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Preliminary investigation of potent thiols in Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi

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ABSTRACT

Polyfunctional thiols have previously been shown to be key aroma compounds in Sauvignon blanc and more recently in Chardonnay wines. Their role in other wine varieties such as those made from three popular indigenous Cypriot grape varieties has remained unexplored. As an extension of a previous project that profiled the sensory and chemical characteristics of Cypriot wines and their comparison to Australian wines, this study aimed to investigate five potent thiols in Xynisteri, Maratheftiko, Giannoudhi, Pinot gris, Chardonnay and Shiraz wines.

Wines were analysed utilising Stable Isotope Dilution Assay (SIDA) with derivatisation and High-Performance Liquid Chromatography–Tandem Mass Spectrometry (HPLC-MS/MS). The varietal thiols measured were 4-methyl-4-sulfanylpentan-2-one (4MSP) that has an aroma of “boxwood” and “cat urine” at high concentration, 3-sulfanylhexas-1-ol (3SH) which has been described as having a “grapefruit/tropical fruit” aroma, and 3-sulfanylhexasyl acetate (3SHA) that has also been described as having an aroma of “passionfruit”. Additionally, two other potent thiols were measured including benzyl mercaptan (BM) that has an aroma of “smoke and meat” and furfuryl thiol (FFT) that has been described as having a “roasted coffee” like aroma. The reason these thiols are known as potent thiols are due to their very low aroma detection thresholds in the low ng/L (ppt) range. Of the thiols that were measured, 3SH was the only varietal thiol detected in the red wine samples. All of the white wine samples contained 3SH, BM and 3SHA, whereas 4MSP was only detected in Pinot gris and three Xynisteri wines. The potent thiol, FFT, was detected only in the Chardonnay and four of the Xynisteri wines. Interestingly the thiols that were present in the samples were found at concentrations above their aroma detection thresholds (determined in hydroalcoholic solutions), especially 3SH which was found in an order of magnitude above its aroma detection threshold. These findings provide early knowledge of the presence of these thiols in Cypriot wines, compared with Australian wines and establish any relationships between this chemical data with previous wine sensory profile data.

KEYWORDS

varietal thiols, sensory analysis, chemical analysis, consumer preference, Xynisteri, Maratheftiko, Giannoudhi

INTRODUCTION

The indigenous grape varieties of Cyprus have become the focus of increasing interest in recent times, with several studies investigating the chemical profiles of their juice, must and wines (Copper *et al.*, 2019; Constantinou *et al.*, 2019; Galanakis *et al.*, 2015; Tsiakkas *et al.*, 2020; Kokkinofita *et al.*, 2014). These studies have mainly reported on the phenolic compounds, volatile compounds and sensory characteristics. Varietal thiols are one such group of aroma compounds that have not been examined in wines made from Cypriot grape varieties to date. One possible reason for this is that polyfunctional thiols have a low sensory threshold and are present at trace concentrations (nanograms per litre). They are missed in conventional wine analysis as it is almost impossible for them to be detected directly with Gas Chromatography/Mass Spectrometry (GC/MS) and require specialised methods (Capone *et al.*, 2018). The polyfunctional thiols 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) are one such group of compounds. More recently, Capone *et al.* (2015) have developed a simplified method whereby the free thiols, including benzyl mercaptan (BM) and (furan-2-yl)-methanethiol also referred to as 2-furfurylthiol (FFT), are derivatised and then analysed using HPLC-MS/MS.

These compounds are potent with extremely low detection thresholds and have been described as possessing aromas of “boxwood”, “cat urine”, “passionfruit”, “grapefruit”, “tropical fruit”,

“smoke” and “roasted coffee” (Dubourdieu and Tominaga, 2009; Table 1). They have been shown to be important aroma compounds in Chardonnay, Sauvignon blanc, Chenin blanc, Grechetto, Pinot noir, Pinot gris, Riesling, Gewürztraminer, Sylvaner and some French red wine blends (Capone *et al.*, 2015; Capone *et al.*, 2018; Coetzee *et al.*, 2018; Maslov-Bandić *et al.*, 2018; Cerreti *et al.*, 2017; Rigou *et al.*, 2014). Thiols have also been identified in indigenous Italian varieties Trebbiano di Lugana (Mattivi *et al.*, 2012) as well as Catarratto Bianco Comune and Grillo (Fracassetti *et al.*, 2018).

King *et al.* (2011) reported that not only did all three thiols (3-SH, 3-SHA and 4-MSP) contribute to the “tropical” and “cat urine/sweaty” aroma in wine, in combination with 3-isobutyl-2-methoxypyrazine (IBMP), they can contribute to a “cooked green vegetal” aroma in Australian Sauvignon blanc wines.

Interestingly, Australian consumers preferred wines containing a combination of varietal thiols and IBMP (a cluster containing 43 %) compared with wines possessing higher “tropical” and “confectionery” aromas (a cluster containing 31 %) (King *et al.*, 2011). Given Australian consumer’s liking of “tropical” and “confectionery” characters (Copper *et al.*, 2019) and that they are important in other varieties, their contribution in Cypriot varieties remains unexplored. Therefore, this study aimed to assess the thiol composition of the indigenous Cypriot varieties, which in the future could allow producers to tailor their products to suit consumer preferences.

TABLE 1. Polyfunctional thiols in wine, their characteristic aromas and their aroma detection thresholds (determined in hydroalcoholic or model solutions).

Thiols	4MSP	3SHA	3SH	BM	FFT
Sensory descriptor	Boxwood, blackcurrant	Passionfruit, tropical, boxwood	Grapefruit, tropical, passionfruit	Smoke, toasty, struck flint	Roasted Coffee
Odour detection threshold (ng/L)	0.8	4	60	0.3	0.4
Concentration range in commercial Chardonnay wines (ng/L)	0 ^a –23 ^b	0 ^a –100 ^b	10 ^b –1368 ^a	30–40 ^c	14 ^c
Concentration range in commercial Sauvignon Blanc wines (ng/L)	0–88 ^d	0–106 ^d	350–5664 ^d	0.6–5.5 ^d	1–36 ^d

a: Capone *et al.* (2018); b: Mateo-Vivaracho *et al.* (2010); c: Gambetta *et al.* (2014); and d: Capone *et al.* (2015). 4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol; 3SH: 3-sulfanylhexan-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

MATERIALS AND METHODS

1. Wine samples

The wines used in this study were previously reported Copper *et al.* (2019). They included 2016 Xynisteri (n = 5), 2015 Xynisteri (n = 1), 2017 Australian Pinot gris (n = 1) and 2017 Australian Chardonnay (n = 1). The red wines 2015 Maratheftiko (n = 2), 2013 Maratheftiko (n = 1), 2014 Cypriot Giannoudhi (n = 1) and 2014 Australian Shiraz (n = 1). The wines were chosen as they were common brands, readily available at wine retailers and were spread across a range of price points (5-20 € for Cypriot wines. \$20-\$25AUD for Australian wines). Further detailed information on the wines are provided in Table 2.

2. Chemicals and materials

Analytical reagents were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) unless otherwise specified. Unlabelled and deuterium-labelled standards were synthesised as previously described in Capone *et al.* (2015). This included: 3-SH, [²H₁₀]-3-sulfanylhexan-1-ol (d₁₀-3-SH), 3-SHA, [²H₅]-3-sulfanylhexyl acetate (d₅-3-SHA), 4-MSP, [²H₁₀]-4-methyl-4-sulfanylpentan-2-one (d₁₀-4-MSP) (Howell *et al.*, 2004; Swiegers *et al.*, 2007; Pardon *et al.*, 2008), [²H₅]-2-furfurylthiol (d₅-FFT) and [²H₅]-benzyl mercaptan (d₅-BM) (Capone *et al.*, 2015). Bond Elut C18 (500 mg, 6 mL) solid-phase extraction (SPE) cartridges were purchased from Agilent Technologies (Mulgrave, Vic, Australia).

3. Chemical analysis of wine thiols

Samples were prepared as described by Capone *et al.* (2015), an aliquot of wine (20 mL) was added into a 22 mL glass screw cap vial with a Teflon lined cap, labelled standards, d₁₀-4-MSP, d₁₀-3-SH, d₅-3-SHA, d₅-FFT and d₅-BM, each with a final concentration of 500 ng/L was added. An addition of ethylenediaminetetraacetic acid disodium salt (20 mg), 50 % acetaldehyde (80 µL) and freshly thawed 4,4'-dithiodipyridine reagent (10 mmol, 200 µL) proceeded. After 30 min, the sample was passed through a pre-conditioned Bond Elut C18 cartridge (6 mL, 500 mg, Agilent Technologies, Forest Hill, Vic., Australia) as previously detailed. The eluate was collected, concentrated and reconstituted with 10 % ethanol (200 µL) and analysed on an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) using a 250 × 2.1 mm i.d., 5 µm, 100-Å Alltima® C18 column (Grace Davison Discovery Sciences, Rowville, Vic., Australia) coupled to an Agilent 6410A Triple Quad MS (Agilent, Santa Clara, CA, USA) in electrospray ionisation mode as described in Capone *et al.* (2015). Data acquisition was performed using Agilent Mass Hunter Workstation software (version 10.0). For accurate quantification, duplicate standards in the appropriate wine matrix were prepared at the time of analysis with 4-MSP at concentrations of 0, 11.5, 38, 50, 75 and 100 ng/L; 3-SH at 0, 620, 1824, 2400, 3650 and 5000 ng/L; 3-SHA at 0, 120, 380, 500, 775 and 1025 ng/L; FFT and BM at 0, 12.5, 37.5, 50, 200 and 400 ng/L.

TABLE 2. Basic wine chemical data, oak treatment use and other information of wines used in thiol analysis.

Code	Vintage	Wine	pH	TA (mg/L)	Alc (%)	Oak
M1	2015	Maratheftiko	3.43	5.86	14.8	Yes
M2	2013	Maratheftiko	3.62	5.45	13.2	Yes
M3	2015	Maratheftiko	3.44	5.88	14.5	Yes
SH	2014	Shiraz	3.57	6.13	14.5	Yes
Yia	2014	Giannoudhi	3.65	5.5	13.4	Yes
CH	2017	Chardonnay	3.33	7.35	12.9	No
PG	2017	Pinot Gris	3.54	6.65	12.5	No
X1	2016	Xynisteri	3.21	5.93	12.8	No
X2	2015	Xynisteri	3.26	5.94	12.8	Yes
X3	2016	Xynisteri	3.22	5.52	13.7	No
X4	2016	Xynisteri	3.35	5.44	12.8	No
X5	2016	Xynisteri	3.16	4.72	12.6	No
X6	2016	Xynisteri	3.42	5.02	12.6	No

X4-contained 5 % Muscat in addition to Xynisteri. Table adapted from Copper *et al.* (2019).

Duplicate quality control checks were also prepared with 4-MSP at concentrations of 0, 25, 50, 75 and 100 ng/L; 3-SH at 0, 1200, 2400 and 5000 ng/L; 3-SHA at 0, 250, 500 and 1025 ng/L; FFT and BM at 0, 25, 50 and 400 ng/L.

4. Sensory analysis

The Rate-All-That-Apply (RATA) technique was utilised for descriptive sensory profiling of the wines. RATA is a rapid sensory profiling method used to describe the intensity of only the sensory characteristics actually perceived in wines employing a rating scale (Danner *et al.*, 2017). Wines were presented sequentially, monadically, blind and in a random order to the tasters to overcome serving order effects. Wines were served in clear International Standards Organisation (ISO) tasting glasses at 15 °C. Tasters were required to select only the attributes that were applicable to the wine and additionally indicate the perceived intensity of these sensory attributes using a seven-point rating scale as previously reported by Copper *et al.* (2019).

5. Statistical analysis

Basic chemical data were processed with Microsoft Excel 2010. Chemical data are presented as mean values with standard deviation from duplicate determinations and analysed by one-way ANOVA (sample) using XLSTAT (version 2018.7, Addinsoft SARL, Paris, France). The significantly different means of the chemical and sensory data were subjected to partial least squares (PLS) regression using The Unscrambler (version 10.5.1, CAMO Software AS, Oslo, Norway) with chemical parameters (X-variables) and sensory data from Rate-All-That-Apply (RATA) (Y-variables). All variables were normalised before analysis and significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The only thiol detected in red wines was 3SH. Maratheftiko (M3) had a significantly higher concentration than the other two Maratheftiko wines (M1 and M2) and was similar in concentration to Shiraz (SH) and Giannoudhi (Yia) (Table 3). The levels of 3SH ranged from 164–172.8 ng/L, which was lower than that detected by Rigou *et al.* (2014) in French red wine blends (Syrah, Grenache, Mourvedre, Cinsault, Carignana, Grenache noir) which ranged from 678–11,487 ng/L. Regardless of this, the concentration of 3SH measured in the samples was above the aroma detection threshold (60 ng/L in hydroalcoholic solution), meaning this thiol could

contribute to “blackcurrant” aroma as described by Rigou *et al.* (2014) or as “berry”, “jam”, “earthy”, “plum” and “soy” aromas as recently reported by Garrido-Bañuelos *et al.* (2020). Although these compounds were identified in Bordeaux red wines several years ago, there is limited quantitative and sensory data available for red varieties, most likely due to previous difficulties in measuring these compounds at threshold concentrations (Capone *et al.*, 2015). Recently, Mafata *et al.* (2018) quantified 4 thiols (3SH, 3SHA, 4MSP and FFT) using ultraperformance convergence chromatography-tandem mass spectrometry in South African Shiraz, Cabernet-Sauvignon and Pinotage, the reported 3SH levels for all three varieties ranged from 76–363 ng/L.

TABLE 3. Concentration of thiols in red and white wine samples (ng/L).

Code	4MSP	3SHA	3SH	BM	FFT
M1	nd	nd	165b	nd	nd
M2	nd	nd	164b	nd	nd
M3	nd	nd	172a	nd	nd
SH	nd	nd	167ab	nd	nd
Yia	nd	nd	167ab	nd	nd
	nd ≤ 1	nd ≤ 3		nd ≤ 2	nd ≤ 3
CH	nd	47b	486d	9.9a	3.7c
PG	3.6cd	54a	663a	7.7b	nd
X1	nd	39d	642b	6.7cd	nd
X2	nd	39d	444f	7.1c	12.9a
X3	3.9bc	39d	426g	8b	nd
X4	5.8a	40d	546c	5.7e	4c
X5	4.2b	39d	407h	6e	5.1b
X6	3.5d	42c	464e	6.5d	3.4c
	nd ≤ 3.4				nd ≤ 3

Different letters next to the concentration of each compound indicate significant differences ($p < 0.05$), between white or red wines.

Concentrations containing the same letters are not statistically significantly different. nd — not detected. M1 = 2015 Maratheftiko; M2 = 2013 Maratheftiko; M3 = 2015 Maratheftiko; SH = 2014 Shiraz; Yia = 2014 Giannoudhi; CH = 2017 Chardonnay; PG=2017 Pinot gris; X1 = 2016 Xynisteri; X2 = 2015 Xynisteri; X3, X4, X5, X6 = 2016 Xynisteri.

4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol, 3SH: 3-sulfanylhexas-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

In the white wine samples, 4MSP was only detected in Pinot gris (PG) and four Xynisteri wines. Xynisteri (X3, X4, X5) had the highest levels, Pinot gris had the second-lowest levels with Xynisteri (X6) the lowest, ranging from 3.5–5.8 ng/L. These were all above the detection threshold of 0.8 ng/L (in hydroalcoholic solution) and above the 1 ng/L threshold (determined in a dearomatised white wine, with added flavour compounds), reported by Mateo-Vivaracho *et al.* (2010) required to contribute to “green” and “fruity” aromas.

The highest level of 3SHA was detected in Pinot gris followed by Chardonnay (CH), whereas all Xynisteri were present at lower concentrations. 3SHA ranged from 38.6–53.7 ng/L which was above the detection threshold and consistent with levels seen by Coetzee *et al.* (2018) in South African Sauvignon blanc wines, which ranged from 23–151 ng/L.

3SH was highest in Pinot gris followed by Xynisteri (X1) and ranged from 426.7–663.7 ng/L and were both above the detection threshold. This range was similar to those reported by Coetzee *et al.* (2018) in South African Sauvignon blanc.

BM was highest in Chardonnay followed by Xynisteri (X3) then Pinot gris, which ranged from 5.7–9.9 ng/L this concurred with Capone *et al.* (2015) who reported that Chardonnay and Pinot gris had higher levels when compared to some sparkling wines and may contribute to “smoky”, “struck flint” characteristics. Capone *et al.* (2015) also suggested that BM may be a varietal character of importance to Pinot gris. Consequently, BM has the potential to be important in Xynisteri wines as well and warrants further investigation.

FFT was only detected in Chardonnay and four Xynisteri wines and ranged from 3.4–12.9 ng/L. The highest level was in Xynisteri (X2) and may be attributable to its fermentation and ageing in oak barrique. Capone *et al.* (2015) reported that Chardonnay wines aged in oak also had higher levels of FFT and attributed this to it being a Maillard reaction product and associated with exposure to toasted oak. Interestingly, no FFT was detected in any of the red wine samples despite being aged in oak, possibly due to the binding of melanoidin and FFT as has been shown to occur in coffee (Hofmann and Schieberle, 2002).

Volatile composition, basic wine chemical parameters and sensory data from the previous

work by Copper *et al.* (2019) were re-analysed with thiol data included. PLS regression was utilised to explore the underlying relationship and determine if the model was improved with the inclusion of the thiol data. In the red wine samples, the first two factors explained 75 % of the variation in wine composition (x-variables) and 63 % of the variation in sensory properties (Y-variables), Figure 1a and b. The Maratheftiko sample M3 had the highest concentration of 3SH and was correlated with the attributes of smooth mouthfeel, fruity after taste, jammy aroma and jammy palate, which are similar to the blackcurrant aromas descriptor reported by Rigou *et al.* (2014).

In the white wines, the first two factors explained 58 % of the variation in the chemical composition (x-variables) and 61 % of the variation in sensory properties (y-variables), Figure 2a and b. However, utilising three factors explained an additional 12 % of the sensory variation (data not shown), now explaining 73 % of the sensory difference. Including the additional thiols slightly improved the previous model reported by Copper *et al.* (2019) from 60 % (x-variables) and 62 % (y-variables). Xynisteri (X2) correlated with FFT and “bread”, “wood”, “toasty” and “buttery” characteristics and these associations can be further demonstrated in Figure 3, by the regression coefficients for “bread” and “wood” aroma and flavour as well as “butter” aroma at or very close to 0.1. The remaining Xynisteri wines (X1, X3, X4, X5, X6) correlated with 4MSP aroma and had more astringent and bitter characteristics. Pinot gris and Chardonnay correlated with BM, 3SHA and “stone fruit”, “sweet”, “confectionery”, “tropical”, “floral”, “herbaceous”, “citrus”, “apple” and “pear” characteristics. 3SH in the lower right quadrant did not correlate with any of the wines, but correlated with “grassy”, “floral”, “apple/pear”, “confectionery” and “citrus” aromas. Regression coefficients of “grassy”, “citrus” and “herbal” aroma showed a strong association with 3SH (Figure 4a, 4c, 4d, respectively), whereas “tropical” aroma was strongly associated with 3SHA (Figure 4b).

The thiols that were detected in all the Cypriot white wines were above threshold levels; however, the Australian Pinot gris and Chardonnay had better correlation with the desirable thiol 3SHA and its “passionfruit/tropical” characteristics. One explanation for this could be the age difference of the wines. Apart from Xynisteri (X2), which was aged in oak for 6 months, the remaining Xynisteri wines were at least six months older

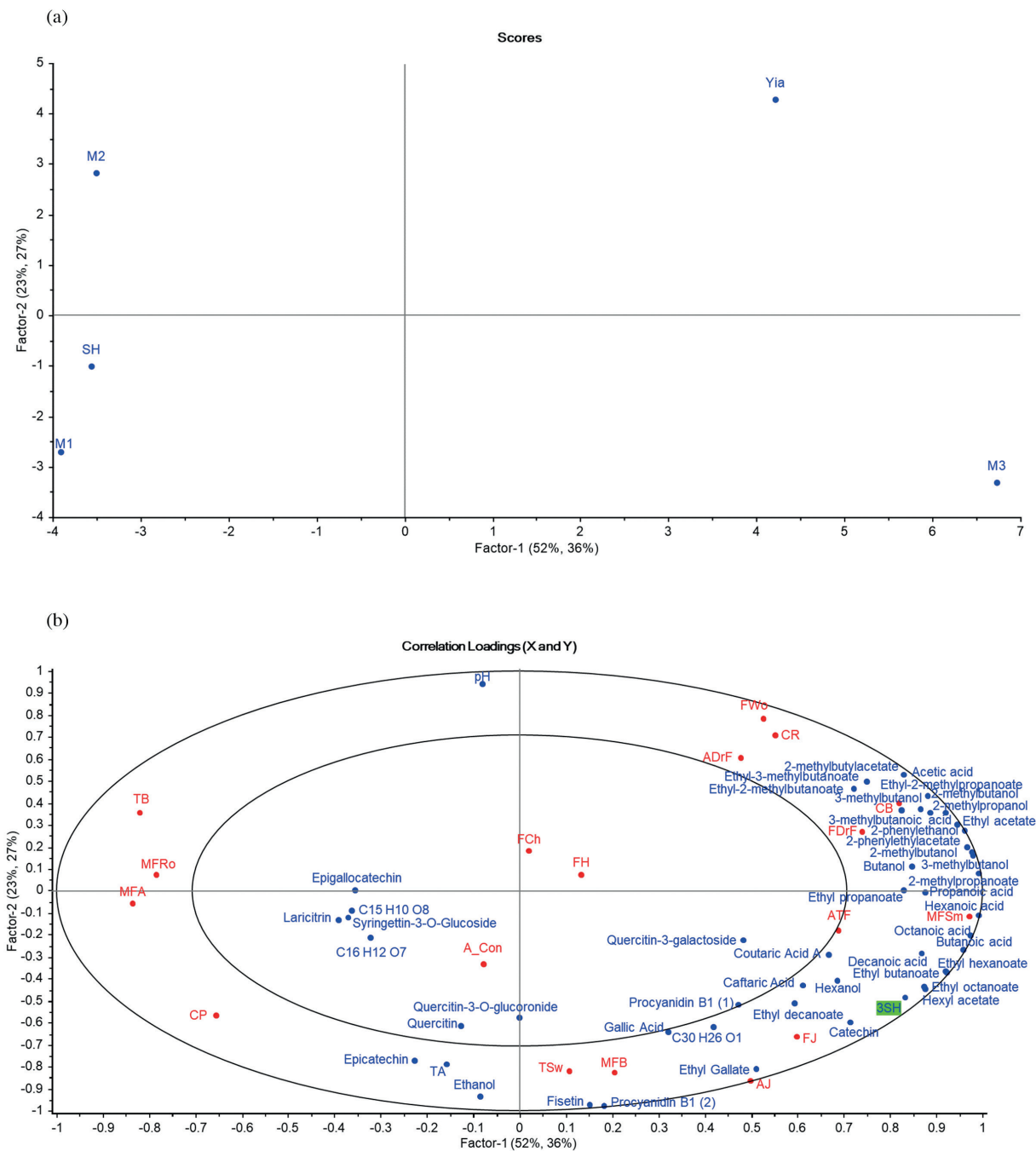


FIGURE 1. Adapted from Copper *et al.* (2019). (a) PLS Regression plots of standardised volatile aroma and thiol compounds (green) in red wines. (b) Correlation loadings between chemical (blue) and sensory (red) data 50 % (inner), 100 % (outer) explained variance limits.

Chemical compounds (Blue), Sensory attributes (Red). Colour Red (CR), Colour Purple (CP), Colour Brown (CB), Aroma Dried Fruit (ADrF), Aroma Jammy (AJ), Aroma Confectionery (ACon), Taste Bitter (TB), Taste Sweet (TSw), Flavour Dried Fruit (FDrF), Flavour Jammy (FJ), Flavour Chocolate (FCh), Flavour Herbal (FH), Flavour Wood (FWo), Mouth Feel Bitter (MFB), Mouth Feel Astringent (MFA), Mouth Feel Smooth (MFSm), Mouth Feel Rough (MFRo), After Taste Fruit (ATF). 3SH: 3-sulfanylhexan-1-ol. Maratheftiko (M1, M2, M3), Giannoudhi (Yia), Shiraz (SH).

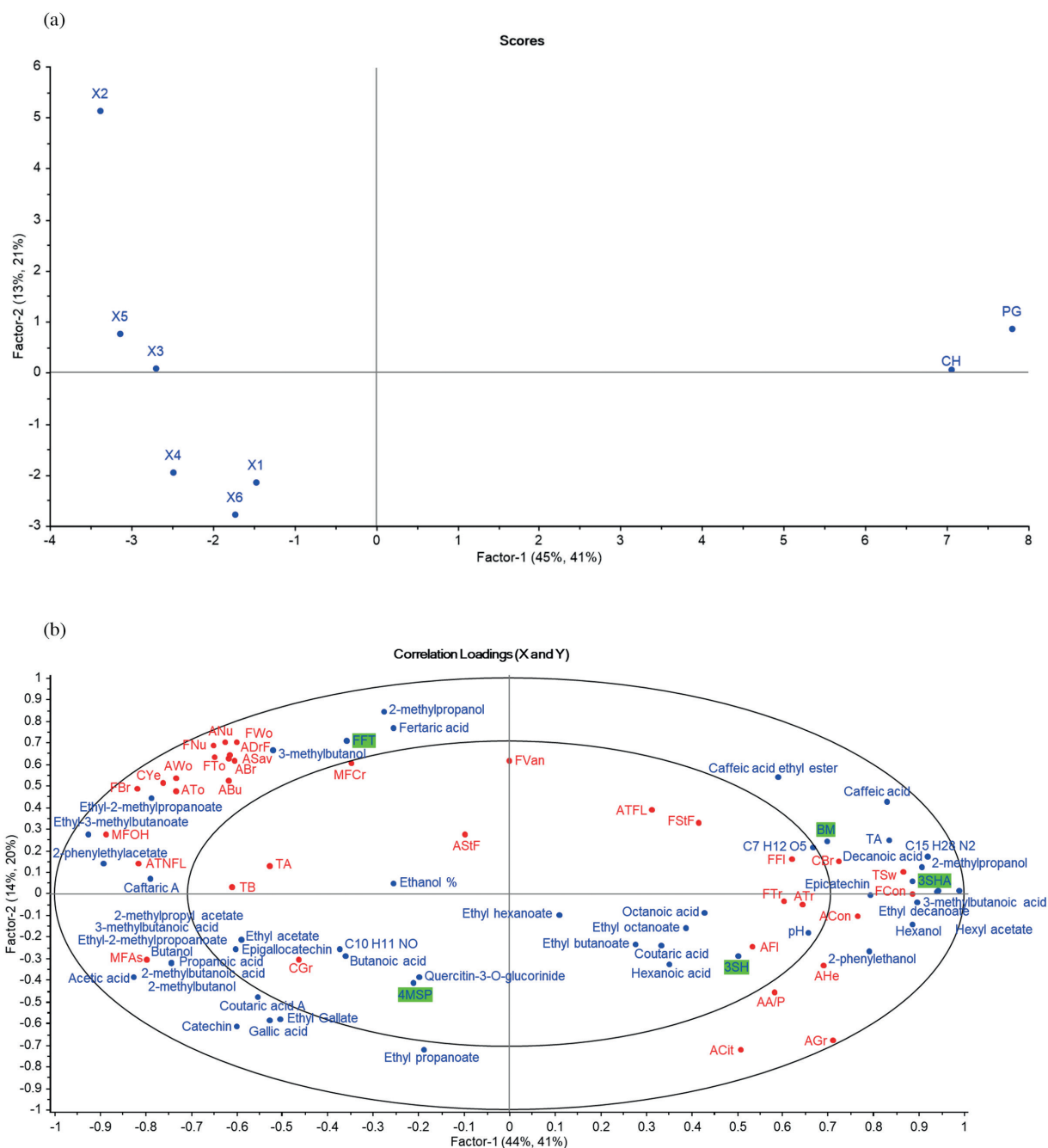


FIGURE 2. Adapted from Copper et al. (2019). (a) PLS Regression plots of standardised volatile aroma and thiol compounds (green) in white wines. (b) Correlation loadings between chemical (blue) and sensory (red) data, 50 % (inner), 100 % (outer) explained variance limits.

Colour Brown (CB), Colour Green (CGr), Colour Yellow (CYe), Aroma Apple Pear (AA/P), Aroma Citrus (ACit), Aroma Dried Fruit (ADrF), Aroma Stone Fruit (AStF), Aroma Confectionery (ACon), Aroma Tropical (ATr), Aroma Floral (AFI), Aroma Grass (AGr), Aroma Herbal (AHe), Aroma Butter (Abu), Aroma Nutty (ANu), Aroma Savoury (ASav), Aroma Toast (ATo), Aroma Wood (AWo), Aroma Bread (ABr), Taste Bitter (TB), Taste Sweet (TSw), Taste Acid (TA), Flavour Stone Fruit (FStF), Flavour Confectionery (FCon), Flavour Tropical (FTr), Flavour Floral (FFI), Flavour Nutty (FNu), Flavour Toast (FTo), Flavour Wood (FWo), Flavour Vanilla (FVan), Flavour Bread (FBr), Mouth Feel Alcohol (MFOH), Mouth Feel Astringent (MFAs), Mouth Feel Creamy (MFCr), After Taste Fruit Length (ATFL), After Taste Non-Fruit Length (ATNFL). 4MSP: 4-methyl-4-sulfanylpentan-2-one, FFT: furfuryl thiol, 3SH: 3-sulfanylhexasan-1-ol, BM: benzyl mercaptan, 3SHA: 3-sulfanylhexasyl acetate.

Xynisteri (X1, X2, X3, X4, X5, X6), Pinot gris (PG), Chardonnay (CH)

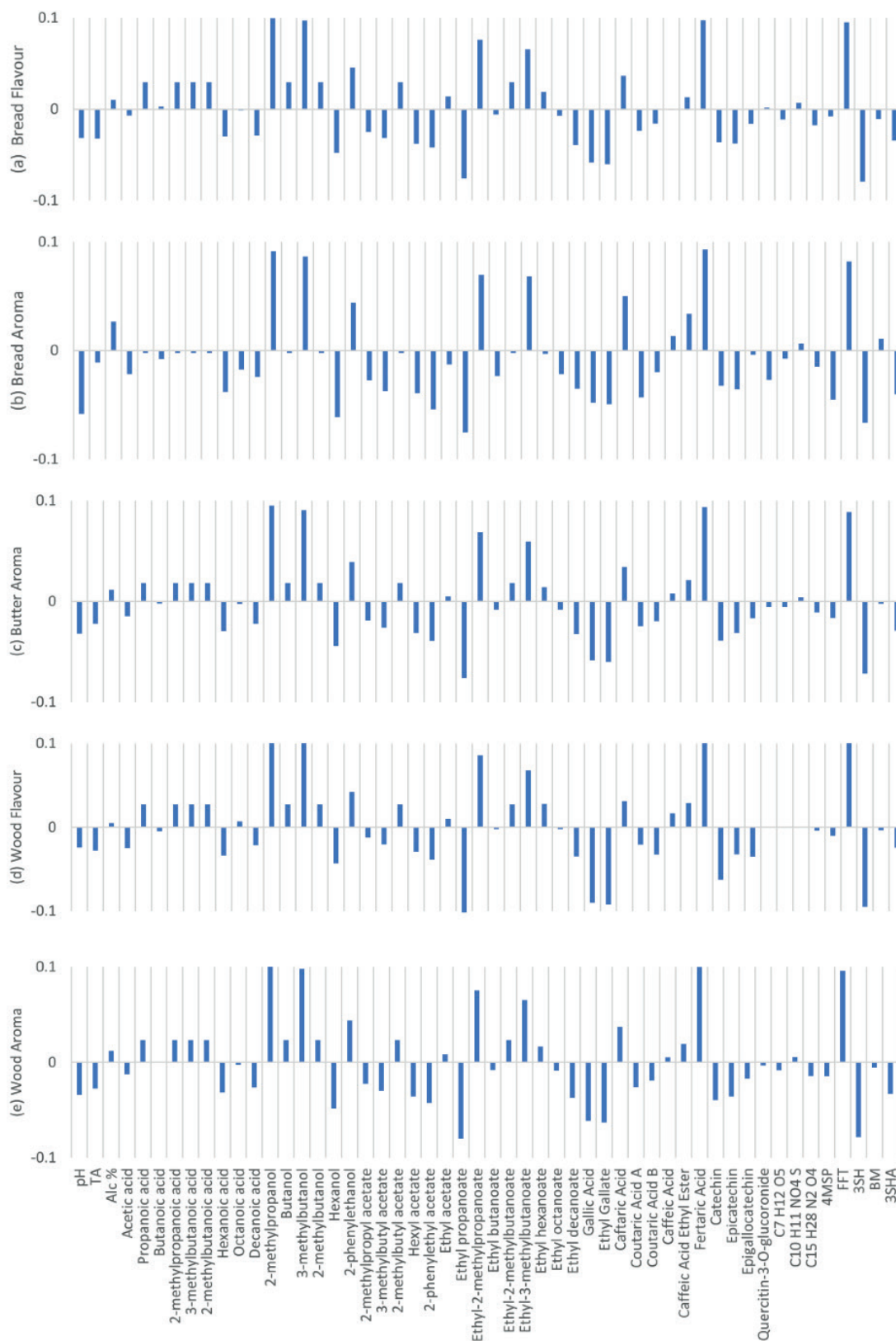


FIGURE 3. Regression coefficients from partial least squares model, relating relative volatile composition for the white wines: (a) bread flavour, (b) bread aroma, (c) butter aroma, (d) wood aroma and (e) wood flavour.

4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol; 3SH: 3-sulfanylhexan-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

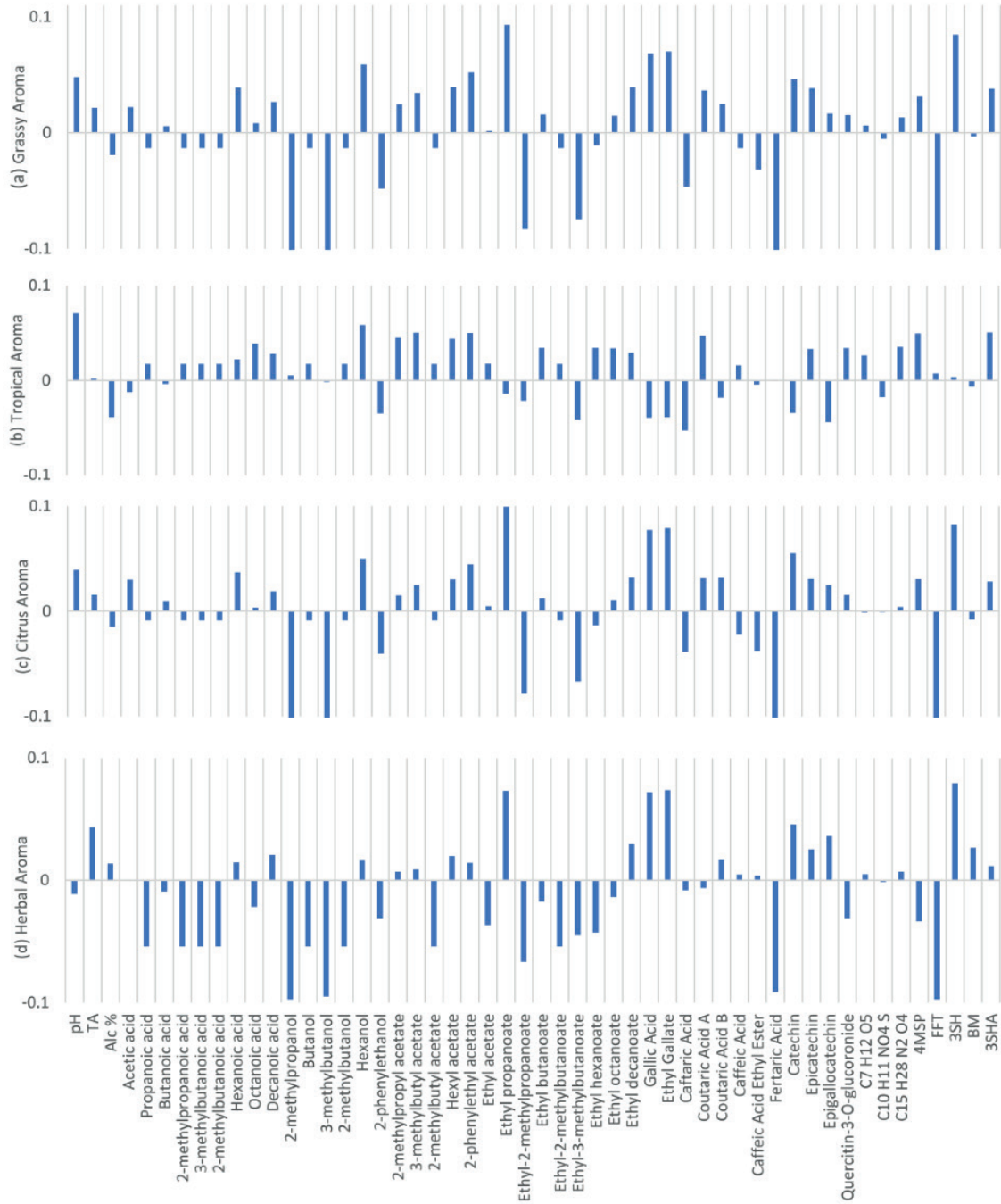


FIGURE 4. Regression coefficients from partial least squares model, relating relative volatile composition for the white wines: (a) grassy aroma, (b) tropical aroma, (c) citrus aroma and (d) herbal aroma.

4MSP: 4-methyl-4-sulfanylpentan-2-one; FFT: furfuryl thiol; 3SH: 3-sulfanylhexas-1-ol; BM: benzyl mercaptan; 3SHA: 3-sulfanylhexyl acetate.

than the Australian wines, having been bottled in late 2016 or early 2017, alternatively, this may also be attributable to masking/suppression and synergistic effects as has been shown previously in Chenin blanc (Wilson *et al.*, 2019) and in red wine (Garrido-Bañuelos *et al.*, 2020). Herbst-Johnstone *et al.* (2011) report that thiol concentrations in Sauvignon blanc wines were not stable and that between 62 % and 76 % of 3SHA had been lost seven months after bottling. Therefore, for the preservation of these compounds, bottle storage conditions can be an important issue. Thiol concentration in wines can also be altered pre-fermentation for example they can be enhanced with particular treatments of grapes and must prior to fermentation. Additionally, Capone *et al.* (2012) report that storing fruit at 10 °C prior to crushing can lead to an increase in 3SH precursors. Chen *et al.* (2019) also demonstrated that freezing grapes and musts to -20 °C prior to fermentation dramatically increased varietal thiol precursors and thiol levels in the finished Sauvignon blanc wine. Furthermore, Maggu *et al.* (2007) demonstrated that increasing skin contact time, less clarified juice (higher turbidity) and a greater press pressure also resulted in higher concentrations of 3SH precursors, most likely due to a greater concentration of precursors located in the skins (Roland *et al.*, 2011).

CONCLUSION

This study measured the concentration of varietal thiols in a small selection of Cypriot wines and the concentration determined in these wines was comparable to those found in popular Australian wines such as Chardonnay and Sauvignon blanc. These varietal thiols are important compounds in certain varieties when “fruity”, “tropical” and “citrus” aromas are desired. While this study was a preliminary investigation, it highlights the importance of thiols in white wines, however, their role in red wines is not well understood.

Further investigation into the role of thiols in Cypriot wines could involve analysis of a much greater number of samples and vintages to evaluate its distribution. Winemaking practices that affect thiols, such as the handling of the grapes prior to fermentation, could be applied to these varieties to be able to meet the desired wine style, whether it be to enhance or reduce these characteristics.

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REFERENCES

- Capone, D. L., Black, C. A., & Jeffery, D. W. (2012). Effects on 3-mercaptohexan-1-ol precursor concentrations from prolonged storage of Sauvignon blanc grapes prior to crushing and pressing. *Journal of Agricultural and Food Chemistry* 60(13), 3515–3523. <https://doi.org/10.1021/jf300054h>
- Capone, D. L., Ristic, R., Pardon, K. H., & Jeffery, D. W. (2015). Simple quantitative determination of potent thiols at ultra-trace levels in wine by derivatization and High-Performance Liquid Chromatography–Tandem Mass Spectrometry (HPLC-MS/MS) analysis. *Analytical Chemistry* 87(2), 1226-1231. <https://doi.org/10.1021/ac503883s>
- Capone, D. L., Barker, A., Williamson, P. O., & Francis, I. L. (2018). The role of potent thiols in Chardonnay wine aroma. *Australian Journal of Grape and Wine Research* 24(1), 38-50. <https://doi.org/10.1111/ajgw.12294>
- Cerreti, M., Ferranti, P., Benucci, I., Liburdi, K., De Simone, C., & Esti, M. (2017). Thiol precursors in Grechetto grape juice and aromatic expression in wine. *European Food Research and Technology* 243(5), 753-760. <https://doi.org/10.1007/s00217-016-2789-7>
- Chen, L., Capone, D. L., Nicholson, E. L., & Jeffery, D. W. (2019) Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for *Vitis vinifera* Sauvignon blanc. *Food Chemistry* 295, 637–645. <https://doi.org/10.1016/j.foodchem.2019.05.126>
- Coetzee, C., Schulze, A., Mokwena, L., du Toit, W. J., & Buica, A. (2018). Investigation of thiol levels in young commercial South African Sauvignon blanc and Chenin blanc wines using propiolate derivatization and GC-MS/MS. *South African Journal of Enology and Viticulture* 39, 180-184. <https://doi.org/10.21548/39-2-2683>

- Constantinou, S., Gómez-Caravaca, A. M., Goulas, V., Fernandez-Gutierrez, A., Koundouras, S., & Manganaris, G. A. (2019). Leaf removal at veraison stage differentially affects qualitative attributes and bioactive composition of fresh and dehydrated grapes of two indigenous Cypriot cultivars. *Journal of the Science of Food and Agriculture* 99(3), 1342-1350. <https://doi.org/10.1002/jsfa.9309>
- Copper, A. W., Johnson, T. E., Danner, L., Bastian, S. E. P., & Collins, C. (2019). Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers. *OENO One* 53(2), 253-273. <https://doi.org/10.20870/oeno-one.2019.53.2.2423>
- Danner, L., Crump, A. M., Croker, A., Gambetta, J. M., Johnson, T. E. & Bastian, S. E. (2017). Comparison of Rate-All-That-Apply (RATA) and Descriptive Analysis (DA) for the sensory profiling of wine. *American Journal of Enology and Viticulture* 56, 55-68. <https://doi.org/10.5344/ajev.2017.17052>
- Dubourdieu, D., & Tominaga, T. (2009). Polyfunctional thiol compounds. In: *Wine chemistry and biochemistry* (pp. 275-293). Springer, New York, NY. ISBN : 978-0-387-74116-1
- Fracassetti, D., Stuknytė, M., La Rosa, C., Gabrielli, M., De Noni, I., & Tirelli, A. (2018). Thiol precursors in Catarratto Bianco Comune and Grillo grapes and effect of clarification conditions on the release of varietal thiols in wine. *Australian Journal of Grape and Wine Research* 24(1), 125-133. <https://doi.org/10.1111/ajgw.12311>
- Galanakis, C. M., Kotanidis, A., Dianellou, M., & Gekas, V. (2015). Phenolic content and antioxidant capacity of Cypriot wines. *Czech Journal of Food Sciences* 33, 126-136. <https://doi.org/10.17221/335/2014-CJFS>
- Gambetta, J. M., Bastian, S. E. P., Cozzolino, D., & Jeffery, D. W. (2014). Factors influencing the aroma composition of Chardonnay wines. *Journal of Agricultural and Food Chemistry* 62(28), 6512-6534. <https://doi.org/10.1021/jf501945s>
- Garrido-Bañuelos, G., Panzeri, V., Brand, J., & Buica, A. (2020). Evaluation of sensory effects of thiols in red wines by projective mapping using multifactorial analysis and correspondence analysis. *Journal of Sensory Studies* 35(4), e12576. <https://doi.org/10.1111/joss.12576>
- Herbst-Johnstone, M., Nicolau, L., & Kilmartin, P. (2011). Stability of varietal thiols in commercial Sauvignon blanc wines. *American Journal of Enology and Viticulture* 62, 495-50. <https://doi.org/10.5344/ajev.2011.11023>
- Hofmann, T., & Schieberle, P. (2002). Chemical interactions between odour-active thiols and melanoidins involved in the aroma staling of coffee beverages. *Journal of Agricultural and Food Chemistry* 50(2), 319-326. <https://doi.org/10.1021/jf010823n>
- Howell, K. S., Swiegers, J. H., Elsey, G. M., Siebert, T. E., Bartowsky, E. J., Fleet, G. H., Pretorius, I. S., & de Barros Lopes, M. A. (2004). Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *Fems Microbiology Letters* 240, 125-129. <https://doi.org/10.1016/j.femsle.2004.09.022>
- King, E. S., Osidacz, P., Curtin, C., Bastian, S. E. P., & Francis, I. L. (2011). Assessing desirable levels of sensory properties in Sauvignon blanc wines – consumer preferences and contribution of key aroma compounds. *Australian Journal of Grape and Wine Research* 17 (2), 169-180. <https://doi.org/10.1111/j.1755-0238.2011.00133.x>
- Kokkinofta, R., Tzioni, E., Ioannou-Kakouri, E., Kanari, P., Economidou, N., Damianou, K., Prokopiou, E., Krashia, A., Frantzi, M., & Kakouri, E. (2014). Phenolic antioxidants in Cypriot wines as a tool for their varietal discrimination: Preliminary results. *Journal of Chemistry and Chemical Engineering* 8, 810–818.
- Mafata, M., Stander, M. A., Thomachot, B., & Buica, A. (2018). Measuring thiols in single cultivar South African red wines using 4,4-dithiodipyridine (DTDP) derivatization and Ultraperformance Convergence Chromatography-Tandem Mass Spectrometry. *Foods* 7(9), 138. <https://doi.org/10.3390/foods7090138>
- Maggu, M., Winz, R., Kilmartin, P., Trought, M., & Nicolau, L. (2007). Effect of Skin Contact and Pressure on the Composition of Sauvignon blanc Must. *Journal of Agricultural and Food Chemistry* 55(25), 10281–10288. <https://doi.org/10.1021/jf072192o>
- Maslov-Bandić, L., Viskić, M., Korenika, A. M. J., & Jeromel, A. (2018). Varietal thiols in grape and wine. In: *A closer look at grapes and winemaking*. New York, USA, Nova Science Publishers, 43-73.
- Mateo-Vivaracho, L., Zapata, J., Cacho, J., & Ferreira, V. (2010). Analysis, occurrence, and potential sensory significance of five polyfunctional mercaptans in white wines. *Journal of Agricultural and Food Chemistry* 58, 10184– 10194. <https://doi.org/10.1021/jf101095a>
- Mattivi, F., Fedrizzi, B., Zenato, A., Tiefenthaler, P., Tempesta, S., Perenzoni, D., Cantarella, P., Simeoni, F., & Vrhovsek, U. (2012). Development of reliable analytical tools for evaluating the influence of reductive winemaking on the quality of Lugana wines. *Analytica Chimica Acta* 732, 194-202. <https://doi.org/10.1016/j.aca.2011.11.051>
- Pardon, K. H., Graney, S. D., Capone, D. L., Swiegers, J. H., Sefton, M. A., & Elsey G. M. (2008). Synthesis of the individual diastereomers of the cysteine conjugate of 3-mercaptohexanol (3-MH). *Journal of Agricultural and Food Chemistry* 56, 3758-3763. <https://doi.org/10.1021/jf8000444>

Rigou, P., Triay, A., & Razungles, A. (2014). Influence of volatile thiols in the development of blackcurrant aroma in red wine. *Food Chemistry* 142, 242-248. <https://doi.org/10.1016/j.foodchem.2013.07.024>

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M., & Razungles, A. (2011). Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon blanc grapes. *Food Chemistry* 125(1), 139-144. <https://doi.org/10.1016/j.foodchem.2010.08.050>

Swiegers, J.H., Capone, D.L., Pardon, K.H., Elsey, G.M., Sefton, M. A., Francis, I. L., & Pretorius, I. S. (2007). Engineering volatile thiol release in *Saccharomyces cerevisiae* for improved wine aroma. *Yeast* 24, 561-574. <https://doi.org/10.1002/yea.1493>

Tsiakkas, O., Escott, C., Loira, I., Morata, A., Rauhut, D., & Suárez-Lepe, J. A. (2020). Determination of anthocyanin and volatile profile of wines from varieties Yiannoudi and Maratheftiko from the Island of Cyprus. *Beverages* 6(1), 1-13. <https://doi.org/10.3390/beverages6010004>

Wilson, C., Brand, J., du Toit, W. & Buica, A. (2019). Matrix effects influencing the perception of 3-mercaptohexan-1-ol (3-MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin blanc wines by projective Mapping (PM) with Ultra Flash profiling (UFP) intensity ratings. *Food Research International* 121, 633-640. <https://doi.org/10.1016/j.foodres.2018.12.032>