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Influence of Grape-Harvesting Steps on Varietal Thiol Aromas in Sauvignon blanc Wines

Thomas Allen, Mandy Herbst-Johnstone, Melanie Girault, Paul Butler, Gerard Logan, Sara Jouanneau, Laura Nicolau, and Paul A. Kilmartin*

Wine Science Programme, School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

ABSTRACT: The intense tropical fruit aroma of Sauvignon blanc wines has been associated with the varietal thiols 3-mercaptohexanol (3MH), derived from odorless precursors in the grape, and 3-mercaptohexyl acetate (3MHA), arising from 3MH during fermentation. Grapes and juice were sourced from five locations in Marlborough, New Zealand, taking hand-picked grapes and samples at four stages during the mechanical harvesting process and pressing, which were then fermented in replicated 750 mL bottles. With each set of juices, the highest concentrations of Cys-3MH and Glut-3MH were found in the juices pressed to 1 bar, but these juices produced wines with lower 3MH and 3MHA concentrations. With three of the juices, there was an increase in varietal thiol content for wines made from juices that had been machine harvested compared to the hand-picked samples, which matched earlier findings of lower 3MH and 3MHA levels in wines made from hand-picked grapes. Juices that were more oxidized, and which showed a higher absorbance at 420 nm, were found to produce wines with lower 3MH and 3MHA concentrations.

KEYWORDS: Sauvignon blanc, wine aroma, varietal thiols, 3-mercaptohexanol, 3-mercaptohexyl acetate, harvesting

INTRODUCTION

A range of aroma profiles is presented by Sauvignon blanc wines, from greener capsicum and asparagus notes, flinty and mineral characters, through to the more tropical and fruity aromas typical of grapefruit and passion fruit. The varietal thiols 3-mercaptohexanol (3MH) and 3-mercaptohexyl acetate (3MHA) are of particular interest for their contributions to the tropical and passion fruit descriptors in white wines.[1−3] Also known as polyfunctional thiols, these compounds are present at quite variable concentrations in commercial Sauvignon blanc wines, with odor activity values at times in excess of 100.[4]

3MH is largely released from odorless precursors during fermentation, and a certain fraction is converted to 3MHA also during fermentation. The first potential precursor identified in Sauvignon blanc grapes by researchers at the University of Bordeaux was *S*-3-(hexan-1-ol)-L-cysteine (Cys-3MH),[5] which was found to be present at higher concentrations in the skins than in the juice, leading to continued release with prolonged skin contact.[6] The identification of a further precursor, *S*-3-(hexan-1-ol)-glutathione (Glut-3MH), in Sauvignon blanc grape must suggested that glutathione detoxification systems in the plant were producing Glut-3MH via glutathione-S-transferase activity, with subsequent catabolism to Cys-3MH.[7] Greatly increased production of Cys-3MH was also seen in grapes exposed to noble rot (*Botrytis cinerea*), and higher concentrations of 3MH were noted in botrytized wines.[8] It was also recognized that aroma precursors are formed under postharvest conditions through grape metabolism. At Bordeaux, the effect of botrytis on Cys-3MH was further confirmed using *Vitis vinifera* cell cultures, as was the conjugation of (*E*)-2-hexenal, released during plant pathogen attack, with glutathione to produce Glut-3MH.[9]

Issues about the conversion efficiencies of precursors such as Cys-3MH, found to be <1% in one study from Montpellier, France, suggested that Cys-3MH is not the major precursor of 3MH in Sauvignon blanc wine.[10] Likewise, the direct addition of a sulfur compound, possibly H_2S, to (*E*)-2-hexenal had been previously examined as an alternative pathway,[11] but this was also shown to contribute only a small amount of the 3MH present in the final wine.[12] In a recent study from the Montpellier group it was confirmed that Cys-3MH and Glut-3MH are located preferentially in the grape skin, leading to greater extraction at the end of the commercial pressing cycle (at ca. 50−100 μg/L for both compounds in Sauvignon blanc juices) and also to higher varietal thiols in the end-pressed wine (above 2 atm), compared to wines from juices from the beginning of the pressing cycle (below 1 atm).[12] In one study from Yamanashi in Japan, the formation of Cys-3MH and Glut-3MH in grape berries was increased through various environmental stresses, such as cold shock, heat shock, UV-C radiation, and biochemical stimulation, linked to increased glutathione-*S*-transferase activity and the production of Glut-3MH from glutathione and hexenal.[13]

Analysis of individual diastereomers of Cys-3MH and Glut-3MH has shown remarkably high concentrations of these compounds in a range of white grape juices, with some of the highest values seen in Sauvignon blanc juices. From research undertaken at the Australian Wine Research Institute, the individual Cys-3MH diastereomers were present in the 7−40 μg/L range and the Glut-3MH diastereomers in the 35−550 μg/L range, with the *S*-form predominating over the *R*-form in each case.[14] Significant amounts of Cys-3MH and Glut-3MH were also found in bottled wine samples. In recent studies from the Australian group, it has been shown that the concentration of Cys-3MH and Glut-3MH in the berries can rise by up to 10-fold in the period leading up to

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harvest.[15] However, a comparison of Cys-3MH and Glut-3MH concentrations with free 3MH in wines made on a small scale from different clones did not show a straightforward relationship. Furthermore, long-distance transportation of machine-harvested Sauvignon blanc grape juices by truck was shown to lead to a 10-fold increase in the concentrations of the Cys-3MH diastereomers (to >200 μg/L) and a doubling in the concentration of Glut-3MH diastereomers.[16] Additions of sulfur dioxide to the grapes prior to transportation, to help prevent oxidation, led to lower Cys-3MH and Glut-3MH accumulation, which could be due to SO_2 binding any (*E*)-2-hexenal present, inhibiting enzymes involved in (*E*)-2-hexenal formation or inhibiting the transformation of Glut-3MH into Cys-3MH. Some hand-harvested samples collected several days before the machine-harvesting also had lower Cys-3MH and Glut-3MH concentrations, which the authors ascribed to more minor berry damage in comparison with grapes derived from machine-harvesting.[16]

In our previous studies at the University of Auckland, we have found that higher Cys-3MH concentrations were obtained in juices later in the commercial pressing cycle.[17] However, in a follow-up study, wines made from juices pressed to 0.25 and 1 bar showed lower 3MH and 3MHA concentrations compared to wines made from the free-run juices.[18] The free-run juices were also characterized by higher glutathione concentrations and lower oxidative development, reflected in the polyphenol profiles. We have also found that the 3MH concentrations in experimental Sauvignon blanc wines made from hand-picked grapes are typically 5−10 times lower than the values obtained in surveys of commercial Marlborough Sauvignon blanc wines, in nearly all cases made from machine-harvested fruit.[19] To examine the impact of hand- versus machine-harvesting on juice 3MH conjugates and wine varietal thiols in Sauvignon blanc, we have taken grape and juice samples from five sites in the Marlborough grape-growing region of New Zealand and at various points in the commercial harvesting operation, including hand-picked grapes taken immediately prior to machine harvesting. The varietal thiol concentrations in wines made under controlled fermentation conditions in the laboratory have then been compared with the original juice chemical parameters, including Cys-3MH and Glut-3MH concentrations.

MATERIALS AND METHODS

Chemicals. Potassium metabisulfite came from Redox Chemicals (Christchurch, New Zealand). d_3-(*R*/*S*)-Cys-3MH, d_3-(*R*/*S*)-CysGly-3MH, d_3-(*R*/*S*)-Glut-3MH, and d_3-(*E*)-2-hexenal (with all three deuteriums on the C6 position, the terminal methyl, with 95−98% deuterium incorporation starting from methyl iodide from Cambridge Isotope laboratories) were supplied by Buchem B.V. (The Netherlands), along with unlabeled Cys-3MH and Glut-3MH. 4-Mercapto-4-methylpentan-2-one was purchased from Interchim (France), 3-mercaptohexan-1-ol from Acros Organics (Geel, Belgium), and 3-mercapthexyl acetate from Oxford Chemicals (U.K.). As internal standards, 4-methoxy-2-methyl-2-mercaptobutane was supplied by Frutarom (U.K.), and 3-mercapto-[1-$^2H_2$]hexan-1-ol ([1-$^2H_2$]3-MH) and 3-mercapto[1-$^2H_2$]hexyl acetate ([1-$^2H_2$]3-MHA) were synthesized at the University of Auckland.[20] Hydrochloric acid (37%, reagent grade) and sodium hydroxide (pellets, ≥99%, reagent grade), disodium hydrogen phosphate dihydrate (≥99.5%), sodium acetate trihydrate (99.5−100.5%), and sodium sulfate anhydrous (powder, extra pure, 98.5−100.5%) were obtained from Scharlau (Barcelona, Spain). 4-Hydroxymercuribenzoic acid sodium salt (≥95.0% Hg), and L-cysteine hydrochloride hydrate (99%) and butylated hydroxyanisole (BHA) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). TRIS (ultrapure, ≥99.9%) was provided by Applichem (Darmstadt, Germany), and DOWEX (1X2, Cl^--form, strongly basic, 50−100 mesh) was a product of Sigma-Aldrich (Castle Hill, NSW, Australia). 5,5′-Dithiobis(2-nitrobenzoic acid) (99%) was from Acros Organics. Ethyl acetate (≥99.7%, LC-MS CHROMASOLV, Fluka, Castle Hill, NSW, Australia) and dichloromethane (for gas chromatography, SupraSolv, Merck, Darmstadt, Germany) were used as solvents. Helium (instrument grade) and nitrogen (food grade) were supplied by BOC Gases NZ Ltd. (Auckland, New Zealand). All water was of Milli-Q grade (resistivity = 18.2 MΩ cm at 25 °C), processed from a Millipore water purification system (Millipore Australia Pty Ltd., North Ryde, Australia).

Juice Samples. The five sets of grape and juice samples were obtained from the Marlborough grape-growing region of New Zealand during April 2010. Juices A and B were obtained from the Awatere Valley, sample A from a location within 2 km of the Clifford Bay coast and sample B from a site 20 km along the valley at an altitude of 180 m above sea level. Juices C, D, and E were from the Wairau Valley, sample C from the foothills of the Wither Hills and samples D and E on flat land in the south Wairau Valley subregion. In each case five sampling points were taken and are described in the following paragraphs. These are referred to below as "hand-picked", "harvester", "winery hopper", "free-run", and "pressed to 1 bar" samples.

On the day of harvesting, 15 kg of grapes was hand-picked with secateurs and placed gently in a picking bin, keeping fruit damage to a minimum, to provide the first "hand-picked" sample. A short time later the mechanical harvester came through the same row, and a second 15 kg "harvester" sample was taken as the grapes came off the harvester into the accompanying gondola. The harvester for samples A−C was a Pellenc 4560 with sorting table and on-board storage tank; for samples D and E a New Holland Braud SB58 was employed with a direct over-row side conveyor arm into the gondola bypassing the on-board storage tank. These samples contained broken grape bunches without rachii and some free juice. Ten milliliters of a 100 g/L solution of potassium metabisulfite was added to the two sets of samples to supply 33 mg of SO_2/kg of grapes. As the commercial harvesting operation continued, the gondola tipped grapes into a truck for transport to the winery. This transfer operation exposes the grapes to considerable air contact, and additions of SO_2 were made by the wine company, at a rate of 34 mg/kg, by using a 2 L addition of a 12% solution of potassium metabisulfite to each truck load of about 4 tonnes of grapes. The time of transport was noted in each case and is indicated in Table 1. The third 15 kg sample was taken as the truck tipped the grapes and juice into the winery crusher receival hopper, at a point about halfway through the unloading in each case, to provide the "winery hopper" sample. This lot of grapes was pressed soon after.

For the first three samples, the fruit was pressed using an 80 L basket press with an inner water bag. Main water pressure was applied at 2.5−3 bar and held in this position for 20 min. The juice was collected in a plastic bucket and then transferred to a 5 L PVC bottle, filled to the brim to minimize subsequent oxygen exposure. Two further 5 L juice samples were taken from the commercial pressing operation. The "free-run" sample was taken during the initial draining of the press. A further sample "pressed to 1 bar" was taken as this pressure was achieved to provide a relatively hard-pressed juice sample. Free-run juice typically accounts for about 500−650 L/ton of grapes. This value varies considerably on the basis of many factors, including fruit condition, sugar concentration, harvester and transport conditions, the use of enzymes, temperature, crusher type, and so on. The juice separated to a 1 bar pressure can vary between 100 and 200 L/ton from the same fruit loads. Winemakers in Marlborough make use of both free-run and pressed juices to make Sauvignon blanc wine, sometimes fermented separately and in other cases after combining the juice fractions. The commercial pressing units were a 15 ton capacity Bücher RPZ 150 for samples A and D, a 32 ton capacity

Table 1. Chemical Analytical Data Obtained from FOSS WineScan and UV−Visible Absorbances for the Five Juices Obtained at Sites across Marlborough, along with Truck Transport Times (in Parentheses)

	°Brix	pH	TA, g/L	YAN, mg/L	A_{280}[a]	A_{320}	A_{420}	A_{420}/A_{320}
Juice A, Awatere Valley (55 min)								
hand-picked	23.8	2.92	13.0	398	6.51	6.24	0.07	0.010
harvester	23.1	3.04	12.2	419	6.06	5.31	0.00	0.00
winery hopper	24.0	3.11	11.9	451	6.23	3.97	0.27	0.067
free run	24.0	3.14	11.5	556	6.63	5.45	0.00	0.00
pressed to 1 bar	25.5	3.44	9.8	513	9.86	4.96	0.85	0.170
Juice B, Awatere Valley (1 h 40 min)								
hand-picked	22.9	2.76	14.1	206	8.68	8.33	0.37	0.045
harvester	23.2	2.69	13.5	269	6.72	5.34	0.16	0.030
winery hopper	22.8	3.03	11.9	310	7.54	7.00	0.03	0.004
free run	23.3	3.00	12.8	339	7.17	3.43	0.46	0.133
pressed to 1 bar	23.3	3.11	11.5	354	7.79	4.76	0.32	0.066
Juice C, Wairau Valley (40 min)								
hand-picked	23.3	2.91	12.7	374	5.95	6.49	0.10	0.015
harvester	22.1	3.08	10.4	417	6.00	4.42	0.05	0.011
winery hopper	22.6	3.12	10.4	434	6.39	2.98	0.45	0.151
free run	22.1	3.15	10.0	380	5.95	3.88	0.00	0.00
pressed to 1 bar	23.0	3.49	8.0	474	7.97	3.59	0.51	0.142
Juice D, Wairau Valley (10 min)								
hand-picked	21.2	2.77	14.3	209	6.12	6.33	0.12	0.019
harvester	21.0	2.82	14.2	273	5.13	5.35	0.12	0.021
winery hopper	21.0	2.92	12.5	223	4.97	4.74	0.12	0.024
free run	20.8	2.93	11.5	190	4.75	4.76	0.00	0.00
pressed to 1 bar	22.5	3.35	8.7	295	6.97	3.63	0.47	0.129
Juice E, Wairau Valley (35 min)								
hand-picked	21.5	2.88	12.7	270	4.68	2.84	0.26	0.092
harvester	22.3	3.03	10.5	277	5.46	2.69	0.28	0.104
winery hopper	21.6	3.04	10.5	289	5.98	2.96	0.44	0.147
free run	22.2	3.07	10.2	306	5.61	2.66	0.36	0.133
pressed to 1 bar	22.6	3.65	7.3	399	7.19	3.27	0.38	0.116

[a] Absorbance at 280 nm, etc.

Diemme Millenium 320 for samples B and C, and a 15 ton capacity Diemme AR80 MFC for sample E. The juices were then cold settled for 36 h in a 12 °C temperature-controlled room. An extra set of 5 L juice samples was taken from site A at the hand-picked and free-run stages. These were frozen and shipped to Auckland for subsequent addition of labeled 3MH conjugates prior to fermentation.

After cold settling, each juice was transferred by siphon into a second 5 L plastic bottle, leaving the sediment behind. At this point a 50 mL juice sample was taken for analysis by FOSS WineScan FT120 (Hillerod, Denmark) for pH, titratable acidity (TA in g/L), °Brix, and yeast-available nitrogen (YAN in mg/L). These results are presented in Table 1. With only four juices the YAN was below 250 mg/L, and an addition of 1.25 g of the yeast nutrient Superfood (Beverage Supply Group) was made to each 5 L juice bottle; this product contains yeast hulls, diammonium phosphate, minerals, and vitamins. Further diammonium phosphate (DAP) was added in different amounts, namely, 0.45 g for juice B hand-picked and to juice D, 0.40 g for hand-picked, 0.12 g for winery hopper, and 0.75 g for the free-run juices. Samples for subsequent analysis were collected at this point, three 15 mL lots and two 5 mL lots in each case, and frozen for transport to the University of Auckland.

Winemaking. The 5 L juice lots were inoculated with 10 mL preparations of Lalvin dried yeast strain, EC1118 *Saccharomyces cerevisiae*, at 0.2 g/L. Green wine bottles (750 mL) were prepared by rinsing with a 200 mL solution of 10 g/L potassium metabisulfite and 10 g/L citric acid, to remove any impurities or micro-organisms, and left upside down to drain dry. The juices were then transferred into triplicate 750 mL bottles using an Enolmatic bottling pump, which itself had been rinsed with 750 mL of the juice in question prior to bottle filling. The bottles were then fitted with a rubber bung with a thin hole, into which was inserted a 100 μL plastic pipet tip filled with glass wool to release CO_2 produced during fermentation.[18] The bottles were labeled, weighed, and placed in a 12 °C temperature storage room to allow the fermentations to begin slowly. The weight of the bottles was measured daily. After 20 days, the bottles were transferred to an 18.5 °C temperature-controlled room to increase the rate of fermentation. Once the weight of the bottles remained steady over 2 days, indicating the end of fermentation, a 50 mg/L SO_2 addition was made using 1 mL of a concentrated potassium metabisulfite solution (70 g/L), and the bottles were capped and shipped to the University of Auckland. Upon arrival at Auckland, the wines were centrifuged at 8000 rpm for 5 min (Eppendorf 5804 centrifuge with a fixed-angle F-34-6-38 rotor) and were frozen for subsequent measurement of varietal thiols by GC-MS.

Addition of Labeled Cys-3MH, CysGly-3MH, and Glut-3MH. Standards were added separately into the site A hand-picked and free-run juices at 500 μg/L for d_3-(*R*/*S*)-Glut-3MH, at 50 μg/L for d_3-(*R*/*S*)-Cys-3MH and d_3-(*R*/*S*)-CysGly-3MH, and at 500 μg/L for d_3-(*E*)-2-hexenal. Aliquots of 187.5 μL of each standard solution were prepared at 2000 times the above concentrations and added to each 375 mL of juice for fermentation in bottles of this volume at 15 °C. With the inclusion of control wines without added labeled compounds, a total of 10 wines were fermented, all in triplicate. The weight of the bottles was measured daily. Once the weight of the bottles remained steady over 3 days, the wines were stored at −20 °C for varietal thiol analysis. The free-run juices were observed to ferment a little more quickly than the hand-picked juices, but only one of the ferments became stuck, and this bottle was not included in the subsequent analyses.

Spectrophotometric Analysis of Juices. The juice were filtered through a 0.45 μm membrane filter (Minisart RC 15, Sartorius, Göttingen, Germany) and diluted 5-fold with ultrapure water. A Cary 50 UV−vis spectrophotometer (Varian Inc., USA) was used to scan the filtered juices from 250 to 800 nm with a 10 mm path length.

***S*-3-(Hexan-1-ol)-L-cysteine (Cys-3MH) and *S*-3-(Hexan-1-ol)-glutathione (Glut-3MH) Analysis.** The concentrations of Cys-3MH and Glut-3MH in the juices prior to fermentation were determined by HPLC-MS after solid phase extraction (SPE) based upon a published procedure, with some modifications.[14] After defrosting, the grape juice samples were clarified by centrifugation for 10 min at 4000 rpm. A 320 μL aliquot of an aqueous solution containing both diastereomers of d_3-(*R*/*S*)-Cys-3MH, d_3-(*R*/*S*)-CysGly-3MH, and d_3-(*R*/*S*)-Glut-3MH, to give final concentrations of 50 μg/L for each diastereomer, was added to 32 mL of the clarified grape juice. The labeled standards were previously prepared volumetrically in Milli-Q water and aliquots stored at −80 °C until required. Strata SDB-L cartridges (500 mg/6 mL, Phenomenex) were first conditioned with two lots of 3 mL of methanol (for cleanup) and followed by two lots of 3 mL Milli-Q water (to establish polarity). The juice sample was then passed through the cartridges, which were then flushed with two lots of 5 mL of Milli-Q water (to remove the sugars). The cartridge was then dried under air for 5 min and eluted four times with 1 mL of methanol and once with 0.5 mL of methanol. The eluate was collected in 2 mL Eppendorf tubes. The samples were concentrated, lids opened, in a centrifugal vacuum concentrator for 45 min at 45 °C at 10 mmHg (to remove methanol) and then for 45 min at 45 °C at 0.1 mmHg (to remove water). Once they were dry, the

Eppendorf tubes were closed and placed in a −40 °C freezer or analyzed immediately by HPLC-MS. The SPE cartridges could be reused four or five times without losses in the chromatographic peaks.

For HPLC-MS analyses, the contents of the five Eppendorf tubes collected for each sample were redissolved in 250 μL of Milli-Q water and combined. The aqueous extract was filtered through a 0.45 μm RC4 syringe filter into a vial, using a two-piece 1 mL disposable plastic syringe. All HPLC-MS analyses were carried out using a Dionex Ultimate 3000 instrument equipped with a binary pump and connected in series to a micrOTOF-QII mass spectrometer (Hybrid Quadrupole, Bruker Daltonics, Madison, WI). The column was a 150 × 3.0 mm, 5 μm ZORBAX SB-C18 column (Agilent Technologies) operating at 25 °C. The solvents were 45 mM NH_4-HCO_2 (solvent A), adjusted to pH 4.50, and 100% acetonitrile (solvent B), with a flow rate of 0.5 mL/min. The gradient for solvent B was as follows: 0 min, 9%; 1.067 min, 9%; 6 min, 40%; 8.3 min, 80%; 8.5 min, 100%; 10 min, 100%; 11.5 min, 9%; 15 min, 9%. The column was equilibrated with NH_4-HCO_2 prior to each injection. The column was flushed with 1 μg/mL of solution containing the internal standard for 3 min prior to each injection, which assisted with mass calibration of the detector. A 10 μL injection was employed. All mass spectrometric data were obtained in positive-ion mode. Nitrogen gas was used in the nebulizer, 4 bar, and for dry gas, 8.5 L/min at 200 °C; argon was used as the collision gas. The collision energy was set at 6.0 eV with ion energy at 1.3 eV, and the capillary was set at 3500 V, using an electrospray ionization source. Data acquisition and processing were performed using HyStar software. The mass ions (m/z) used to quantify the compounds were as follows: (R/S)-Cys-3MH, 222.1, with qualifiers at 205.1, 159.0, 101.1, and 83.1; (R/S)-Glut-3MH, 408.2, with qualifiers at 333.1, 279.1, 262.1, and 162.0. We also ran the instrument at 279.2 to look for (R/S)-CysGly-3MH in the juices and for the internal standards: d_3-(R/S)-Cys-3MH, 225.1, with qualifiers at 208.1, 162.1, 104.1, and 86.1; d_3-(R/S)-CysGly-3MH, 282.2, with qualifier at 254.2; and d_3-(R/S)-Glut-3MH, 411.2.

Calibration curves were also prepared with different concentrations of Cys-3MH from 1.25 to 250 μg/L in a Sauvignon blanc grape juice to which 200 μg/L of total d_3-(R/S)-Cys-3MH had been spiked. The ratio of Cys-3MH to d_3-(R/S)-Cys-3MH versus Cys-3MH concentration had the equation $y = 0.00498x + 0.072$, with $r^2 = 0.9994$. The x-axis intercept indicated that the juice naturally contained 14.4 μg/L Cys-3MH. Likewise, additions of Glut-3MH from 50 to 600 μg/L were made in the Sauvignon blanc grape juice to which 100 μg/L of total d_3-(R/S)-Glut-3MH had been spiked. The ratio of Glut-3MH to d_3-(R/S)-Glut-3MH versus Glut-3MH concentration had the equation $y = 0.0102x + 2.524$, with $r^2 = 0.9967$; the x-axis intercept here indicated a natural Glut-3MH concentration in the juice of 247 μg/L. These calibration curves showed that the instrument gave similar responses for both labeled and labeled 3MH conjugates in each case and that an excellent linear response was obtained over the concentration ranges employed.

Varietal Thiol Analysis. The thiol concentrations were assayed once for each triplicate bottle according to the method originally developed by Tominaga et al.,[5] with some modifications. Five milliliters of *p*-HMB (1 mM in a 0.1 M TRIS solution) and 0.5 mL of a 2 mM BHA solution were added to 50 mL of wine containing 20 nmol/L of 4-methoxy-2-methyl-2-mercaptobutane as an internal standard for 4MMP, 10 nmol/L of [1-$^2H_2$]3-MH for 3MH, and 5 nmol/L of [1-$^2H_2$]3-MHA for 3MHA. After pH adjustment to 7.00 ± 0.05 (10 N, 1 N NaOH; 1 N HCl), the sample was loaded onto a strongly basic anion exchange column (DOWEX), which had been previously activated using 0.1 M HCl and then rinsed with ultrapure water. After percolation of the sample, the column was washed with 50 mL of a 0.1 M sodium acetate buffer (pH 6.00). The varietal thiols were released from the thiol-*p*-HMB complex fixed onto the column by percolating 50 mL of a 50 mM L-cysteine solution (400 mg in 0.1 M sodium acetate buffer), adjusted to pH 6.00. The eluate was extracted twice with dichloromethane (4 and 2 mL) after addition of 0.5 mL of ethyl acetate. The collected organic phase was dried over sodium sulfate anhydrous, filtered through silanized glass wool (Supelco, Bellefonte, PA), and then concentrated under nitrogen flow to ∼25 μL. The gas chromatographic analysis of varietal thiols was carried out using an Agilent 6890N gas chromatograph (Santa Clara, CA) equipped with a 7683B automatic liquid sampler, a G2614A autosampler, and a 5973 mass selective detector. Samples were placed into a tray cooled to 9 °C for automated injection. The inlet temperature was held at 240 °C. One microliter of the sample was injected in pulsed splitless mode and delivered onto an Agilent HP-INNOWax capillary column (60 m × 0.252 mm i.d., 0.25 μm film) using helium (BOC) as carrier gas (112 kPa) at an initial flow rate of 1 mL/min (for 43.60 min), raised to 2.4 mL/min for 7 min after separation of the compounds of interest, and dropping to 1 mL/min for 2 min. The initial oven temperature (50 °C for 5 min) was ramped to 162 °C at a rate of 3 °C/min, then raised to 250 °C at 70 °C/min, and held for 10 min, before dropping to 50 °C. The temperature of the interface line was set to 250 °C. The ion source, operating in electron impact mode at 70 eV, was held at 250 °C. The quadrupole temperature was set at 150 °C. The varietal thiols and internal standards were detected in SIM mode selecting the following ions (m/z) for identification (besides the retention time given by an injection of each individual standard); the quantifier ions are listed first: 134/75 for 4-methoxy-2-methyl-2-mercaptobutane, 132/75/99 for 4MMP, 119/104 for [1-$^2H_3$]3-MHA, 118/103 for [1-$^2H_2$]3MHA, 116/101 for 3MHA, 137/103 for [1-$^2H_3$]3-MH, 136/102 for [1-$^2H_2$]3MH, 134/100 for 3MH. 5,5′-Dithiobis(2-nitrobenzoic acid) was used to determine the concentration of the thiol standards for calibration purposes.[21] Standard curves were obtained with nine calibration points by adding increasing quantities of the reference standards to 50 mL of Sauvignon blanc wine (10−500 ng/L for 4MMP; 40−1400 ng/L for 3MHA; 250−5000 ng/L for 3MH). The linear regressions were found to be very good for all thiols (4MMP, r^2 = 0.9993; 3MHA, r^2 = 0.9999; 3MH, r^2 = 0.9963) with recoveries close to 100%.

Statistical Analysis. One-way analysis of variance (ANOVA) was performed for all varietal thiols using IBM SPSS Statistics 19.0 (SPSS Inc., Chicago, IL). When significant differences ($p < 0.05$) were indicated, the Tukey honestly significant difference (HSD) test ($p < 0.05$) was used to evaluate differences between harvesting treatments for each Sauvignon blanc wine.

RESULTS AND DISCUSSION

Sauvignon blanc Juice Analysis. The five sets of juices showed general chemical parameters typical of Sauvignon blanc grapes from the Marlborough grape-growing region (Table 1). In most cases the yeast-available nitrogen levels were quite adequate, and additions of Superfood and DAP were made to only four juices. The acid content was also quite high, whereas higher pH and lower titratable acidity values were obtained in the pressed to 1 bar juices, a trend observed in our previous Sauvignon blanc pressing trial.[18] As expected, the juices pressed to 1 bar were visibly browner than the other four juices within each set. All five of the juice E samples were also found to be quite brown. The polyphenol and oxidative development was further characterized by recording the UV−visible spectra of the juices (Table 1). The 420 nm absorbance, a common measure of wine and juice browning, and indicative of the formation of oxidized polyphenol products, was quite high in the pressed to 1 bar juices, being >0.3 unit in each case. Many of the other juices had 420 nm absorbances of <0.12 unit, with some exceptions, including all of the juice E samples. Values that were very close to a zero absorbance reading at 420 nm, and even small apparently negative values, have been labeled "0.00" in Table 1. A clear peak at 320 nm was also seen in many of the juices, as seen in the hand-picked juice C spectrum presented in

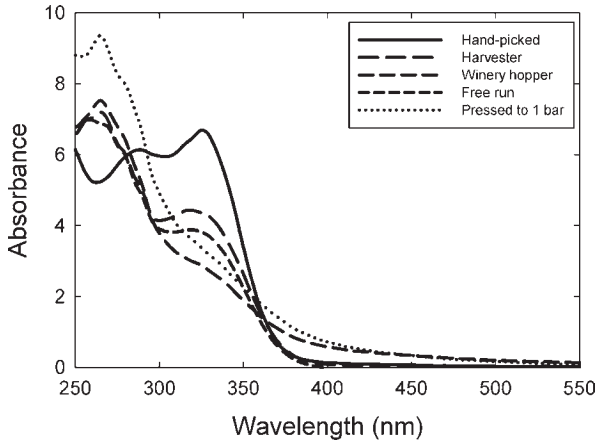

Figure 1. UV−visible spectra of the five juice C samples.

Figure 1. This peak can be ascribed to hydroxycinnamic acids such as caftaric acid and *S*-glutathionyl caftaric acid, the phenolic compounds known to be present at the highest concentrations in Sauvignon blanc juices.[17,18] The 320 nm peak was highest for the juices from the hand-picked grapes, for which the least enzymatic oxidation was expected, and lower values were obtained with juices from fruit that had been machine-harvested.

With the pressed to 1 bar juices, and all of the juice E samples, only a slight shoulder was seen at 320 nm against a broad absorbance that extended into the visible region. A further peak at around 285 nm was seen with the hand-picked juices, which can be ascribed to further phenolic compounds such as gallic acid and some flavonoids contributing at this wavelength. The peak at 265 nm, which was most intense with the pressed to 1 bar juice, is expected to be largely due to nonphenolic material such as nucleotides and other cellular material.[22,23] Given that oxidative development was marked by a decline in the 320 nm absorbance, as hydroxycinnamic acids were consumed, and an increase in the 420 nm absorbance, we have tabulated the ratio of 420 to 320 nm (A_{420}/A_{320}) as an oxidative index in Table 1. Values in excess of 0.05 were associated with the more oxidized juices.

3MH Conjugates Present in the Sauvignon blanc Juices. LC-MS analyses were undertaken to determine the concentrations of Cys-3MH and Glut-3MH in the juices. Figure 2A shows chromatograms of the three labeled standards, d_3-(R/S)-Cys-3MH, d_3-(R/S)-CysGly-3MH, and d_3-(R/S)-Glut-3MH, prepared at 1000 μg/L. Similar retention times were obtained for the 3MH conjugates prepared in water or in Sauvignon juices and for labeled and unlabeled compounds. In each case the pairs of diastereomers were not fully separated, and a total diastereomer response was obtained from the area under the appropriate single-ion chromatograms. A lower peak height was given by Glut-3MH than by the other two 3MH conjugates.

Figure 2B shows chromatograms corresponding to the unlabeled 3MH conjugates present in a typical juice sample. The concentration of Glut-3MH, with a range of 22−541 μg/L, was in each case greater than that of Cys-3MH, at 7.3−111 μg/L (Table 2) and consistent with values published previously for Sauvignon blanc juices.[14] With each sample set, the highest concentrations of Cys-3MH and Glut-3MH were found in the pressed to 1 bar juices, as already observed for Cys-3MH.[17] On the other hand, the trend among the remaining samples was quite variable, with higher values in the hand-picked grapes in some cases (juices A and D) and lower values in others (juices C and E).

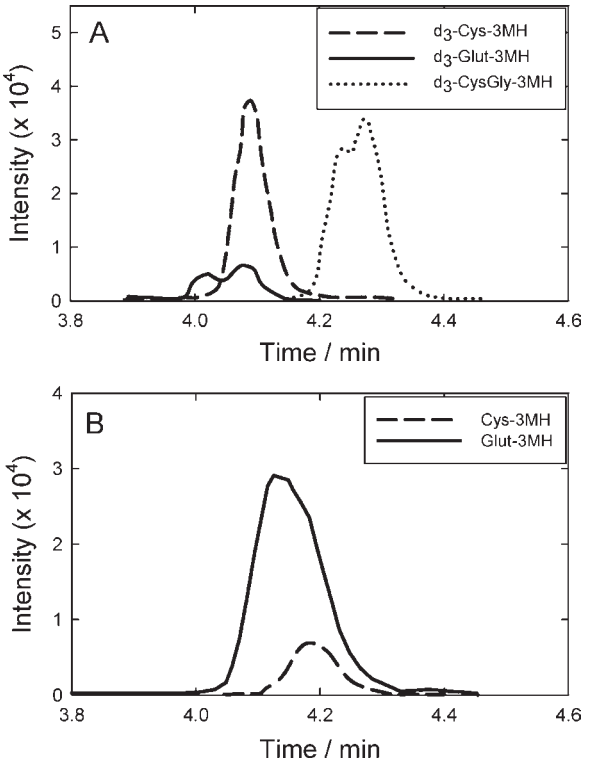

Figure 2. HPLC-MS selected ion chromatograms of 3MH conjugates: (A) deuterated standards, with each diastereomer at a concentration of 1000 μg/L, using m/z values of 225.1 for d_3-(R/S)-Cys-3MH, 282.2 for d_3-(R/S)-CysGly-3MH, and 411.2 for d_3-(R/S)-Glut-3MH; (B) unlabeled Cys-3MH and Glut-3MH present in a typical Sauvignon blanc juice, using m/z values of 222.1 for (R/S)-Cys-3MH and 408.2 for (R/S)-Glut-3MH, along with m/z 279.2 to look for the presence of (R/S)-CysGly-3MH.

Likewise, the concentration of Glut-3MH varied between 2 and 32 times the Cys-3MH concentration, on a mass basis. The formation mechanisms of the 3MH conjugates have yet to be fully characterized, but may involve a complex interplay of (E)-2-hexenal formation and the enzymatic coupling of glutathione to (E)-2-hexenal, each step with its own promoters and inhibitors.[9,12−15] The particular chemical and enzymatic composition of each of the juices may have favored Glut-3MH formation in a variable manner, whereas differences in extraction efficiency and rates of degradation could also have come into play. Under the more oxidative conditions of the pressed to 1 bar juices, and greater extraction of components located in the skins, Glut-3MH formation was intensified, where glutathione is also rapidly consumed and free SO_2 depleted.[18] Cys-3MH may have been formed already in the grapes, or from the breakdown of Glut-3MH,[7,9,16] with variable effects again across the different juice samples, and the higher concentrations in the pressed to 1 bar juices may also relate to increased extraction of compounds located in the skins. On the other hand, there was no CysGly-3MH detected in any of the juices, expected to produce a peak in chromatograms run at m/z 279.2, a further 3MH conjugate that has been suggested as an intermediate in the conversion of Glut-3MH to Cys-3MH.[13]

Wine Varietal Thiol Content. The varietal thiol content was measured in each of the 25 wines (Table 2). The compounds were found to be present at concentrations higher than their respective perception thresholds, namely, 60 ng/L for 3MH,

Table 2. LC-MS of Thiol Conjugates in the Sauvignon blanc Juices (n = 3) and GC-MS Results for Varietal Thiols in the Finished Wines, Using Triplicate Bottle Ferments (n = 3)[a]

	Cys-3M, μg/L	Glut-3M, μg/L	3MH, ng/L	3MHA, ng/L	4MMP, ng/L
	Juice A			**Wine A**	
hand-picked	57.0 (±1.3)	224 (±8)	914 (±46)a	172 (±16)c	11.0 (±0.1)a
harvester	25.8 (±1.3)	87 (±13)	2565 (±120)b	446 (±31)d	17.9 (±1.1)b
winery hopper	26.6 (±1.0)	177 (±7)	766 (±79)a	90 (±4)b	9.6 (±0.7)a
free run	39.5 (±3.7)	322 (±30)	791 (±138)a	200 (±6)c	10.1 (±0.5)a
pressed to 1 bar	111 (±35)	541 (±9)	751 (±41)a	38 (±3)a	16.2 (±2.7)b
	Juice B			**Wine B**	
hand-picked	12.0 (±1.3)	94	744 (±95)c	121 (±8)b	18.3 (±1.0)a
harvester	10.5 (±0.8)	56 (±3)	649 (±4)bc	147 (±2)b	11.3 (±1.3)a
winery hopper	19.4 (±4.5)	124 (±5)	551 (±71)c	128 (±11)b	14.9 (±5.7)a
free run	13.6 (±3.0)	33 (±2)	502 (±41)c	126 (±15)b	17.3 (±0.9)a
pressed to 1 bar	19.7 (±1.1)	200 (±8)	198 (±45)a	35 (±2)a	16.7 (±1.5)a
	Juice C			**Wine C**	
hand-picked	24.4 (±0.3)	44 (±4)	688 (±116)b	139 (±20)a	11.8 (±2.1)a
harvester	15.9 (±0.6)	74 (±1)	3570 (±118)d	1012 (±167)c	11.4 (±2.0)a
winery hopper	14.6 (±0.8)	84 (±5)	281 (±31)a	91 (±26)a	10.9 (±0.6)a
free run	14.4 (±1.6)	91 (±3)	1522 (±72)c	623 (±37)b	9.6 (±1.5)a
pressed to 1 bar	41.2 (±1.4)	406 (±22)	263 (±31)a	55 (±21)a	9.8 (±2.8)a
	Juice D			**Wine D**	
hand-picked	12.8 (±0.8)	130 (±24)	2257 (±198)bc	240 (±69)bc	17.9 (±2.1)b
harvester	7.3 (±0.5)	22 (±8)	3051 (±179)c	366 (±32)cd	14.7 (±0.3)ab
winery hopper	11.1 (±0.2)	85 (±7)	3314 (±626)c	187 (±21)ab	24.8 (±1.3)c
free run	9.6 (±0.5)	80 (±4)	1618 (±71)b	438 (±5)d	13.1 (±0.1)ab
pressed to 1 bar	16.5 (±0.6)	534 (±21)	215 (±63)a	80 (±16)a	11.0 (±1.7)a
	Juice E			**Wine E**	
hand-picked	7.6 (±0.7)	33 (±2)	337 (±76)b	ND[b]a	10.8 (±0.1)a
harvester	11.4 (±0.8)	84 (±10)	335 (±2)b	96 (±8)b	12.3 (±1.0)ab
winery hopper	10.1 (±1.3)	88 (±5)	387 (±51)b	ND[b]a	11.0 (±1.6)a
free run	11.2 (±1.1)	117 (±1)	171 (±29)a	77 (±7)b	12.1 (±0.9)ab
pressed to 1 bar	34 (±3)	490 (±23)	170 (±1)a	ND[b]a	15.0 (±1.4)b

[a] Standard deviations are given in parentheses after each value. Means of analyte in a column sharing the same letter(s) do not differ significantly between harvesting treatments for that juice by Tukey's HSD test (p < 0.05). [b] ND, not detected.

4 ng/L for 3MHA, and 0.8 ng/L for 4MMP,[24] with the exception of three of the sample E wines, for which the concentration of 3MHA was below the detection limit for this procedure (i.e., <10 ng/L). In three of the wine sets from the current trial (A, C, and D), there was an increase in 3MH and 3MHA concentrations for wines made from grapes taken from the harvester compared to the hand-picked samples. This result matches anecdotal accounts from a number of New Zealand wineries that machine-harvested wines exhibit more thiol-related tropical and fruity aromas compared to trial wines made from hand-picked grapes. We have also previously obtained wines with lower varietal thiol concentrations when using hand-picked grapes to produce research-scale wines for regional trials. In one set of 12 such wines, the 3MH concentration ranged from 122 to 1235 ng/L and 3MHA from 86 to 161 ng/L.[19] These lower values can be compared with an average of 6600 ng/L for 3MH and 486 ng/L for 3MHA in one survey of 16 commercial Marlborough Sauvignon blanc wines[3] and a mean 3MH value of 3164 ng/L for 14 New Zealand Sauvignon blanc wines, compared to a 1775 ng/L global average, in another survey.[4] The commercial New Zealand wines are produced mainly from machine-harvested grapes.

The bulk of the 3MH content is formed by yeast activity from odorless precursors in the grape juice, with a certain fraction then converted to 3MHA, and the formation of precursors to 3MH, whatever their identity, is likely to be enhanced by the increased enzymatic activity that occurs following damage to the grapes during the mechanical harvesting process.[13,15,16] In the other two cases (B and E), there was little effect from harvesting points upon the relative concentrations of 3MH and 3MHA. However, these two wines also showed lower 3MH and 3MHA concentrations across all five samples and, for one reason or another, may have lacked the propensity to form higher concentrations.

Each of the pressed to 1 bar juices produced wines with lower 3MH and 3MHA concentrations, although for two of the wines

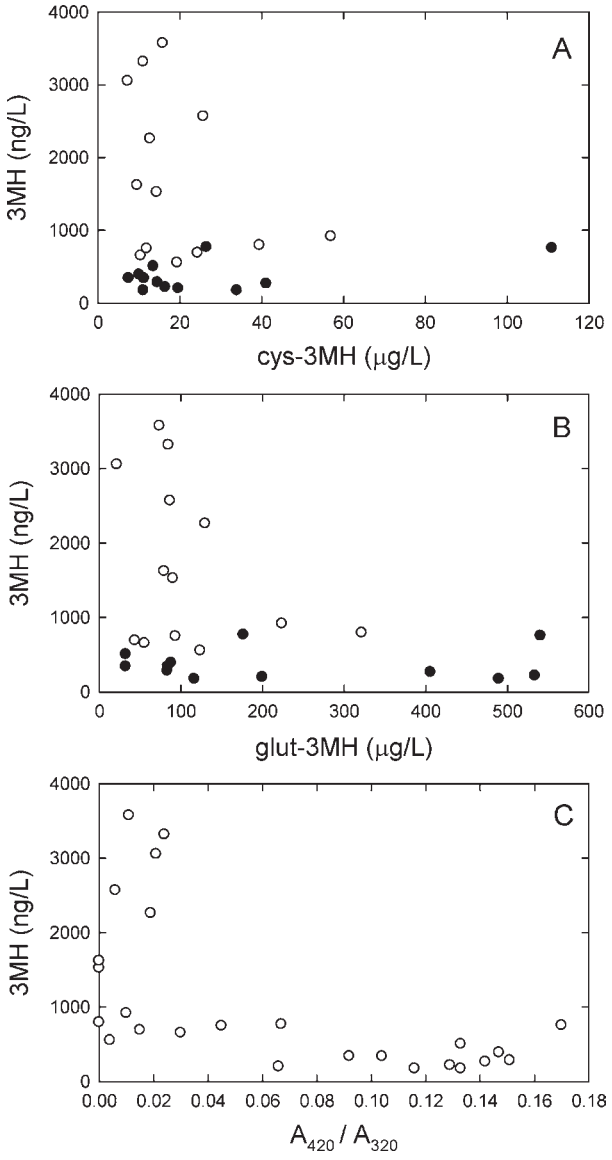

Figure 3. Wine 3MH concentrations for the 25 Sauvignon blanc research wines, compared to (A) Cys-MH concentrations and (B) Glut-3MH concentrations in the grape juice sources, with the more oxidized juices indicated by solid circles (defined as having an A_{420}/A_{320} value >0.05); (C) wine 3MH concentrations compared to the A_{420}/A_{320} value of the juices.

(A and E), the free-run and pressed to 1 bar wines had similar 3MH contents (Table 2). This trend to lower 3MH and 3MHA in wines from heavier pressed juices was seen in our previous pressing trial[18] and occurred even though the pressed to 1 bar juices had the highest concentrations of Cys-3MH and Glut-3MH within each sample set. We note that in a further trial, higher varietal thiols were seen in end-pressed wine (>2 atm),[12] but it is possible that the juices obtained in this study at the beginning of the pressing cycle (low pressure, <1 atm) were closer in character to the wines from light pressing (at 0.25 bar) in our previous study,[18] which gave similar and even lower thiol concentrations compared to the heavy pressing wines, as opposed to the wines from the earlier free run that led to wines with higher 3MH and 3MHA concentrations. The 3MH and 3MHA contents in the intermediate wines were somewhat variable, and in two cases (wines A and C), quite low concentrations were obtained in the wines from grapes sourced at the winery hopper, compared to wines from grapes taken immediately off the harvester. In all of the B wines, and particularly all of the E wines, the 3MH content was low in each case (<750 ng/L). These lower thiol wines did not match any particular pattern for the concentrations of Cys-3MH and Glut-3MH in the grape source. Furthermore, there was no correlation between Cys-3MH or Glut-3MH grape content and the resulting wine 3MH concentrations across the complete data set (Figure 3, panels A and B, respectively), an observation supported by a recently published study.[15] This applied even when the more oxidized juices were separated out, marked with solid circles in Figure 3A,B, and considering only the open circle values relating to the juices showing little early oxidation. Instead, the only wines that produced 3MH concentrations >1000 ng/L were from juices with Cys-3MH of <30 μg/L and Glut-3MH of <150 μg/L. These findings indicate that some care is needed when grape sampling followed by small-scale winemaking is used for the likes of trials on the regional and viticultural impacts upon Sauvignon blanc 3MH and 3MHA derived aroma. The differences seen in the present study between juice sets suggest that as yet unknown factors in the handling operations can have a large impact upon final wine 3MH and 3MHA concentrations.

By contrast, the concentration of 4MMP in the research wines was less affected by harvesting and pressing conditions. Most of the wines contained 10−18 ng/L of 4MMP (Table 2), which is expected to make an important contribution to the box tree note of these Sauvignon blanc wines.[4] An occasional sample was higher in 4MMP than the others within a data set, most notably the winery hopper wine D sample, which had the highest value seen in this trial of 24.8 ng/L. Of note is the finding that the pressed to 1 bar juices did not produce wines with particularly high or low 4MMP values, unlike the consistently low 3MH/3MHA concentrations seen in these wines. The relationship between 4MMP and its precursors may be quite different from the 3MH case, and a stronger connection with Cys-4MMP and Glut-4MMP in the juice and the role of yeast species has been indicated in previous research.[25,26] A more consistent contribution of 4MMP to wine aroma is therefore expected and may balance the differences seen in 3MH and 3MHA concentrations to a certain extent. Future research with the inclusion of sensory analysis is needed in this area.

Fermentation with Labeled 3MH Conjugates. To examine further the origin of 3MH and 3MHA in the Sauvignon blanc wines, d_3-labeled versions of Cys-3MH or CysGly-3MH at 50 μg/L and of Glut-3MH or (*E*)-2-hexenal at 500 μg/L were added separately to hand-picked and free-run sample A juices prior to fermentation. The GC-MS detector was then used in selective ion mode to search for the corresponding d_3 versions of 3MH and 3MHA in the resulting wines. However, for all of the samples, no d_3-3MH or d_3-3MHA was detected, despite typical values being recorded for the unlabeled thiols (551 ± 72 ng/L for 3MH and 105 ± 27 ng/L for 3MHA in the hand-picked wines (across 15 bottles) and 660 ± 41 ng/L for 3MH and 261 ± 6 ng/L for 3MHA in the free-run wines.) A small peak on the m/z 137 trace, a few seconds after the 3MH m/z 134 peak, was observed, but this was not matched by a peak on the m/z 103 chromatogram comparable to the sizable peak seen at m/z 100 due to unlabeled 3MH. Instead, the m/z 137 and 103 chromatograms were very similar for control wines and for wines fermented in the presence of labeled 3MH conjugates. We have been able to

observe peaks for d_3-3MH and d_3-3MHA using this GC-MS methodology in further research trials including modified yeasts and fermentations in which the same labeled conjugates have been added, and these results will be reported elsewhere. It can be noted that starting with 500 μg/L Glut-3MH (1.2 μmol/L), a 1% conversion would have afforded 1640 ng/L of 3MH (at 0.012 μmol/L), which would have generated a sizable peak in the chromatograms. A 3MH yield of 3% has been reported using labeled Glut-3MH added at 3000 μg/L to 250 mL of a model fermentation medium and fermented with VIN13 yeast at 22 °C.[27] However, this conversion yield was only obtained with a specially modified VIN13 yeast, and with a commercial VIN13 strain no 3MH was detected. In a further study, the conversion of Glut-3MH to 3MH and 3MHA was examined using VL3 yeast and a chemically defined grape juice medium for 1 L ferments undertaken at 20 °C. A conversion rate of 0.5% from Glut-3MH (initially 925 μg/L) and 1% from Cys-3MH (initially 500 μg/L) to 3MH and 3MHA was reported.[28] Using Sauvignon blanc grape musts, a conversion yield of 4.4% has been reported from deuterated Glut-3MH to the corresponding labeled 3MH.[29]

Some minimal conversion of the labeled conjugates in the present study cannot be excluded, as oxidative loss of the resulting thiols remains a possibility with the small-scale winemaking employed, even though the wines were frozen for later GC-MS analyses as soon as possible after fermentation was complete. However, Glut-3MH and Cys-3MH do not appear to be the major sources of 3MH and 3MHA in these Sauvignon blanc wines using EC1118 yeast, given the absence of labeled 3MH and 3MHA in wines fermented from juices to which d_3 labeled versions of Cys-3MH or Glut-3MH had been added. Additional grape juice components not present in model fermentation medium may affect the conversion efficiency, and further studies are required to examine the conversions efficiencies of Glut-3MH and Cys-3MH to 3MH (and onto 3MHA) in real grape juices, on both small and large scales. A similar conclusion could be drawn regarding (*E*)-2-hexenal. However, the most important formation steps toward 3MH precursors from (*E*)-2-hexenal could be occurring soon after harvesting, and the late addition of the labeled form just prior to fermentation may overlook those important processes. It may also happen that much higher total amounts of (*E*)-2-hexenal are produced in grapes postharvest than are detected by the analysis of grapes and juices at any particularly point in time, owing to their reactivity with various sulfur-containing compounds.[11,13] Future experiments with labeled (*E*)-2-hexenal may need to involve higher concentrations and additions immediately after harvesting.

Juice Oxidative Status. One grape juice factor that did influence the capacity of the juice to produce 3MH and 3MHA was the extent of juice oxidation. A higher degree of juice oxidation is a normal feature of pressed to 1 bar juices, which in this work and in previous trials[18] were shown to produce lower yields of thiols in wine. In this particular trial, certain other juices were also more advanced in terms of juice oxidation, notably all of the juice E samples, along with the winery hopper juice C sample. This last sample was more oxidized than the harvester or free-run juices for some unknown reason, but likely related to the specific chemical composition of the juice and the handling of the sample on that day. The extent of oxidation (browning of juices) was measured by the 420 nm absorbance and further assessed by comparing the 420 nm value to the 320 nm absorbance attributable to the hydroxycinnamic acids present (Table 1). These six oxidized juices, as well as the 1 bar pressed samples, all produced relatively low levels of thiols in their fermented wines. Figure 3C compares the 3MH wine content with the values of A_{420}/A_{320} for all of the grape juices. Only in the case of A_{420}/A_{320} values <0.03, corresponding to the least oxidized juices, were 3MH concentrations >1000 ng/L observed. By contrast, all of the juices with A_{420}/A_{320} values >0.07 produced wines with a 3MH content <750 ng/L. A good example is the 12-fold lower 3MH content in the wnery hopper wine C, compared to the harvester wines, matched by an increased A_{420}/A_{320} ratio in the grape juices to 0.151 unit for winery hopper juice compared to 0.011 unit for the harvester juice.

The changes in grape composition that are important to 3MH formation and are affected by oxidation in the grapes need to be investigated further, but the binding of S-containing compounds to polyphenol quinones may have a role. The pool of juice glutathione, an antioxidant compound that can bind with quinones, could also be very important in this regard. The depletion of glutathione in the heavier pressed[18] and more oxidized juices may have an important influence on varietal thiol formation, as more quinones may build up in the juice when the concentration of glutathione is low. At the same time there is potential for grape growers and winemakers to make use of the simply obtained spectrophotometric measure of grape juice oxidative development to provide information about the potential for 3MH and 3MHA formation from each juice. Juices that are already advanced in oxidative terms and exhibit a higher 420 nm absorbance might not be capable of producing wines with high concentrations of 3MH/3MHA, and winemakers may prefer to ferment these juices separately, with the expectation that wines with lower thiol-related characters will be produced, a trend that needs to be checked in future studies. Should a cause for varietal thiol suppression in the more oxidized juices be identified, there would be scope for managing this effect to gain more varietal thiol expression in the resulting wines when this character is sought by the winemaker.

3MH Precursor Question. The nature and reactivity of the precursors to 3MH and 3MHA in Sauvignon blanc grape juice remain unclear. Among the potential precursor compounds involved, (*E*)-2-hexenal and other C6 compounds are known to be produced during grape processing, promoted by cell wounding and by some grape oxidation.[15,30] Considering the trends obtained in Figure 3A,B, some consideration could be given to the idea that the formation of Glut-3MH and Cys-3MH is somehow in competition with pathways that lead to the 3MH/3MHA precursors ultimately converted to free thiols during alcoholic fermentation. The addition of H_2S to an alkene such as (*E*)-hexenal during fermentation has been examined,[11] but the earlier addition of a sulfur source such as SO_2 during grape harvesting and pressing, leading to an intermediate sulfonate compound that could be reduced to the 3MH thiol during fermentation, can also be considered. Such a process would compete for the C6 alkene substrates with the glutathione that leads to Glut-3MH. More studies are required in this area.

A further implication in the wide variations seen between the five sets of juices in the present trial is that multiple sites and grape samples need to be examined to establish how widespread the various trends will be with regard to 3MH precursors and thiol concentrations in the resulting wines. A single grape juice set may not be representative or typical of the majority of Sauvignon blanc grape lots in a given region.

Machine Harvesting of Sauvignon blanc Grapes. Of more practical importance to winemakers is the trend to higher 3MH and 3MHA concentrations in wines made from machine-harvested versus hand-picked grapes. This was confirmed to some

extent in the present study, although two sets of juices (B and E) exhibited low 3MH and 3MHA levels regardless of the harvesting procedure. However, in light of further trial results,[19] there is every reason to believe that machine-harvesting of Sauvignon blanc grapes is an important part of the high 3MH and 3MHA content of wines from the Marlborough region. This finding runs somewhat counter to the common industry understanding that higher quality wines are obtained from grapes that have been hand-picked. The effects of berry damage during machine-harvesting have typically been linked to a deterioration in grape quality in studies that have examined the role of grape juice oxidation during machine-harvesting.[31,32] Note that higher 3MH and 3MHA concentrations should not be seen as simply equivalent to greater wine quality in Sauvignon blanc. Instead, high-quality wines with low varietal thiol contents can certainly be found, and certain quality styles of Sauvignon blanc will typically be characterized by a lower thiol content; conversely, some higher 3MH/3MHA wines may suffer from some wine defect. However, to obtain a Sauvignon blanc wine style with a more tropical and fruity character, with an accompanying intense green edge, high 3MH and 3MHA concentrations are an important, if not essential, component.[1,3] In the case of Sauvignon blanc grapes, the enhanced enzymatic activity that follows mechanical harvesting may be very important in the formation of 3MH precursors in many grape lots and the subsequent release of the free thiols during fermentation.

However, the inherent variability of Sauvignon blanc juices in their ability to produce 3MH needs to be recognized, and more trials need to be undertaken in the future of a more replicated nature. There can also be cases in which wines with moderate to high 3MH concentrations can be obtained from hand-picked grapes, as seen in the value of 2257 ng/L obtained for the hand-picked wine D sample. Likewise, the highest 3MH concentration we have measured to date at the University of Auckland, involving several thousand samples over the past six years using the same GC-MS methodology, was in a commercial Sauvignon blanc wine, for which a 3MH concentration of 45000 ng/L was observed, accompanied by 2500 ng/L 3MHA. This high concentration was seen in a trophy-winning 2004 Sauvignon blanc wine from the Gisborne area, produced from grapes grown on bud wood to provide vine cuttings for grafting, and thus very different from the plantings in Marlborough responsible for the major part of the New Zealand Sauvignon blanc production. These grapes were also hand-picked.

AUTHOR INFORMATION

Corresponding Author

*Phone: (64-9) 3737999, ext. 88324. Fax: (64-9) 3737422. E-mail: p.kilmartin@auckland.ac.nz.

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ABBREVIATIONS USED

3MH, 3-mercaptohexanol; 3MHA, 3-mercaptohexanol acetate; 4MMP, 4-mercapto-4-methylpentan-2-one; Cys-3MH, *S*-3-(hexan-1-ol)-L-cysteine; CysGly-3MH, *S*-3-(hexan-1-ol)-L-cysteine-glycine; Glut-3MH, *S*-3-(hexan-1-ol)-L-glutathione; *R*/*S*, 50:50 mixture of two diastereomers; YAN, yeast-available nitrogen; TA, titratable acidity; DAP, diammonium phosphate; SPE, solid phase extraction; A_{280}, absorbance at 280 nm; A_{420}/A_{320}, absorbance at 420 nm divided by absorbance at 320 nm.

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