Do varietal thiols matter in red wine?

The varietal thiols 3-mercaptohexanol (3-MH), 3-mercaptohexyl acetate (3-MHA) and 4-mercapto-4-methylpentan-2-one (4-MMP) are well known impact aroma compounds in Sauvignon Blanc and other white wines, giving 'tropical', 'box hedge', 'grapefruit' and 'passionfruit' aromas. These compounds have been well studied in white wines but their role in red wine aroma and flavour is not so clear.

Varietal thiols in wine are produced during fermentation. Initially, thiol-amino acid conjugates (odourless precursors of 3-MH and 4-MMP) form in the grapes, especially after crushing, following any skin contact or with *Botrytis* infection. Increasing the levels of nitrogen and sulfur in the vineyard can also lead to more 3-MH precursors forming in the grapes. During fermentation, yeast enzymes release 3-MH and 4-MMP from their conjugates, while 3-MHA is produced directly from 3-MH by yeast metabolism. Some yeast strains have been shown to be better at releasing 3-MH and 4-MMP than others, with some strains producing more 3-MHA. Many of the studies investigating precursor formation and the subsequent release of varietal thiols have been conducted with Sauvignon Blanc and have been summarised recently (Bandić et al. 2018, Jeffery 2016). There is good evidence that the thiols also can contribute strongly to rosé flavour.

In red wines, 3-MH and 3-MHA were first identified in Bordeaux blends in 1998 (Bouchilloux et al. 1998) and 4-MMP was first reported in Spanish red wine blends in 2004 (Culleré et al. 2004). However, only a limited number of studies over the past 20 years have measured varietal thiols in red wines, and it is not known whether they are important flavour compounds in red wines.

Table 1 gives some insight into the presence of varietal thiols in a few red wine varieties and red wine blends, summarising previously published data. From the measurements made, many red wines have concentrations of these thiols above the reported sensory aroma detection thresholds. However, the concentration needed to give a sensory effect is not clear. In addition, these compounds are notoriously difficult to quantify in wine because they are not very stable and are at trace levels in a complex matrix. Several very different analytical methods have been used to measure them in wine by different research groups. This situation makes it difficult to accurately compare the results between studies and varieties.

Survey in Australian wines

To better assess the contribution of these thiols to Australian red wines, they were measured in 105 commercially produced Australian wines of ten different varieties: Shiraz, Cabernet

Table 1. Varietal thiols measured in commercial or bottled experimental red wines.

Varieties	Number of wines	min – max (ng/L)			Analytical method	Reference
		3-MH	3-MHA	4-MMP		
Cabernet Sauvignon, Merlot and blends (France)	12	10–5,000	1–200	nq	Vacuum distilled -*p*HMB GC-MS	Bouchilloux et al. (1998)
Cabernet Sauvignon, Cabernet Franc, Merlot blends (France)	10	68–1,362	0–8.6	nq	LLE-*p*HMB SPE GC-MS	Murat et al. (2001)
Blends (Spain)	6	163–328	26–95	4–11	SPE *p*HMB-LLE GC-MS	Culleré et al. (2004)
Negrette (France)	5*	909–1,617	8–22	2–4	SIDA Deriv SPE SPME GC-NCI-MS	Rodríguez-Bencomo et al. (2009)
Carménère (Chile)	6	667 (mean)	373 (mean)	nq	*p*HMB-SPE LLE GC-MS	Domínguez and Agosin (2010)
Blends (France)	10	675–3,423 [11,487]	5–26 [153]	5–54	SIDA Deriv SPE SPME GC-NCI-MS	Rigou et al. (2014)
Cabernet Sauvignon, Cabernet Franc, Merlot blends (France)	24	100–634	nq	3–20	*p*HMB-SPE LLE GC-MS	Picard et al. (2015)
	22	50–525	nq	nq		
Pinot Noir (Australia)	34	250–1,250	0–16	0–16	SIDA Deriv SPE LC-MS/MS	Capone et al. (2015)
Cabernet Sauvignon (USA)	20	300–1,161	39–91	< LOD	SIDA Deriv LLE SPME GC-MS	Musumeci et al. (2015)
Carménère (Chile)	2*	422–760	8–22	nq	SIDA LLE *p*HMB-SPE SAFE GC-MS	Pavez et al. (2016)
Gamay (France)	1	190	16	nq	SIDA Deriv SPE nano-LC-MS/MS	Roland et al. (2016)
Shiraz (South Africa)	16	76–363	5–8	0–3	Deriv SPE UPC²-MS/MS	Mafata et al. (2018)
Cabernet Sauvignon (South Africa)	16	77–147	23–24	3–3		
Pinotage (South Africa)	16	127–311	7–12	0–2		
Sensory detection threshold (in model wine)		60	4	0.8		Tominaga et al. (2000)

* Bottled experimental wines; nq, not quantified; < LOD, below limit of detection; [ ], outlier; *p*HMB, *p*-hydroxymercuribenzoic acid; LLE, liquid-liquid extraction; SPE, solid-phase extraction; Deriv, derivatisation; SIDA, stable isotope dilution assay; UPC², ultra-performance convergence chromatography

Sauvignon, Grenache, Merlot, Pinot Noir, Malbec, Durif, Tempranillo, Mataro and Petit Verdot, using a validated and accurate analytical method (Capone et al. 2015). The wines were selected based on percentage of Australian production and retail sales data across multiple price points and regions, and included a minimum of three wines per variety. They ranged in retail value from AUD $4 to $76 and were purchased from several wine retail outlets or directly from wineries. The wines were mostly one to four years old, with six wines five to six years old and one older wine (11 years). Alcohol content ranged from 12.5 to 16.0% (v/v).

In this survey, surprisingly, all wines had a higher concentration of 3-MH than the sensory detection threshold measured in model wine (Figure 1), indicating the possible contribution of this compound to red wine flavour. Neither 3-MHA nor 4-MMP were detected in any of the wines. Pinot Noir wines were found to have a wide concentration range and the highest concentrations of 3-MH. Grenache wines were the next highest, although only three Grenache wines were measured. As Cabernet Sauvignon is the offspring of Sauvignon Blanc and Cabernet Franc, it was thought that the Cabernet Sauvignon wines might contain high levels of the varietal thiols. A few Cabernet Sauvignon wines had higher levels of 3-MH, but otherwise the levels were similar to Shiraz. One Mataro wine had a much higher concentration of 3-MH than the other two, while one Shiraz was exceptionally high at 1,500 ng/L of 3-MH (not shown on Figure 1). No significant links were found between 3-MH concentration in the wines and regional variables, for example mean January temperature, or other parameters, such as price or ethanol content. However, most of the Pinot Noir wines were from cooler climate regions.

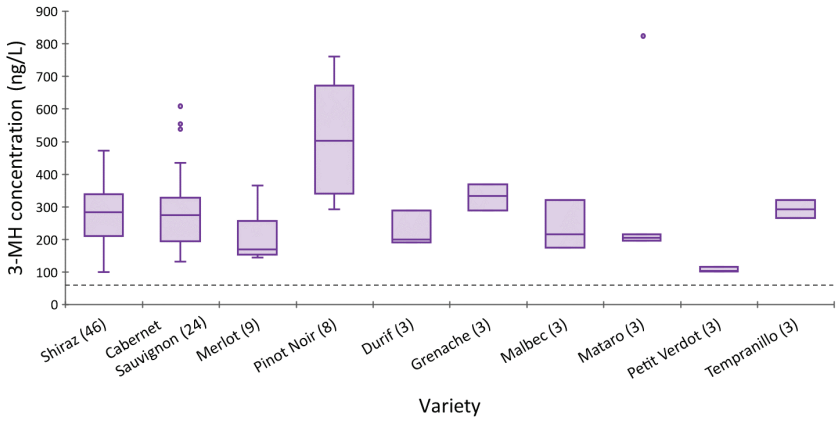

Figure 1. Concentrations (ng/L) of the thiol compound 3-MH for a set of 105 commercially available single variety red wines. Box plots show median (horizontal line in box), upper and lower quartile, minimum and maximum value (vertical bars) and outliers (small circles); the values in parentheses indicate the number of wines analysed. The sensory detection threshold of 3-MH in aqueous ethanol (60 ng/L) is shown as the dashed line.

From the results of this survey, it is clear that 3-MH is found in red wines, but at much lower levels than that often found in Sauvignon Blanc wines (1,530–7,080 ng/L) (Jeffery 2016). White wines with high 3-MH levels are often described as having a 'passionfruit' or 'grapefruit' aroma, but does this compound do anything for red wine aroma?

Do varietal thiols contribute to the 'red fruit' character of red wines?

Higher intensity 'red fruit' characters are often desirable in red wines, especially in some styles of Pinot Noir and Grenache. Last year, Grenache wines from an AWRI yeast strain study were assessed by a descriptive sensory analysis panel (Cordente et al. 2018). One of the yeast strains gave Grenache wines that were rated significantly higher in 'red fruit' aroma, and these wines also had higher concentrations of 3-MH and 3-MHA (700 and 20 ng/L, respectively) than wines made with the other strains (mean 3-MH = 380 ng/L).

To investigate the possible contribution of 3-MH and 3-MHA to 'red fruit' aroma, base red wines were spiked with increasing amounts of 3-MH with or without 3-MHA added. Four varieties were chosen for this preliminary study: Pinot Noir because of higher concentrations of 3-MH found in the survey; Grenache due to the interesting increase in 'red fruit' aroma seen in the yeast study; Cabernet Sauvignon as it is genetically related to Sauvignon Blanc; and Shiraz since it is Australia's major red wine variety. The base wines contained very low concentrations of naturally present 3-MH and 3-MHA. The wines were spiked at levels from 500 up to 1,500 ng/L of 3-MH and 3-MHA at 0 or 20 ng/L (Figure 2). The compound 3-MHA was not spiked into the Shiraz because it was not present in any of the Shiraz wines in the survey, even in the Shiraz wine with the very high concentration of 3-MH (1,500 ng/L). The aroma of the base wines and spiked wines was assessed by an experienced sensory panel (n = 9).

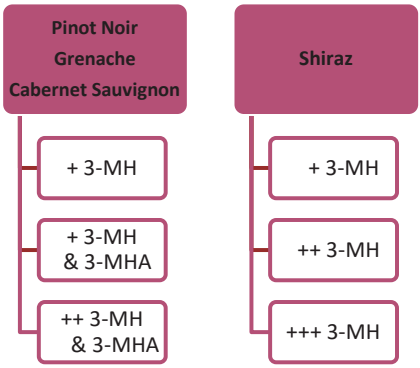

Figure 2. Spiking protocol of the thiol compounds 3-MH and 3-MHA into four base red wines. For 3-MH, each + represents an addition of 500 ng/L. For 3-MHA, & represents an addition of 20 ng/L.

Aroma differences across the spiking levels were seen by most panellists. Little difference was evident compared to the base wines when only 3-MH was added at the lowest level. More intense aromas of 'red fruit' and 'lolly' were noted when both 3-MH and 3-MHA were added to the Pinot Noir and Grenache, which then changed to 'tropical' for the highest spiked level. For Cabernet Sauvignon, 'blackcurrant' and 'tropical' characters were indicated. 'Sweaty' and 'tropical' descriptors were given for the Shiraz wines with the higher spiked levels of 3-MH (1,000 and 1,500 ng/L).

To confirm these preliminary findings of enhanced 'red fruit' aroma with moderately high concentrations of 3-MH and 3-MHA, a full descriptive sensory study with spiked Pinot Noir and Grenache wines is planned in the coming year. If the results are confirmed, then further work could investigate the thiol precursors in Pinot Noir and Grenache grapes and must as well as yeast strain comparisons, using the lessons already learnt from research with Sauvignon Blanc.

Furthermore, in a more recent AWRI study, foliar applications of nitrogen (N, urea) and sulfur (S, wettable-sulfur) to Shiraz vines significantly enhanced 'tropical' flavours and aromas in the resulting Shiraz wines. Even with the lower application rate (10 kg/na N and 5 kg/ha S), the concentration of 3-MH increased five-fold, to 1,500 ng/L, and 3-MHA increased two-fold, to 16 ng/L (Solomon et al. 2019). This result in Shiraz shows the potential of using foliar applications of nitrogen and sulfur on other red grape varieties, for example Pinot Noir and Grenache, as another option for grapegrowers and winemakers to tailor wine style.

Summary

A survey found that the varietal thiol compound 3-MH is present in Australia's most commercially important red wine varieties at concentrations well above its aroma detection threshold. The thiol was found in all the red wines tested, with the highest median concentration and widest range found in Pinot Noir wines. A spiking sensory study showed that a moderately high concentration of 3-MH (700 ng/L) and 3-MHA (20 ng/L) increased the 'red fruit' character in Pinot Noir and Grenache wines, without giving less desirable 'tropical fruit' or 'sweaty' aromas. Further sensory work is planned to confirm these interesting results.

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