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ARTICLE

Influence of Juice Pressing Conditions on Polyphenols, Antioxidants, and Varietal Aroma of Sauvignon blanc Microferments

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The impact of juice press fractions upon the content of varietal thiols in Sauvignon blanc has been examined for wines fermented at the laboratory scale (750 mL). Wines made from pressed juices (taken at 0.25 and 1.0 bar) contained less than half the concentration of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), important contributors to the tropical and passion fruit character of Sauvignon blanc wines, compared to wines made from free run juices. The pressed juices and wines exhibited lower acidity values, more rapid decline in glutathione content, and more advanced polyphenol oxidation. Supplementation of the juices with glutathione (at 67 mg/L) prior to fermentation led to lower varietal thiol concentrations in the finished wines, typically by several percent, whereas treatment with polyvinylpolypyrrolidone (PVPP) made no impact on wine parameters. Pasteurization of pressed juices increased 3MHA content in the finished wines, but also led to a decline in 3MH concentrations.

KEYWORDS: Sauvignon blanc; wine aroma; varietal thiols; pressing; glutathione; PVPP; polyphenols

INTRODUCTION

Winemakers are very aware of the higher quality of free run juice for the fermentation of a range of white wines, and Sauvignon blanc is no exception to this rule. Although known for higher polyphenol content and a greater tendency for oxidative browning, pressed fractions can still be used to make quality wines. At the same time, the amount of pressure applied and the period of skin contact will affect the extraction of aroma compounds and precursors.

In Sauvignon blanc grapes, 2-methoxy-3-isobutyl pyrazine (IBMP), with a herbaceous, capsicum-like aroma, is largely located in the skin. IBMP is easily released into the free run juice and resulting wines, although longer skin contact time in pressed juices may yield slightly higher concentrations (*1*−*3*). The varietal thiols with descriptors such as broom, grapefruit, and passion fruit, namely, 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), are almost totally absent from the juice, but are released from odorless precursors during yeast fermentation (*4*, *5*). Interest in cysteinylated compounds, such as *S*-3-(hexan-1-ol)-L-cysteine (3MH-S-cys) as a precursor for 3MH and 3MHA, led to studies which showed that 3MH-S-cys was more highly concentrated in the skins than the juice and continued to be released with prolonged skin contact (*6*) or with higher pressure applied in pressed fractions (*3*). More recently, the role of 3MH-S-cys as the major precursor for 3MH and 3MHA has been called into question (*7*), whereas that of the contribution of a glutathionylated precursor (*8*), or for glutathione as an activator of 3MH release (*7*), has been raised. Glutathione already has established roles in limiting the effects of oxidation in juice and wine through its reaction with polyphenol quinones (*9*−*11*) and has been found to protect a range of volatile compounds in white wines (*12*). Whereas the concentration of glutathione can increase following fermentation (*13*) due to its uptake and release by wine yeasts, the concentration has been found to decline with more oxidative juice treatments (*14*) and as a wine ages (*15*, *16*).

Past studies have also demonstrated that pressing conditions affect glutathione and polyphenolic compounds in white wines (*17*, *18*). With Sauvignon blanc, a higher concentration of flavonoids has been observed with greater skin contact time (*1*,*19*,*20*). In our previous paper, free run juices were characterized by high glutathione and caftaric acid concentrations, both of which had largely disappeared in the higher press fractions to be replaced by 2-*S*-glutathionyl caftaric acid, known as the grape reaction product (GRP), along with higher concentrations of *cis*-coutaric acid and certain flavonol compounds (*3*). This represents an overall increase in oxidative potential in the higher press fractions, which is important given the ready oxidation of such polyphenols to *o*-quinones able to react with thiols, including 3MH (*21*,*22*), either via Michael addition reactions (*23*) or via the generation of peroxides (*24*).

The aim of the present study was to ferment research-scale wines and determine final 3MH and 3MHA concentrations, using juices sourced from commercial pressing operations, taking the free run, light (0.25 bar), and heavy (1.0 bar) pressings. In addition, two pressed juices were analyzed before and after a pasteurization treatment. The concentration of 3MHA is of particular interest due to its contribution to the "tropical fruit"

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descriptor and suppression of the impact of methoxypyrazines at concentrations greater than about 50 ng/L (*25*). 3MHA concentrations were found to be particularly high in Marlborough Sauvignon blanc wines, averaging 486 ng/L, and to correlate strongly with "tropical" and "sweet sweaty passion fruit" descriptors (*26*, *27*). The glutathione and polyphenol contents of the juices and wines were also examined, along with the impact of glutathione supplementation and of polyvinylpolypyrrolidone (PVPP) fining treatments on the juices prior to fermentation.

MATERIALS AND METHODS

Chemicals. 3-Mercaptohexan-1-ol was purchased from Interchim (France) and 3-mercaptohexyl acetate from Oxford Chemicals (U.K.). L-Glutathione reduced (≥98%), caffeic acid (≥98%), *trans-p*-coumaric acid (≥98%), quercetin-3-*β*-D-glucoside (≥90%), rutin hydrate (≥94%), and quercetin dihydrate (≥98%) were purchased from Sigma (St. Louis, MO). Potassium metabisulfite came from Redox Chemicals (Christchurch, New Zealand). 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. All juice samples were obtained from the Marlborough grape-growing region of New Zealand during the 2008 harvest. Juice A was obtained from the Brancott valley, whereas juices B and C were sourced from sites in the Rapaura subregion and were donated by wine companies from their commercial pressing operations. The Sauvignon blanc grapes were machine-harvested and handled according to the respective wineries' standard practices. The juices were collected sequentially at the free run, light press (0.25 bar), and heavy press (1.0 bar) stages. The procedures and equipment varied to some extent between wineries and in the additions of SO_2 during the operation. The grapes for juices A and B were loaded into 75 tonne tippy tanks, which were drained for some 5 h before being transferred into a bag press, to which a series of pressing steps were applied. For juice C, two truckloads of grapes were loaded at a time into a Bucher RPZ150 press over a period of 30−60 min, followed by a sequential pressing cycle. The initial rollovers and drainage lasted for 40 min, during which the pressure was below 0.2 bar and the free run juice was collected. Further pressings and rollovers were involved in a further 100 min cycle during which the pressure was raised progressively to 2 bar and during which the pressed fractions were collected. Two additional sets of juices (D and E) came from holding tanks that contained heavier and quite brown press fractions and were obtained before and after a commercial pasteurization operation at 83 °C for 15 s.

General wine analytical parameters for the juices were measured in the laboratories at the winery sites (free and bound SO_2, free amino nitrogen (FAN), °Brix, pH, and titratable acidity (values presented in **Table 1**)). The juices were held in 20 L carboys with screw-cap lids in a cool room at 4 °C to cold settle for 2 days, and then the clear juice was siphoned into new 20 L plastic containers and adjusted to 20 mg/L of free SO_2 by the addition of potassium metabisulfite. The containers were filled to the brim to minimize headspace and oxygen exposure. The juices were dispatched under chilled conditions to the University of Auckland Wine Hall.

Winemaking. Upon arrival at the Wine Hall, the juices were again analyzed for °Brix, pH, and titratable acidity, and similar values were obtained as for the analyses undertaken at the wineries in Marlborough. Free and bound SO_2 were also measured at this point. Additions of Superfood (Pacific Rim Oenology Services, Blenheim, New Zezland), at 0.25 g/L, and of the Lalvin dried yeast strain, EC1118 *Saccharomyces cerevisiae*,at 0.2 g/L were then made to the juices in the 20 L containers, which were then transferred into multiple 750 mL wine bottles. Additions of 50 mg of glutathione (67 mg/L) or 200 mg of PVPP in solid form (Siha-Optipur brand from Begerow, Germany) were made to selected bottles, with all treatments and controls set up in triplicate. This level of PVPP fining is in the middle range recommended by the suppliers for a moderate adjustment to a white wine. The PVPP remained in the bottles throughout the trial, and no attempt was made to filter and remove the PVPP, owing to the additional exposure of the wine to oxygen that would result. Each bottle was then sealed 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. The bottles were then labeled and weighed, and transferred in crates to a temperature-controlled room set at 15 °C. During fermentation the escape of CO_2 was expected to counter O_2 entry and maintain an anaerobic environment, but some small amount of O_2 could have entered the wine at the end of alcoholic fermentation.

The weight of the individual bottles was measured each day to follow the progress of the fermentation, which was generally complete (to a steady bottle weight) after 8−14 days. After a further 5−10 days, samples of the wines were frozen at −20 °C for analysis of varietal thiols by GC-MS and wine composition at a later date.

Wine Analyses. A FOSS WineScan FT120 operated at the Pernod Ricard winery in Glen Innes, Auckland, New Zealand, was used to determine several wine parameters, including percent ethanol, pH, titratable acidity (TA), volatile acidity, and ethyl acetate content, using duplicate bottles and duplicate instrument injections.

3-Mercaptohexan-1-ol and 3-Mercaptohexyl Acetate Analysis. The quantification of 3MH and 3MHA was carried out according to the method described by Tominaga et al. (*27*, *28*), with some modifications, including the use of the deuterated analogues 3-mercaptohexanol (3-mercapto(1-$^2H_2$)hexanol) and 3-mercaptohexyl acetate (3-mercapto(1-$^2H_2$)-hexyl acetate) as internal standards (*29*). The thiols were extracted from 50 mL of wine using *p*-hydroxymercuribenzoic acid, which was then fixed onto an anion exchange column before the thiols were eluted with cysteine and extracted into dichloromethane prior to concentration and injection of 5 μL in split mode with a split ratio of 5:1 and split flow of 5 mL/min onto an Agilent 6890N GC with a 5973 MS detector (Agilent, Santa Clara, CA). The thiols were separated on a HP-Innowax column from J&W Scientific (60 m × 252 μm × 0.25 μm) using He carrier gas at a flow rate of 1 mL/min and an oven temperature ramping at a rate of 3 °C/min from 50 °C (held for 3 min) to 115 °C (held for 22 min), then raised to 150 °C at 40 °C/min and held for 3 min, followed by a further increase to 173 °C at 3 °C/min, and finally to 250 °C at 70 °C/min (held for 17 min) before dropping to 50 °C at 40 °C/min (held for 3 min). The interface temperature of the detector was kept at 230 °C, and the ion source working in electron impact mode at 70 eV was held at 230 °C. The quadrupole temperature was set at 150 °C. The ions *m*/*z* 116, 118, 134, and 136 were used as quantifiers for 3MHA, 3-mercapto(1-$^2H_2$)hexyl acetate, 3MH, and 3-mercapto(1-$^2H_2$)-hexanol, respectively. The ions *m*/*z* 101, 103, 100, and 102 were qualifiers for 3MHA, 3-mercapto(1-$^2H_2$)hexyl acetate, 3MH, and 3-mercapto-(1-$^2H_2$)hexanol, respectively. Standard curves were obtained by adding increasing quantities of the two varietal thiols to a Sauvignon blanc wine (70−2500 ng/L 3MHA; 500−18000 ng/L 3MH). The regression equation obtained for 3MH was $y = 2443.1x - 113.58$ with $R^2 = 0.999$ and for 3MHA was $y = 782.55x - 55.537$ with $R^2 = 0.999$. Relative standard deviations of 2.5 and 3% were obtained for 3MH and 3MHA, respectively, by assessing five samples of the same Sauvignon blanc wine. All of the samples were analyzed in duplicate.

Polyphenols and Glutathione Analysis. Monomeric wine polyphenols were determined on the basis of a RP-HPLC method described previously (*3*, *30*), with the inclusion of a Coulochem III electrochemical detector with model 5010 analytical cell (ESA Laboratories, Chelmsford, MA) for the quantification of glutathione in the same run. In brief, about 2 mL of wine was filtered through a 0.45 μm cellulose filter (Minisart RC-4), of which 20 μL was injected into a Phenomenex Luna C18 column (4.6 × 250 mm, 5 μm particle size) (Torrence, CA) on an Agilent 1100 series instrument (Waldbronn, Germany). A ternary solvent was run at a flow rate of 0.8 mL/min employing (A) water, (B) 5% aqueous acetic acid, and (C) acetonitrile. The initial gradient composition was 45% A and 55% B, and over the course of 20 min the gradient was changed linearly to 100% B. From 20 to 50 min the gradient moved to 90% B and 10% C and from there to 55% B and 45% C by 70 min. While 55% B was maintained constant over the next 20 min, the gradient shifted from 45% C to 45% A, giving a total run time of 90 min. The diode array detector was set at 320 nm (for hydroxycinnamic acids) and at 365 nm (for flavonols), and the polyphenols were identified using a combination of commercial standards and the UV−visible spectra of peaks in comparison with published procedures, as described previously (*3*). The electrochemical detector was set at +0.75 V, which enabled a peak for glutathione to be obtained after about 5.4 min; a typical chromatogram and further experimental details for Sauvignon blanc juices are provided in ref *3*. External standard calibration was made using 0.2−50 mg/L standards of glutathione, caffeic

Table 1. Chemical Analytical Data for the Five Juices Obtained at the Winery Sites in Marlborough[a]

	free SO_2, mg/L	FAN, mg/L	°Brix	pH	TA, g/L	ethanol, % vol	pH	TA, g/L	volatile acidity, g/L	ethyl acetate, μg/L
	Juice A							**Wine A**		
free run, control	20.8	116	21.2	3.11	7.9	12.88 (0.2%)	3.15 (0.2%)	8.04 (0.0%)	0.46 (0.0%)	1499 (0.3%)
Glut						12.87 (0.3%)	3.17 (0.2%)	8.18 (0.0%)	0.48 (0.0%)	1523 (0.6%)
PVPP						12.86 (0.2%)	3.18 (0.0%)	8.14 (0.4%)	0.51 (1%)	1540 (0.7%)
light press, control	ND[b]	140	21.5	3.33	6.2	12.94 (0.2%)	3.49 (0.1%)	6.63 (0.3%)	0.21 (11%)	475 (2.5%)
Glut						12.93 (0.1%)	3.49 (0%)	6.46 (0.4%)	0.23 (0.0%)	415 (2.7%)
PVPP						NA[c]	NA	NA	NA	NA
heavy press, control	4.8	154	21.4	3.38	5.8	12.75 (0.2%)	3.66 (0.1%)	6.43 (1.2%)	0.23 (2.1%)	337 (3.6%)
Glut						12.75 (0.2%)	3.63 (0%)	6.40 (0.3%)	0.28 (3.6%)	345 (7.5%)
PVPP						12.71 (0.1%)	3.63 (29%)	6.46 (0.7%)	0.23 (2.2%)	300 (4.6%)
	Juice B							**Wine B**		
free run, control	15.1	200	21.8	3.22	8.1	13.14 (0.3%)	3.33 (0.3%)	7.86 (0.2%)	0.41 (0.0%)	1306 (0.5%)
Glut						13.15 (0.0%)	3.32 (0.3%)	7.92 (0.5%)	0.41 (3.6%)	1321 (0.4%)
PVPP						13.15 (0.1%)	3.34 (0.0%)	7.95 (0.1%)	0.44 (4.5%)	1322 (0.2%)
light press, control	ND	233	22.0	3.48	6.9	13.03 (0.1%)	3.82 (0.4%)	6.42 (0.2%)	0.33 (3.1%)	252 (1.0%)
Glut						13.03 (0.1%)	3.84 (0.4%)	6.41 (0.1%)	0.37 (5.4%)	251 (3.5%)
PVPP						13.02 (0.2%)	3.81 (0.1%)	6.44 (0.3%)	0.32 (3.1%)	256 (2.6%)
heavy press, control	1.6	258	21.8	3.62	6.9	12.59 (0.1%)	4.12 (0.5%)	6.84 (0.6%)	0.28 (3.5%)	93 (5.3%)
Glut						12.58 (0.1%)	4.10 (0.5%)	6.81 (0.4%)	0.25 (9.8%)	58 (21.9%)
PVPP						12.61 (0.3%)	4.10 (0.2%)	6.80 (0.2%)	0.28 (0.0%)	66 (49.1%)
	Juice C							**Wine C**		
free run, control	23	112	21.9	3.07	9.8	13.29 (0.3%)	3.03 (0.5%)	8.85 (0.1%)	0.44 (1.2%)	1930 (0.1%)
Glut						13.33 (0.1%)	3.06 (0.3%)	8.91 (0.6%)	0.56 (0.1%)	1912 (0.4%)
PVPP						13.32 (0.1%)	3.05 (0.0%)	8.99 (0.2%)	0.38 (0.0%)	1953 (0.1%)
light press, control	14	110	22.1	3.28	7.5	13.54 (0.1%)	3.44 (0.4%)	6.63 (0.2%)	0.36 (1.4%)	710 (2.4%)
Glut						13.54 (0.2%)	3.45 (0.3%)	6.66 (0.1%)	0.39 (1.3%)	720 (0.6%)
PVPP						13.55 (0.2%)	3.47 (0.3%)	6.58 (0.1%)	0.38 (2.7%)	726 (2.2%)
heavy press, control	10	93	22.0	3.38	6.4	13.54 (0.1%)	3.60 (0.6%)	6.22 (0.1%)	0.32 (0.0%)	407 (0.1%)
Glut						13.54 (0.2%)	3.60 (0.1%)	6.27 (0.5%)	0.32 (3.1%)	399 (3.4%)
PVPP						13.56 (0.2%)	3.60 (0.1%)	6.32 (0.2%)	0.31 (3.2%)	418 (6.4%)
	Juice D							**Wine D**		
unpasteurized, control	ND	215	21.9	3.40	7.7	12.78 (0.1%)	3.69 (0.1%)	6.69 (0.5%)	0.39 (1.3%)	354 (0.6%)
Glut						12.75 (0.1%)	3.70 (0.1%)	6.65 (0.2%)	0.36 (5.6%)	331 (1.3%)
pasteurized, control	3.2	207	21.9	3.38	7.6	12.77 (0.2%)	3.71 (0.1%)	6.62 (0.1%)	0.44 (2.3%)	442 (1.4%)
Glut						12.74 (0.1%)	3.69 (0.1%)	6.66 (0.3%)	0.43 (2.3%)	445 (2.8%)
	Juice E							**Wine E**		
unpasteurized, control	3.2	163	20.8	3.39	7.4	12.43 (0.25)	3.60 (0.3%)	6.68 (0.6%)	0.30 (1.7%)	457 (1.4%)
Glut						12.46 (0.1%)	3.61 (0.1%)	6.66 (0.1%)	0.31 (1.7%)	448 (0.4%)
pasteurized, control	ND	155	20.9	3.43	7.9	12.40 (0.1%)	3.65 (0.4%)	6.74 (0.1%)	0.35 (1.5%)	578 (0.1%)
Glut						12.39 (0.0%)	3.64 (0.3%)	6.76 (0.2%)	0.33 (7.5%)	542 (2.5%)

[a] Analytical results obtained for the finished wines obtained with a FOSS WineScan calibrated for Sauvignon blanc wines and using duplicate bottles ($n = 2$). Percent of variation to the means are given in parentheses after each value. [b] ND, not detected. [c] NA, not available (sample lost).

acid (for the hydroxycinnamic acids), and rutin (for the flavonols) at five different concentrations. Juice samples were analyzed in duplicate, and wine samples were analyzed in triplicate (single injection from the triplicate fermentation bottles).

Statistical Analysis. The duplicate analysis of the varietal thiol data from triplicate fermentations ($n = 6$) and the triplicate analysis (single injection of the triplicate fermentations) of the polyphenols and glutathione data ($n = 3$) were subjected to single factor analysis of variance (ANOVA) to calculate Fisher's least significant difference (LSD) with 95% confidence level ($\alpha = 0.05$). For the comparison of the individual means of the different treatments, Microsoft Office Excel 2003 software (Add-ins, Analysis ToolPak) was used.

RESULTS AND DISCUSSION

The three free run juices (A−C in **Table 1**) were quite typical of Marlborough Sauvignon blanc and showed FAN values from 112 to 258 mg/L, °Brix values from 21.2 to 21.9, pH values from 3.07 to 3.22, and TA from 7.9 to 9.8 g/L. In each case, the pressed juices were of a similar °Brix to the free run juices, but the pressed juices were consistently higher in pH (by 0.23 unit in the light press and by 0.33 unit in the heavy press, on average) and lower in TA (by 15−35%), a well-known trend that reflects the higher concentration of acids in the grape pulp as opposed to the skins or seeds. The light press samples of two of the juices (A and B) were collected prior to SO_2 additions during the pressing, leading to low levels in the pressed juices, whereas juice C received extra SO_2 additions during the pressing cycle, leading to free SO_2 concentrations of 10−14 mg/L in the pressed fractions. The two juices examined for the influence of juice pasteurization (D and E) showed acid values typical of the other pressed Sauvignon blanc juices and were also low in free SO_2 at the time of sampling. All juices were adjusted to a free SO_2 content of 20 mg/L before shipping to the University Wine Hall in Auckland. The free SO_2

content of the free run juices was measured at 17−20 mg/L, and the free SO_2 content of the pressed juices had declined to concentrations in the 0−6 mg/L range after shipment. No measurements of O_2 concentrations were made on the juices, but this is clearly an area in need of further investigation for the effects of oxygen on Sauvignon blanc juices, particularly on the small laboratory scale employed here.

The juices were analyzed for glutathione and polyphenol contents by HPLC upon arrival at Auckland (**Table 2**); duplicate HPLC injections provided very consistent results with only a few exceptions (glutathione and caftaric acid levels in the pasteurized juices). As observed previously (*3*), the concentrations of glutathione (20−48 mg/L) and caftaric acid (20.4−22.4 mg/L) were quite high in the free run juices, but these concentrations declined in the higher press fractions. Conversely, the concentration of 2-*S*-glutathionyl caftaric acid increased in the light pressing, whereas lower values were seen in two of the three juices (B and C) in the heavy pressing. With juice C, a higher concentration of 2-*S*-glutathionyl caftaric acid was already present in the free run juice (and lower glutathione), pointing to greater conversion to the GRP in prior juice handling, but the subsequent decline in glutathione and caftaric acid was less with this juice compared to juices A and B, likely related to the higher concentration of free SO_2 maintained during the press cycle. The concentrations of glutathione and caftaric acid were low in the juices (D and E) used in the pasteurization experiments, but higher concentrations of 2-*S*-glutathionyl caftaric acid remained (although some loss was seen in the unpasteurized juice E).

The remaining polyphenol analyses also followed previously observed trends (*3*), with only a small free caffeic acid content (<1.6 mg/L) and increasing concentrations of two flavonol compounds in the higher pressings (at low levels in the free run). The first flavonol had the same retention time as a quercetin-3-glucoside standard, but the identity of the two closely eluting flavonols remains to be confirmed (λ_{max} = 354 nm for both). Particularly high concentrations of the flavonols were seen in juices D and E, and more so following pasteurization where enzyme inactivation may have lessened oxidative degradation processes. A few milligrams per liter of catechin and epicatechin was also observed in most of the juices and wines (data not included). Juice C followed previous trends with an increase in *cis*-coutaric acid concentration in the higher pressings, whereas the *trans*-coutaric acid concentration was higher in the light press and lower in the heavier pressing, reflecting the location of coutaric acids largely in the grape skins and the greater susceptibility of the trans form to oxidative degradation (*3*,*31*). However, these trends were not followed in the light press for juice A and both pressings for juice B, where very low concentrations of *trans*-coutaric acid, and of *cis*-coutaric acid in juice B, were seen. The latter three pressed juices were noted to be very brown in color and may have been subject to additional enzymatic oxidation including laccase activity.

After fermentation, settling, and bottling, the wines were analyzed for a range of standard chemical parameters using a FOSS WineScan FT120 (**Table 1**). The ethanol content was generally proportional to the juice °Brix values and ranged from 12.4 to 13.6%. Likewise, the acid values of the wines were consistent with the juice trends, with lower pH and higher TA values in the free run and higher pH values in the wines made from pressed juices. The volatile acidity (VA) was also lower in wines made from the pressed juices, but with no values in excess of 0.6 g/L. Similar trends of low volatile acidity in pressed wines were observed in past studies (*32*) and attributed to a higher extraction of polyphenolic compounds and unsaturated fatty acids from grape skins and seeds. Likewise, the concentration of ethyl acetate, expected to develop as a wine fault under conditions of high VA (more than about 0.9 g/L), did not exceed 2000 μg/L, and lower values were again seen in the wines made from pressed juices, particularly the wines from juice B, which were also higher in pH. No consistent effect was seen in these chemical parameters arising from supplementation of the juices with glutathione or through PVPP additions prior to fermentation.

Several trends also emerged in the glutathione and polyphenol contents of the finished wines (**Table 3**). The concentration of glutathione dropped considerably from the high values seen in the free run juices and remained a few milligrams per liter higher in the juices to which 67 mg/L additions of glutathione had been made. These results were consistent with past studies of glutathione in Sauvignon blanc and other juices, where a decline in glutathione concentration during fermentation has been noted (*14*) and where a considerable decline in glutathione content was observed after 3 months in the bottle (*15*). For most of the juices supplemented with glutathione, the concentration of 2-*S*-glutathionyl caftaric acid was higher in the final wines and the concentration of caftaric acid a little lower, with some exceptions (e.g., wine C, light press). The concentration of 2-*S*-glutathionyl caftaric acid remained quite high in the wines made from pressed juices, even for wine B, where the browner and more oxidized juice led to quite low concentrations of other hydroxycinnamic acids (e.g., caftaric and caffeic acids both <1 mg/L), except *cis*-coutaric acid, which appeared to be less susceptible to oxidative degradation. Higher caftaric and coutaric acids were reported in a previous study on glutathione juice additions (*15*), but in the present study only the 2-*S*-glutathionyl caftaric acid content was higher at times, which may reflect differences in the winemaking procedures between studies. The concentrations of free caffeic acid and coumaric acid were higher in the wines compared to the original juices, indicating some hydrolysis of the corresponding hydroxycinnamate ester forms. Higher concentrations of caffeic and coumaric acids were obtained in wines from juice C that also had greater protection against oxidation from a higher free SO_2 content maintained during the pressing cycle. It is expected that the wines will contain a range of polyphenol oxidation products of unknown structure that will also contribute to polyphenol-related properties such as wine mouthfeel and various interactions with wine aroma compounds (*33*). The approach to PVPP fining undertaken in this trial had little influence on the polyphenol content of the wines, and no consistent trends were observed.

The two unidentified flavonol compounds that made a major contribution to the polyphenol content of the pressed juices were largely absent from the final wines (**Table 3**). Trace amounts of flavonol 2 were seen in some of the heavier pressings, and around 7 mg/L of flavonol 1 remained in the wines from the pasteurized juices D and E that had particularly high initial levels (>11 mg/L, **Table 1**). By contrast, some polyphenols that had been more concentrated in the pasteurized juices were also more abundant in subsequent wines. More caftaric and *trans*-coutaric acids, along with twice the concentration of glutathione (following supplementation), remained after pasteurization. Pasteurization can therefore be concluded to have lowered the extent of oxidation in the juices and wines, likely through deactivation of polyphenol oxidase enzymes. This result can be contrasted with some losses in phenolic content observed in Muscadine grapes (with prior thermal enzyme deactivation) subject to thermal pasteurization at 75 °C for 15 s, with losses ascribed to byproduct formation during the heat treatment (*34*). Likewise, pasteurization at 85 °C lowered the flavanol content of previously hot-pressed (60 °C) Concord juices, but maintained significantly higher flavanol concentrations in cold-pressed juices, with the suggestion that

Table 2. Glutathione and Polyphenol Composition of the Juices, with Duplicate Injections on the HPLC ($n = 2$)[a]

	glutathione, mg/L	caftaric acid, mg/L CAE	*S*-glutathionyl caftaric acid, mg/L CAE	caffeic acid, mg/L CAE	*cis*-coutaric acid, mg/L CAE	*trans*-coutaric acid, mg/L CAE	coumaric acid, mg/L CAE	flavonol 1, mg/L RE	flavonol 2, mg/L RE
					Juice A				
free run	32.6 (0.9%)	20.4 (0.3%)	7.5 (0.5%)	0.8 (1.7%)	1.7 (1.8%)	3.5 (1.9%)	0.3 (0.6%)	0.4 (0.3%)	0.5 (1.2%)
light press	ND[b]	1.9 (0.2%)	8.1 (0.4%)	0.7 (0.3%)	3.1 (0.4%)	0.1 (1.9%)	0.2 (0.3%)	0.8 (0.2%	6.8 (0.6%)
heavy press	0.6 (5.9%)	4.3 (0.3%)	12.0 (0.2%)	0.9 (2.4%)	5.7 (0.1%)	2.5 (0.15)	0.7 (0.8%)	0.5 (0.2%)	7.3 (1.5%)
					Juice B				
free run	47.9 (0.1%)	22.4 (0.3%)	9.2 (1.6%)	1.1 (1.6%)	1.5 (0.1%)	3.3 (0.2%)	0.4 (1.3%)	0.4 (0.3%)	0.5 (1.7%)
light press	ND	1.1 (9.0%)	9.8 (0.6%)	0.6 (0.3%)	0.5 (5%)	ND	0.1 (3.4%)	0.8 (0.4%)	4.8 (0.4%)
heavy press	ND	0.9 (1.4%)	5.4 (0.2%)	0.6 (0.0%)	0.5 (3.8%)	ND	ND	0.5 (0.1%)	5.7 (0.4%)
					Juice C				
free run	20.5 (3.9%)	21.6 (1.7%)	18.2 (0.8%)	1.6 (0.3%)	2.5 (0.8%)	4.2 (1.3%)	1.0 (1.1%)	0.7 (0.2%)	1.9 (4.1%)
light press	4.5 (3.0%)	16.0 (0.3%)	27.1 (0.1%)	0.9 (0.4%)	6.7 (0.1%)	9.0 (0.7%)	1.7 (1.0%)	2.2 (0.1%)	6.0 (1.4%)
heavy press	1.1 (4.7%)	12.6 (0.5%)	22.0 (0.4%)	0.8 (0.8%)	9.4 (0.5%)	6.8 (0.6%)	1.0 (1.2%)	2.3 (0.1%)	12.1 (1.0%)
					Juice D				
unpasteurized	ND	ND	25.2 (0.1%)	ND	5.0 (0.9%)	ND	0.2 (0.7%)	6.2 (0.4%)	18.2 (2.7%)
pasteurized	2.0 (17.8%)	3.2 (9.6%)	24.5 (0.7%)	ND	6.0 (1.7%)	1.5 (2.3%)	0.2 (0.7%)	13.5 (1.5%)	22.6 (1.3%)
					Juice E				
unpasteurized	ND	ND	4.4 (0.4%)	ND	2.3 (3.7%)	ND	ND	3.5 (1.75)	10.8 (1.6%)
pasteurized	0.5 (38%)	3.2 (2.1%)	16.4 (0.4%)	ND	6.3 (1.7%)	1.3 (3.2%)	0.2 (4.4%)	11.5 (2.3%)	17.4 (1.3%)

[a] Pecent of variation to the means are given in parentheses after each value. [b] ND, not detected.

Table 3. Glutathione and Polyphenol Composition of the Wines, Including Control, Glutathione, and PVPP Treatments and from Triplicate Bottles (Single HPLC Injections) ($n = 3$)[a]

	glutathione, mg/L	caftaric acid, mg/L CAE	*S*-glutathionyl caftaric acid, mg/L CAE	caffeic acid, mg/L CAE	*cis*-coutaric acid, mg/L CAE	*trans*-coutaric acid, mg/L CAE	coumaric acid, mg/L CAE	flavonol 1, mg/L RE	flavonol 2, mg/L RE
Wine A									
Fischer $LSD_{(0.05)}$	0.2	0.3	0.7	0.24	0.2	0.05	0.1		
free run, control	0.5 (±0.0)	3.5 (±0.4)	5.0 (±0.3)	2.3 (±0.1)	1.2 (±0.0)	0.7 (±0.0)	1.1 (±0.0)	ND[b]	ND
Glut	2.8 (±0.1)	3.3 (±0.3)	6.0 (±0.7)	2.4 (±0.1)	1.2 (±0.0)	0.7 (±0.0)	1.1 (±0.1)	ND	ND
PVPP	0.3 (±0.0)	2.9 (±0.1)	5.4 (±0.4)	2.3 (±0.1)	1.3 (±0.1)	0.6 (±0.0)	1.2 (±0.1)	ND	ND
light press, control	0.2 (±0.0)	2.0 (±0.0)	5.1 (±0.2)	2.0 (±0.1)	3.5 (±0.0)	0.6 (±0.0)	0.7 (±0.0)	ND	ND
Glut	1.2 (±0.2)	1.9 (±0.1)	5.1 (±0.3)	1.8 (±0.1)	3.4 (±0.1)	0.6 (±0.0)	0.8 (±0.0)	ND	ND
PVPP	0.4 (±0.1)	1.9 (±0.1)	5.3 (±0.3)	2.0 (±0.2)	3.3 (±0.1)	0.6 (±0.1)	0.7 (±0.0)	ND	ND
heavy press, control	0.1 (±0.1)	1.3 (±0.1)	4.7 (±0.1)	1.9 (±0.1)	4.8 (±0.2)	0.5 (±0.0)	1.2 (±0.1)	ND	ND
Glut	1.1 (±0.1)	1.2 (±0.0)	5.0 (±0.4)	1.7 (±0.1)	4.7 (±0.1)	0.5 (±0.0)	1.2 (±0.0)	ND	ND
PVPP	0.1 (±0.0)	1.1 (±0.0)	4.5 (±0.2)	1.5 (±0.1)	4.4 (±0.2)	0.4 (±0.0)	0.9 (±0.1)	ND	ND
Wine B									
Fischer $LSD_{(0.05)}$	0.5	0.1	1.17	0.6	0.8		0.2		
free run, control	1.8 (±0.3)	1.6 (±0.1)	4.9 (±0.1)	6.1 (±0.3)	1.0 (±0.0)	ND	1.2 (±0.0)	ND	ND
Glut	7.0 (±0.7)	1.5 (±0.1)	5.8 (±0.6)	5.9 (±0.5)	1.0 (±0.0)	ND	1.2 (±0.0)	ND	ND
PVPP	2.2 (±0.2)	1.5 (±0.0)	5.4 (±0.6)	5.9 (±0.6)	1.0 (±0.1)	ND	1.1 (±0.1)	ND	ND
light press, control	0.4 (±0.1)	0.7 (±0.1)	11.2 (±0.6)	0.9 (±0.0)	2.9 (±0.1)	ND	0.5 (±0.0)	ND	ND
Glut	1.7 (±0.2)	0.6 (±0.0)	11.1 (±0.7)	0.8 (±0.1)	3.0 (±0.0)	ND	0.5 (±0.0)	ND	ND
PVPP	0.5 (±0.1)	0.7 (±0.1)	10.6 (±0.8)	0.9 (±0.0)	2.5 (±0.1)	ND	0.4 (±0.0)	ND	ND
heavy press, control	0.2 (±0.1)	0.6 (±0.0)	11.0 (±0.3)	0.8 (±0.2)	4.4 (±1.1)	ND	0.3 (±0.1)	ND	ND
Glut	0.5 (±0.1)	0.7 (±0.1)	11.7 (±0.6)	0.7 (±0.0)	5.1 (±0.1)	ND	0.3 (±0.1)	ND	ND
PVPP	0.2 (±0.1)	0.6 (±0.0)	10.9 (±0.6)	0.8 (±0.1)	4.5 (±0.6)	ND	0.3 (±0.1)	ND	ND
Wine C									
Fischer $LSD_{(0.05)}$	0.3	0.2	1.49	0.8	0.35	0.07	0.2		0.04
free run, control	0.5 (±0.2)	1.0 (±0.1)	7.7 (±0.7)	9.1 (±1.1)	1.2 (±0.1)	ND	0.8 (±0.1)	ND	ND
Glut	5.3 (±0.4)	1.0 (±0.1)	7.7 (±0.4)	9.3 (±0.3)	1.2 (±0.0)	ND	0.9 (±0.0)	ND	ND
PVPP	0.7 (±0.1)	0.9 (±0.0)	7.4 (±0.5)	8.3 (±0.2)	1.2 (±0.0)	ND	0.9 (±0.0)	ND	ND
light press, control	0.3 (±0.1)	2.0 (±0.1)	10.8 (±0.9)	6.7 (±0.1)	4.2 (±0.2)	0.2 (±0.0)	2.3 (±0.2)	ND	ND
Glut	4.7 (±0.1)	1.8 (±0.1)	9.4 (±1.2)	5.5 (±0.3)	3.6 (±0.1)	0.2 (±0.0)	1.9 (±0.1)	ND	ND
PVPP	0.5 (±0.1)	1.6 (±0.1)	9.9 (±0.9)	9.9 (±0.9)	3.8 (±0.2)	0.2 (±0.0)	1.9 (±0.1)	ND	ND
heavy press, control	1.1 (±0.0)	4.1 (±0.0)	7.5 (±0.5)	7.5 (±0.5)	6.0 (±0.0)	0.3 (±0.0)	2.0 (±0.0)	ND	0.4 (±0.0)
Glut	3.2 (±0.2)	4.6 (±0.1)	8.2 (±0.5)	8.2 (±0.5)	6.5 (±0.3)	0.4 (±0.0)	2.2 (±0.1)	ND	0.4 (±0.1)
PVPP	0.3 (±0.0)	4.0 (±0.3)	7.4 (±0.8)	7.4 (±0.8)	6.1 (±0.4)	0.3 (±0.0)	1.9 (±0.1)	ND	0.5 (±0.0)
Wine D									
Fischer $LSD_{(0.05)}$	0.7	0.2	0.9		0.2	0.03	0.04	0.8	0.04
unpasteurized, con	0.4 (±0.1)	0.9 (±0.1)	8.9 (±0.3)	ND	3.2 (±0.2)	0.2 (±0.0)	0.4 (±0.0)	ND	ND
Glut	2.4 (±0.2)	0.8 (±0.1)	9.1 (±0.4)	ND	3.4 (±0.0)	0.2 (±0.0)	0.4 (±0.0)	ND	ND
pasteurized, control	0.4 (±0.0)	2.5 (±0.2)	8.3 (±0.1)	ND	3.9 (±0.1)	0.9 (±0.0)	ND	7.2 (±1.3)	0.4 (±0.0)
Glut	6.2 (±0.6)	2.6 (±0.1)	8.8 (±0.7)	ND	3.9 (±0.0)	0.9 (±0.0)	ND	7.8 (±0.3)	0.4 (±0.0)
Wine E									
Fischer $LSD_{(0.05)}$	0.9	0.2	0.6		0.25	0.09	0.05	0.6	0.04
unpasteurized, control	0.3 (±0.1)	1.0 (±0.1)	6.0 (±0.1)	ND	3.7 (±0.1)	0.3 (±0.0)	0.5 (±0.0)	ND	ND
Glut	2.9 (±0.4)	0.9 (±0.0)	6.4 (±0.3)	ND	3.8 (±0.1)	0.4 (±0.0)	0.5 (±0.0)	ND	ND
pasteurized, control	0.5 (±0.3)	2.5 (±0.1)	6.9 (±0.1)	ND	4.5 (±0.2)	0.8 (±0.0)	ND	7.3 (±0.6)	0.4 (±0.0)
Glut	5.6 (±0.7)	2.6 (±0.1)	6.7 (±0.5)	ND	4.5 (±0.1)	0.8 (±0.1)	ND	7.4 (±0.1)	0.4 (±0.0)

[a] Standard deviations are given in parentheses after each value. [b] ND, not detected.

monomeric flavanols could have been produced by depolymerization of oligomeric procyanidins (*35*).

The concentrations of 3MH and 3MHA in the finished wines are presented in **Figure 1**. Perception thresholds of 60 and 4.2 ng/L have been reported for 3MH and 3MHA, respectively (*5*, *28*). The three commercial free run juices (A−C) produced wines with a wide range of 3MH concentrations, typical of the variation seen in Marlborough Sauvignon blanc wines (*26*, *27*). 3MH at 4200, 11100, and 980 ng/L was determined in the control wines, respectively, with the highest and lowest values from grapes sourced in the Rapaura area. The concentration of 3MHA was found to be 8−14-fold lower, with values of 360, 1310, and 70 ng/L for control wines A−C taken from the free run juices. The consistency in the results for duplicate analyses of the triplicate bottle ferments was generally excellent, with relative standard deviations for both 3MH and 3MHA typically of $< 5\%$ and of $> 10\%$ only for some of the lower 3MHA wines (i.e., less than about 150 ng/L, seen in the heavier pressings and for wine C).

In our earlier study, the concentration of 3MH-S-cys, a potential precursor of 3MH and 3MHA, was found to be higher in the pressed juices compared to the free run and more so for the highest applied pressures (up to 2 atm) (*3*). The expectation was that the higher pressings would produce wines with more of the varietal thiols 3MH and 3MHA, although the higher oxidative

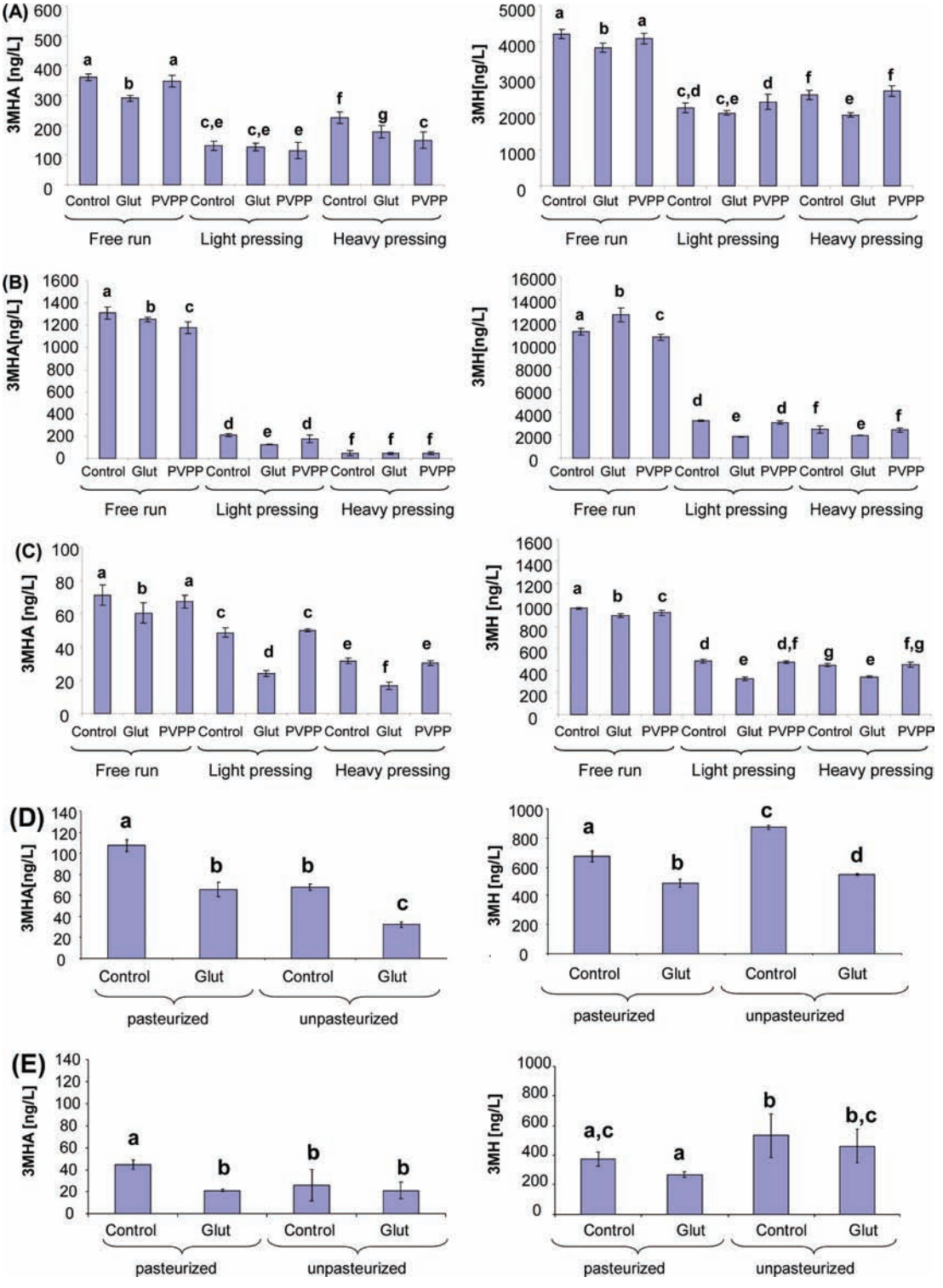

Figure 1. Average concentrations of 3MH and 3MHA in wines made from five different commercial Sauvignon blanc juices. Juices A−C were taken at the free run, light (0.25 bar), and heavy (1.0 bar) pressings, including control wines, wines made from juice supplemented with glutathione (Glut), and wines following PVPP treatment (PVPP). Juices D and E were taken before and after a commercial pasteurization procedure, including control wines and wines made from juice supplemented with glutathione (Glut). The standard deviations ($n = 6$, duplicate analyses of triplicate fermentations) are represented by the *Y*-error bars. Samples marked by the same letter are not significantly different by Fischer's $LSD_{(0.05)}$.

potential of the pressed juices was also recognized. However, wines made from press fraction of juices A and C showed concentrations of 3MH half those of wines made from corresponding free run juices, and pressing of juice B reduced 3MH to a fourth of its free run juice (**Figure 1**). An even greater drop in 3MHA concentration was typically seen in the pressed juices, with only 36−44% remaining in the wines from pressed juices for wine A, 10−16% for wine B, and 40−68% for wine C, compared to the free run wines. Wine B was marked by very high concentrations of both 3MH and 3MHA, but the greater decline in varietal thiols for the pressed fractions may relate to the more oxidative conditions noted above as affecting the polyphenol content, including higher pH conditions, and the general browner and more oxidized character of the pressed juices. However, the

reductions in the concentrations of 3MH and 3MHA in the light and heavy pressings were not consistently different, being a little lower in the heavy pressing for wines B and C and a little higher in the heavier pressing for wine A.

Glutathione supplementation of the juice prior to fermentation was undertaken in an attempt to minimize the effects of nonenzymatic oxidation on polyphenol and varietal thiol loss (*10*). Glutathione supplementation did not lead to an increase in the concentrations of 3MH and 3MHA, but rather to a decrease; in nearly all cases the varietal thiol content was lower by 5−40% (with the exception of a 13% increase in 3MH for wine B free run). These results suggest that additional glutathione in grape juice slightly represses thiol production. This conclusion does not support the concept that glutathione acts as an activator of 3MH release, as suggested previously on the basis of a lowering of thiol production in a yeast mutant that cannot take up glutathione (*7*). The repression could be interpreted as competition by glutathione for uptake by the yeast of the thiol precursor(s), which is consistent with the alternative concept of Subileau et al. (*7*) that some part of the precursor is in a glutathionylated form. However, other explanations for this glutathione repression are also possible. For example, it might occur as a result of nitrogen catabolite repression (NCR), which has been shown to affect thiol yields (*36*, *37*); however, the amount of glutathione added (67 mg/L) corresponds to only an additional 9 mg/L of total nitrogen, which is unlikely to be enough to affect NCR in a these juices (given that each of the juices already contained >90 mg/L of free amino nitrogen; see **Table 1**).

The second juice treatment, namely PVPP fining, was undertaken to remove some of the polyphenol compounds, important initial oxidation substrates in wine, with the aim of minimizing oxidative losses. Instead, the concentrations of 3MH and 3MHA were not significantly different for PVPP fined and control wines for most of the treatments except for the 3MHA levels of the heavy pressing of the wine A, both 3MHA and 3MH level in the free run of the wine B, and the 3MH level in the free run of the wine C. This trend was consistent with the minimal influence of PVPP fining on the polyphenol content of the wines in relation to varietal thiol aroma formation. Subsequent tests on Sauvignon blanc juices showed an 8−12% drop in polyphenol content, as measured by the 280 nm absorbance, for PVPP additions in the 200−800 mg/L range.

Juice pasteurization was found to improve juice and wine oxidative stability, as seen above in higher glutathione and polyphenol retention. However, the concentration of 3MH was lower and the 3MHA content higher in both cases following pasteurization (**Figure 1**). The ratio of 3MH to 3MHA was as high as 6:1 in pasteurized wine D (**Figure 1D**), indicating that the effects of pasteurization and the changes that resulted in juice components may have had a bearing on conversion of 3MH to 3MHA during yeast fermentation. Knowledge of juice parameters that affect the thiol acetylation ratio is minimal, although it is clear that there are significant differences between juices (*38*). Although it is clear that two yeast genes, ATF1 and IAH1, increase or decrease the acetylation ratio (*39*), little is known of the factors that regulate the activity of these two genes during fermentation. An alternative explanation for the different acetylation ratios of the thiols is that juice pasteurization may have affected the relative stability of the two compounds, 3MH or 3MHA, in the wine. For example, 3MHA is known to be converted back to 3MH by hydrolysis (*40*, *41*), adding to the quantity of 3MH already present, as well as by the action of yeast enzymes. Glutathione supplementation again led to lower values for both varietal thiols after pasteurization of each juice.

The effect of pressing conditions has been shown to have significant consequences for the finished wines, with lower acidity, more rapid glutathione loss, changes in polyphenol content, and lower 3MH and 3MHA content. The large variation in 3MHA content is particularly important given the impact that this varietal thiol has on tropical and passion fruit aromas for Sauvignon blanc wines (*25*−*27*) and its low perception threshold of 4.2 ng/L (*5*); concentrations ranging between 20 and 1300 ng/L were recorded in the research wines of the present study. The 3MHA content is equally important for its negative impact on the floral descriptor and the contribution of methoxypyrazines (*25*), where the content of pyrazines had to be increased by >50% to have an impact when 3MHA was present at 500 ng/L, an average value for Marlborough Sauvignon blanc wines (*26*, *27*). The decline in 3MHA concentration with wine age (>70% can be lost during the first year in the bottle (*41*)) is particularly relevant for wines made from pressed juices and already lower in 3MHA content.

The juice treatments undertaken in the present study, involving additions of glutathione or PVPP prior to fermentation, did not lead to increased concentrations of 3MH and 3MHA, but instead to lower levels in the case of glutathione supplementation. Pasteurization was beneficial for increasing 3MHA content, but not for 3MH. A greater understanding of the influence of juice components upon the production of the varietal aromas 3MH and 3MHA in Sauvignon blanc wines, including an identification of the main precursors in the grape, is required to allow the development of more effective means to improve the aroma profile and stability of wines made from pressed juices.

ABBREVIATIONS USED

3MH, 3-mercaptohexanol; 3MHA, 3-mercaptohexanol acetate; 3MH-S-cys, *S*-3-(hexan-1-ol)-L-cysteine; Con, control; Glut, glutathione; PVPP, polyvinylpolypyrrolidone; CAE, caffeic acid equivalents; RE, rutin equivalents; FAN, free amino nitrogen; TA, titratable acidity; VA, volatile acidity.

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LITERATURE CITED

(1) Marais, J. Effect of grape temperature, oxidation and skin contact on Sauvignon Blanc juice and wine composition and wine quality. *S. Afr. J. Enol. Vitic.* **1998**, *19* (1), 10–16.

(2) Roujou de Boubee, D.; Cumsille, A. M.; Pons, M.; Dubordieu, D. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *Am. J. Enol. Vitic.* **2002**, *53* (1), 1–5.

(3) Maggu, M.; Winz, R.; Kilmartin, P. A.; Trought, M. C. T.; Nicolau, L. Effect of skin contact and pressure on the composition of Sauvignon Blanc must. *J. Agric. Food Chem.* **2007**, *55*, 10281–10288.

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

(5) Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification de l'acetate de 3-mercaptohexanol, compose a forte odeur de buis, intervenant dans l'arome des vins de Sauvignon. *Vitis* **1996**, *35* (4), 207–210.

(6) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Localisation of S-cysteine conjugates in the berry: effect of skin contact on aromatic potential of *Vitis vinifera* L. cv. Sauvignon Blanc must. *Am. J. Enol. Vitic.* **2002**, *53* (2), 144–146.

(7) Subileau, M.; Schneider, R.; Salmon, J.-M.; Degryse, E. New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon Blanc wines: Cys-3MH and (*E*)-hexen-2-al are not the major precursors. *J. Agric. Food Chem.* **2008**, *56*, 9230–9235.
(8) Des Gachons, C. P.; Tominaga, T.; Dubourdieu, D. Sulfur aroma precursor present in *S*-glutathione conjugate form: identification of *S*-3-(hexan-1-ol)-gluathione in must from *Vitis vinfiera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **2002**, *50*, 4−76–4079.
(9) Cheynier, V. F.; Masson, G.; Rigaud, J.; Moutounet, M. Estimation of must oxidation during pressing in Champagne. *Am. J. Enol. Vitic.* **1993**, *44*, 393–399.
(10) Dubourdieu, D.; Moine-Ledoux, V.; Lavigne-Cruege, V.; Blanchard, L.; Tominaga, T. Recent advances in white wine aging: the key role of the lees. Presented at the *ASEV 50th Anniversary Meeting*, Seattle, WA; American Society for Enology and Viticulture: Davis, CA, **2000**.
(11) Makhotkina, O.; Kilmartin, P. A. Uncovering the influence of antioxidants on polyphenol oxidation in wines using an electrochemical method: cyclic voltammetry. *J. Electroanal. Chem.* **2009**, *633*, 165–174.
(12) Roussis, I. G.; Lambropoulos, I.; Tzimas, P. Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or gluathione. *Am. J. Enol. Vitic.* **2007**, *58*, 274–278.
(13) Park, S. K.; Boulton, R. B.; Noble, A. C. Automated HPLC analysis of glutathione and thiol-containing compounds in grape juice and wine using pre-column derivatization with fluorescence detection. *Food Chem.* **2000**, *68*, 475–480.
(14) Du Toit, W. J.; Lisjak, K.; Stander, M.; Prevoo, D. Using LC-MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. *J. Agric. Food Chem.* **2007**, *55*, 2765–2769.
(15) Penna, N. C.; Daudt, C. E.; Brendel, M.; Henriques, J. A. P. Evolución de los niveles de glutathiona y ésteres hidroxicinámicos durante la vinificación de Sauvignon Blanc, Chenin Blanc, Niágara y Tannat. *Alimentaria* **2001**, *326*, 147–151.
(16) Lavigne, V; Pons, A.; Dubourdieu, D. Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection. Changes in concentration in dry white wines during alcoholic fermentation and aging. *J. Chromatogr., A* **2007**, *1139*, 130–135.
(17) Singleton, V. L.; Zaya, J.; Trousdale, E. White table wine quality and polyphenol composition as affected by must SO_2 content and pomace contact time. *Am. J. Enol. Vitic.* **1980**, *31*, 14–20.
(18) Cheynier, V. F.; Rigaud, J.; Souquet, J. M.; Barillère, J. M.; Moutounet, M. Effect of pomace contact and hyperoxidation on the phenolic composition and quality of Grenache and Chardonnay wines. *Am. J. Enol. Vitic.* **1989**, *40*, 36–42.
(19) Darias-Martín, J.; Díaz-González, D.; Díaz-Romero, C. Influence of two pressing processes on the quality of must in white wine production. *J. Food Eng.* **2004**, *63*, 335–340.
(20) Gómez-Míguez, M. J.; González-Miret, M. L.; Hernanz, D.; Fernández, M. A.; Vicario, I. M.; Heredia, F. J. Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. *J. Food Eng.* **2007**, *78*, 238–245.
(21) Blanchard, L.; Darriet, P.; Duboudieu, D. Reactivity of 3-mercaptohexanol in red wine: impact of oxygen, phenolic fractions, and sulfur dioxide. *Am. J. Enol. Vitic.* **2004**, *55*, 115–120.
(22) Danilewicz, J. C.; Seccombe, J. T.; Whelan, J. Mechanism of interaction of polyphenols, oxygen, and sulfur dioxide in model wine and wine. *Am. J. Enol. Vitic.* **2008**, *59*, 128–136.
(23) Cheynier, V. F.; Trousdale, E. K.; Singleton, V. L.; Salgues, M.; Wylde, R. Characterization of 2-*S*-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. *J. Agric. Food Chem.* **1986**, *34*, 217–221.
(24) Wildenradt, H. L.; Singleton, V. L. The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Vitic.* **1974**, *25*, 119–126.
(25) Campo, E.; Ferreira, V.; Escudero, A; Cacho, J. Prediction of wine sensory properties related to grape variety from dynamic-headspace gas chromatography−olfactometry data. *J. Agric. Food Chem.* **2005**, *53*, 5682–5690.
(26) Nicolau, L.; Benkwitz, F.; Tominaga, T. Characterizing the aroma of New Zealand Sauvignon blanc wines. *Aust. N.Z. Grape Winegrowers J.* **2006**, *509*, 46–49.
(27) Lund, C. M.; Thompson, M. K.; Benkwitz, F.; Wohler, M. W.; Triggs, C. M.; Gardner, R.; Heymann, H.; Nicolau, L. New Zealand Sauvignon Blanc distinct flavor characteristics: sensory, chemical, and consumer aspects. *Am. J. Enol. Vitic.* **2009**, *60*, 1–12.
(28) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J.* **1998**, *13*, 159–162.
(29) Hebditch, K. R.; Nicolau, L.; Brimble, M. A. Synthesis of isotopically labelled thiol volatiles and cysteine conjugates for quantification of Sauvignon Blanc wine. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 237–243.
(30) Kilmartin, P. A.; Zou, H.; Waterhouse, A. L. Correlation of wine phenolic composition versus cyclic voltammetry response. *Am. J. Enol. Vitic.* **2002**, *53*, 294–302.
(31) Vrhovšek, U. Extraction of hydroxycinnamoyltartaric acids from berries of different grape varieties. *J. Agric. Food Chem.* **1998**, *46*, 4203–4208.
(32) Delfini, C.; Pessione, E.; Moruno, E. G.; Giunta, C. Localization of volatile acitidy reducing factors in grape. *J. Ind. Microbiol. Biotechnol.* **1992**, *11*, 19–22.
(33) Lund, C. M.; Nicolau, L.; Gardner, R. C.; Kilmartin, P. A. Effect of polyphenols on the perception of key aroma compounds from Sauvignon blanc wine. *Aust. J. Grape Wine Res.* **2009**, *15*, 18–26.
(34) Del Pozo-Insfran, D.; Balaban, M. O.; Talcott, S. T. Microbial stability, phytochemical retention, and organoleptic attributes of dense phase CO_2 processed muscadine grape juices. *J. Agric. Food Chem.* **2006**, *54*, 5468–5473.
(35) Fuleki, T.; Ricardo Da Silva, J. M. Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *J. Agric. Food Chem.* **2003**, *51*, 640–646.
(36) Thibon, C.; Marullo, P.; Claisse, O.; Cullin, C.; Dubourdieu, D.; Tominaga, T. Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. *FEMS Yeast Res.* **2008**, *8*, 1076–1086.
(37) Subileau, M.; Schneider, R.; Salmon, J. M.; Degryse, E. Nitrogen catabolite repression modulates the production of aromatic thiols characteristic of Sauvignon Blanc at the level of precursor transport. *FEMS Yeast Res.* **2008**, *8*, 771–780.
(38) Lee, S. A.; Rick, F. E.; Dobson, J.; Reeves, M.; Clark, H.; Thoomson, M.; Gardner, R. C. Grape juice juice is the major influence on volatile thiol aromas in Sauvignon blanc. *Aust. N.Z. Grape Winegrower. J. Tech. Iss.* **2008**, 78–86.
(39) Lilly, M.; Bauer, F. F.; Lambrechts, M. G.; Swiegers, J. H.; Cozzolino, D.; Pretorius, I. S. The effect of increased yeast alcohol acetyltransferase and esterase activity on the flavour profiles of wine and distillates. *Yeast* **2006**, 641–659.
(40) Tominaga, T.; Masneuf, I.; Dubourdieu, D. Powerful aromatic volatile thiols in wines made from several Vitis Vinifera grape varieties and their releasing mechanism. *ACS Symp. Ser.* **2004**, *No. 871*, 314–337.
(41) Herbst, M.; Nicolau, L.; Kilmartin, P. A. Aroma stability in Sauvignon blanc wines. *Aust. N.Z. Grape Winegrower J. Tech. Iss.* **2008**, 66–72.

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