glycosidase 1	18	<1	1.62	3.25	Yes, flavour only
Glycosidases 2-4	19	23	3.22	4.42	Yes, aroma and flavour
Pinot Noir wine 1					
Control	9	81	1.58	1.90	-
Glycosidase 1	22	<1	1.39	3.17	Yes, flavour only
Glycosidases 2-4	29	29	2.03	3.17	Yes, flavour only
Pinot Noir/Chardonnay wine					
Control	15	119	1.00	1.51	-
Glycosidase 1	32	<1	2.57	4.04	Yes, aroma and flavour
Glycosidases 2-4	29	39	2.73	4.18	Yes, aroma and flavour
Pinot Noir wine 2					
Control	43	55	1.18	2.20	-
Glycosidase 1	43	21	1.77	2.75	No
Tempranillo wine					
Control	26	126	0.89	1.39	-
Glycosidase 1	25	57	1.58	2.87	Yes, flavour only
Durif wine					
Control	24	395	0.15	0.75	-
Glycosidase 1 + Fenol Free	31	189	1.39	2.51	Yes, aroma and flavour
Shiraz wine					
Control	56	161	0.42	1.19	-
Glycosidase 3 + PROVGREEN	56	92	1.12	1.56	No
Muscat wine					
Control	36	230	1.27	1.25	-
Glycosidase 1 + Granucol GE	36	162	1.68	2.18	Yes, flavour only

*Based on the sensory ratings for smoke aroma and smoke flavour;
Glycosidase 1 = Erbsloh Trenolin Bouquet Plus 15 mL/hL; Glycosidase 2 = DSM Rapidase Revelation Aroma 3 g/hL; Glycosidase 3 = Vason Zimarom 3 g/hL; Glycosidase 4 = Martin Vialatte Viazym Aroma 5 g/hL;
Glycosidases 2-4 refer to a blended wine containing an equal proportion of wines individually treated with glycosidases 2, 3 and 4.
Sum of volatile phenols = additive concentrations (in µg/L) of 4-methylguaiacol, guaiacol, *o*-cresol, *p*-cresol, *m*-cresol, syringol and 4-methylsyringol; sum of phenolic glycosides = additive concentrations (in µg/L SyGG equivalents) of Syringol gentiobioside (SyGG), methylsyringol gentiobioside (MSyGG), phenol rutinoside (PhRG), cresol rutinoside (CrRG), guaiacol rutinoside (GuRG) and 4-methylguaiacol rutinoside (MGuRG).
Fenol Free, PROVGREEN and Granucol GE were dosed at 0.5, 0.4 and 0.5 g/L, respectively.

Despite the reductions in phenolic glycoside concentrations observed in all wines, an increase in the concentrations of volatile phenols was only seen for 50% of the wines. As the six phenolic glycosides reported are disaccharides (two sugar units attached to one volatile phenol molecule) it is possible that the observed reductions in their concentrations without a corresponding increase in the concentrations of volatile phenols may have resulted from cleavage of one sugar unit to form the corresponding monoglucosides (one sugar unit attached to one volatile phenol molecule) rather than cleavage of both sugars to release the volatile phenols. This phenomenon has been observed in previous investigations (Culbert et al. 2020) but why it occurs in some wines and not others is unknown.

It should also be highlighted that the direct influence of the glycosidase treatment alone on smoke compounds in the Durif, Shiraz and Muscat wines is unknown as they underwent further treatment with products that can absorb volatile phenols (see Table 2). For these wines, the volatile phenol concentrations post-glycosidase treatment were likely higher than those measured after the secondary treatment. The ratings for smoke aroma and smoke flavour were higher for all wines treated with glycosidases, and in most instances these increases were statistically significant for one or both sensory attributes.

Impact of dose rate and wine temperature

The length of time needed for glycosidase enzymes to reduce the concentrations of phenolic glycosides in smoke-affected wine is influenced by the enzyme dose rate and wine temperature, with performance accelerated as both factors increase. At a wine temperature of 17°C, most glycosidase enzyme activity occurs within three weeks at the highest recommended dose. Contact time beyond three weeks can provide further benefit for the slower acting glycosidases (i.e. glycosidases 2-4) but is less beneficial for the faster-acting glycosidase 1.

Similar glycosidase activity was observed in smoke-affected wine after one week at 25°C compared to three weeks at 17°C, indicating that eventually the glycosidases ‘catch up’ at lower wine temperature. Therefore, while higher wine temperatures can reduce the required contact time for the glycosidase enzymes, it may be more practical to keep wine at lower temperature (15-17°C) and extend contact times and/or increase enzyme dose rate to ensure wine integrity is maintained.

Conclusions/recommendations

In all instances where glycosidases were the sole treatment, there was an increase in ratings for smoke aroma and smoke flavour compared to the control wine and in most instances these increases were statistically significant. An increase in the perception of smoke characters following the use of glycosidases was often associated with an increase in the concentration of volatile phenols. Unless glycosidases are used prior to an additional treatment to reduce the concentrations of volatile phenols, glycosidase treatment alone may exacerbate the perception of smoke taint. Further research is being undertaken to determine the stability of phenolic glycosides during bottle ageing to further evaluate potential benefits from reducing the concentration of these compounds in wine.

References and further reading

Culbert, J.A., Jiang, W., Krstic, M.P., Herderich, M.J. 2020. Mitigation of climate change impacts on the national wine industry by reduction in losses from controlled burns and wildfires and improvement in public land management. Final report.

Parker, M., Osidacz, P., Baldock, G., Hayasaka, Y., Black, C., Pardon, K., Jeffery, D., Geue, J., Herderich, M., Francis, I. 2012. Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine. *J. Agric. Food Chem.* 60: 2629–2637.

Mayr, C.M., Parker, M., Baldock, G.A., Black, C.A., Pardon, K.H., Williamson, P.O., Herderich, M.J., Francis, I.L. 2014. Determination of the importance of in-mouth release of volatile phenol glycoconjugates to the flavor of smoke-tainted wines. *J. Agric. Food Chem.* 62: 2327−2336.

Parker, M., Onetto, C., Hixson, J., Bilogrevic, E., Schueth, L., Pisaniello, L., Borneman, A., Herderich, M., de Barros Lopes, M., Francis, L. 2020. Factors contributing to interindividual variation in retronasal odor perception from aroma glycosides: the role of odorant sensory detection threshold, oral microbiota, and hydrolysis in saliva. *J. Agric. Food Chem.* 68: 10299−10309.

Acknowledgements

This work was supported through funding from the Wine Victoria Bushfire Technical Response Program which aims to deliver a suite of technical support activities for Victorian grape and wine producers affected by the 2020 bushfires. Wine Victoria has received funding from the Victorian Government to facilitate wine making remediation trials. Wine Network Consulting is thanked for collaboration on this project.

The AWRI's communications are supported by Australian grapegrowers and winemakers through their investment body, Wine Australia, with matching funding from the Australian Government. The AWRI is a member of the Wine Innovation Cluster.

WINE VICTORIA
AUSTRALIA

wine Network Consulting

Contact

For further information, please contact: AWRI helpdesk

Phone 08 8313 6600 **Fax** 08 8313 6601 **Email** helpdesk@awri.com.au

Website https://www.awri.com.au/industry_support/winemaking_resources/smoke-taint/

Address Wine Innovation Central Building, Corner of Hartley Grove & Paratoo Rd, Urrbrae (Adelaide), SA 5064