Wine Victoria

Bushfire technical package of programs

Program activity: cross-validation of 2020 grape and wine smoke analytical data between Vintessential and AWRI Commercial Services laboratories

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Comparative results for grape and wine smoke testing from Vintessential and AWRI Commercial Services laboratories

Introduction

During the 2019/2020 bushfire season in Australia extensive testing of grape and wine samples for smoke markers was conducted by both the Vintessential laboratories based in Victoria and the AWRI Commercial Services laboratories based in South Australia. A number of observers commented that the results from the two laboratories appeared to be inconsistent and a greater understanding of how they related was needed. For this reason, a comparative study was commissioned by Wine Victoria.

It should be noted that the two laboratories, at least in part, use different technologies and methods to determine the levels of free and bound smoke markers in grapes and wine. These differences are driven not by simple choice but rather by the available equipment and resources at each organisation, as well as the underlying background datasets each organisation has developed. The core technology used for determination of free smoke volatiles (gas chromatography mass spectrometry, GCMS) is very similar between the two laboratories, with some minor differences in extraction protocols, standards and experimental design, driven by each laboratory's processes. The determination of glycosidically bound smoke markers, however, is done differently in each organisation:

- Vintessential uses a hydrolysis step to cleave the free volatiles from the entities they are bound to and then measures the released free volatiles by GCMS analysis.
- AWRI Commercial Services uses liquid chromatography mass spectrometry (LCMS) to directly measure a group of six bound volatiles, without conducting any hydrolysis step.

These two methods will inherently provide different (but valid) results, which can each be compared to data from non-smoke-affected samples analysed using the same method. It should be noted that there is no international standard for the measurement of smoke markers and given that the choice of method is largely driven by the available instruments, such a standard is unlikely to be adopted in the near future. Efforts are underway, however, to develop agreed standards and protocols to make comparing and developing methods more consistent.

It is not within the scope of this report to determine if any given approach is better or worse than the other. A number of references are listed at the end of this report which discuss the processes of determining which compounds are most appropriate as smoke markers and the strengths and weaknesses of different analysis methods. Instead, this study sets out to determine if any of the analytes from the two laboratories show reasonable correlation and how they relate, potentially allowing users to make reasonable comparisons of results received from the two laboratories.

Comparison conducted

Using grapes independently gathered from the 2020 harvest as part of an ongoing AWRI study, 50 grape samples (16 Chardonnay, 19 Shiraz and 15 Pinot Noir) were randomly subsampled and sent to each laboratory using blind identifiers. The grapes used had been previously determined to cover a range of low, high and medium smoke impacts; however, neither laboratory was aware of any previous results or the source or variety of the samples. Once again, as part of an independent study, the same grapes were used to make a series of wines in small-scale ferments and subsamples of each of the wines were sent to each laboratory.

Each laboratory analysed the samples as per their normal protocols and submitted their results independently to Professor Leigh Schmidtke at Charles Sturt University. Prof. Schmidtke conducted a statistical comparison of the results, the full text of which is available on the AWRI and Vintessential websites (Schmidtke 2020). The results outlined in this document represent a summary of the relevant information in the full statistical report (Schmidtke 2020).

When comparing the results for free smoke markers, the same analytes were measured at each laboratory via very similar methods, so the following pairs of results were compared:

Free volatile measured by AWRI Commercial Services - analysis codes shown in brackets	**Free volatile measured by Vintessential – analysis codes shown in brackets**
4-methylguaiacol (4.Methylguaiacol)	4-methylguaiacol (4.MG)
Guaiacol (Guaiacol)	Guaiacol (Guaiacol)
m-cresol (m.CRESOL)	*m*-cresol (m.Cresol)
4-methylsyringol (Methyl.Syringol)	4-methylsyringol (4.MS)
o-cresol (o.CRESOL)	*o*-cresol (o.Cresol)
p-cresol (p.CRESOL)	*p*-cresol (p.Cresol)

When comparing the results for bound smoke markers, which were analysed using different methods and technologies between the two laboratories, the following pairs of results were compared:

Bound volatile measured by AWRI Commercial Services – analysis codes are the same as the compound name	**Free volatile measured following hydrolysis step by Vintessential – analysis codes shown in brackets**
Cresol rutinoside	Total cresol (Total cresol)[1]
Guaiacol rutinoside	Guaiacol (Guaiacol)
Methylguaiacol rutinoside	4-methylguaiacol (4.MG)
Methylsyringol gentiobioside	4-methylsyringol (4.MS)
Syringol gentiobioside	Syringol (Syringol)

[1] Note that total cresol for Vintessential results was calculated for use in the statistical comparison and is not generally reported to clients, as the individual cresol results are instead reported to clients.

It's important to understand that the different methods used by the two laboratories when analysing bound smoke markers are not measuring exactly the same thing. For example, when considering a bound syringol smoke marker, the Vintessential method is measuring syringol released by hydrolysis from all bound syringol sources and the AWRI Commercial Services method is measuring syringol gentiobioside specifically. This difference in approach means that direct correlations between results from the two laboratories for bound smoke markers are less likely than for free smoke markers, where the two laboratories use very similar methods.

Results for grape analysis

A summary of the statistical analysis of the direct comparison of grape analysis results from the two laboratories is provided in Table 1. It should be noted that while both laboratories analysed all 50 grape samples, where the results were below limit of quantification (LOQ) for one or both laboratories, they could not be used in the comparison. This is most obvious in the case of the free volatiles 4-methylsyringol and syringol, which were below the LOQ for all samples for the AWRI Commercial Services results and hence no comparison was possible. The table contains several different statistical measures, which are described briefly below:

- **R-squared** is a statistical measure of correlation (the extent to which two variables are related). R-squared values can range from 0 to a maximum of 1, with higher values indicating a stronger correlation. A value of 1 does not mean that the results compare exactly one to one, just that they are correlated, and that this correlation can be described mathematically. In Table 1, analytes with an R-squared value greater than 90% (0.9) are highlighted in green, as an arbitrary indication of a reasonable correlation.
- **Gradient** is the gradient (or slope) for the correlation, and it is this which indicates how the results scale against each other in absolute terms. An ideal gradient of 1 implies that the results compare one to one along the range of values; a value of 0.7 would suggest that the AWRI result would be (on average) 0.7 times the Vintessential results for the same sample.
- The **intercept** represents the constant offset between the results across the range.
- The **RMSE** is essentially a measure of the average difference of the real results from the correlation.
- **n** is the number of samples for which results could be included in the statistical analysis.

Table 1. Summary of the statistical comparison of results of analysis of free and bound smoke markers in grapes conducted by Vintessential and AWRI Commercial Services. Analytes with an R-squared value greater than 0.9 are shown in green as an arbitrary indication of a reasonable correlation between the results from the two laboratories. Analytes with an R-squared value below 0.9 are shown in red, indicating a less good correlation; n represents the number of samples where results could be used in the statistical comparison.

Comparison of results for free smoke markers in grapes						
AWRI Commercial Services analysis code	**Vintessential analysis code**	**n**	**R^2**	**Intercept**	**Gradient**	**RMSE**
4.Methylguaiacol	4.MG	19	0.73	0.67	0.81	1.47
Guaiacol	Guaiacol	45	0.92	2.07	0.98	3.14
m.CRESOL	m.Cresol	11	0.9	0.31	0.98	0.806
o.CRESOL	o.Cresol	44	0.87	0.76	0.7	1.73
p.CRESOL	p.Cresol	8	0.9	-0.01	1.14	0.47
Comparison of results for bound smoke markers in grapes						
AWRI Commercial Services analysis code	**Vintessential analysis code**	**n**	**R^2**	**Intercept**	**Gradient**	**RMSE**
Cresol rutinoside	Total cresol	45	0.18	1.43	0.13	3.03
Guaiacol rutinoside	Guaiacol	46	0.47	5.78	0.79	11
Methylguaiacol rutinoside	4.MG	45	0.49	0.88	0.08	1.9
Methylsyringol gentiobioside	4.MS	40	0.92	8.84	1.31	17.1

Comparison of results for free smoke markers in grapes						
AWRI Commercial Services analysis code	**Vintessential analysis code**	**n**	**R^2**	**Intercept**	**Gradient**	**RMSE**
Syringol gentiobioside	Syringol	49	0.91	25.27	0.71	55.2

An example of the statistical report's graphical output for a free volatile smoke marker and a bound smoke marker in grapes where the R-squared values were greater than 0.9 is shown in Figure 1. The graphs for all the other analytes can be found in the full statistical report (Schmidtke 2020).

Free volatile smoke markers in grapes

The free volatiles guaiacol, *m*-cresol and *p*-cresol all showed correlations above 90%. The gradients for guaiacol and *m*-cresol were within 2% of the ideal value of 1 and while not 0, the intercepts were relatively small in comparison to the range of results. The gradient for *p*-cresol was 1.14, suggesting that on average AWRI Commercial Services results were ~14% higher than those from Vintessential. Based on this analysis, results for all three analytes (guaiacol, *m*-cresol and *p*-cresol) from the two laboratories can be meaningfully compared for grape samples. The root mean square error (RMSE), was also acceptable for these three analytes, given the range of results observed.

Results for the volatile analyte *o*-cresol also showed a positive correlation (87%), just below the arbitrary cut-off described above. The gradient for this analyte was, however, only 0.7, which is significantly different from the ideal value of 1. This is most likely driven by a predominance of low results near the LOQ. Results for the analyte 4-methylguaiacol did not correlate well between the two laboratories, but this is most likely due to the fact that only 19 results were available for correlation (the rest being below LOQ) and because those results that were measured were relatively low.

Bound smoke markers in grapes

For the bound analytes in grapes, only the pairs of methylsyringol gentiobioside/4-methylsyringol and syringol gentiobioside/syringol showed correlations above 90%, with the correlations for all the other analytes below 50%. The gradients for the two pairs with correlations above 90% demonstrated significant variations from 1 (1.31 and 0.71). It's important to remember that the methods for bound smoke markers are not measuring identical things (e.g. one method is measuring syringol released by hydrolysis from all bound syringol sources and the other is measuring syringol gentiobioside specifically). The strong correlations for these two pairs of analytes suggests that the methods do give equivalent information; however, care must be taken in comparing absolute values.

The other three bound analytes compared did not show meaningful correlations and therefore efforts to compare results for these pairs between laboratories cannot be recommended. This does not mean that either method is providing inaccurate or inconsistent results, instead that they are again measuring different things. For both laboratories, comparison of results for these three analytes against internally generated background data is still valid, but comparisons between laboratories or their respective background datasets for non-smoke-affected fruit would not be valid.

Summary for grape analysis

In summary, for grape analysis the direct comparison of results from the two laboratories is possible for the volatile analytes guaiacol, *m*-cresol and *p*-cresol (and perhaps *o*-cresol with some care). For bound analytes there is a strong correlation between results for the pairs methylsyringol gentiobioside/4-methylsyringol and syringol gentiobioside/syringol, although care must be taken in comparing absolute values as the correlations are not a simple one to one relationship. Comparison to an internal dataset (generated using the same method) is valid, which is important because research suggests these two analytes are two of the more sensitive markers for smoke exposure in

Australia. No correlations were identified for the other bound analytes. As such, comparisons between results from the two laboratories for those other bound analytes cannot be recommended.

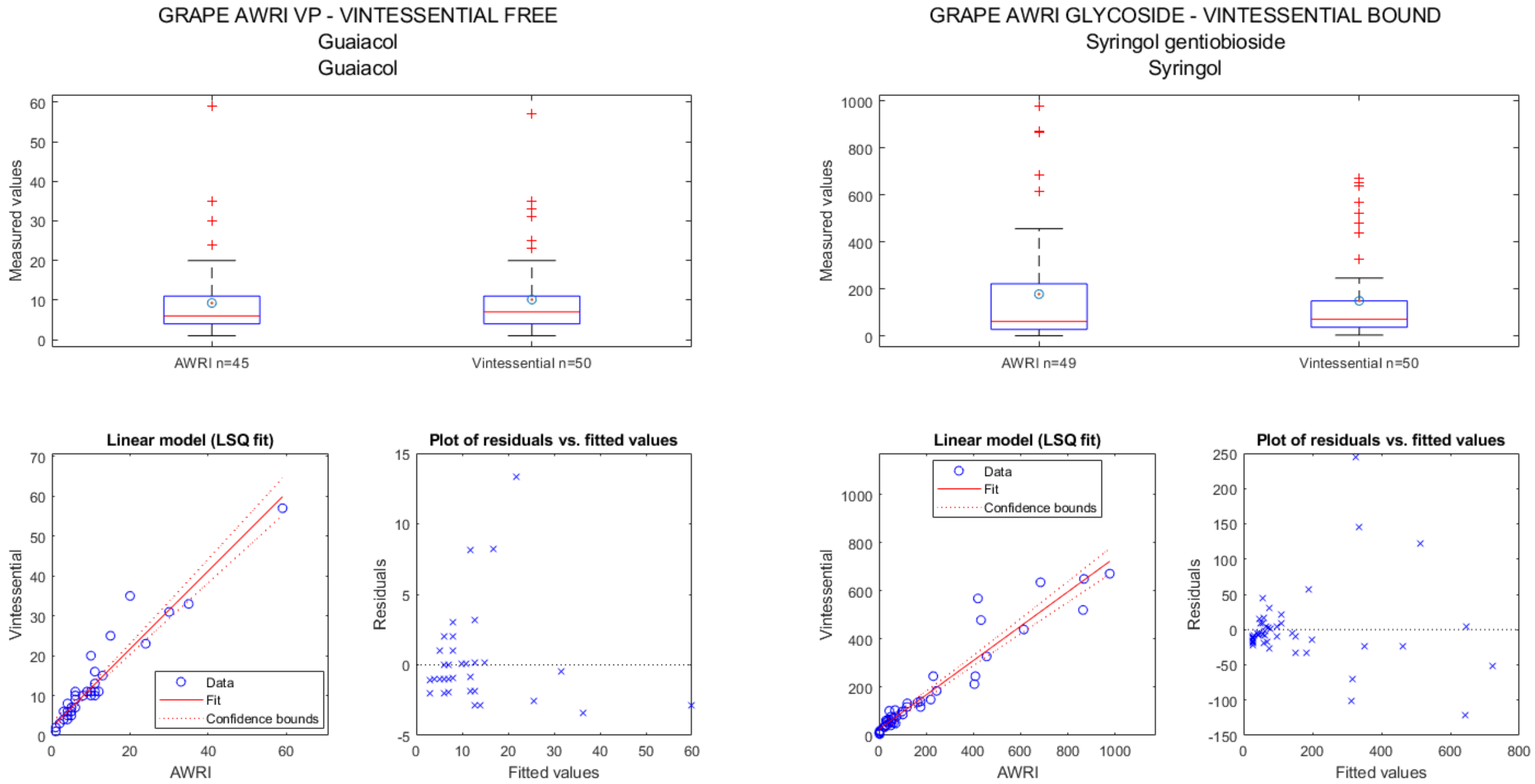


Figure 1. Example graphs from the full statistics report (Schmidtke 2020) demonstrating the positive correlation between results from both laboratories for the free volatile smoke marker guaiacol and for the bound smoke marker syringol gentiobioside (AWRI Commercial Services) and bound syringol results (Vintessential) in grapes. The graphs for all analytes are included in the report by Schmidtke (2020).

Results for wine analysis

A summary of the statistical analysis of the direct comparison of results from the two laboratories for wine samples is provided in Table 2. It should be noted that while both laboratories analysed all 50 samples, where the results were below LOQ for one or both laboratories they could not be used in the comparison. In Table 2, analytes with an R-squared value greater than 90% (0.9) are highlighted in green. As in Table 1, 90% has been chosen as an arbitrary indication of a reasonable correlation. An example of the statistical report's graphical output of a free volatile smoke marker and a bound smoke marker that meet this criterion is shown in Figure 2. The graphs for all the other analytes can be found in the report by Schmidtke (2020).

Table 2. Summary of the statistical comparison of results of analysis of free and bound smoke markers in wines conducted by Vintessential and AWRI Commercial Services. Analytes with an R-squared value greater than 0.9 are shown in green as an arbitrary indication of a reasonable correlation between the results from the two laboratories. Analytes with an R-squared value below 0.9 are shown in red, indicating a less good correlation.

Comparison of results for free smoke markers in wine						
AWRI Commercial Services analysis code	**Vintessential analysis code**	**n**	**R^2**	**Intercept**	**Gradient**	**RMSE**
4.Methylguaiacol	4.MG	29	0.99	0.19	1.2	0.61
Guaiacol	Guaiacol	46	1	0.82	1.26	1.37
m.CRESOL	m.Cresol	37	0.94	0.09	0.95	1.36
Methyl.Syringol	4.MS	20	0.98	1.06	1.32	0.98
o.CRESOL	o.Cresol	46	0.99	-0.07	1.14	0.78
p.CRESOL	p.Cresol	31	0.98	0.58	0.89	0.43
Comparison of results for bound smoke markers in wine						
AWRI Commercial Services analysis code	**Vintessential analysis code**	**n**	**R^2**	**Intercept**	**Gradient**	**RMSE**
Cresol rutinoside	Total cresol	47	0.54	0.84	0.44	5.62
Guaiacol rutinoside	Guaiacol	49	0.55	10.23	1.17	17.2
Methylguaiacol rutinoside	4.MG	47	0.75	1.81	0.21	3.34
Methylsyringol gentiobioside	4.MS	38	0.88	13.11	4.31	31.7
Syringol gentiobioside	Syringol	50	0.97	23.55	1.22	37.4

Free volatile smoke markers in wine

All the wine free volatiles showed strong correlations above 94%, with only *m*-cresol being below 98%. The gradients for the volatile analytes varied between 0.89 and 1.32 compared to the ideal value of 1. Results for the analytes 4-methyl guaiacol, guaiacol, methyl syringol and *o*-cresol tended on average to be higher for AWRI Commercial Services while m-cresol and p-cresol tended on average to be higher for Vintessential. The intercepts for all volatile analytes were within the range of experimental error and suggest a limited offset between the two sets of results. The root mean square error (RMSE) was also seen to be within the range of expected analytical variation. Results for all five volatile smoke markers can, based on these results, be meaningfully compared between the two laboratories for wine, if some care is taken to account for the differences in absolute values that are demonstrated by the differences in gradient.

Bound smoke markers in wine

For the bound analytes in wine, only the syringol gentiobioside/syringol pair showed a correlation above 90%. The gradient for this combination was 1.22, which suggested that results from AWRI Commercial Services tended to be approximately 20% higher on average. The intercept and RMSE for this comparison were 24 and 37 respectively but given that the results ranged between 0 and more than 800 this is considered acceptable, but should be noted. The strong correlation suggests that the two different methods give equivalent information and within reason it should be possible to compare results between the two laboratories for this combination, with a degree of care.

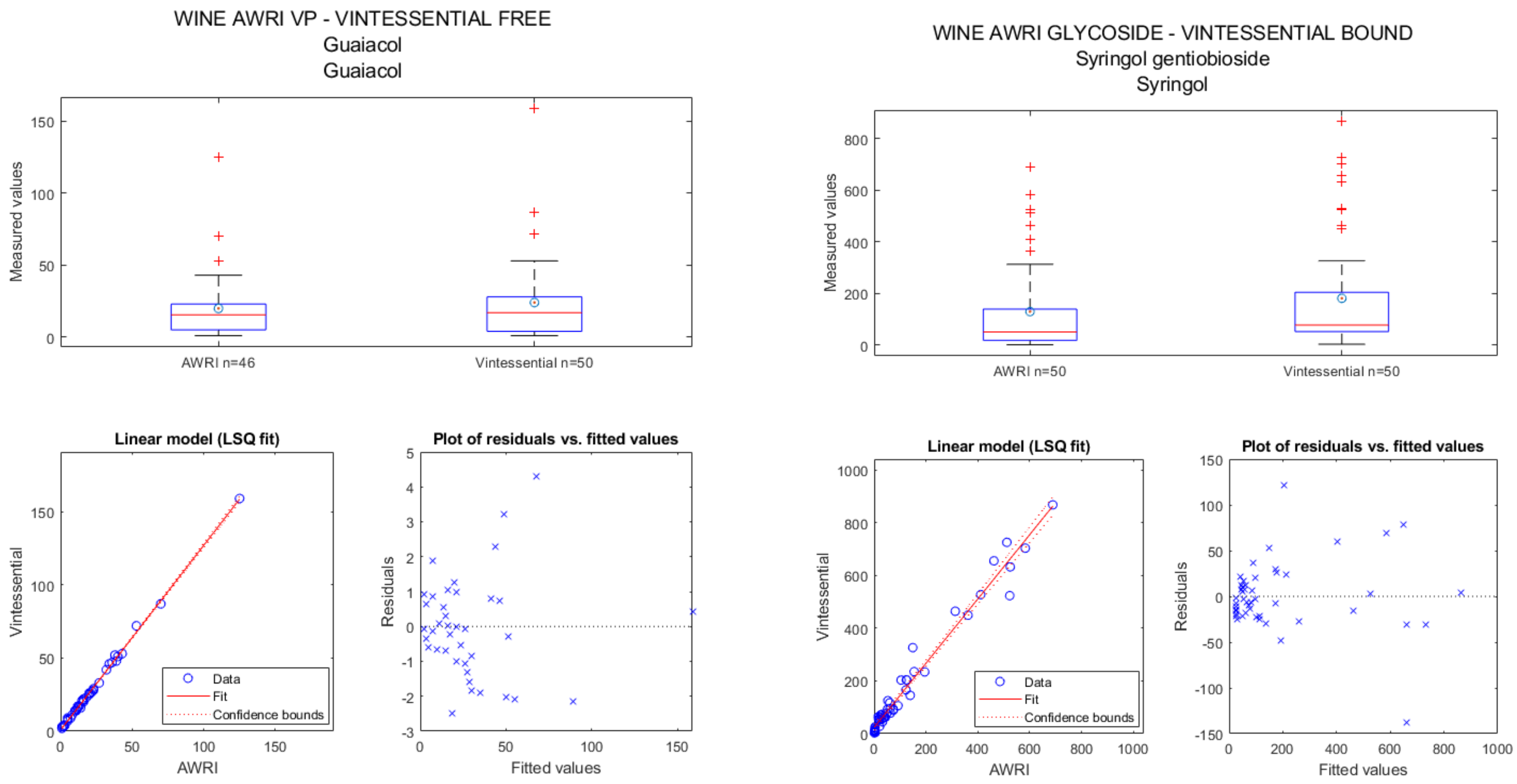
Weaker correlations were observed for methylsyringol gentiobioside/4 methylsyringol (88%) and methylguaiacol rutinoside/4 methylguaiacol (75%), and the gradients for the two sets were significantly far from the ideal value of 1 at 4.31 and 0.21 respectively, making direct comparisons between laboratories for these analytes questionable. The correlations for the other two analyte pairs were even weaker (54 and 55%) suggesting that direct comparison is not appropriate. Once again, given the differences in methods used, this lack of direct correlation should not be considered surprising. The two methods are not measuring identical things (i.e. the Vintessential method measures guaiacol released by hydrolysis from all bound guaiacol sources and the AWRI Commercial Services method measures guaiacol rutinoside specifically).

As discussed for the grape results, this lack of strong correlation for all the bound data, except for syringol gentiobioside/syringol, does not mean that either method provides inaccurate or inconsistent results, rather that they are measuring different things. For both laboratories, comparison of results for these bound analytes against internally generated background data is still valid, but comparisons between laboratories or their respective background datasets for non-smoke-affected fruit would not be valid. This can be seen in the graphs of bound analytes, where, even though the actual correlations are weak, the general trends between the two laboratories are consistent.

Summary for wine analysis

In summary, for wine analysis the direct comparison of results from the two laboratories is possible for all the volatile analytes, with care taken to recognise different trends in absolute values. For bound analytes in wine, there is a strong correlation between results from the two laboratories only for syringol gentiobioside/syringol and care must be taken in comparing absolute values for this analyte, as the correlations are not simply one to one. Comparison to an internal dataset (generated using the same method) is valid, which is important because research suggests this analyte is a sensitive marker for smoke exposure in Australia. No correlations were identified for the other bound analytes. As such, comparisons between results from the two laboratories for the other bound analytes in wine cannot be recommended.

Figure 2. Example graphs from the full statistics report (Schmidtke 2020) demonstrating the positive correlation between results from both laboratories for the free volatile smoke marker guaiacol and for the bound smoke marker syringol gentiobioside (AWRI Commercial Services) and bound syringol results (Vintessential) in wine. The graphs for all analytes are included in the full statistical report (Schmidtke 2020).



References and further reading

Hayasaka, Y., Parker, M., Baldock, G., Pardon, K., Black, C., Jeffery, D., Herderich, M. 2013. Assessing the impact of smoke exposure in grapes: development and validation of a HPLC-MS/MS method for the quantitative analysis of smoke-derived phenolic glycosides in grapes and wine. *J. Agric. Food Chem.* 61(1): 25-33.

Dungey, K., Hayasaka, Y., Wilkinson, K. 2011. Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. *Food Chem.* 126(2): 801-806.

Hayasaka, Y., Dungey, K., Baldock, G., Kennison, K., Wilkinson, K. 2010. Identification of a β-d-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke *Analyt. Chim. Acta* 660(1-2): 143-148.

Schmidtke, L., 2020. *AWRI and Vintessential Grape and Wine Analysis Smoke Method Comparison*. National Wine and Grape Industry Centre, Charles Sturt University. Available from: https://www.vintessential.com.au/smoke-taint-test/ or https://www.awri.com.au/wp-content/uploads/2020/11/AWRI-and-Vintessential-Grape-and-Wine-Analysis-method-comparison.pdf

Wilkinson, K., Ristic, R., Pinchbeck, K., Fudge, A., Singh, D., Pitt, K., Downey, M., Baldock, G., Hayasaka, Y., Parker, M., Herderich, M. 2011. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine. *Aust. J. Grape Wine Res.* 17(2): S22-S28.