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INTRAREGIONAL PROFILES OF VARIETAL THIOLS AND PRECURSORS IN SAUVIGNON BLANC JUICES AND WINES FROM THE ADELAIDE HILLS

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Abstract

Aims: To investigate the intraregional variation of varietal thiol precursors and free thiols in Sauvignon blanc grape juices and experimental wines arising from the Adelaide Hills Geographical Indication (GI) in South Australia.

Methods and Results: *Vitis vinifera* L. cv Sauvignon blanc grape parcels (n = 21, approx. 8 kg each, encompassing 5 clones) were hand harvested from different blocks within seven commercial vineyards in the Adelaide Hills GI during the 2018 vintage. Parcels were divided into subsets for winemaking and freezing experiments. Amino acid (AA) and thiol precursor concentrations in juice were determined using high performance liquid chromatography (HPLC) with fluorescence detection and stable isotope dilution assay (SIDA) using HPLC with tandem mass spectrometry (MS/MS), respectively, and free thiols in wine were quantified by SIDA with HPLC-MS/MS, after derivatisation with 4,4'-dithiodipyridine. Intraregional variations in grape ripeness were evident according to total soluble solids content, pH, and titratable acidity, even within single locations or for the same clones. Significant differences in the glutathionylated precursor to 3-sulfanylhexan-1-ol (3-SH) were found among several locations whereas for the cysteinylated variant of 3-SH, one location was distinct from the rest. Variation in precursor concentrations was also noted from different blocks within a single vineyard location but was not dependent on grape ripeness. Fermentations progressed without any obvious relationship to location, and wines that were high in 3-SH were also usually high in 3-sulfanylhexyl acetate (3-SHA). One location had significantly higher levels of thiols in wine despite the juice not being the highest for grape-derived precursors, and also gave a substantial concentration of 4-methyl-4-sulfanylpentan-2-one in comparison to other locations within the GI. The AA profile of juices was found to vary according to location, and certain AAs were strongly correlated to thiol precursor concentrations, but relationships of AAs with free thiols in wine were generally weak. Additionally, enhancements in the concentrations of precursors in juice (up to 19-fold) and free thiols in wine (up to 10-fold) were revealed from freezing whole grape bunches in contrast to using fresh juice.

Conclusions: Intraregional variation was noted for thiols in wine, and precursors and amino acids in juice, for 21 Sauvignon blanc samples collected from within the Adelaide Hills region. The effects of terroir were implicated in explaining the differences in grape composition, and the potential interactions among grape amino acids and thiol precursors in berries and thiols in wine were revealed.

Significance and Impact of the Study: Sauvignon blanc is a significant variety produced in the Adelaide Hills GI but no information was available on the effects of location within the GI on grape and wine composition with respect to varietal thiols. This was the first study of intraregional variations of thiol precursors, amino acids, and free thiols in Sauvignon blanc juices and wines that were produced in a consistent manner. A remarkable enhancing effect of freezing was noted for thiol precursors in juice, and importantly, free thiols in wine.

Keywords: *Vitis vinifera*, terroir, chemical composition, polyfunctional thiols, wine aroma, winemaking

Introduction

Sauvignon blanc is a globally-important white grape variety and one that expresses the terroir of the region in which it is grown (Berna *et al.*, 2009; Lund *et al.*, 2009). Sensory characters are driven most notably by varietal thiols such as 3-sulfanylhexan-1-ol (3-SH), 3-sulfanylhexyl acetate (3-SHA) and 4-methyl-4-sulfanylpentan-2-one (4-MSP), which impart 'tropical fruit' and 'citrus' characters, and by methoxypyrazines in some instances, especially 3-isobutyl-2-methoxypyrazine (IBMP), which contributes 'green capsicum' attributes (Coetzee and Du Toit, 2012). Although not specific to Sauvignon blanc, fermentation-derived volatiles such as esters can also be important (including for consumer liking) (King *et al.*, 2011), and the action of fermentation is certainly a necessity to enzymatically release the varietal thiols form their grape-derived, non-volatile precursors (Jeffery, 2016).

Many factors influence the composition of grapes and thus the volatile and sensory profiles of wine. Importantly, biophysical aspects of terroir such as climate and soil have strong influences, as do the water and nitrogen statuses of the vines (Peyrot Des Gachons *et al.*, 2005). These factors influence not only thiol precursors in the grape berry but also other metabolites such as amino acids. In turn, both of these compound classes are transformed during fermentation, producing volatiles like the varietal thiols and esters mentioned above. Thus, wine composition and sensory qualities are intimately linked with the terroir (inclusive of cultural practices) where the grapes are produced.

Considering the scales of terroir and that biophysical factors can vary at macro and meso levels (Priori *et al.*, 2019), this study aimed to investigate the presence of intraregional variation of varietal thiols and precursors and their relationship with amino acid composition, using Sauvignon blanc fruit harvested from 21 vineyards within the Adelaide Hills Geographical Indication (GI) of South Australia. The impact on the concentration of thiols in wine due to pre-fermentation freezing of grapes or juice was also explored.

Materials and Methods

Parcels of *Vitis vinifera* L. cv Sauvignon blanc grapes (n = 21, approx. 8 kg for each) encompassing five clones were hand-harvested in February and March of 2018 from commercial vineyards (n = 7, Figure 1) located in the cool-climate Adelaide Hills GI of South Australia (Chen *et al.*, 2019). Approx. 5 kg of each sample was hand crushed and juices were collected for triplicate fermentation (Control) and frozen (one month) for the frozen juice experiments. Approx. 3 kg of each sample was carefully stored frozen as whole grape bunches for one month, then thawed, crushed and the juices were fermented as per the other treatments, using VIN13 yeast strain at 16 °C. Amino acids, varietal thiols and their precursors were analysed according to literature procedures, and data analysis was undertaken as previously reported (Chen *et al.*, 2019).

Results and Discussion

Grape parcels were collected from 21 sites in the Adelaide Hills at commercial harvest, with a targeted grape juice total soluble solids value of 20-21 °Brix. Most values ranged between 19 to 22 °Brix, with slight differences in ripeness (data not shown) within single locations (including for the same clone type) and across the GI attributed to variability in ecophysiology and cultural practices (Chen *et al.*, 2019). Fermentations proceeded to dryness and did not present any obvious differences according to GI.

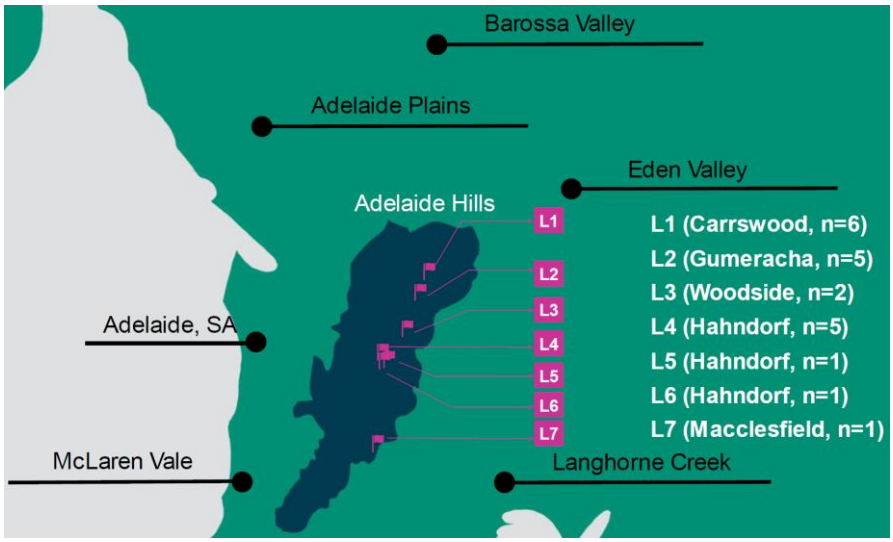

Figure 1: South Australian GI showing the Adelaide Hills wine region where the grapes were sampled from (Locations 1–7). Reproduced from Food Chemistry, 295, Chen, L., Capone, D. L., Nicholson, E. L., & Jeffery, D. W., Investigation of intraregional variation, grape amino acids, and pre-fermentation freezing on varietal thiols and their precursors for *Vitis vinifera* Sauvignon blanc, 637-645, Copyright 2019, with permission from Elsevier.

The profiles of glutathionylated and cysteinylated thiol precursors in juice (GSH-3-SH and Cys-3-SH) and varietal thiols in wines (3-SH, 3-SHA and 4-MSP) were investigated for the different sites (Table 1). Thiol precursors were detected in all juices, with GSH-3-SH (34–171 µg/L) being more abundant than Cys-3-SH (11–45 µg/L) in the same juice, as is frequently the case (Capone *et al.*, 2010; Roland *et al.*; 2011, Pinu *et al.*; 2012, Fracassetti *et al.*; 2018). Concentrations of precursors generally aligned with the literature (Jeffery, 2016) and there was a strong positive correlation (r = 0.98) between the two precursor types, as expected based on their biochemical relationship (Chen *et al.*, 2019).

Significant differences occurred between locations for GSH-3-SH, with the means for Carrswood (118 µg/L) and Woodside (112 µg/L) both being higher than Gumeracha (58 µg/L) and Hahndorf (50 µg/L). For Cys-3-SH, only Carrswood (34 µg/L) was significantly different from the group means of other locations (ranging from 13 to 20 µg/L). Within locations containing several blocks, Cys-3-SH varied by a factor of around 1.4–1.7 whereas GSH-3-SH varied by 1.3–3.6 (data not shown), seemingly independent of ripeness of the berries (known to affect precursor concentrations (Capone *et al.*, 2012)). This variation might imply that ecophysiological effects and grapevine genetics play a larger role in accumulation of GSH-3-SH as opposed to Cys-3-SH, given that post-harvest processing was identical. Precursor concentrations were previously found to vary by one or two orders of magnitude for Sauvignon blanc juices from different locations in New Zealand (including sub-regions of Marlborough) (Pinu *et al.*, 2012), although that study was not specifically investigating regional influences and involved commercial juices that were unlikely to have been handled consistently. This is important to consider when aiming to evaluate the role of terroir, given the various impacts of post-harvest treatment of grapes and the prospective ability to increase the juice concentration of GSH-3-SH more markedly than Cys-3-SH (Jeffery, 2016; Allen *et al.*, 2011).

Table 1: Mean concentrations (± standard deviation) for thiol precursors (µg/L) in Sauvignon blanc juices and varietal thiols (ng/L) in resulting wines from samples (n = 21) collected within the Adelaide Hills GI.

		Precursors		Varietal thiols		
Entry	**Location**	**GSH-3-SH**	**Cys-3-SH**	**3-SH**	**3-SHA**	**4-MSP**
1	**Carrswood**	171 ± 4	45 ± 4	74 ± 4	10 ± 3	n.d.
2		111 ± 4	30 ± 0	42 ± 12	5 ± 1	n.d.
3		119 ± 8	30 ± 4	75 ± 30	12 ± 4	n.d.
4		89 ± 3	33 ± 0	54 ± 6	6 ± 2	n.d.
5		95 ± 3	32 ± 2	72 ± 8	5 ± 1	4 ± 1
6		122 ± 4	33 ± 9	126 ± 69	21 ± 14	3[a]
7	**Gumeracha**	36 ± 0	11 ± 0	53 ± 21	9 ± 4	6 ± 3
8		123 ± 11	18 ± 0	197 ± 104	19 ± 10	36 ± 12
9		34 ± 3	11 ± 0	514 ± 14	36 ± 4	40 ± 3
10		53 ± 2	11 ± 0	192 ± 26	22 ± 4	29 ± 4
11		46 ± 3	12 ± 0	46 ± 3	4 ± 0	6 ± 1
12	**Woodside**	146 ± 7	24 ± 1	69 ± 27	12 ± 5	n.d.[b]
13		78 ± 14	16 ± 1	52 ± 3	8 ± 2	n.d.
14	**Hahndorf**	52 ± 1	18 ± 2	442 ± 66	24 ± 0	46 ± 4
15		44 ± 2	13 ± 1	151 ± 85	20 ± 8	20 ± 5
16		50 ± 3	13 ± 0	293 ± 25	18 ± 5	30 ± 4
17		48 ± 1	16 ± 1	195 ± 31	18 ± 2	6 ± 1
18		57 ± 2	17 ± 2	97 ± 14	11 ± 2	5[a]
19		80 ± 4	17 ± 2	386 ± 12	47 ± 6	94 ± 4
20		72 ± 3	17 ± 0	40 ± 1	6 ± 1	4 ± 1
21	**Macclesfield**	65 ± 1	15 ± 1	386 ± 12	11 ± 1	24 ± 12

[a] Only one replicate was found with a detectable amount of the analyte. [b] n.d., not detected (limit of detection < 3 ng/L); for statistical purposes the value was set to half the limit of detection.

Varietal thiols in wines from the respective juices were determined (Table 1) and ranged from 40–514 ng/L for 3-SH, 5–36 ng/L for 3-SHA, and from undetectable to a relatively high value of 94 ng/L for 4-MSP at one Hahndorf location (Entry 19). In the main, the thiols were present above their respective odour detection thresholds, meaning they would be expected to contribute 'tropical fruit' aromas to the wines. Although tending to be lower than found in commercial Sauvignon blanc wines (Lund *et al.*, 2009), the concentrations of 3-SH and 3-SHA were consistent with other studies on experimental wines produced with Sauvignon blanc from the Adelaide Hills (Capone *et al.*, 2011; Chen *et al.*, 2018). Notably, 3-SH correlated strongly with 3-SHA (r = 0.86), as could be expected based on the acetylation pathway that yields 3-SHA from 3-SH (Roland *et al.*, 2011).

Variations for the three thiols were similar across the locations, especially with respect to 3-SHA and 4-MSP. One Hahndorf site (Entry 19, Table 1) stood out with significantly higher values for thiols compared to other locations. On the other hand, Woodside locations and another Hahndorf site (Entry 20) were lower in thiols. Despite the likely importance of thiol precursors in the formation of thiols in wine, previous research has shown no correlation between thiols and known precursors (e.g. Pinu *et al.*, 2012; Jeffery, 2016), and there was little resemblance in their patterns of variation in the present work. Carrswood was a noteworthy example, whereby relatively high precursor concentrations did not translate into wines with the highest amounts of thiols among the locations. Correlations between the precursor types and 3-SH/3-SHA ended up being weak and negative (around r = −0.2 to −0.4; weaker when considering 3-SHA than 3-SH). Evaluation of other datasets showed that 3-SH was correlated positively and weakly with GSH-3-SH (r = 0.32 and 0.40) whereas essentially no correlation existed with Cys-3-SH (r = −0.05 and −0.11), as discussed previously (Chen *et al.*, 2019). These outcomes highlight the complicated nature of varietal thiol formation during winemaking and the need to conduct further studies on the fate of precursors and thiols.

Amino acids (AAs) are involved in the formation of varietal thiol precursors in grape berry and in the biochemical pathways associated with alcoholic fermentation. Both aspects have potential implications for varietal thiol concentrations in wine but few publications have dealt specifically with the impact of AAs on thiol production (Alegre *et al.*, 2017; Pinu *et al.*, 2014; Pinu *et al.*, 2019). The AA profiles of the 21 Sauvignon blanc juices obtained

from within the Adelaide Hills GI were determined (data not shown) so that insight into precursor formation and thiol production could be further addressed. Total AA concentrations were calculated to be 390–1091 mg/L, with the main AAs being arginine (mean of 146 mg/L), proline (124 mg/L), glutamic acid (124 mg/L), γ-amino butyric acid (75 mg/L), and α-alanine (52 mg/L) (Chen *et al.*, 2019). Individual amino acids varied among the different locations (having differences in elevation that impact mean January temperature and rainfall), which may be attributable to factors associated with terroir, such as climate, irrigation and fertilisation (Ortega-Heras *et al.*, 2014).

Total AAs in juice correlated only weakly with thiols in wine ($r < 0.2$), whereas the correlations of 3-SH and 3-SHA with glutamic acid and proline ranged from $r = 0.32$ to $r = 0.42$. Glutamic acid (as well as glutamine and GABA) has previously been correlated with thiol production and shown a significant enhancing effect (Pinu *et al.*, 2014). It is a preferred nitrogen source for fermentation and its relevance may in general relate to yeast metabolism (including acetylation activity) and/or thiol precursor uptake (Alegre *et al.*, 2017; Cordente *et al.*, 2015). The importance of proline was not determined, although it is a non-preferred nitrogen source for yeast. Notably and apparently for the first time, it was shown that thiol precursors correlated better with a wider number of amino acids in comparison to the free thiols. Glutamic acid was again prominent, albeit with a strong negative correlation to precursors ($r \leq -0.73$). In addition, glycine ($r \geq 0.62$), GABA ($r \geq 0.59$), alanine ($r \leq -0.55$), and isoleucine ($r \geq 0.55$) had important correlations. Together, the results were indicative of biochemical interactions among AAs and precursors in grape berry during ripening, especially considering that glutamic acid and glycine are AA residues within the tripeptide glutathione, which is a natural plant antioxidant (Galant *et al.*, 2011) and metabolically-related to both glutathionylated and cysteinylated thiol precursor types (Chen *et al.*, 2019). Principal component analysis (data not shown) reinforced some overall trends with regard to AAs, thiols, and precursors with respect to location (Chen *et al.*, 2019). Carrswood and Woodside samples were typically characterised by higher amounts of thiol precursors along with glycine and GABA, whereas Gumeracha and Hahndorf samples tended to be higher in thiols but relatively low in most AAs. Nonetheless, within sub-region variability was also noticeable, highlighting that the influence of terroir might be discernible within a GI.

Although less about addressing aspects of terroir, the study also assessed the influence on thiols in wine as a result of freezing either grape bunches or juice prior to fermentation. As alluded to earlier, there can be post-harvest changes to thiol precursor concentrations, and the enhancing effect of freezing grape bunches has already been reported (Capone *et al.*, 2011). Additionally, cryomaceration of Sauvignon blanc must with dry ice (−20 °C to 15 °C over 24 h) had been found to increase varietal thiol concentrations (Olejar *et al.*, 2015). In the present study, grape bunches and grape juice from five clones grown at a single location were frozen for 30 days at −20 °C, for comparison with wine made from fresh juice (Chen *et al.*, 2019). Compared to fresh juice (Entries 14-18, Table 1), there were significant changes in precursor concentrations as a result of freezing grape bunches, with increases of up to 19 and 6 times, respectively, for GSH-3-SH (mean of 724 µg/L) and Cys-3-SH (mean of 73 µg/L). 3-SH in the subsequent wines was also significantly elevated as a result of freezing bunches (mean of 1139 ng/L) more so than freezing juice (mean of 526 ng/L), with both treatments being higher than the wine prepared with fresh juice (mean of 223 ng/L). Similar trends were observed for 3-SHA and 4-MSP, with larger increases in concentration as a result of freezing bunches rather than juice. These findings reinforced the notion of being able to augment varietal thiol concentrations in wine by undertaking pre-fermentation freezing of fruit.

Conclusions

This was the first study of intraregional variations of thiol precursors, amino acids, and free thiols in Sauvignon blanc juices and wines that were produced in a consistent manner, using fruit collected from 21 sites within the Adelaide Hills wine region. Differences in precursor and thiol concentrations were evident among the sub-regions, with the effects of terroir being implicated in explaining the differences in grape composition. The impacts of terroir extend to amino acid profiles, with potential interactions between grape amino acids being revealed with respect to thiol precursor formation in berries and thiol production during fermentation. In addition, pre-fermentation freezing of grape bunches led to dramatic increases in precursor concentrations in juice and thiol concentrations in the subsequent wines. This may open the way for further investigation of cryomacerative conditions that can enhance varietal thiol production during winemaking, which could add a further cultural element to the expression of terroir in wines of provenance.

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