Role of extraction procedures on the concentration of varietal thiol precursors in Grillo white grape must

A. TIRELLI[1], I. DE NONI[1], M. STUKNYTÉ[2], V. PICA[1] and D. FRACASSETTI[1]

[1] Department of Food Environmental and Nutritional Sciences, Università degli Studi di Milano, Milan 20133, Italy; [2] Unitech COSPECT—University Technological Platforms Office, Università degli Studi di Milano, Milan 20133, Italy
Corresponding author: Professor Antonio Tirelli, email antonio.tirelli@unimi.it

Abstract

Background and Aims: Varietal thiol precursors (VTPs) decrease strongly after the pre-fermentative operations. The effect of grape must extraction steps on the concentration of S-3-(hexan-1-ol)-L-glutathione (G-3SH), S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) and S-3-(hexanal)-glutathione (G-3SHal) was investigated.

Methods and Results: Must produced on a commercial scale was sampled throughout the pre-fermentation steps. Must was also prepared in the laboratory for assessing the role of copper, sulfite and air. In commercial must fractions, over 95% of G-3SHal was lost because of sulfite addition, while the concentration of G-3SH and Cys-3SH increased along with must yield, and it exceeded the concentration found in berries. No Cys-3SH was found in the intact grape; instead a trace concentration of G-SHal, and a high concentration of glutathione and even cysteine was determined when sulfite was added. A large amount of G-3SHal was formed when copper was depleted.

Conclusions: Our data suggest that Cys-3SH and G-3SHal are promptly and mainly formed in grape must upon grape pressing. Sulfite and copper prevented the formation of VTPs, while settling of must led to a loss of VTPs.

Significance of the Study: Varietal thiol precursors can be preserved through the proper management of pressing with particular attention to must contact with air, extraction conditions, contact time with solids and addition of sulfite.

Keywords: *air, copper, glutathione, grape juice, sulfur dioxide, thiol precursors*

Introduction

The varietal volatile thiols, 4-sulfanyl-4-methyl-2-pentanone (4MMP), 3-sulfanyl-1-hexanol (3SH) and its acetate ester, are flavour compounds present in some white and rose wines. These compounds are characterised by a low olfactory perception threshold corresponding to nanograms per litre (Darriet et al. 1995, Tominaga et al. 1998, Roland et al. 2011, Coetzee and Du Toit 2012). These compounds are released in wine as a result of the β-lyase activity of the yeast during the alcoholic fermentation. In grape and must, varietal thiols occur in odourless forms bound with glutathione (G) or cysteine (Cys), as S-3-(hexan-1-ol)-L-glutathione (G-3SH), S-3-(hexan-1-ol)-L-cysteine (Cys-3SH), S-4-(4-methyl-pentan-2-one)-L-glutathione and S-4-(4-methyl-pentan-2-one)-L-cysteine, as well as with the dipeptides, as S-3-(hexan-1-ol)-glutamyl-cysteine (GluCys-3SH) and S-3-(hexan-1-ol)-cysteine-glycine (CysGly-3SH) (Bonnaffoux et al. 2017, 2018). Recently, Thibon et al. (2016) identified the S-3-(hexanal)-glutathione (G-3SHal) as an additional precursor of G-3SH. The pathway of Cys-3SH, the direct precursor of 3SH in wine, and the main chemical factors affecting its formation in grape and must are summarised in Figure 1. Linolenic and linoleic acids are released by lipase enzymes from the grape berry phospholipids and then oxidised and split by lipoxygenase and hydroperoxide lyase enzymes, respectively, giving six-carbon aliphatic aldehydes (Joslin and Ough 1978, Iglesias et al. 1991, Hatanaka et al. 1995, Qian et al. 2017). The peroxidation of linolenic acid gives rise to (E)-2-hexenal which can undergo an electrophilic addition (i.e. Michael reaction) with glutathione (GSH) to produce G-3SHal (Clark and Deed 2018). The latter can be then reduced to G-3SH by alcohol dehydrogenase enzymes of grape berries (Fedrizzi et al. 2012) and also by yeasts (Thibon et al. 2016). Eventually, the yeast can hydrolyse G-3SH to Cys-3SH and split the latter to release 3SH (Roland et al. 2010, Kobayashi et al. 2011). S-3-(Hexan-1-ol)-L-cysteine can be released from G-3SH in grape berries as well, and widely variable yields have been reported for different grape cultivars and grape must extraction conditions (Peña-Gallego et al. 2012).

Several factors can affect the concentration of varietal thiol precursors (VTPs) in grape must. Copper ions can hinder the formation of G-3SHal as it is able to oxidise GSH (Kachur et al. 1998). Moreover, copper can also bind GSH, as it occurs with other thiol compounds (Kreitman et al. 2016) or even induce reactions with other compounds as recently reviewed by Clark et al. (2015). Both (E)-2-hexenal and G-3SHal as well as GSH can bind SO_2 thus preventing the yeast-mediated formation of G-3SH (Arapitsas et al. 2016, Thibon et al. 2016, Jastrembski et al. 2017). Clark and Deed (2018), however, suggested that the sulfonated (E)-2-hexenal binds GSH generating G-3SHal more easily than the free aldehyde.

Both vine growing and winemaking practices affect the concentration of varietal thiols (VTPs) (Coetzee and Du Toit 2012, Aleixandre-Tudo et al. 2015, Olejar et al. 2015). Fermenting yeast strains, belonging to both *Saccharomyces* and non-*Saccharomyces* genera, strongly influence the release of varietal thiols (Fracassetti and Vigentini 2018, Ruiz et al. 2019). Among the oenological practices, the use of exogenous enzymes (claimed to contain secondary

doi: 10.1111/ajgw.12514

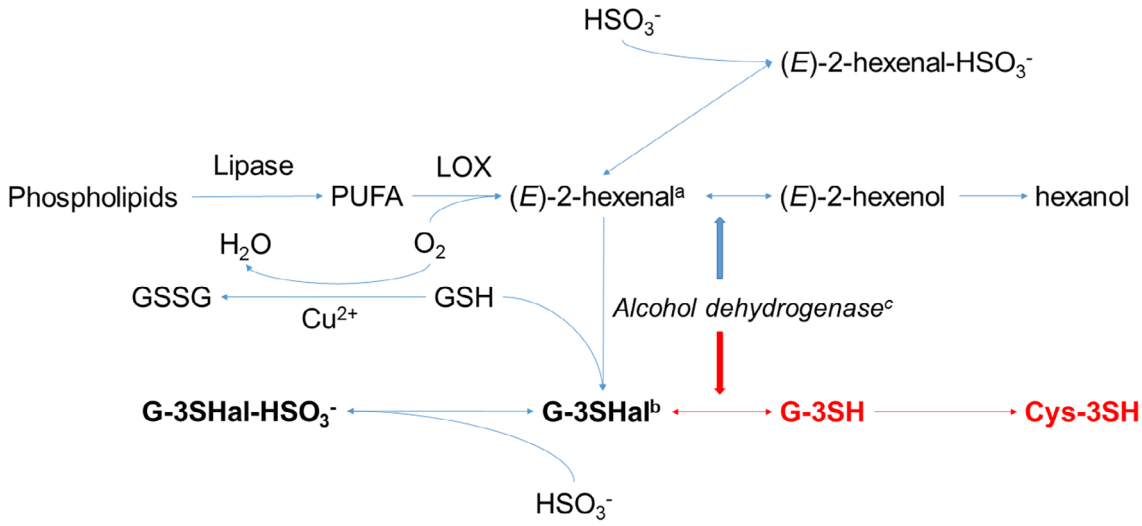

Figure 1. Pathway for the formation of S-3-(hexan-1-ol)-L-cysteine in grape and must. The release of 3-sulfanyl-1-hexanol occurring in wine is shown in green. PUFA, polyunsaturated fatty acids; LOX, lipoxygenase; GSSG, glutathione disulfide; GSH, glutathione; G-3SHal, S-3-(hexanal)-glutathione; G-3SHal-HSO_3^-, sulfonated S-3-(hexanal)-glutathione; G-3SH, S-3-(hexan-1-ol)-L-glutathione; Cys-3SH, S-3-(hexan-1-ol)-L-cysteine; 3SH, 3-sulfanyl-1-hexanol. [a]Joslin and Ough (1978), Iglesias et al. (1991), Hatanaka et al. (1995), Qian et al. (2017); [b]Clark and Deed (2018); [c]Fedrizzi et al. (2012).

enzymatic activities along with pectinase) appeared to enhance the concentration of varietal thiols, while the nutrients for yeast did not (Chen et al. 2018). The grape clone plays an important role in the concentration of VTPs in must (Chen et al. 2018). Recently, it was demonstrated that freezing of grape and juice led to an increase in the concentration of both VTPs and varietal thiols (Chen et al. 2019). Nonetheless, few research studies have investigated the role of grape must extraction steps on the formation of the VTPs. Fracassetti et al. (2018) reported that up to 95% of the VTPs are lost in the grape cultivars Catarratto Bianco Comune and Grillo after the pre-fermentative steps. Therefore, a major goal is to understand the role of the technological factors affecting the concentration of VTPs in grape must in order to protect the flavour of wine.

This research aimed to monitor the concentration of VTPs during the grape must extraction steps until must clarification under commercial winemaking conditions. Musts were also produced in the laboratory from the Grillo grape from the same batch obtained under different conditions in terms of presence of oxygen, addition of SO_2 and depletion of copper ions. The production of musts under laboratory conditions can allow the clarification of results arising from the commercial trials and to establish the factors affecting the concentration of VTPs.

Materials and methods

Chemicals and reagents

Methanol, ethanol, acetonitrile, formic acid, anhydrous tetrahydrofuran, sodium fluoride, EDTA, glutathione (GSH), *p*-benzoquinone (pBQ), 3-mercaptopropanoic acid (3MPA), tartaric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Sodium metabisulfite was purchased from J.T. Baker (Deventer, Holland). 3-(Hexan-1-ol)-L-glutathione (G-3SH), G-3SHal, G-4MMP, Cys-3SH and d_1-G-3SH were synthesised and used for quantification of G-3SH, G-3SHal, Cys-3SH; the deuterated G-3SH was used as an internal standard (IS) (Fracassetti et al. 2018). All chemicals were at least of analytical grade. A Milli-Q system (Merck, Darmstadt, Germany) provided liquid chromatography-grade water.

Grape samples

The white grape cultivar Grillo was used for this study. The 5.5 ha vineyard Sant'Anna at Tenuta Regaleali, Sclafani Bagni, Sicily, Italy, was planted at 520 m above sea level average altitude and the row orientation was south-east at GPS HESI coordinates: 37.71°N, 13.85°E). Grape sampling and harvest were carried out in vintage 2018. The mass of bunches was 170–320 g, while that of berries was 3.4–3.8 g. Grapes were hand harvested in a good sanitary state when ripe (24.0 ± 0.2°Brix) into small containers (20 kg) in order to prevent berry damage.

Determination of varietal thiol precursors in berry fractions

Varietal thiol precursors were determined in the grape fractions, skins, pulp and seeds. Three aliquots of 20 grape berries were peeled and the skins, pulp and seeds were kept separately and placed in tubes maintained in ice. The VTPs were extracted with a tartrate buffer solution (5 g/L of tartaric acid adjusted to pH 3.2 with sodium hydroxide). Pulp, seeds and skins were supplemented with 5, 10 and 20 mL of tartrate buffer solution, respectively, and homogenised by a high-speed Ultra-Turrax T25 (IKA-Werke, Staufen, Germany) for 1 min, maintaining the samples in ice and under N_2 flow. The grape juice generated during the peeling was also collected in ice and under N_2 flow. The samples were immediately frozen at −20°C after preparation. The VTPs were determined in solid phase extraction-purified samples as described below.

Grape must extraction at commercial scale

Grillo grapes (6000 kg) were hand harvested when ripe, and the winemaking was carried out following the procedures usually adopted by the winery. In detail, grape bunches were destemmed on arrival at the winery and then supplemented with 3 g pectolytic enzyme (AST, Enartis San Martino Trecate, Italy)/100 kg of grapes and with 1 g commercial preparation containing 50% of potassium metabisulfite, 30% of ascorbic acid and 20% of nut gall tannins (AST)/100 kg of grapes. The berries were pressed in a closed-tank membrane press without air removal at room temperature (20 ± 2°C) in three sequential steps at

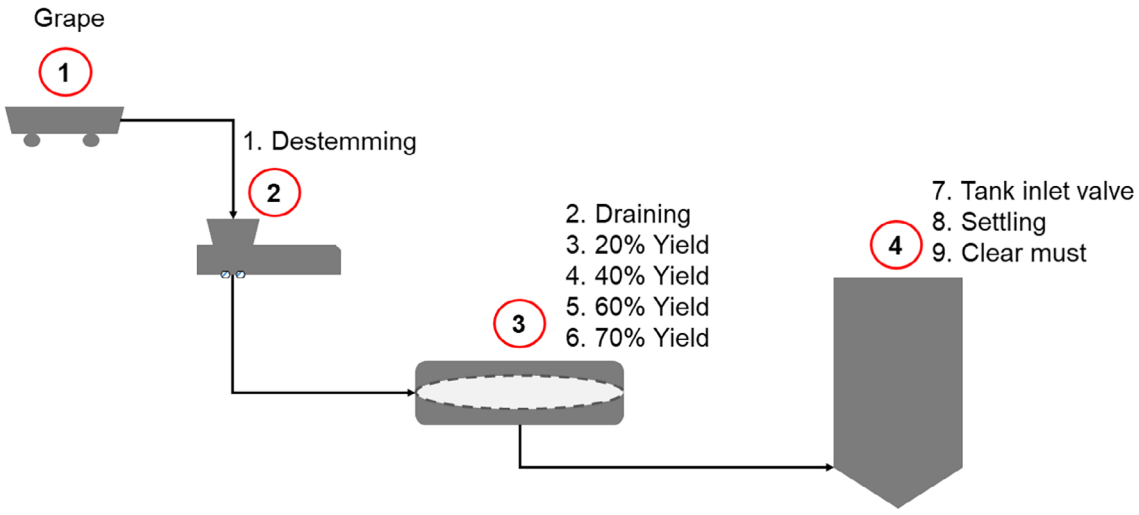

Figure 2. Sampling scheme of must fractions produced in the commercial winery.

20, 40 and 60 kPa for 45 min each obtaining, respectively, a must yield of about 20, 40 and 60%. The must was sampled again after pressing at 120 kPa for 120 min in which the must yield was about 70%. The must (50 hL) was supplemented with 30 mg/L of SO_2 to prevent oxidation, pumped into the clarification tank through plastic pipes, cooled down to 7°C, in order to limit enzymatic activity, and settled for 12 h. The must was extracted either under air-exposure [air-exposed musts (AEM)] or under air-free conditions [air-free must (AFM)], the latter obtained by saturation of the press (reductive pressing), pipes and tank with carbon dioxide. Must was sampled under N_2 flow.

The effect of must extraction steps on the concentration of VTPs in juice was evaluated in samples collected into 500 mL plastic bottles filled with N_2 at the following steps: after crushing (Destemming); after press-loading (Draining) and at must extraction yield steps of 20% (20% yield), 40% (40% yield), 60% (40% yield) and 70% (70% yield); during pumping to the clarification tank (Tank inlet valve); in the clarification tank (Settling); and after settling (Clear must). The nine samples of must (Figure 2) were promptly frozen to −18°C until analysis.

Preparation of must in the laboratory

The experimental design for must samples prepared in the laboratory included four conditions: (i) addition of sodium fluoride and EDTA (2 mL 0.12 mol/L NaF and 0.5 mL 0.06 mol/L EDTA) (Fracassetti and Tirelli 2015); (ii) washing and gentle drying of berry skins; (iii) addition of SO_2 (50 mg/kg); and (iv) Control (without addition of NaF and EDTA, and SO_2).

About 20 kg of Grillo grapes were randomly collected from the hand-harvested grapes and cooled to 4°C overnight. The berries were taken from bunches by cutting the pedicel to prevent any berry damage and divided into four equal samples. Each sample was further divided in two subsamples in order to obtain the experimental musts through: (i) pressing under air-exposed conditions; and (ii) pressing under air-free conditions. Subsamples were hand pressed either in a beaker in the case of the air-exposed condition or in gas-tight plastic bags packed under vacuum in the case of air-free condition. For the air-free condition, must samples were transferred into flasks filled with N_2 and under N_2 flow. Each replicate for both air-exposure conditions was washed separately with about 250 mL of 100 µmol/L citric acid solution. Each condition was prepared in triplicate, and thus 24 laboratory-made must samples were produced. All flasks were filled with N_2 and immediately frozen at −18°C until analysis. In addition to the juices obtained from cooled grape berries, triplicate juice samples were prepared in the presence of sodium fluoride and EDTA (2 mL 0.12 mol/L NaF and 0.5 mL 0.06 mol/L EDTA) using uncooled grapes pressed in gas-tight plastic bags packed under vacuum (Fracassetti and Tirelli 2015).

Determination of cysteine, glutathione and grape reaction product

The concentration of Cys, GSH and grape reaction product (GRP) in grape, must and wine samples was assessed as described by Fracassetti and Tirelli (2015). Briefly, must samples were centrifuged at 5000*g* for 10 min at 10°C in a bench top centrifuge (Hettich, Tuttlingen, Germany). Two mL of supernatant were collected and derivatised with *p*-benzoquinone. The concentration of Cys, GSH and GRP was determined by HPLC/UV at 303 nm following the separation conditions described by Fracassetti and Tirelli (2015).

Determination of varietal thiol precursors

Varietal thiol precursors were assessed in must samples and in extracts of grape berry fractions after SPE-purification using Strata X-polymeric cartridges (200 mg, Phenomenex, Torrance, CA, USA). After SPE activation, 2 mL of sample, spiked with d_1-G-3SH (500 µg/L; exact mass $[M + H^+]^+$: 409.159) IS, was loaded onto the SPE column and eluted with 4 mL of methanol after a washing step with 2 mL water. The solvent was evaporated under N_2 flow to 400 µL (Fracassetti et al. 2018). Thiol precursors were detected and quantified by UPLC/electrospray ionisation (ESI) high-resolution MS [Acquity UPLC separation module (Waters, Milford, MA, USA) coupled to a Q Exactive hybrid quadrupole-Orbitrap MS through an heated (H) ESI-II probe for ESI (Thermo Scientific, Waltham, MA, USA)] analysis following the separation and detection conditions reported by Fracassetti et al. (2018).

Determination of chemical parameters

The chemical parameters [density, TA, tartaric acid, malic acid, pH, yeast assimilable nitrogen (YAN), potassium] were

determined by an UNI CEI EN ISO/IEC 17025-accredited laboratory through Fourier-transform infrared spectroscopy. Copper was quantified according to the reference method MA-F-AS322-06-CUIVRE (Organisation Internationale de la Vigne et du Vin 2010) by atomic absorption spectroscopy (detection limit: 0.05 mg/L). The reproducibility and repeatability of the determination of chemical parameters were, respectively, ±0.00024 and ±0.00010 g/mL for density, ±0.03 and ±0.10 g/L for TA, ±0.5 and ±0.15 g/L for tartaric acid, ±0.40 and ±0.12 g/ L for malic acid, ±30 and ±5 mg/L for YAN, ±100 and ±25 mg/L for potassium and ±0.15 and ±0.05 mg/L for copper.

Statistical analysis

Statistica 12 software (Statsoft, Tulsa, OK, USA) was used for one-way ANOVA. Post-hoc Tukey test was performed and significant differences were judged using a 5% significance level ($P < 0.05$). Correlation indexes were determined among G-3SH, Cys-3SH, G-3SHal, GSH, Cys and GRP for both the commercial-scale and laboratory-made must through the Pearson correlation. Moreover, factor analysis was carried out for the commercial must samples in order to understand the distribution of variables considering the samples and the air exposure conditions. The two considered factors were 'must extraction step' and 'air exposure condition'. Principal component analysis (PCA) was performed on auto-scaled data related to G-3SH, Cys-3SH, G-3SHal, GSH, Cys and GRP for the laboratory-made must for an overall overview of the different conditions of sample preparation (AEM and AFM conditions).

Results and discussion

Varietal thiol precursors in commercial-scale musts

The chemical composition (Table 1) of grape must fractions was assessed in order to compare the grape batches processed under AEM and AFM conditions, as well as to monitor the changes occurring in the consecutive fractions. Minor differences were found among the corresponding must fractions collected under AEM and AFM conditions, except for YAN and potassium which showed some major differences when either the air-exposure conditions or must fractions were compared to each other. The mean values, however, of the concentration of YAN (246 mg/L and 256 mg/L) and potassium (1238 mg/L and 1243 mg/L), respectively, in AEM and AFM were comparable with each other, thus confirming the homogeneity of the processed grape batches. The copper concentration fluctuated slightly along with the must fractions extracted; however, all the values were below 1 mg/L. The highest copper concentration (0.86–0.87 mg/L) was detected in the must fractions arising from destemming, being significantly lower in comparison to samples of clear musts obtained in both AEM and AFM conditions, thus suggesting some copper contamination of grape skins derived possibly from treatment in the vineyard (e.g. copper sulfate). The values, however, were far lower than those usually found in grape and in juice even when rinsed grapes were considered (García-Esparza et al. 2006, Li et al. 2018). As expected, the highest pH value was detected in the fractions related to extensive grape skin damage (destemming and high must extraction yield) even if no significant difference was found among the different must fractions collected. A similar trend was observed for the concentration of malic acid. Overall, the data obtained were consistent with values found in Grillo grape in the previous year (Fracassetti et al. 2018) and indicate that the variability in composition is mainly because of the regular variability occurring in different must fractions from the same pressed grapes.

We have also assessed the concentration of VTPs in grapes and must during the must extraction steps under commercial conditions. As reported by Fracassetti et al. (2018), no 4MMP precursors as well as GluCys-3SH and CysGly-3SH were detected in the samples of grape juice and must. Meanwhile, the concentration of G-3SHal (13.2 μmol/L) in grape was much higher than that of G-3SH (0.43 μmol/L) and Cys-3SH (0.12 μmol/L), but it decreased by over 90% after grape pressing, likely because of the addition of SO_2 at destemming, as discussed earlier (Figure 3). We detected a lower rate of loss of G-3SH and Cys-3SH throughout the must extraction steps. In most of the samples, an increase of the concentration of G-3SH was found in parallel with an increase of that of Cys-3SH as the must extraction yield rose, especially under AEM conditions

Table 1. Effect of air exposure on the composition of the grape must fractions of the Grillo cultivar prepared on a commercial scale.

		Must fraction					
Parameter	**Air exposure condition**	**Destemming**	**Draining**	**20% Yield**	**40% Yield**	**60% Yield**	**70% Yield**
Density (g/mL)	AEM	1.106a	1.098a	1.097a	1.095a	1.105a	1.097a
	AFM	1.107a	1.091a	1.104a	1.105a	1.104a	1.096a
TiA (g/L of tartaric acid)	AEM	4.6a	4.4a	4.4a	4.1a	4.5a	3.6a
	AFM	4.4a	4.0a	4.4a	4.2a	4.3a	3.7a
Tartaric acid (g/L)	AEM	5.15a	5.31ab	5.57ab	5.27ab	5.21a	4.58b
	AFM	5.06a	5.30ab	5.22ab	5.21ab	5.62ab	4.99b
Malic acid (g/L)	AEM	1.56a	0.96a	1.08a	1.08a	1.16a	1.13a
	AFM	1.42a	1.13a	1.11a	1.12a	1.45a	1.19a
pH	AEM	3.59a	3.38a	3.4a	3.45a	3.55a	3.65a
	AFM	3.58a	3.45a	3.45a	3.51a	3.54a	3.65a
YAN (mg/L)	AEM	314a	229b	228bc	208bc	293a	201bc
	AFM	249b	166c	292a	330a	283a	217bc
Potassium (mg/L)	AEM	1400b	1160a	1150a	1180a	1330ab	1210ab
	AFM	1370ab	1260ab	1190a	1060a	1220ab	1360ab
Copper (mg/L)	AEM	0.87a	0.58ab	0.61ab	0.76a	0.82ab	0.49b
	AFM	0.86a	0.56ab	0.76ab	0.85a	0.79ab	0.50b

Different letters mean a significant difference (*t*-test, $P < 0.05$). AEM, air-exposed must; AFM, air-free must; YAN, yeast assimilable nitrogen.

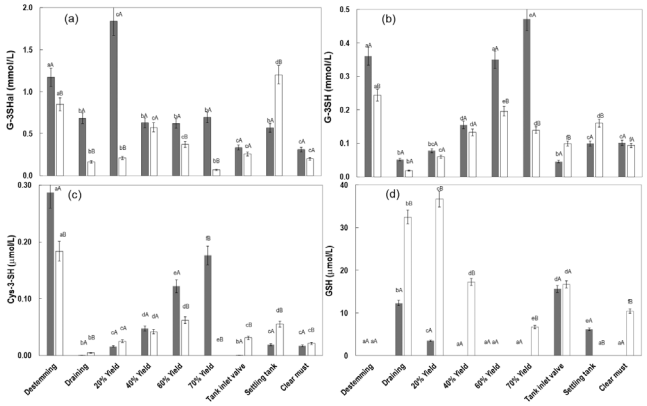

Figure 3. Concentration of (a) S-3-(hexanal)-glutathione (G-3SHal), (b) S-3-(hexan-1-ol)-L-glutathione (G-3SH), (c) S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) and (d) glutathione (GSH) in Grillo grape must fractions obtained from commercial winemaking and collected under air exposure (■) or under air-free conditions (□). The concentration in the grape juice was: G-SHal, 5342 ± 493 µg/L; G-3SH, 24.3 ± 3.7 µg/L; Cys-3SH, 176.2 ± 9.4 µg/L; and GSH, 1.69 ± 0.18 mg/L. A trace concentration of Cys-3SH was detected in the air-exposed must-drained sample and in the air-free must-70% yield sample. Different lowercase letters indicate significant differences among the samples collected in the same air exposure condition ($P < 0.05$). Different capital letters indicate a significant difference within the two air exposure conditions investigated ($P < 0.05$).

(Figure S1). Roland et al. (2010) observed a similar increase in G-3SH in hand-pressed must prepared in the presence of air. Under our conditions, Cys-3SH was not present in the grape and present only as a trace in grape berry skins, whereas both G-3SH and G-3SHal were mostly concentrated in the skins, and their concentration decreased in the inner layers of berries, especially close to the seeds (Table 2). Therefore, the increasing yield of grape must led to increased extraction of G-3SH from the skin. Our results agree with those of Roland et al. (2011) who found that the concentration of VTPs was higher in the grape must fractions from the highest pressure because of a greater concentration of VTPs in the skin of Sauvignon Blanc and Melon du Bourgogne grapes. It is noteworthy that the concentration of the glutathionyl-containing VTPs, especially G-3SHal, detected in the free-run juice fraction (2010 µg/kg of the fraction) was higher than that found in the skin (891 µg/kg of the fraction) (Table 2). Comparable distribution of 3SH precursors was observed in the Merlot grape (Murat et al. 2001). In contrast, our findings disagree with those reported in Sauvignon Blanc (Peyrot Des Gachons et al. 2002) as Cys-3SH was not detected in Grillo grape fractions. In the must fractions collected during the pre-fermentation steps, the concentration of Cys-3SH increased (Figure 3a). We can propose that the peptidolysis of G-3SH occurred in the must as soon as the extraction of must began because Cys-3SH was not present in the grape (Table 2). The concentration of G-3SH detected in AFM samples, however, was consistent with that found in the berry layers considering its concentration in both skins and pulp (Table 2), while its concentration was significantly higher in AEM samples (Figure 3b,c). In contrast, the concentration of G-3SHal substantially decreased upon grape crushing and randomly fluctuated among the grape must fractions. Nevertheless, the concentration of G-3SHal was higher in AEM, except in the sample from the clarification tank (Figure 3c). The significant loss of G-3SHal after crushing (−95%) could be because of the addition of SO_2 at the Destemming step. The loss of G-3SHal cannot be quantitatively explained as a consequence of its reduction generating G-3SH (Figure 3a,b). The addition of SO_2 led to a lower concentration of G-3SHal especially in AFM samples (Figure 3c, Table 2), probably because of the limited oxidative pathways occurring in the presence of SO_2. These results are in agreement with those reported by Thibon et al. (2016) who highlighted the importance of SO_2 addition at pressing. Similarly, considering the data related to the Control laboratory-made must samples (Table 3), the role of air exposure was even clearer when the G-3SHal was considered, as it accounted for up to 4.4 µmol/L under the AEM condition but only for 0.84 µmol/L under the AFM condition. This may be related to significant lipoxygenase activity improving the availability of (E)-2-hexenal as well as the resulting formation of G-3SHal by addition of GSH, as previously reported (Roland et al. 2010, Thibon et al. 2016, Clark and Deed 2018). This assumption could be supported by the undetectable free GSH in the sample Destemming (Figure 3d) and in the Control laboratory-made must samples (Table 3). Cysteine was detected only in AEM 60% yield (0.1 mg/L) and in Clear must (0.44 mg/L) samples.

The factor analysis showed that the two variables considered (must extraction step and AEM condition) explained 69% of variance (50% for factor 1 (extraction step) and 19% for factor 2 (air exposure)], suggesting that other factors (e.g. copper, SO_2, enzymatic activities) could have also affected the concentration of VTPs in must (Figure 4a). While G-3SH, Cys-3SH, GSH and Cys were more dependent on the extraction step, GRP was more affected by AEM conditions. It appears that both variables influenced the concentration of G-3SHal. A further confirmation was obtained related to the negligible impact of AEM conditions on VTPs with the exception of G-3SHal (Figure 3).

Varietal thiol precursors in laboratory-made musts

The availability of GSH to produce VTPs is mainly affected by its concentration in the grape, as well as by its high

Table 2. Concentration of varietal thiol precursors in the skins, pulp, seeds and juice of Grillo berries.

		G-3SHAl		G-3SH		Cys-3SH
Berry fraction	**Mass (%)**	**(µg/kg of fraction)**	**(µg/kg of berry)**	**(µg/kg of fraction)**	**(µg/kg of berry)**	**(µg/kg of fraction)**
Skins	30.3	890.7 ± 8.4a	269.5 ± 2.5a	62.6 ± 0.2a	18.9 ± 0.1a	trace
Pulp	48.5	521.0 ± 13.5b	252.9 ± 6.5b	26.6 ± 0.2b	12.9 ± 0.1b	n.d.
Seeds	5.6	158.9 ± 10.6c	8.9 ± 6.0c	trace	trace	n.d.
Juice[†]	15.6	2009.7 ± 9.9d	313.3 ± 1.5d	105.7 ± 1.6c	16.5 ± 0.3c	n.d.

Data are expressed as average ± SD. Different letters mean a significant difference (*t*-test, $P < 0.05$); n.d., not detectable. [†]Juice obtained during the separation and collection of the skins, pulp and seeds. Cys-3SH, S-3-(hexan-1-ol)-L-cysteine; G-3SH, S-3-(hexan-1-ol)-L-glutathione; G-3SHal, S-3-(hexanal)-glutathione.

Table 3. Effect of air exposure on the concentration of volatile thiol precursors, cysteine, glutathione and grape reaction product, in Grillo grape juices produced under laboratory conditions.

Juice extraction condition	Air exposure condition	Concentration (μmol/L)					
		G-3SHAl	G-3SH	Cys-3SH	Cys	GSH	GRP
Uncooled grape	–	13.2 ± 1.2 g	0.43 ± 0.02b	0.12 ± 0.02d	n.d.	5.50 ± 0.59b	5.13 ± 0.97a
Cooled grape							
No addition/ treatment	AEM	4.4 ± 4.2a	0.23 ± 0.05a	0.57 ± 0.03a	trace	trace	3.61 ± 0.86a
	AFM	0.84 ± 0.51c	0.21 ± 0.00a	0.36 ± 0.01b	trace	n.d.	4.40 ± 3.00a
Addition of sodium fluoride and EDTA	AEM	35.9 ± 2.2b	0.27 ± 0.00a	0.44 ± 0.00 ac	trace	8.34 ± 4.23b	3.00 ± 0.26a
	AFM	23.3 ± 1.4d	0.21 ± 0.07a	0.30 ± 0.00b	trace	4.00 ± 0.72b	6.18 ± 1.05b
Washing of berry skins	AEM	0.84 ± 0.17c	0.40 ± 0.04b	0.46 ± 0.00a	n.d.	n.d.	5.54 ± 1.32a
	AFM	7.8 ± 1.7e	0.37 ± 0.06b	0.66 ± 0.01c	n.d.	n.d.	6.18 ± 1.05b
Addition of SO_2	AEM	0.04 ± 0.04f	0.26 ± 0.01a	0.38 ± 0.00a	0.86 ± 0.25a	85.8 ± 16.6a	0.81 ± 0.05c
	AFM	trace	0.26 ± 0.02a	0.38 ± 0.00a	10.1 ± 6.8b	106.8 ± 10.1b	6.06 ± 2.30b

Data are expressed as average ± SD; different letters mean significant differences (*t*-test, $P < 0.05$). n.d., not detectable. 3MHal, S-3-(hexanal)-glutathione; AFM, air-free; Cys, cysteine; Cys-3MH, S-3-(hexan-1-ol)-L-cysteine; G-3MH, S-3-(hexan-1-ol)-L-glutathione; G-AEM, air-exposed; GSH, glutathione.

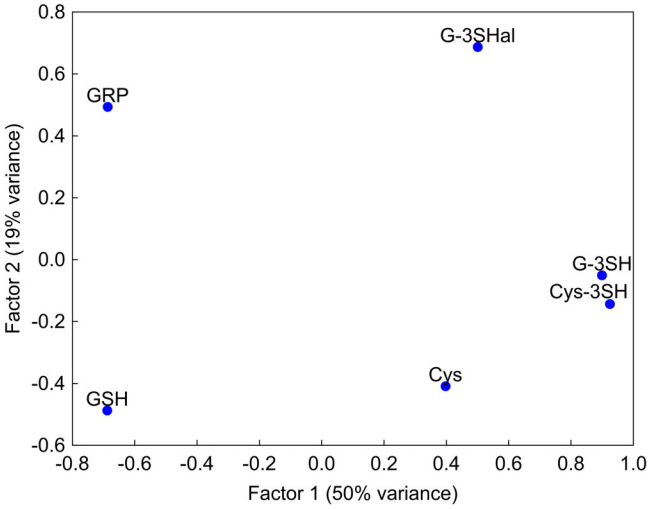

Figure 4. Factor loading obtained for the commercial scale must (factor 1, sample; factor 2, air exposure conditions). G-3MHal, S-3-(hexanal)-glutathione; G-3MH, S-3-(hexan-1-ol)-L-glutathione; Cys-3MH, S-3-(hexan-1-ol)-L-cysteine; GSH, glutathione; Cys, cysteine.

reactivity with other compounds present in the must. The quinone of caffeoyl tartaric acid should be included among them, as it leads to the formation of 2-S-glutathionyl-caffeoyl tartaric acid (GRP) (Singleton et al. 1985, Cheynier et al. 1986, Nikolantonaki and Waterhouse 2012). The oxidative activity of grape polyphenol oxidase enzyme strongly favours the formation of quinones. Addition of SO_2 into the must, as well as AFM condition, inhibits this enzyme and prevents the oxidation (Singleton 1987, Sayavedra-Soto and Montgomery 2006). Indeed, the addition of SO_2 to grape just before juice extraction in laboratory-made preparation led to the highest concentration of GSH and the lowest concentration of GRP in AEM samples. Moreover, the addition of SO_2 hampered the formation of G-3SHal (Table 3) as the hydrogen sulfite addition-product was favoured over the free aldehyde (Clark and Deed 2018). The addition of SO_2 allowed the detection of Cys otherwise occurring as a trace concentration or not detected in all other considered pressing conditions (Table 3). Grape reaction product was found in the concentration range 4.4–6.2 μmol/L. Despite this compound having an oxidative origin, the GRP concentration in AFM samples was comparable, or even higher, than those revealed in AEM samples. In particular, after addition of SO_2, a negligible concentration of GRP was found under AEM conditions. It is worth noting that the loss of GSH is far from being explained by the low GRP concentration detected in AEM treatments, but it was quantitatively comparable to the formation of G-3SHal when the grape juice extraction occurred in the absence of SO_2 (Table 3). Therefore, even though the addition of SO_2 prevents the polyphenol oxidase activity, it can limit the formation of G-3SHal and, consequently, of G-3SH, as also reported by Thibon et al. (2016).

The presence of copper ions (II) is a further potential source of G-3SH loss. As for other thiol compounds, GSH can promptly bind copper to form barely insoluble stable copper sulfide complexes, the formation of which is oxygen independent (Ahmed 2014, Ngamchuea et al. 2016). Under AEM conditions, the copper–GSH complex could be slowly formed and GSH could be oxidised by the copper (II) ion to give oxidised glutathione (GSSG) under oxidative conditions. Both the copper-GSH complex and GSSG cannot bind (E)-2-hexenal (Kachur et al. 1998). In order to assess the role of copper ions on the concentration of VTPs, a laboratory-made must was obtained after the addition of the metal ion-chelating compounds, NaF and EDTA (Janovitz-Klapp et al. 1990, Fracassetti and Tirelli 2015). The data highlight the remarkable effect of copper ions on the formation of VTPs (Table 3). Thus, chemical removal of copper allowed the significant formation of G-3SHal, especially under AEM conditions (35.9 μmol/L). This might be because of the higher concentration of (E)-2-hexenal produced (Roland et al. 2010). In the samples where NaF and EDTA were added, the formation of G-3SH was prevented, with the exception of Control samples in which G-3SH concentration was comparable to that found in samples supplemented with NaF and EDTA. The formation of G-3SHal in the juice also increased when the copper occurring on the grape skin was removed by a citric acid solution but to far less extent than it occurred with chelating salts (Table 3). Up to 7.8 μmol/L G-3SHal was found following grape washing and juice extraction under AFM conditions, but only 0.84 μmol/L was measured under AEM conditions (Table 3). Under the latter conditions, however, the highest concentration of G-3SH and Cys-3SH was detected indicating that grape washing prevented the copper on the skin from interfering in the synthesis pathway of VTPs. Further,

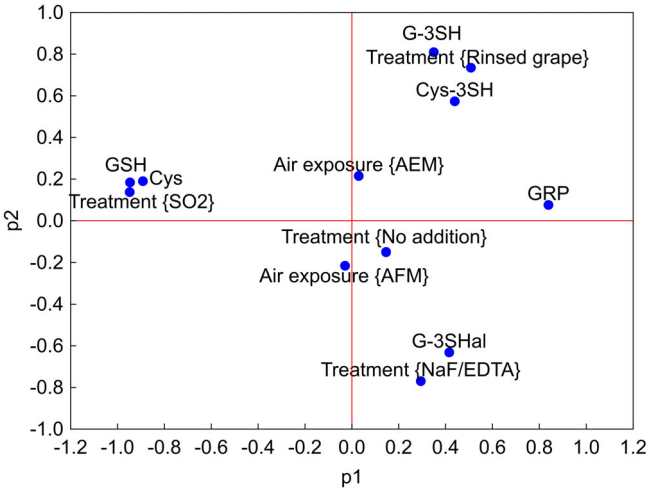

Figure 5. Bi-plot loading scatterplot (p1 vs p2) obtained for laboratory-made must. G-3MHal, S-3-(hexanal)-glutathione; G-3MH, S-3-(hexan-1-ol)-L-glutathione; Cys-3MH, S-3-(hexan-1-ol)-L-cysteine; GSH, glutathione; Cys, cysteine.

the amount of copper ions occurring inside the berry was sufficient (García-Esparza et al. 2006, Li et al. 2018) to likely deplete all the GSH when the grape must was exposed to air (Table 3). These results indicate the role of GSH during the grape must extraction, as it could promptly bind either (E)-2-hexenal, to give VTPs or copper (II). The reaction with the copper (II) ion likely prevailed on the formation of G-3SHal, as a large concentration of this VTP was obtained even under AFM conditions, and GSH was still detectable in the juice when copper-chelating salts were added (Table 3). The role of oxygen on the availability of GSH in grape juice was clearly highlighted in the commercial grape must fractions where a higher concentration of GSH was detected in AFM. The higher GSH concentration, however, did not produce a higher concentration of VTPs suggesting the important role of oxygen in the formation of (E)-2-hexenal during grape pressing, as it was observed in AEM samples produced on the commercial scale (Figure 3). Interestingly, GSH depletion was strongly prevented when SO_2 was added to the juice, thus suggesting the chemical and enzymatic interactions involving GSH were prevented as well as the nucleophilic substitution generating a sulfite adduct of GSH (Jastrembski et al. 2017) (Table 3). The loss of VTPs could not be explained by the conversion of VTPs to dipeptidic precursors (Thibon et al. 2016), as GluCys-3SH and CysGly-3SH were not detected. Finally, whatever the grape must extraction conditions applied in the laboratory, in most of the overnight cooled grapes the concentration of Cys-3SH was higher in comparison to that in the must obtained with grapes that were not cooled, except in the presence of NaF and EDTA. The concentration of Cys-3SH exceeded that of G-3SH under all the extraction conditions applied (Table 3). Such a difference, when data related to the commercial musts and laboratory-made musts are compared, is difficult to explain. The overnight cooling of grapes, not applied under commercial conditions, might favour the concentration of Cys-3SH; hand pressing might favour the hydrolysis of G-3SH. Therefore, further investigations are necessary to clarify this finding.

The PCA showed that three components explained 59% of the variance. Principal component (PC) 1, PC2 and PC3 explained 35, 23 and 17% of the variance, respectively. As shown in Figure 5, both G-3SH and Cys-3SH depended on washing of the grape, thus suggesting the influence of copper on the concentration of VTPs. Moreover, the enzymatic activities involved in the VTPs pathway have not been completely clarified, and one of the unknown enzymatic activities might have increased the concentration of the VTPs. In contrast, the air-free condition exerted only a limited impact, as observed in this investigation in commercial-scale musts and in previous research studies (Larcher et al. 2013, Fracassetti et al. 2018). The concentration of both GSH and Cys was related to the addition of SO_2 which limits the oxidative reaction. The effective removal of copper, and maybe other heavy metals, by chelants was found to be related to G-3SHal.

Overall, data clearly underline the influence of oxygen on the formation of the VTPs in the first steps of must production, as a higher concentration of G-3SH was detected in the commercial AEM fractions despite the presence of a low G-3SHal concentration. Under the same conditions, the concentration of both G-3SH and Cys-3SH increased together with the yield of must extraction. These results suggest that G-3SH was promptly formed during the must extraction; its formation might be because of either the fast production of (E)-2-hexenol or the rapid activity of grape alcohol dehydrogenase enzyme on G-3SHal. The role of this enzymatic activity could be revealed under commercial conditions. Indeed, an increased level of G-3SH was observed when must yield exceeded 40% despite free GSH not being detected, especially in the AEM condition (Figure 3). A large loss of VTPs occurred in the commercial musts during cold settling, mainly under AFM conditions, probably because of adsorption onto the settled grape solids. In clear musts, the concentration of VTPs was comparable for both AEM or AFM conditions. These data are in accordance with the results reported by Larcher et al. (2013) and Fracassetti et al. (2018) who detected no significant increase of VTPs in air-exposed musts.

Conclusions

This investigation highlights that the conditions of grape must extraction can both promptly and significantly affect the concentration of VTPs in grape must. Most of the G-3SHal, and even Cys-3SH, was formed just after the grape pressing. The presence of SO_2 and copper ions hampered the formation of the G-3SHal available for the reduction to G-3SH. Oxygen had a favourable role in the formation of VTPs; this might be because of an increased synthesis of (E)-2-hexenal, the formation of which requires further investigation. These aspects take up a major role at the end of pressing, when the mechanical damage of grape skins can promote a more intense extraction of the lipidic fraction and lipoxygenases, as well as of VTPs that are concentrated in the grape skin. Therefore, the careful handling of must contact with air, the addition of SO_2, the extraction conditions of must and the contact time between grape juice and solids are crucial to preserve or achieve a high concentration of VTPs, which can enhance the tropical, citrus and passionfruit notes in white wine arising from varietal thiols. Further research could also include the investigation of pre-fermentation maceration, an operation eventually playing a positive role to preserve or achieve a higher concentration of VTPs.

Acknowledgements

The authors thank the winery Conti Tasca d'Almerita, Vallelunga Pratameno, Italy, for providing the grape and

must samples and Mr Andrea Servetti for his technical support.

References

Ahmed, A. (2014) Synthesis and structural analysis of copper (II) glutathione complexes via Cu-S linkage. World Applied Sciences Journal **29**, 1357–1362.

Aleixandre-Tudo, J.L., Weightman, C., Panzeri, V., Nieuwoudt, H.H. and du Toit, W.J. (2015) Effect of skin contact before and during alcoholic fermentation on the chemical and sensory profile of South African Chenin Blanc white wines. South African Journal of Enology and Viticulture **36**, 366–377.

Arapitsas, P., Ugliano, M., Perenzoni, D., Angeli, A., Pangrazzi, P. and Mattivi, F. (2016) Wine metabolomics reveals new sulfonated products in bottled white wines, promoted by small amounts of oxygen. Journal of Chromatography A **1429**, 155–165.

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**, 877–886.

Bonnaffoux, H., Roland, A., Rémond, E., Delpech, S., Schneider, R. and Cavelier, F. (2017) First identification and quantification of S-3-(hexan-1-ol)-γ-glutamyl-cysteine in grape must as a potential thiol precursor, using UPLC-MS/MS analysis and stable isotope dilution assay. Food Chemistry **237**, 877–886.

Chen, L., Capone, D.L., Nicholson, E.L. and 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.

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**, 4674–4682.

Cheynier, V.F., Trousdale, E.K., Singleton, V.L., Salgues, M.J. and Wylde, R. (1986) Characterization of 2-S-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. Journal of Agricultural and Food Chemistry **34**, 217–221.

Clark, A.C. and Deed, R.C. (2018) The chemical reaction of glutathione and trans-2-hexenal in grape juice media to form wine aroma precursors: the impact of pH, temperature, and sulfur dioxide. Journal of Agricultural and Food Chemistry **66**, 1214–1221.

Clark, A.C., Wilkes, E.N. and Scollary, G.R. (2015) Chemistry of copper in white wine: a review. Australian Journal of Grape and Wine Research **21**, 339–350.

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**, 287–298.

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J. and Dubourdieu, D. (1995) Identification of a powerful aromatic compound of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. Flavour and Fragrance Journal **10**, 385–392.

Fedrizzi, B., Guella, G., Perenzoni, D., Gasperotti, M., Masuero, D., Vrhovsek, U. and Mattivi, F. (2012) Identification of intermediates involved in the biosynthetic pathway of 3-mercaptohexan-1-ol conjugates in yellow passion fruit (*Passiflora edulis* f. flavicarpa). Phytochemistry **77**, 287–293.

Fracassetti, D. and Tirelli, A. (2015) Monitoring of glutathione concentration during winemaking by a reliable high-performance liquid chromatography analytical method. Australian Journal of Grape and Wine Research **21**, 389–395.

Fracassetti, D. and Vigentini, I. (2018) Occurrence and analysis of sulfur compounds in wine. Jordão, A.M. and Cosme, F., eds. Grapes and wines—advances in production, processing, analysis and valorization (InTechOpen: London, England) pp. 225–251.

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 release of varietal thiols in wine under different clarification conditions of the must. Australian Journal of Grape and Wine Research **24**, 125–133.

García-Esparza, M.A., Capri, E., Pirzadeh, P. and Trevisan, M. (2006) Copper content of grape and wine from Italian farms. Food Additives and Contaminants **23**, 274–280.

Hatanaka, A., Kajiwara, T. and Matsui, K. (1995) The biogeneration of green odour by green leaves and its physiological functions—past, present and future. Zeitschrift für Naturforschung **50c**, 467–472.

Iglesias, M.J.L., Dabila, H.F., Marino, J.I.M., Gorrdillo, C.d.M. and Exposito, J.M. (1991) Biochemical aspects of the lipids in *Vitis vinifera* grapes (Macabeo variety). Part 1. Linoleic and linolenic acids as aromatic precursors. Die Nahrung **35**, 705–710.

Janovitz-Klapp, A.H., Richard, F.C., Goupy, P.M. and Nicolas, J.J. (1990) Inhibition studies on apple polyphenol oxidase. Journal of Agricultural and Food Chemistry **38**, 926–931.

Jastrembski, J.A., Allison, R.B., Friedberg, E. and Sacks, G.L. (2017) Role of elemental sulfur in forming latent precursors of H_2S in wine. Journal of Agricultural and Food Chemistry **65**, 10542–10549.

Joslin, W.S. and Ough, C.S. (1978) Cause and fate of certain C_6 compounds formed enzymatically in macerated grape leaves during harvest and wine fermentation. American Journal of Enology and Viticulture **29**, 11–17.

Kachur, A.V., Koch, C.J. and Biaglow, J.E. (1998) Mechanism of copper-catalyzed oxidation of glutathione. Free Radical Research **28**, 259–269.

Kobayashi, H., Takase, H., Suzuki, Y., Tanzawa, F., Takata, R., Fujita, K., Kohno, M., Mochizuki, M., Suzuki, S. and Konno, T. (2011) Environmental stress enhances biosynthesis of flavor precursors, S-3-(hexan-1-ol)-glutathione and S-3-(hexan-1-ol)-L-cysteine, in grapevine through glutathione S-transferase activation. Journal of Experimental Botany **62**, 1325–1336.

Kreitman, G.Y., Danilewicz, J.C., Jeffery, D.W. and Elias, R.J. (2016) Reaction mechanisms of metals with hydrogen sulfide and thiols in model wine. Part 1: copper-catalyzed oxidation. Journal of Agricultural and Food Chemistry **64**, 4095–4104.

Larcher, R., Nicolini, G., Tonidandel, L., Román Villegas, T., Malacarne, M. and Fedrizzi, B. (2013) Influence of oxygen availability during skin contact maceration on the formation of precursors of 3-mercaptohexan-1-ol in Müller-Thurgau and Sauvignon Blanc grapes. Australian Journal of Grape and Wine Research **19**, 342–348.

Li, X., Dong, S. and Su, X. (2018) Copper and other heavy metals in grapes: a pilot study tracing influential factors and evaluating potential risks in China. Scientific Reports **8**, 17407.

Murat, M.-L., Tominaga, T. and Dubourdieu, D. (2001) Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. Journal of Agricultural and Food Chemistry **49**, 5412–5417.

Ngamchuea, K., Batchelor-McAuley, C. and Compton, R.G. (2016) The copper(II)-catalyzed oxidation of glutathione. Chemistry A European Journal **22**, 15937–15944.

Nikolantonaki, M. and Waterhouse, A.L. (2012) A method to quantify quinone reaction rates with wine relevant nucleophiles: a key to the understanding of oxidative loss of varietal thiols. Journal of Agricultural and Food Chemistry **60**, 8484–8491.

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.

Organisation Internationale de la Vigne et du Vin (2010) Compendium of international methods of analysis. Method OIV-MA-F-AS322-06-CUIVRE (Organisation Internationale de la Vigne et du Vin: Paris, France).

Peña-Gallego, A., Hernández-Orte, P., Cacho, J. and Ferreira, V. (2012) S-Cysteinylated and S-glutathionylated thiol precursors in grapes. A review. Food Chemistry **131**, 1–13.

Peyrot Des Gachons, C., Tominaga, T. and Dubourdieu, D. (2002) Localisation of S-cysteine conjugates in the berry: effect of skin contact on aromatic potential of *Vitis vinifera* L. cv. Sauvignon Blanc must. American Journal of Enology and Viticulture **53**, 144–146.

Qian, X., Sun, L., Xu, X.-Q., Zhu, B.-Q. and Xu, H.-Y. (2017) Differential expression of VvLOXA diversifies C_6 volatile profiles in some *Vitis vinifera* table grape cultivars. International Journal of Molecular Sciences **18**, E2705.

Roland, A., Vialaret, J., Razungles, A., Rigou, P. and Schneider, R. (2010) Evolution of S-cysteinylated and S-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. Journal of Agricultural and Food Chemistry **58**, 4406–4413.

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**, 139–144.

Ruiz, J., Kiene, F., Belda, I., Fracassetti, D., Marquina, D., Navascués, E., Calderón, F., Benito, A., Rauhut, D., Santos, A. and Benito, S. (2019) Effects on varietal aromas during wine making:

a review of the impact of varietal aromas on the flavor of wine. Applied Microbiology and Biotechnology **103**, 7425–7450.

Sayavedra-Soto, L.A. and Montgomery, M.W. (2006) Inhibition of polyphenoloxidase by sulfite. Journal of Food Science **51**, 1531–1536.

Singleton, V.L. (1987) Oxygen with phenols and related reactions in must, wines and model systems: observations and practical implications. American Journal of Enology and Viticulture **38**, 69–77.

Singleton, V.L., Salgues, M., Zaya, J. and Trousdale, E. (1985) Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. American Journal of Enology and Viticulture **36**, 50–56.

Thibon, C., Böcker, C., Shinkaruk, S., Moine, V., Darriet, P. and Dubourdieu, D. (2016) Identification of S-3-(hexanal)-glutathione and its bisulfite adduct in grape juice from *Vitis vinifera* L. cv. Sauvignon blanc as new potential precursors of 3SH. Food Chemistry **199**, 711–719.

Tominaga, T., Furrer, A., Henry, R. and Dubourdieu, D. (1998) Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. Flavour and Fragrance Journal **13**, 159–162.

Manuscript received: 21 April 2021
Revised manuscript received: 17 June 2021
Accepted: 21 June 2021

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12514/abstract.

Figure S1. Correlation between the concentration of S-3-(hexan-1-ol)-L-glutathione (G-3SH) and S-3-(hexan-1-ol)-L-cysteine (Cys-3SH) detected in commercial-scale musts.