Liang Chen, Dimitra L. Capone, Emily L. Nicholson, David W. Jeffery

Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for Vitis vinifera Sauvignon blanc

Food Chemistry, 2019; 295:637-645

Final publication at http://dx.doi.org/10.1016/j.foodchem.2019.05.126

Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for *Vitis vinifera* Sauvignon blanc

Liang Chen [a], Dimitra L. Capone [a, b], Emily L. Nicholson [c], David W. Jeffery [a, b, *]

[a] Department of Wine and Food Science, The University of Adelaide (UA), PMB 1, Glen Osmond, South Australia 5064, Australia

[b] Australian Research Council Training Centre for Innovative Wine Production, UA, PMB 1, Glen Osmond, South Australia 5064, Australia

[c] CSIRO Agriculture and Food, Locked Bag 2, Glen Osmond, SA 5064, Australia

[*] Corresponding author.

E-mail address: david.jeffery@adelaide.edu.au (D.W. Jeffery).

Running title: Intraregional and freezing effects on varietal thiols and precursors

Abstract

Sauvignon blanc grape samples (n = 21) from across a single Geographical Indication of South Australia were analysed for thiol precursors and amino acids, and fermented in an identical laboratory-scale fermentation trial to investigate the intraregional pattern of varietal thiols in the wines. Precursors and thiols exhibited obvious intraregional diversity, and notably, stronger correlations were observed between a number of amino acids and thiol precursors (especially with glutamic acid, $r \leq -0.73$) rather than free thiols. Additionally, pre-fermentation freezing (−20 °C, 1 month) was applied to five selected fresh grape samples and their juices, followed by identical fermentation. In comparison to wines from fresh grapes or frozen juices, significant elevation of varietal thiols (up to 10-fold) occurred in the wines derived from frozen grapes, with parallel increases of precursors (up to 19-fold) in juices from frozen berries. These novel results may lead to new strategies for thiol enhancement during winemaking.

Keywords:

3-sulfanylhexan-1-ol; 3-sulfanylhexyl acetate; 4-methyl-4-sulfanylpentan-2-one; aroma enhancement; wine aroma; winemaking.

Chemical compounds studied in this article:

3-sulfanylhexan-1-ol (PubChem CID: 521348); 3-sulfanylhexyl acetate (PubChem CID: 518810); 4-methyl-4-sulfanylpentan-2-one (PubChem CID: 88290); arginine (PubChem CID: 6322), proline (PubChem CID: 145742), glutamic acid (PubChem CID: 33032); γ-aminobutyric acid (PubChem CID: 119); α-alanine (PubChem CID: 5950).

1. Introduction[1]

Sauvignon blanc (*Vitis vinifera*) is one of the most widely cultivated grapevine varieties in all major wine-producing countries (OIV, 2017). According to the International Organisation of Vine and Wine, Sauvignon blanc is the only top white variety that had a significant increase (> 3%) in annual change of vineyard area worldwide from 2000 to 2015 (OIV, 2017). The success and popularity of Sauvignon blanc wine undoubtedly relate to its distinctive and characteristic "grassy", "citrus", and "tropical fruit" aromas, which are largely contributed by potent volatile compounds with odour thresholds in the nanogram-per-litre range, such as methoxypyrazines and varietal thiols (Coetzee & du Toit, 2012; Jeffery, 2016).

In relation to varietal thiols, 3-sulfanylhexan-1-ol (3-SH), 3-sulfanylhexyl acetate (3-SHA), and 4-methyl-4-sulfanylpentan-2-one (4-MSP) are well recognised as the fundamental volatile compounds imparting aromas of "passionfruit", "grapefruit", "guava", and "box tree" to Sauvignon blanc wine as well as wines made from several other *Vitis vinifera* grape varieties (Roland, Schneider, Razungles, & Cavelier, 2011). 3-SH and 4-MSP are formed through alcoholic fermentation by the action of yeast enzymes from their non-volatile precursors extracted from grapes, and 3-SHA is formed enzymatically from 3-SH (Roland, Schneider, Razungles, & Cavelier, 2011). However, precursors identified in grape juice so far, involving glutathione, dipeptide and cysteine conjugates, and α,β-unsaturated carbonyls, can only partially account for the amounts of the varietal thiols found in wine, primarily for 3-SH (Bonnaffoux, Delpech, Rémond, Schneider, Roland, & Cavelier, 2018; Roland, Schneider, Razungles, & Cavelier, 2011). Furthermore, no consistent correlations have been seen between varietal thiols and their putative precursors (Chen, Capone, Tondini, & Jeffery, 2018; Jeffery, 2016; Pinu, Jouanneau, Nicolau, Gardner, & Villas-Boas, 2012), which suggests that other

[1] **Abbreviations:** 3-SH, 3-sulfanylhexan-1-ol; 3-SHA, 3-sulfanylhexyl acetate; 4-MSP, 4-methyl-sulfanyl 4-pentan-2-one; ANOVA, analysis of variance; Cys-3-SH, 3-*S*-cysteinylhexan-1-ol; DTDP, 4,4′-dithiodipyridine; GABA, γ-aminobutyric acid; GI, Geographical Indication; Glut-3-SH, 3-*S*-glutathionylhexan-1-ol; IS, internal standard; PC, principal component; PCA, principal component analysis; PFF, pre-fermentation freezing; SD, standard deviation; SIDA, stable isotope dilution assay; SPE, solid-phase extraction; TA, titratable acidity; TSS, total soluble solids.

varietal thiol precursors or alternative biogenesis and fate pathways are still waiting to be revealed. Apart from precursor availability, varietal thiol production during fermentation also depends on grape composition (Pinu, Tumanov, Grose, Raw, Albright, Stuart, et al., 2019), such as the profile of amino acids and certain organic acids (Alegre, Culleré, Ferreira, & Hernández-Orte, 2017; Pinu, Edwards, Jouanneau, Kilmartin, Gardner, & Villas-Boas, 2014). However, other than studies involving thiol precursors, literature linking grape composition to varietal thiol formation is limited, and although the enhancive or suppressive roles of amino acids (amounts and ratios) on varietal thiol production have been demonstrated as outlined already, such effects and relationships require further elucidation.

With the incomplete picture of biogenesis of varietal thiols and complex relationship to other grape metabolites, controllable management of the production of varietal thiols and the related sensory quality of a wine through viticultural or oenological practices is still not easy to achieve. In recent years, vineyard practices (application of nitrogen and sulfur), grapes (maturity, clones, grape metabolites), berry processing (harvest, crush, press etc.), and fermentation choices (yeast, commercial additives) have been investigated for their impacts on varietal thiols and/or their precursors (Chen, Capone, Tondini, & Jeffery, 2018; Jeffery, 2016; Roland, Schneider, Razungles, & Cavelier, 2011; Santiago & Gardner, 2015). However, most of the practices exhibited mixed effects (grape-dependent or product-specific) and the modulation of precursors in grapes was not always reflected in the production of varietal thiols in wine. As such, vineyard and/or winemaking practices for enhancing thiol concentrations in wines are still required. Low temperature treatment of grapes maybe a useful option based on the use of cryogenic processing technology in the beverage industry (Brown, 1975; Pando Bedriñana, Picinelli Lobo, & Suárez Valles, 2018). The first indication of its potential utility for thiol management in Sauvignon blanc was revealed in a study of thiol precursors 3-*S*-cysteinylhexan-1-ol (Cys-3-SH) and 3-*S*-glutathionylhexan-1-ol (Glut-3-SH), whereby Glut-3-SH increased by around four times in frozen grapes stored at -20 °C for 2 months compared to frozen or fresh juices (Capone, Sefton, & Jeffery, 2011). In a subsequent study, pre-fermentative cryomaceration, undertaken by adding dry ice to crushed Sauvignon blanc grape must and leaving it to thaw over a 24-h period, was found to increase 3-SH and 3-SHA concentrations in the wine (Olejar, Fedrizzi, & Kilmartin, 2015). However, the effect of cryogenic storage on thiol production during fermentation remained to be further investigated, and influences of cryogenic treatments on grape precursors and wine thiols have never been shown in parallel.

The present work sought to investigate a number of hypotheses related to varietal thiols and precursors, which included: i) the presence of intraregional variation; ii) relationship with grape amino acids; iii) pre-fermentation freezing (PFF) as a tool to enhance thiols in wine. Parcels of Sauvignon blanc grapes (n = 21) were hand harvested from commercial vineyards within the Geographical Indication (GI) of the Adelaide Hills wine region. Amino acids and thiol precursors were measured in grape juices and laboratory-scale fermentation trials were conducted with a high throughput automated fermentation platform. Varietal thiols were analysed in the finished wines by HPLC–MS/MS after derivatisation. Intraregional variations of precursors in juices and varietal thiols in wines were examined and correlated with amino acids in grapes. To test the potential applicability for thiol enhancement during winemaking, PFF treatment (−20 °C, 1 month) was applied for the first time to the fermentation of a subset of fresh whole grape bunches and matched juices that were obtained from the fresh grapes.

2. Material and methods

2.1. Chemicals and solutions

The following chemicals and consumables were obtained from commercial suppliers: 4,4′-dithiodipyridine (DTDP), formic acid, acetaldehyde, and EDTA 2Na (Sigma-Aldrich, Castle Hill, NSW, Australia); Merck liquid chromatography-grade ethanol, methanol, and acetonitrile (VWR International, Tingalpa, QLD, Australia); Bond Elut C18 (500 mg, 6 mL) solid-phase extraction (SPE) cartridges (Agilent, Mulgrave, VIC, Australia); polymeric Strata-X-C (30 mg, 1 mL) and Strata SDB-L (500 mg, 6 mL) SPE cartridges (Phenomenex, Lane Cove, NSW, Australia); AccQ-Fluor amino acid reagent kit and AccQ-Tag eluent A (Waters, Rydalmere, NSW, Australia). Water used was purified through a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Thiol and precursor standards and internal standards (IS) were prepared as previously reported (Chen, Capone, Tondini, & Jeffery, 2018). Standard and IS solutions were prepared volumetrically either in Milli-Q water (for mixtures of precursors) or in absolute ethanol (for mixtures of thiols). Stock solutions were kept at −20 °C and working solutions were stored at 4 °C until required. DTDP solution (10 mM) was prepared as detailed previously (Capone, Ristic, Pardon, & Jeffery, 2015).

2.2. Grape and juice

Parcels of *Vitis vinifera* L. cv Sauvignon blanc grapes (n = 21, abbreviated in Table S1 of the Supporting Information) encompassing five clones were hand-picked from seven commercial vineyards located in the Adelaide Hills GI of South Australia (L1–L7, mapped in Fig. S1 of the Supporting Information) on 27th February (n = 9), 28th February (n = 7), and 7th March (n = 5) during the 2018 vintage. For each sample, ≈8 kg of whole grape bunches were collected from both sides of the vines across multiples rows within each vineyard, temporarily stored in food-grade resealable plastic bags (≈2 kg/bag), transported to the laboratory (<2 h) and stored at 4 °C overnight. Grape bunches were then gently randomised in a plastic sample tray and divided into two subsets (≈5 kg + ≈3 kg).

The first subset of fresh grape bunches (≈5 kg) was sulfured (50 mg/kg SO_2 added as potassium metabisulfite) and crushed immediately under dry ice protection following a previously reported procedure (Chen, Capone, Tondini, & Jeffery, 2018). The resultant juices were collected in food-grade plastic storage bottles (1 L), cold settled at 4 °C for 12 h, and the clear juices were divided into two groups: the first group of juices (n = 21) was subjected to laboratory-scale fermentation immediately, acting as the Control wines (non-PFF); the other group of clear juices was stored in PET bottles (500 mL, protected by dry ice during filling) at −20 °C, and used as the frozen juice treatment (PFF-juice).

The second subset of fresh bunch grapes (≈3 kg,) was carefully sealed in food-grade resealable plastic bags and wrapped in aluminium foil, and stored at −20 °C as the frozen grape treatment (PFF-grape). After 1 month, only the frozen juices and matching grape bunches from co-located Sauvignon blanc clones (L4, n = 5, Table S1 of the Supporting Information) were assessed to highlight this concept. Juices were thawed at 4 °C overnight, and defrosted grape bunches were crushed and the resultant juices were collected in the same manner as for non-PFF wine, undergoing cold settling at 4 °C overnight. Fermentation of the thawed juices and juices obtained from frozen grape bunches was conducted in an identical manner to the Control wines.

2.3. Fermentation

Laboratory-scale fermentations were performed in triplicate on an automated fermentation platform (TEE-BOT) as detailed previously (Chen, Capone, Tondini, & Jeffery, 2018). Yeast *Saccharomyces cerevisiae* strain VIN13 (after culturing in liquid YPD for 24 h at 28 °C) was used for inoculation (1 mL of culture). Fermentation temperature was set at 16 °C. Residual sugars were sampled daily and measured using an enzymatic assay (Chen, Capone, Tondini, & Jeffery, 2018). Fermentation was considered to be completed when residual sugar <2.5 g/L. Finished ferments were cold settled at 4 °C for about 1 week before being opened for varietal thiol analysis.

2.4. Basic juice parameter measurement

Total soluble solid (TSS), pH, and titratable acidity (TA) were measured in freshly obtained juice samples in duplicate according to the previously reported methods (Chen, Capone, Tondini, & Jeffery, 2018).

2.5. High-performance liquid chromatography (HPLC) analysis for amino acids in juices

Freshly thawed juice obtained from fresh whole bunches (n = 21) was centrifuged at 14462 *g* for 10 min and 60 μL of supernatant was collected and mixed with 60 μL of α-aminobutyric acid (0.5 mM in MilliQ water). Mixed samples (100 μL) were loaded onto Strata-X-C cartridges preconditioned with 1 mL of methanol followed by 1 mL of water. After sample loading, the column was washed with 1 mL of 80% aq. methanol solution and eluted with 1 mL of freshly prepared 25% ammonium hydroxide:methanol (1:1) and the eluate was dried under nitrogen flow at room temperature using an Alltech drying lid attachment for a vacuum manifold (Grace Davison Discovery Sciences, Rowville, VIC, Australia). The dried extract was reconstituted with 1 mL of sodium borate buffer (0.2 M, pH = 8.8), derivatised according to the manufacturer's instructions using an AccQ-Fluor reagent kit, and analysed by HPLC with a fluorescence detector following a published procedure and using the same instrumentation and HPLC parameters (Culbert, McRae, Condé, Schmidtke, Nicholson, Smith, et al., 2017).

2.6. Stable isotope dilution assay (SIDA) using high-performance liquid chromatography and tandem mass spectrometry (HPLC–MS/MS) for thiol precursors in juices

Freshly thawed juice obtained from fresh whole bunches (n = 21) was cold settled at 4 °C for 2 hours and aliquot was analysed for thiol precursors (Cys-3-SH, Glut-3-SH) in duplicate according to a previously reported method with modified reconstitution procedure (Capone & Jeffery, 2011). Analysis was performed on a Thermo Finnigan Surveyor HPLC fitted with an Alltima C18 HPLC column (250 × 2.1 mm i.d., 5 µm, 100 Å, Grace Davison Discovery Sciences, Rowville, VIC, Australia) connected to a Thermo Finnigan LCQ Deca XP Plus mass spectrometer using electrospray ionisation in positive ion mode. Chromatographic conditions and ion pairs were as described previously (Capone, Sefton, Hayasaka, & Jeffery, 2010) and helium was used as collision gas with the following source and mass spectrometer conditions: spray voltage of 4.5 kV, respective sheath and aux/sweep gas flow rates of 30 and 19, capillary voltage of 36 V, capillary temperature of 250 °C, single reaction monitoring mode with activation Q of 0.250, activation time of 30 ms, normalised collision energy of 35%, and isolation width m/z = 1.50. Xcalibur software (Thermo Finnigan, version 1.3) was used for instrument control and data acquisition. Cys-3-SH and Glut-3-SH concentrations were reported as the sum of the two respective diastereomers.

2.7. SIDA HPLC–MS/MS analysis for thiols in wines

Thiol extracts were prepared and analysed following a previously published method (Capone, Ristic, Pardon, & Jeffery, 2015). After cold settling, ferment bottles were opened and an aliquot of wine (20 mL) was accurately pipetted into a 22 mL glass vial for sample preparation according to the previously reported derivatisation and isolation steps. Extracts were reconstituted with 10% aq. ethanol solution (200 µL) and stored at −20 °C pending analysis. A batch of calibration and quality control samples was prepared in the same manner with the wine samples for quantitation. HPLC–MS/MS analysis was performed with an Agilent 1200 Series HPLC connected to an Agilent 6410A Triple Quad MS (Agilent, Santa Clara, CA, USA) as reported previously (Chen, Capone, & Jeffery, 2018).

2.8. Statistics

Data reduction, mean values, standard deviation (SD), and Pearson correlation were performed with Microsoft Excel 2016. Unpaired t-test (two tailed) and one-way analysis of variance (ANOVA) was conducted with $\alpha = 0.05$ using Prism 7 (GraphPad Software, CA, USA). Principal component analysis (PCA) was undertaken on all significantly different variables after standardisation using The Unscrambler X (CAMO Software, Oslo, Norway).

3. Results and discussion

Regional investigations of varietal thiols and precursors have been previously reported in few instances and mostly focused on Sauvignon blanc from the world famous Marlborough region of New Zealand (Jouanneau, Weaver, Nicolau, Herbst-Johnstone, Benkwitz, & Kilmartin, 2012; Pinu, Jouanneau, Nicolau, Gardner, & Villas-Boas, 2012) although other regions and varieties have also been evaluated (Capone, Barker, Williamson, & Francis, 2017; Fracassetti, Stuknytė, La Rosa, Gabrielli, De Noni, & Tirelli, 2018). The cool climate Adelaide Hills wine region in South Australia was the focus for the present work, with a total of 21 Sauvignon blanc grape parcels sourced from vineyard blocks in seven locations (Fig. S1 of the Supporting Information) during the 2018 vintage to investigate the intraregional variations of precursors in juices and thiols in wines. Grape samples were harvested by hand at around the same maturity levels (Table S1 of the Supporting Information) and fermented in triplicate under identical winemaking conditions at laboratory-scale using an automated fermentation platform (Chen, Capone, Tondini, & Jeffery, 2018).

3.1. Basic juice parameters and fermentation

The results for TSS, pH, and TA for freshly obtained juices of the 21 Sauvignon blanc grape parcels are summarised in Table S1 of the Supporting Information. A TSS of around 20-21 °Brix was targeted but sampling had to occur within the constraints of the commercial vineyards. TSS values generally ranged from 19 to 22 °Brix (L1_1 and L3_2 were ≤ 17 °Brix), pH varied from 2.53 to 3.32, and TA was between 6.5 and 13.9 g/L. Except for the higher TA values in 2018, the basic juice parameters of L4 were similar to the data from the 2017 vintage for grapes from the same vines (Chen, Capone, Tondini, & Jeffery, 2018). Slight differences in ripeness within single locations (even for the same clones) and across the GI were considered to result from complex ecophysiological responses and/or viticulture practices (Dai, Ollat, Gomes, Decroocq, Tandonnet, Bordenave, et al., 2011). For all fermentation trials, cold-settled clear juices were fermented in triplicate in an identical manner without any adjustments to composition using commercial yeast strain VIN13 at 16 °C. Fermentations all proceeded to dryness (<2.5 g/L) within 3 weeks and no obvious patterns of fermentation duration across grape samples were noticed.

3.2. Overview of intraregional variation on precursors in juices and thiols in wines

Data from quantitative analysis of juice precursors (Glut-3-SH and Cys-3-SH, sum of respective diastereomers) and wine varietal thiols (3-SH, 3-SHA, and 4-MSP) are presented in Fig. 1a-f and Table S2 of the Supporting Information. The two precursors were detected in all juice samples with Glut-3-SH (33.7 – 170.7 µg/L) dominating over Cys-3-SH (7.9 – 44.7 µg/L) (Fig. 1a, Table S2 of the Supporting Information). There was a strong positive correlation between Cys-3-SH and Glut-3-SH (r = 0.98, Fig. 1f). The higher abundance of Glut-3-SH and the strong correlation between precursors were in accord with previous studies (Capone, Sefton, Hayasaka, & Jeffery, 2010; Fracassetti, Stuknytė, La Rosa, Gabrielli, De Noni, & Tirelli, 2018; Pinu, Jouanneau, Nicolau, Gardner, & Villas-Boas, 2012), and is reflective of an enzymatic degradation pathway of Glut-3-SH to Cys-3-SH as detailed previously (Jeffery, 2016). The overall concentrations of precursors were well in line with data from the previous vintage for grapes from the Adelaide Hills (samples from L4) (Chen, Capone, Tondini, & Jeffery, 2018).

Previous studies have demonstrated variations of precursors in Sauvignon blanc juices but they had either been assessed in a smaller sample set (n = 5) (Allen, Herbst-Johnstone, Girault, Butler, Logan, Jouanneau, et al., 2011) or used commercial juices arising from standard practices (Pinu, Jouanneau, Nicolau, Gardner, & Villas-Boas, 2012) that were unlikely to involve consistent grape processing (e.g., transport, maceration, and press cycle). In that latter report, variations of precursors in 55 commercial New Zealand Sauvignon blanc juices from different vintages but mainly from locations within Marlborough were up to 20-fold and 126-fold for Glut-3-SH and Cys-3-SH, respectively (Pinu, Jouanneau, Nicolau, Gardner, & Villas-Boas, 2012). In the present study, 21 grape parcels and corresponding juices were obtained in an identical manner so the results may better reflect possible intraregional variations of precursors.

The concentrations of precursors in grapes from different locations were examined by one-way ANOVA ($\alpha = 0.05$), with results presented in Fig. 1c. In terms of Glut-3-SH, significant differences occurred between L1 (118.0 ± 27.8 µg/L) and L2 (58.3 ± 34.7 µg/L), L1 and L4 (50.3 ± 5.0 µg/L), L2 and L3 (112.2 ± 40.4 µg/L), and L3 and L4. For Cys-3-SH, a significant difference was only present between L1 samples (average 33.9 µg/L) and others (average 12.6–20.2 µg/L). Within the vineyard locations containing different blocks (and clones) that were sampled (i.e., L1 to L4), Cys-3-SH varied almost consistently, around 1.4-fold (L4) to 1.7-fold (L2), whereas Glut-3-SH fluctuated from 1.3-fold (L4) to 3.6-fold (L2), apparently independent of grape ripeness. This variation among grape parcels from within single locations may suggest that the biological accumulation of Glut-3-SH was more affected (e.g., by genetics and/or environment) than Cys-3-SH, as the post-harvest processing conditions were essentially identical.

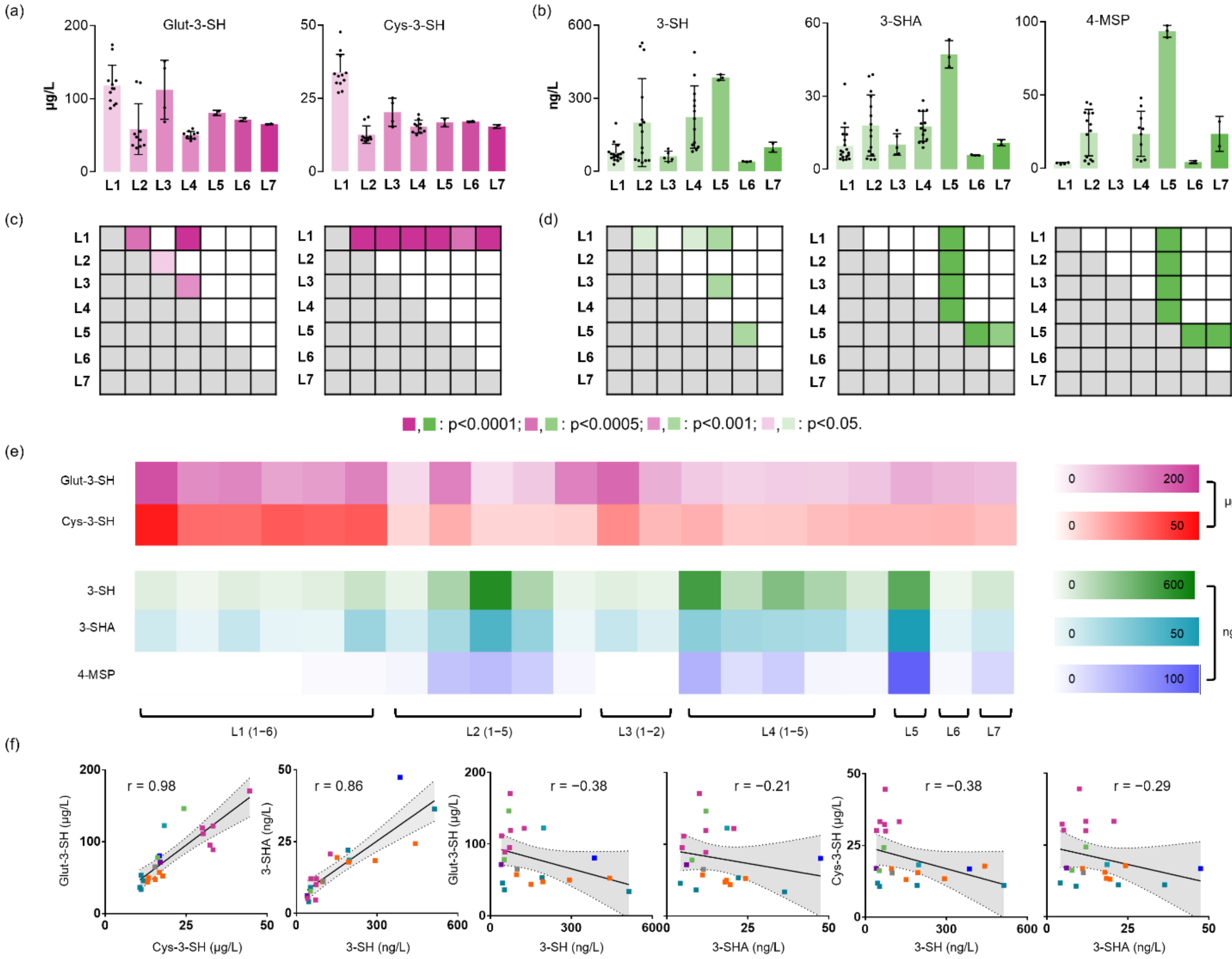

Fig. 1. Overview of the precursors (Glut-3-SH and Cys-3-SH) in juices and varietal thiols (3-SH, 3-SHA, and 4-MSP) in wines from 21 Sauvignon blanc grape parcels from seven locations (L1 to L7) within the Adelaide Hills wine region showing: mean concentrations of (a) precursors (μg/L) and (b) thiols (ng/L), where error bars represent the group SD, and scattered dots in black indicate the measured value of analyte in individual samples; statistically significant differences (coloured) of (c) precursors and (d) thiols across locations, examined by one-way ANOVA ($\alpha = 0.05$); (e) heat maps showing the quantitative results of precursors and thiols by grape parcel; and (f) scatter plots (Glut-3-SH vs. Cys-3-SH, 3-SH vs. 3-SHA, precursors vs. varietal thiols) with shaded areas indicating 95% confidence bands and black lines showing the best-fit lines based on Pearson correlation analysis. For location (L) details, refer to Table S1 and Fig. S1 of the Supporting Information.

3-SH, 3-SHA, and 4-MSP in the resulting wines also occurred at various concentrations (Fig. 1b, Table S2 of the Supporting Information), with 3-SH ranging from 29–528 ng/L (average 152 ng/L, 18-fold variation) and 3-SHA ranging from 4–53 ng/L (average 15 ng/L, 13-fold variation), in agreement with previous data reported for Adelaide Hills Sauvignon blanc wines (Capone, Sefton, & Jeffery, 2011; Chen, Capone, Tondini, & Jeffery, 2018). Wines high in 3-SH were usually high in 3-SHA, with the strong correlation (r = 0.86, Fig. 1f) being consistent with the yeast acetylation pathway linking 3-SHA to 3-SH (Roland, Schneider, Razungles, & Cavelier, 2011). Concentrations of 4-MSP in the finished wines varied from undetectable in six samples up to a notable high of 97 ng/L (Fig. 1b). Compared to reported odour detection thresholds of thiols (Roland, Schneider, Razungles, & Cavelier, 2011), 15 out of 21 Sauvignon blanc wines contained 3-SH above its odour threshold (odour activity value, OAV: 1.1 – 8.8), 17 out of 21 wines had 3-SHA greater than its reported threshold (OAV: 1.0 – 13.3), and all wines containing 4-MSP had concentrations above its odour threshold (OAV: 3.4 – 121.7). The abundances of these thiols at concentrations well-above threshold means they would be expected to contribute perceivable "tropical fruit" aromas in these laboratory scale Adelaide Hills Sauvignon blanc wines.

Regarding intraregional variations, the patterns for 3-SH, and especially 3-SHA and 4-MSP, were similar (Fig. 1b), with L5 standing out with significantly higher thiol levels compared with others based on one-way ANOVA (Fig. 1d). In contrast, L3 and L6 showed lower amounts of all three thiols. In combination with precursor data, no obvious relationship from precursors to thiols was apparent in their patterns of variation. Juices with higher amounts of precursors did not necessarily lead to wines with greater levels of thiols, with L1 being a notable example (Fig. 1e). The opposite could also be said, as was the case for L5, with moderate juice precursor levels but high wine thiol concentrations. Quantitatively, 3-SH and 3-SHA in the wines were both negatively correlated to Glut-3-SH ($r = -0.38$ with 3-SH, $r = -0.21$ with 3-SHA) and Cys-3-SH ($r = -0.38$ with 3-SH, $r = -0.29$ with 3-SHA) in juices (Fig. 1f). These correlations between precursors and varietal thiols contrasted to previously reported correlation results for 55 Sauvignon blanc juices and wines, where little correlation was found for 3-SH and weak but positive correlations to Cys-3-SH, Glut-3-SH and total precursors were evident for 3-SHA (Pinu, Jouanneau, Nicolau, Gardner, & Villas-Boas, 2012).

Due to the limited availability of results that examine correlations between precursors and thiols, several previously reported sets of quantitative data for Sauvignon blanc juice and wine (Allen, et al., 2011; Capone, Sefton, & Jeffery, 2011; Chen, Capone, Tondini, & Jeffery, 2018) were selected and the correlation coefficients were calculated. Interestingly, the calculated correlations were 0.32 (Capone, Sefton, & Jeffery, 2011) and 0.40 (data from hand-picked grapes were selected) (Allen, et al., 2011) for Glut-3-SH to 3-SH, indicating a weak to moderate positive relationship. The correlations between 3-SH to Cys-3-SH were negative but essentially absent (−0.05 and −0.11) (Allen, et al., 2011; Capone, Sefton, & Jeffery, 2011; Chen, Capone, Tondini, & Jeffery, 2018) but 3-SHA was positively related to both Cys-3-SH (r = 0.34) and Glut-3-SH (r = 0.61) (Allen, et al., 2011). The inconsistent correlations demonstrated in the present study and from the abovementioned literature indicate that the relationship between precursors and varietal thiols is even more complicated than perhaps is appreciated, and that ongoing work is required to resolve aspects of varietal thiol biogenesis during winemaking.

3.3. Potential relationship between grape amino acids with precursors and thiols

Grapes from *V. vinifera* cultivars are compositionally complex systems containing numerous chemical components of various categories. In relation to varietal thiols in wine, two major types of precursors to 3-SH and 4-MSP identified in grapes are conjugates of cysteine (Cys-3-SH and Cys-4-MSP) and glutathione (Glut-3-SH and Glut-4-MSP) (Roland, Schneider, Razungles, & Cavelier, 2011). Interestingly, the conjugates all involve amino acid unit(s) (i.e., glycine, glutamic acid, cysteine), which also applies to some recently identified precursors (Bonnaffoux, Delpech, Rémond, Schneider, Roland, & Cavelier, 2018). As a key group of grape metabolites, amino acids have been intensively investigated for their relationship with aroma development during fermentation (Burin, Gomes, Caliari, Rosier, & Bordignon Luiz, 2015; Hernández-Orte, Ibarz, Cacho, & Ferreira, 2006; Park, Boulton, & Noble, 2000) but only a few publications have investigated their influences on varietal thiol production during fermentation (Alegre, Culleré, Ferreira, & Hernández-Orte, 2017; Pinu, Edwards, Jouanneau, Kilmartin, Gardner, & Villas-Boas, 2014; Pinu, et al., 2019). Since previous studies either involved synthetic media or a single Sauvignon blanc juice (Alegre, Culleré, Ferreira, & Hernández-Orte, 2017), or showed inconsistent correlations between amino acids and thiols (Pinu, Edwards, Jouanneau, Kilmartin, Gardner, & Villas-Boas, 2014; Pinu, et al., 2019), the profiles of amino acids in a range of Sauvignon blanc grapes from within a single GI were determined and compared with both precursor and varietal thiol concentrations.

The total amino acid concentrations of the 21 grape juices ranged from 390 to 1091 mg/L (L1_3 and L7, respectively). Compositionally, the major amino acids were arginine (146 ± 84 mg/L), proline (124 ± 52 mg/L), glutamic acid (124 ± 44 mg/L), γ-amino butyric acid (GABA, 75 ± 16 mg/L), and α-alanine (52 ± 21 μg/L) in contrast to minor amino acids such as glycine, asparagine, methionine, lysine, tryptophan, and isoleucine (Table S3 of the Supporting Information), which accords with literature data on amino acids in Sauvignon blanc (Martin, Grose, Fedrizzi, Stuart, Albright, & McLachlan, 2016; Park, Boulton, & Noble, 2000; Spayd & Andersen-Bagge, 1996). The ratio of proline to arginine, a suggested cultivar-dependent index, varied widely from 0.36 to 2.78 in the 21 Sauvignon blanc juices, with such an inconsistency having been observed in a previous multi-cultivar survey (Spayd & Andersen-Bagge, 1996). Variation of individual amino acid concentrations between juices from different locations was apparent, as shown in the heatmap (Fig. 2a). Two samples from L1 (L1_1 and L1_2) and juices from L5 to L7 contained higher amounts of minor amino acids. Greater amounts of aspartic acid, serine, proline, and glutamic acid were seen in juices from L4 to L7. Various factors influence grape amino acid concentrations including fertilisation, irrigation and climatic conditions (Ortega-Heras, Pérez-Magariño, Del-Villar-Garrachón, González-Huerta, Moro Gonzalez, Guadarrama Rodríguez, et al., 2014).

Correlation analysis was performed to investigate the potential relationships between amino acids and both thiols and their precursors (Fig. 2b–d). As a whole, amino acids in juices were only weakly correlated to 3-SH and 3-SHA in the wines ($r < 0.2$, Fig. 2b). Individually, correlations (positive or negative) with 3-SH and 3-SHA ranged from absent to weak ($|r| \leq 0.30$, Fig. 2c) except for glutamic acid ($r = 0.42$ for 3-SH, $r = 0.32$ for 3-SHA) and proline ($r = 0.34$ for 3-SH, $r = 0.36$ for 3-SHA).

Glutamic acid has previously been positively correlated to thiol concentrations in a metabolomic profiling study of Sauvignon blanc, along with GABA and glutamine (Pinu, Edwards, Jouanneau, Kilmartin, Gardner, & Villas-Boas, 2014). As varietal thiol production is the result of yeast metabolism during fermentation, the observed correlations between amino acids and varietal thiols could indicate the impacts of amino acids (especially glutamic acid and proline in the present case) on thiol production or interactions between amino acids and thiol precursors during fermentation. The significant enhancing effects of glutamic acid on 3-SH and 3-SHA production were demonstrated previously (Pinu, Edwards, Jouanneau, Kilmartin, Gardner, & Villas-Boas, 2014). Glutamic acid stands out perhaps because it is a preferred yeast nitrogen source for fermentation but the similar correlation results obtained for proline, a non-preferred nitrogen source, were somewhat intriguing.

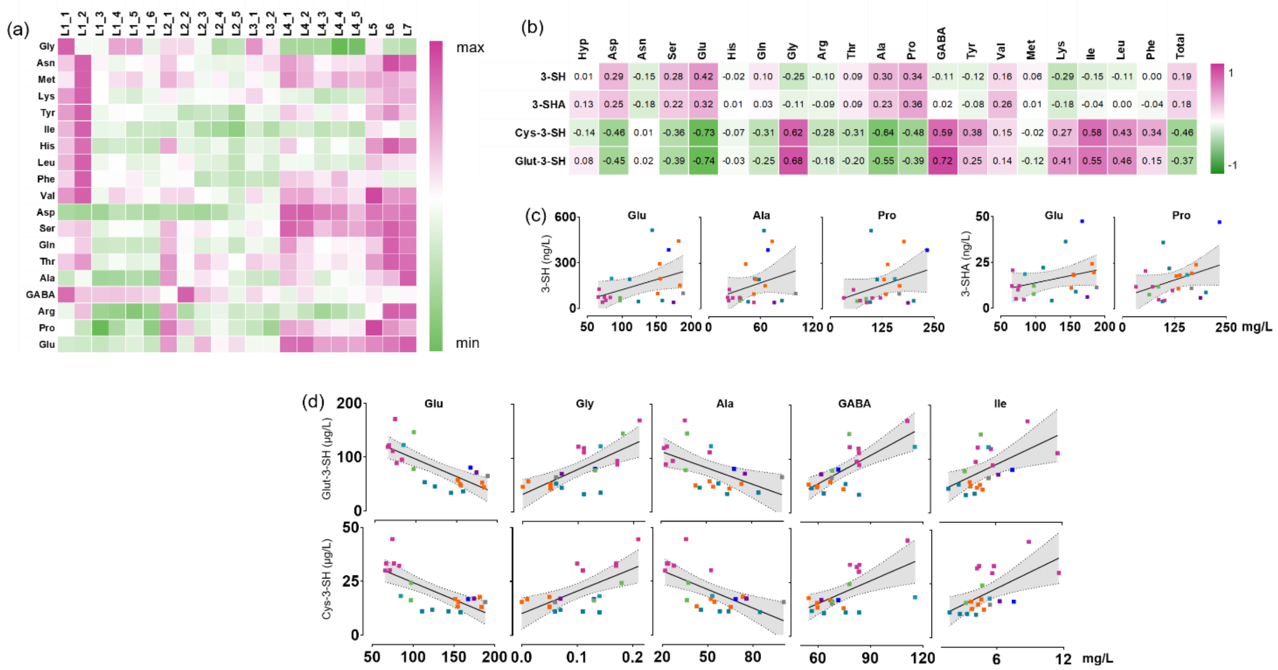

Fig. 2. (a) Relative quantity (%) of amino acids in 21 Sauvignon blanc juices from Adelaide Hills; (b) correlation values between thiols and precursors to amino acids; and (c, d) scatter plots (3-SH and 3-SHA, Glut-3-SH and Cys-3-SH vs. certain grape amino acids) with shaded areas indicating 95% confidence bands and black lines showing the best-fit lines based on Pearson correlation analysis. For location (L) details, refer to Fig. S1 and Table S1 of Supporting Information.

In contrast to the results for the free thiols, precursors were more strongly correlated to a greater number of amino acids ($|r| \geq 0.30$ for thirteen amino acids) (Fig. 2b). Among these apparently novel findings, glutamic acid featured again and had the strongest correlation to both of the precursors ($r \leq -0.73$), followed by glycine ($r \geq 0.62$), GABA ($r \geq 0.59$), alanine ($r \leq -0.55$), and isoleucine ($r \geq 0.55$). The moderate to strong correlations were suggestive of the interaction between the biochemical accumulation/degradation outcomes of thiol precursors and amino acids during grape ripening. Glutamic acid and glycine are component amino acids of glutathione, which plants require to respond to environmental stress (Galant, Preuss, Cameron, & Jez, 2011), so the strong correlations likely relate to promotion (glycine) or inhibition (glutamic acid) of glutathione biosynthesis and thus of glutathione-conjugated thiol precursor Glut-3-SH, which in turn is linked to Cys-3SH formation. The moderate correlations between proline and thiol precursors ($r = -0.39$ for Glut-3-SH, $r = -0.48$ for Cys-3-SH) could also be related to glutamic acid production, which serves as a precursor to proline (Anjum, Aref, Duarte, Pereira, Ahmad, & Iqbal, 2014). Nonetheless, the mechanisms underlying these correlations as well as those of precursors with GABA, alanine, and isoleucine are still unclear and require further investigations. Recent literature suggested that certain ratios of amino acids could also modify thiol production (Alegre, Culleré, Ferreira, & Hernández-Orte, 2017) so the correlations of various amino acid combinations (Glu/GABA, Glu−GABA, Glu+GABA, Glu/Pro, Glu−Pro, Glu+Pro, GABA/Pro, GABA+Pro, GABA−Pro) with thiols and precursors were assessed but no notable correlations were observed (data not shown).

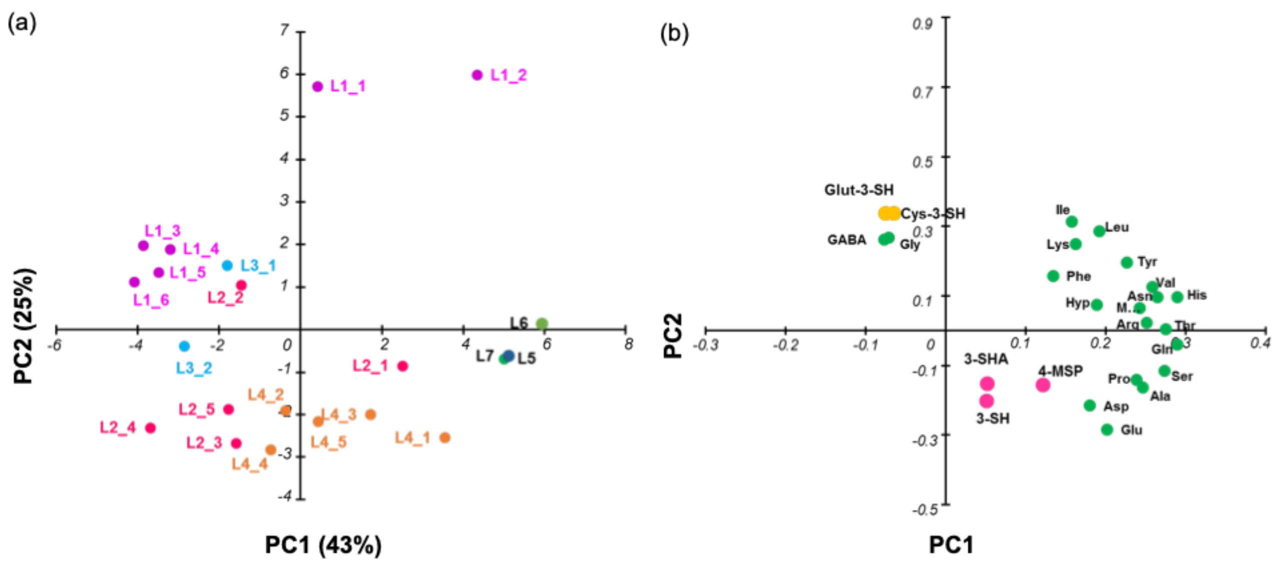

Fig. 3. PCA analysis showing (a) distribution of 21 Sauvignon blanc samples on PC1 vs PC2 and (b) loadings plot based on concentrations of varietal thiols in wines, and precursors and amino acids in juices. For sample codes, refer to Table S1 and Fig. S1 of the Supporting Information.

PCA analysis of quantitative data for varietal thiols, precursors, and amino acids is presented in Fig. 3. The first two principal components (PC) explained a total of 68% variance, with 43% and 25% of the total attributable to PC 1 and PC 2, respectively (Fig. 3a). Samples from L1 were located in the top quadrants of the figure and generally corresponded to higher concentrations of Cys-3-SH, Glut-3-SH, GABA, and glycine (Fig. 3b). Samples from L3 were relatively closely plotted to L1 samples, which indicated similarity between them. Three out of five L2 samples grouped together in the bottom left quadrant, close to the varietal thiols but far away from all amino acids. L4 samples were located together in the bottom quadrants and close to the free thiols (Fig. 3b), indicating relative higher amounts of 3-SH, 3-SHA, and 4-MSP (Fig. 1b). Notably, L5 wine contained the highest amounts of 3-SHA and 4-MSP and was clustered with L6 and almost inseparable from L7, which were dominated by the higher levels of amino acids, indicating the potential impact of amino acids on the variation of thiol metabolism.

3.4. Impact of pre-fermentation freezing (PFF) treatment on precursors and thiols

Cryomaceration (low temperature maceration with solid CO_2 for a period of time) or grape/must freezing can be employed to induce berry damage and enhance extraction of components (Sacchi, Bisson, & Adams, 2005), and has primarily been assessed for its impact on the non-volatile composition (e.g., phenolics or organic acids) of wines or on stability (Álvarez, Aleixandre, García, & Lizama, 2006; Baiano, Terracone, Longobardi, Ventrella, Agostiano, & Del Nobile, 2012). Several studies have considered the impact of cryogenic treatment on volatile compounds in grape or wine (Moreno-Pérez, Vila-López, Fernández-Fernández, Martínez-Cutillas, & Gil-Muñoz, 2013; Ouellet & Pedneault, 2016; Peinado, Moreno, Bueno, Peng, Wen, Tao, & Lan, 2013) but only one report appeared to be available on the potential effect on varietal thiols (Olejar, Fedrizzi, & Kilmartin, 2015). This is despite the technique potentially offering a practical way to increase thiol concentrations in wine through greater extraction of components from grape skin or formation of precursors (Roland, Schneider, Charrier, Cavelier, Rossignol, & Razungles, 2011). Some further insight into the possible impact can be gained from a previous study, whereby frozen storage of fresh grapes increased the concentrations of Cys-3-SH (inconsistently) and Glut-3-SH (substantially) (Capone, Sefton, & Jeffery, 2011). However, the impact of PFF treatment on varietal thiols was not pursued in that work.

In the present study, a period of 30 days of frozen storage (–20 °C) was selected as the PFF treatment on freshly harvested whole grape bunches and their subsequently obtained fresh juices. The conditions for PFF were based on a previous study (Capone, Sefton, & Jeffery, 2011) and were also chosen for convenience, to accommodate other time-sensitive aspects of the experiments. Optimisation of PFF conditions (e.g., temperature, duration, thawing process) was not included but previous work has assessed some conditions and shown an effect on wine volatiles with as little as 6 h of freezing at −20 °C (Peng, Wen, Tao, & Lan, 2013). The concentrations of Glut-3-SH and Cys-3-SH in juices obtained from grapes from L4 with/without PFF treatment and those of 3-SH, 3-SHA, and 4-MSP in subsequent wines from corresponding juices are demonstrated in Fig. 4. After PFF treatment of grape berries, concentrations of Glut-3-SH and Cys-3-SH were 724.3 ± 78.7 µg/L and 73.1 ± 11.7 µg/L, respectively. Compared to grapes without PFF (see L4 in Fig. 1, Glut-3-SH: 50.3 ± 5.0 µg/L, Cys-3-SH: 15.4 ± 2.2 µg/L), Glut-3-SH exhibited a significant 11–19 fold increase and Cys-3-SH increased about 4–6 fold, and with the exception of sample L4_5, all the increments were statistically significant (Fig. 4a). The enhancement of precursors after PFF was much higher than previously reported, in which Glut-3-SH increased by about 5-fold after 1 month of frozen storage but little change was observed for Cys-3-SH (Capone, Sefton, & Jeffery, 2011). The significant increase of Glut-3-SH appeared to be caused by *de novo* formation due to berry damage that occurred during PFF, as explained previously (Capone, Sefton, & Jeffery, 2011). Higher amounts of Cys-3-SH after PFF treatment in the present study suggested a similar formation mechanism might occur for Cys-3-SH, but potential degradation from Glut-3-SH to Cys-3-SH or improved extraction of Cys-

3-SH from damaged cells (Sacchi, Bisson, & Adams, 2005) during the freezing/thawing process could also contribute to the greater amounts of Cys-3-SH observed.

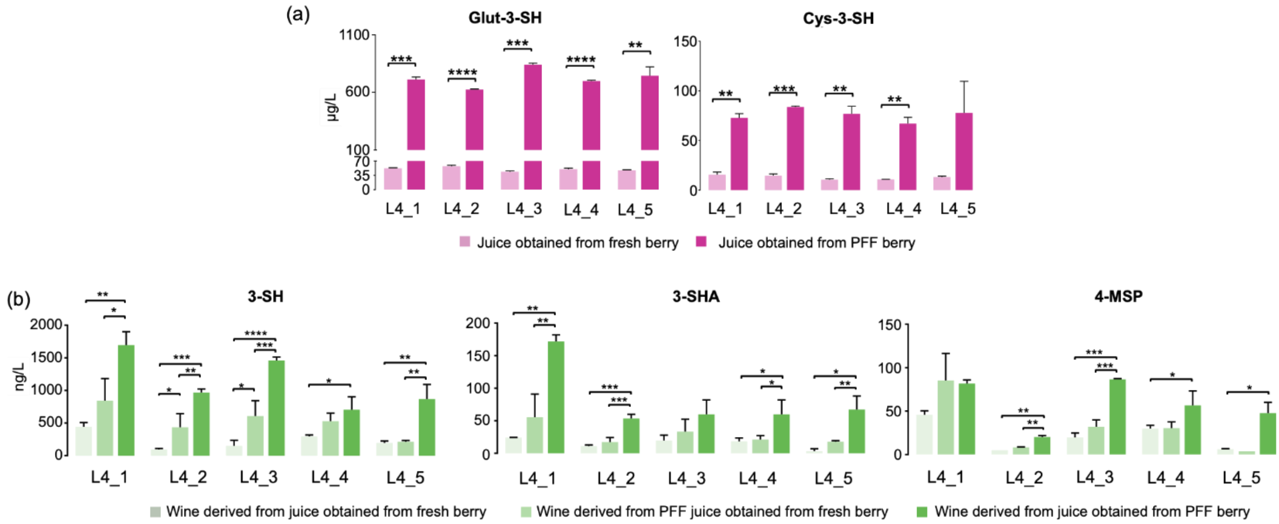

Fig. 4. Comparison of (a) concentrations (μg/L) of precursors (Glut-3-SH, Cys-3-SH) in juices from fresh and PFF treatment grapes, and (b) concentrations (ng/L) of varietal thiols (3-SH, 3-SHA, and 4-MSP) in wines made from juices from fresh grapes, PFF treatment juices, and PFF treatment grapes sampled from Location 4. Error bars represent the SD derived from replicate analysis (n = 2 for precursors, n = 3 for varietal thiols). Precursor data were compared by unpaired t-test and thiol data were evaluated with one-way ANOVA. *: p<0.05, **: p<0.001, ***: p<0.0005, ****: p<0.0001. For sample codes, refer to Table S1 and Fig. S1 of the Supporting Information.

As with precursors in the juices, varietal thiol concentrations were also significantly enhanced in wines with PFF treatment (Fig. 4b) except for 3-SHA in L4_3 wine and 4-MSP in L4_1 wine. Overall, 3-SH concentrations of L4_1 to L4_5 were 1139.0 ± 412.1 and 526.0 ± 279.4 ng/L in wines from PFF treatment of grape bunches and juices, respectively, and both were higher than the average for wine derived from non-PFF treatment (222.8 ± 128.0 ng/L). Stronger increases of thiols were seen in wines from PFF of grapes bunches than PFF of juices and similar trends were observed for 3-SHA and 4-MSP. Compared to wines made from fresh grapes, 3-SH, 3-SHA, and 4-MSP increased by around 2–10, 3–7, and 2–8 times when PFF was applied to grapes. Although lower in magnitude, significant increases of varietal thiols were also noted when comparing wines from PFF grapes to wines arising from PFF juices (Fig. 4b). When considering production from fresh grapes versus frozen juices, significant differences were only observed for 3-SH production in L4_2 and L4_3 wines (approximate 4-fold increase). Notably, even though the increased concentrations from PFF treatments were evident for both precursors and free thiols, with the latter potentially being a reflection of elevated precursor levels, there were much greater relative increases for precursors. Consistent with the weak correlation between precursor and thiol concentrations after PFF treatments (data not shown), this outcome implied that only partial amounts of the enhanced precursor levels induced by PFF treatments were converted to varietal thiols. Nonetheless, whatever the precise mechanism (i.e., from known precursors or some other thiol biogenesis pathway), the significant effects of the freezing treatments showed that remarkable thiol augmentation in wine was possible, which complements the previous work involving dry ice cryomaceration of Sauvignon blanc grape musts (Olejar, Fedrizzi, & Kilmartin, 2015).

4. Conclusion

Intraregional variations of precursors in juice and varietal thiols in wine were characterised for 21 Sauvignon blanc samples from the Adelaide Hills wine region. Obvious intraregional variations were seen in the amounts of precursors in juices and thiols produced in wines. The mixed correlations, weak between grape amino acids and wine varietal thiols but moderate to strong between amino acids and precursors, together with multivariate data analysis, indicated the potential interactions between amino acids and both precursor biosynthesis in grapes and thiol metabolism during fermentation. Notably, pre-fermentation freezing treatment of grape berry parcels induced significant increases in concentrations not only of precursors but also of free thiols, which was revealed for the first time on the same set of grape and wine samples. Pre-fermentation freezing could be a potential approach for winemakers to enhance the production of varietal thiols in wines and this warrants further investigation. In particular, experiments focusing on optimal PFF conditions, for instance, the duration of PFF, storage temperature, thawing process, and single/multiple PFF cycles, could be conducted.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We are immensely grateful to David Coleman from Adelaide Hills Vine Improvement Inc. (AHVI) for assistance of with grape harvesting and facilitating access to vineyards. We acknowledge the time contributions and generous donations of grapes provided by Peter Bird and David Kohl (Casella Family Brands), Chris Mein (Shaw + Smith), David Blows (Spring Dale), Spiro Papadopoulos (Australian Vintage Ltd), and James Thorpe (Nova Vita Wines). We are thankful to our UA colleagues, Michelle Walker and Nick Van Holst for help with fermentation, and Ross Sanders for laboratory assistance. Sue Maffei from CSIRO is acknowledged for providing assistance with HPLC–MS/MS analysis. L.C. was a recipient of the joint scholarship of UA and China Scholarship Council (CSC201506350012), and was also supported by a Wine Australia supplementary scholarship (AGW Ph1512). The Australian Research Council Training Centre for Innovative Wine Production (www.ARCwinecentre.org.au; project number IC170100008) is funded by the Australian Government with additional support from Wine Australia and industry partners. The University of Adelaide and CSIRO are members of the Wine Innovation Cluster.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.

References

Alegre, Y., Culleré, L., Ferreira, V. and Hernández-Orte, P. (2017). Study of the influence of varietal amino acid profiles on the polyfunctional mercaptans released from their precursors. *Food Research International, 100*, 740-747.

Allen, T., Herbst-Johnstone, M., Girault, M., Butler, P., Logan, G., Jouanneau, S., Nicolau, L. and Kilmartin, P. A. (2011). Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon blanc wines. *Journal of Agricultural and Food Chemistry, 59*(19), 10641-10650.

Álvarez, I., Aleixandre, J. L., García, M. J. and Lizama, V. (2006). Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. *Analytica Chimica Acta, 563*(1-2), 109-115.

Anjum, N. A., Aref, I. M., Duarte, A. C., Pereira, E., Ahmad, I. and Iqbal, M. (2014). Glutathione and proline can coordinately make plants withstand the joint attack of metal(loid) and salinity stresses. *Frontiers in Plant Science, 5*, 662.

Baiano, A., Terracone, C., Longobardi, F., Ventrella, A., Agostiano, A. and Del Nobile, M. A. (2012). Effects of different vinification technologies on physical and chemical characteristics of Sauvignon blanc wines. *Food Chemistry*, *135*(4), 2694-2701.

Bonnaffoux, H., Delpech, S., Rémond, E., Schneider, R., Roland, A. and Cavelier, F. (2018). Revisiting the evaluation strategy of varietal thiol biogenesis. *Food Chemistry*, *268*, 126-133.

Brown, M. S. (1975). Wine from frozen grapes. *American Journal of Enology and Viticulture, 26*(2), 103-104.

Burin, V. M., Gomes, T. M., Caliari, V., Rosier, J. P. and Bordignon Luiz, M. T. (2015). Establishment of influence the nitrogen content in musts and volatile profile of white wines associated to chemometric tools. *Microchemical Journal*, *122*, 20-28.

Capone, D. L., Barker, A., Williamson, P. O. and Francis, I. L. (2017). The role of potent thiols in Chardonnay wine aroma. *Australian Journal of Grape and Wine Research*, *24*(1), 38-50.

Capone, D. L. and Jeffery, D. W. (2011). Effects of transporting and processing Sauvignon blanc grapes on 3-mercaptohexan-1-ol precursor concentrations. *Journal of Agricultural and Food Chemistry, 59*(9), 4659-4667.

Capone, D. L., Ristic, R., Pardon, K. H. and Jeffery, D. W. (2015). Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis. *Analytical Chemistry, 87*(2), 1226-1231.

Capone, D. L., Sefton, M. A., Hayasaka, Y. and Jeffery, D. W. (2010). Analysis of precursors to wine odorant 3-mercaptohexan-1-ol using HPLC-MS/MS: resolution and quantitation of diastereomers of 3-*S*-cysteinylhexan-1-ol and 3-*S*-glutathionylhexan-1-ol. *Journal of Agricultural and Food Chemistry, 58*(3), 1390-1395.

Capone, D. L., Sefton, M. A. and Jeffery, D. W. (2011). Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. *Journal of Agricultural and Food Chemistry, 59*(9), 4649-4658.

Chen, L., Capone, D. L. and Jeffery, D. W. (2018). Identification and quantitative analysis of 2-methyl-4-propyl-1,3-oxathiane in wine. *Journal of Agricultural and Food Chemistry*. *66*(41), 10808-10815.

Chen, L., Capone, D. L., Tondini, F. A. and Jeffery, D. W. (2018). Chiral polyfunctional thiols and their conjugated precursors upon winemaking with five *Vitis vinifera* Sauvignon blanc clones. *Journal of Agricultural and Food Chemistry, 66*(18), 4674-4682.

Coetzee, C. and du Toit, W. J. (2012). A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. *Food Research International, 45*(1), 287-298.

Culbert, J. A., McRae, J. M., Condé, B. C., Schmidtke, L. M., Nicholson, E. L., Smith, P. A., Howell, K. S., Boss, P. K. and Wilkinson, K. L. (2017). Influence of production method on the chemical composition, foaming properties, and quality of Australian carbonated and sparkling white wines. *Journal of Agricultural and Food Chemistry, 65*(7), 1378-1386.

Dai, Z. W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.-P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen, C., Vivin, P. and Delrot, S. (2011). Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: A review. *American Journal of Enology and Viticulture*, *62*, 413-425.

Fracassetti, D., Stuknytė, M., La Rosa, C., Gabrielli, M., De Noni, I. and 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.

Galant, A., Preuss, M. L., Cameron, J. C., and Jez, J. M. (2011). Plant glutathione biosynthesis: Diversity in biochemical regulation and reaction products. *Frontiers in Plant Science, 2*, 45.

Hernández-Orte, P., Ibarz, M. J., Cacho, J. and Ferreira, V. (2006). Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. *Food Chemistry*, *98*(2), 300-310.

Jeffery, D. W. (2016). Spotlight on varietal thiols and precursors in grapes and wines. *Australian Journal of Chemistry, 69*(12), 1323-1330.

Jouanneau, S., Weaver, R. J., Nicolau, L., Herbst-Johnstone, M., Benkwitz, F. and Kilmartin, P. A. (2012). Subregional survey of aroma compounds in Marlborough Sauvignon Blanc wines. *Australian Journal of Grape and Wine Research, 18*(3), 329-343.

Martin, D., Grose, C., Fedrizzi, B., Stuart, L., Albright, A. and McLachlan, A. (2016). Grape cluster microclimate influences the aroma composition of Sauvignon blanc wine. *Food Chemistry, 210*, 640-647.

Moreno-Pérez, A., Vila-López, R., Fernández-Fernández, J. I., Martínez-Cutillas, A. and Gil-Muñoz, R. (2013). Influence of cold pre-fermentation treatments on the major volatile compounds of three wine varieties. *Food Chemistry, 139*(1–4), 770-776.

OIV. Distribution of the world's grapevine varieties. (2017) http://www.oiv.int/public/medias/5888/en-distribution-of-the-worlds-grapevine-varieties.pdf Accessed 4 February 2019.

Olejar, K. J., Fedrizzi, B. and Kilmartin, P. A. (2015). Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. *Food Chemistry, 183*, 181-189.

Ortega-Heras, M., Pérez-Magariño, S., Del-Villar-Garrachón, V., González-Huerta, C., Moro Gonzalez, L. C., Guadarrama Rodríguez, A., Villanueva Sanchez, S., Gallo González, R. and

Martín de la Helguera, S. (2014). Study of the effect of vintage, maturity degree, and irrigation on the amino acid and biogenic amine content of a white wine from the Verdejo variety. *Journal of the Science of Food and Agriculture, 94*(10), 2073-2082.

Ouellet, É. and Pedneault, K. (2016). Impact of frozen storage on the free volatile compound profile of grape berries. *American Journal of Enology and Viticulture, 67*(2), 239-244.

Pando Bedriñana, R., Picinelli Lobo, A. and Suárez Valles, B. (2019). Influence of the method of obtaining freeze-enriched juices and year of harvest on the chemical and sensory characteristics of Asturian ice ciders. *Food Chemistry, 274*, 376-383.

Park, S. K., Boulton, R. B. and Noble, A. C. (2000). Formation of hydrogen sulfide and glutathione during fermentation of white grape musts. *American Journal of Enology and Viticulture, 51*(2), 91-97.

Peinado, R. A., Moreno, J., Bueno, J. E., Moreno, J. A. and Mauricio, J. C. (2004). Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry, 84*(4), 585-590.

Peng, C.-T., Wen, Y., Tao, Y.-S. and Lan, Y.-Y. (2013). Modulating the formation of Meili wine aroma by prefermentative freezing process. *Journal of Agricultural and Food Chemistry, 61*(7), 1542-1553.

Pinu, F. R., Edwards, P. J. B., Jouanneau, S., Kilmartin, P. A., Gardner, R. C. and Villas-Boas, S. G. (2014). Sauvignon blanc metabolomics: grape juice metabolites affecting the development of varietal thiols and other aroma compounds in wines. *Metabolomics, 10*(4), 556-573.

Pinu, F. R., Jouanneau, S., Nicolau, L., Gardner, R. C. and Villas-Boas, S. G. (2012). Concentrations of the volatile thiol 3-mercaptohexanol in Sauvignon blanc wines: no correlation with juice precursors. *American Journal of Enology and Viticulture, 63*(3), 407-412.

Pinu, F. R., Tumanov, S., Grose, C., Raw, V., Albright, A., Stuart, L., Villas-Boas, S. G., Martin, D., Harker, R. and Greven, M. (2019). Juice Index: an integrated Sauvignon blanc grape and wine metabolomics database shows mainly seasonal differences. *Metabolomics, 15*, 3.

Roland, A., Schneider, R., Charrier, F., Cavelier, F., Rossignol, M. and 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.

Roland, A., Schneider, R., Razungles, A. and Cavelier, F. (2011). Varietal thiols in wine: Discovery, analysis and applications. *Chemical Reviews, 111*(11), 7355-7376.

Sacchi, K. L., Bisson, L. F. and Adams, D. O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *American Journal of Enology and Viticulture, 56*(3), 197-206.

Santiago, M. and Gardner, R. C. (2015). Yeast genes required for conversion of grape precursors to varietal thiols in wine. *FEMS Yeast Research, 15*(5), 1-10.

Spayd, S. E. and Andersen-Bagge, J. (1996). Free amino acid composition of grape juice from 12 *Vitis vinifera* cultivars in Washington. *American Journal of Enology and Viticulture, 47*(4), 389-402.