ABSTRACT

Guaiacol β-D-glucopyranoside was prepared via a modified Koenigs-Knorr glycosylation method as a reference compound to confirm its presence in smoke affected grapes, using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. The β-D-glucopyranoside of guaiacol was identified in extracts of Sangiovese grapes exposed to bushfire smoke and Chardonnay grapes exposed to smoke under experimental conditions. Only trace levels of the glucoside were identified in the corresponding control (i.e. unsmoked) Chardonnay grapes, indicating glycosylation of smoke-derived guaiacol occurred in response to smoke exposure. The reference compound and the glucoside present in smoked juice samples remained largely unaffected following strong acid hydrolysis but were highly susceptible to β-glucosidase enzyme hydrolysis, providing a plausible explanation for the release of guaiacol during fermentation of smoke affected grapes.

Following the identification of additional guaiacol glycoconjugate precursors, the d_4-labelled analogue of guaiacol β-D-glucopyranoside was synthesised for use as an internal standard in the development of a quantitative stable isotope dilution analysis (SIDA) method, using HPLC-MS/MS. This method was subsequently applied to the analysis of several grape varieties exposed to either experimental or bushfire smoke, to investigate the accumulation of guaiacol glycoconjugates following grapevine smoke exposure. Experimentally smoked grapes contained glycoconjugate concentrations up to 300 µg/kg; whereas grapes affected by bushfire smoke contained up to 2,000 µg/kg glycoconjugates, attributed to the different durations of smoke exposure.

Analysis of separated berry components indicated that the majority of guaiacol glycoconjugates were present in skin and pulp fractions, although approximately 6.7 times higher concentrations were found in the skins by mass. As such,

glycoconjugate extraction from berry homogenate was considered to be more efficient than from juice. To investigate the potential for smoke taint carry over to subsequent growing seasons, grapes were collected from control and smoked Merlot and Viognier grapevines in the season following smoke exposure. Subsequent analysis showed no evidence to suggest grapevine sequestration of glycoconjugates.

The HPLC-MS/MS based SIDA method was adapted for the quantification of guaiacol glycoconjugates in wine and the method applied to several winemaking trials to investigate glycoconjugate metabolism during fermentation. Reduced skin contact achieved using a 'cold soak' winemaking technique, yielded wines with significantly lower concentrations of guaiacol glycoconjugates, compared with traditional red winemaking practices involving extended skin contact at ambient temperature. This suggests winemaking processes which limit precursor extraction might offer opportunities for winemakers to ameliorate the impact of smoke taint in wine. A yeast selection trial demonstrated only partial metabolism of glycoconjugates during fermentation, with glycoconjugate concentrations of finished wines not significantly influenced by choice of yeast strain. The fact that a considerable portion of the glycoconjugate pool remained after fermentation has important implications for industry; i.e. hydrolysis of glycoconjugates after bottling could result in enhanced smoke characters with ageing.

The effect of grapevine smoke exposure on grape berry microflora and the performance of several winemaking yeast in the presence of smoke-derived volatiles were also investigated. The growth of indigenous and winemaking yeast on yeast media agar plates spiked with guaiacol, 4-methylguaiacol or a liquid smoke preparation was investigated to further determine the impact of smoke-derived volatile compounds on yeast performance.