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Effect of Skin Contact and Pressure on the Composition of Sauvignon Blanc Must

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Early white winemaking operations are known to affect the extraction of grape skin compounds into the juice fraction, which will dictate their concentration in the resulting wine. Grape skin contact and the amount of pressure applied during grape pressing affect the extraction of varietal aromas located in the skins. Compounds such as the polyphenols and glutathione, with antioxidant properties involved in juice oxidation processes and white wine stability, are also affected. The present study evaluates how grape skin contact and the amount of pressure applied during grape pressing affect the levels of *S*-(3-hexan-l-ol)cysteine (3MH-*S*-cys, a key grape-derived precursor to the volatile thiol 3-mercaptohexanol (3MH), which is reminiscent of passion fruit aroma); 2-methoxy-3-isobutylpyrazine (IBMP, with a capsicum-like descriptor); phenolic compounds; and glutathione in Sauvignon Blanc juice. The study was conducted using grapes obtained from commercial Marlborough (New Zealand) vineyards, using both commercial and laboratory grape-processing procedures. Immobilized metal ion chromatography was used to isolate the 3MH-*S*-cys precursor from the juices. The isolated precursor was then volatilized by trimethylsilylation and analyzed using gas chromatography/mass spectrometry (GC/MS). IBMP was analyzed by GC/MS after solvent extraction, and a high-performance liquid chromatography method was used for the quantification of phenolic compounds and glutathione. 3MH-*S*-cys levels were seen to increase in juice fractions obtained from a winery press operating at higher pressures. The increase was attributed to the cumulative effect of longer skin contact time and the amount of pressure applied. The highly water-soluble IBMP was less affected by the amount of pressure applied during commercial grape pressing. Additional information was generated by the specific assessment of skin contact and applied pressure during grape pressing in a laboratory trial. In this trial, a long (32 h) skin contact time resulted in a greater release of varietal aroma compounds, 3MH-*S*-cys, and IBMP into the juice, and the concentration was further raised by increasing the pressure applied during pressing. However, for both experiments, the extraction of the varietal aroma compounds was offset by a clear increase in the juice oxidative potential, seen by a decline in glutathione content, a natural grape antioxidant, and an increase in particular oxidizeable polyphenol compounds, which may cause the must or wine to brown and lead to a loss of varietal aromas.

KEYWORDS: Sauvignon blanc; wine aroma; skin contact; pressing; glutathione; polyphenols

INTRODUCTION

The compounds responsible for the varietal aromas of wines reflect the particular grape variety, climate, and soil and play a major role in the quality and regional character of wines. These compounds may differ from those found in the free state in grapes. Early winemaking procedures such as skin contact and the amount of pressure applied during pressing will affect the extraction of aroma compounds and their precursors into the grape juice and ultimately their concentrations in the resulting wine.

The herbaceous, capsicum-like aroma of 2-methoxy-3-isobutyl pyrazine (IBMP), identified in grapes by Bayonove et al. (*1*), is known to be a key part of the varietal character of Sauvignon Blanc wines (*2*–*4*). At harvest time, this volatile compound is located largely (95%) in the skin of the grapes (*5*).

The identification in Sauvignon Blanc wines of traces of highly odorous compounds such as 4-mercapto-4-methylpentan-

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2-one (4MMP), 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA) (*6*–*8*) allowed wine scientists to explain the varietal aroma characters of these wines associated with descriptors such as broom, grapefruit, and passion fruit (*9*). These volatile thiols are almost totally absent from the grape must and are released into wine from their cysteinylated precursors, *S*-4-(4-methylpentan-2-one)-L-cysteine (4-MMP-*S*-cys), *S*-4-(4-methylpentan-2-ol)-L-cysteine (4MMPOH-*S*-cys), and *S*-3-(hexan-1-ol)-L-cysteine (3MH-*S*-cys), during alcoholic fermentation (*10*). In 2002, Peyrot de Gachons et al. (*11*) studied the distribution of these compounds in grape berries during ripening and showed that at harvest time 4MMP-*S*-cys and 4MMPOH-*S*-cys are located for the most part in the juice (about 80%) whereas 3MH-S-cys is distributed equally between the juice and the skins. Consequently, the authors showed that skin contact has only a minor effect on the extraction of 4MMP-*S*-cys and 4MMPOH-*S*-cys but a noticeable effect on the extraction of 3MH-*S*-cys. In a recent study, Nicolau et al. (*12*) showed the importance of 3MH, 3MHA, and IBMP in the distinctive aroma profile of Marlborough Sauvignon Blanc wine.

Skin contact and pressing not only affect the extraction of varietal aromas, but phenolic compounds (hydroxycinnamic acids and some flavonoids) are also dramatically affected (*13*–*16*). In the grape juice, the hydroxycinnamic acids are known to be easily oxidized by grape polyphenol oxidases in the presence of oxygen to caftaric acid *o*-quinone, which can react with glutathione and form 2-*S*-glutathionyl caftaric acid known as the grape reaction product (GRP) (*17*). The grape juice can be protected against this enzymatic oxidation by SO_2 and the presence of glutathione (*15*). During wine maturation and in the bottle, the compositions of phenolics, glutathione, and oxygen and the catalytic action of metals such as iron and copper may be critical to varietal aroma stability. In the presence of oxygen, the hydroxycinnamic acids and catechin present in wine can be oxidized to *o*-quinones, which are known to easily react with the thiols (such as 3MH) involved in varietal aroma (*18*), either via Michael addition reactions (*19*) or via the generation of peroxides (*20*). On the other hand, IBMP was shown to be unaffected by oxidation (*16*).

The purpose of the present paper is to evaluate how typical commercial Marlborough (New Zealand) grape processing and early winemaking procedures affect the concentrations of 3MH-*S*-cys, IBMP, phenolic compounds, and glutathione in Sauvignon Blanc juices. Juice skin contact often begins with damage to the grapes during mechanical harvesting and continues during grape transport and grape pressing at the winery. Two experiments were undertaken. In the first, Sauvignon Blanc juice was obtained from a commercial winery at different stages of the press cycle and the composition of the juice was analyzed. A second laboratory experiment investigated the effects of skin contact time and pressing pressure upon the composition of Sauvignon Blanc juice. Although the effects of skin contact (*11*, *16*) and pressing (*5*) on Sauvignon Blanc juice composition have been studied in the past, this is the first study looking at how these two winemaking procedures affect important Sauvignon Blanc varietal aroma compounds together with glutathione and phenolic compounds under both commercial and laboratory conditions.

MATERIALS AND METHODS

Chemicals. *N*-BOC-L-cysteine, *trans*-2-hexenal, 1–4-dioxane, and potassium dihydrogenphosphate were purchased from Merck (Palmerston North, New Zealand). Pyridine, trimethylchlorosilane, and *N*,*O*-(bistrimethylsilyl)trifluoroacetamide were supplied by Pierce (Rockford, United States). Anhydrous sodium sulfate, hydrochloric acid, sodium hydroxide, and diethyl ether (GC grade) were supplied by Scharlau Chemie S.A. (Barcelona, Spain). 1–4-Dioxane in a 4 M solution of hydrochloric acid, ethyl acetate, glutathione, gallic acid, caffeic acid, coumaric acid, catechin, epicatechin, quercetin-3-glucoside, and quercetin were purchased from Sigma-Aldrich (St. Louis, Mo). Sodium borohydride came from Lancaster (Heysham, United Kingdom). Chelating sepharose fast flow was from Amersham Biosciences (Auckland, New Zealand), and *S*-benzyl cysteine was from Fluka (Buchs, Switzerland). Dipotassium hydrogenphosphate was obtained from BDH (Palmerston North, New Zealand), and potassium metabisulfite was from Redox Chemicals (Christchurch, New Zealand). Copper sulfate was from Riedel-de Haen (Seelze, Germany), 2-methoxy-3-isobutylpyrazine was from Acros Organics (New Jersey), methoxy-3-([$^2H_3$]isobutyl)pyrazine was purchased from CDN Isotopes (Quebec, Canada), and hexane (GC grade) was supplied by Burdick & Jackson (Muskegon, MI). Helium (instrument grade) and nitrogen (food grade) were supplied by BOC Gases NZ Ltd. (Auckland, New Zealand). All water was 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).

Grape Juice Samples. All grape samples were obtained from the Marlborough grape-growing region of New Zealand during the 2005 harvest.

Grape Processing for the Winery Trial. Grapes originating from three different Sauvignon Blanc clones (a mass selection of UCD1, BDX 316, and BDX 317) were machine harvested at around 22 °Brix and 9.5 g/L total acidity (TA). These are labeled as grape samples A, B, and C throughout this report, to emphasize that they were selected to replicate the winery trial rather than to examine differences between the clonal types. A Bucher RTZ150 (15T) pneumatic press was used to process the grapes. For one press cycle, several gondola trucks of grapes were required to fill the press over a period of 4–6 h. Typically, the temperature of the fruit arriving at the winery was 10−18 °C (depending on the time of day) and the whole press cycle took about 5 h from the time that the press was filled. The free run juice was collected on the arrival of the first truck. The must with 1 h of skin contact was obtained from the press by draining a few liters of must 1 h after the first truckload was pumped into the press. The juice fractions at different pressures were obtained only after the press was filled completely. For this experiment, the pressing fractions at around 0.4, 1.2, and 2 atm were sampled. From the pneumatic press, the juice was drained into an intermediate holding tank and pumped into a tank for settling and further processing. The samples were drawn from the intermediate tank. The free run juice, the 1 h skin contact, and each pressing fraction were collected sequentially, to these was added 50 mg/L of potassium metabisulfite, and they were frozen at −20 °C until analysis.

Grape Processing for the Laboratory Trial. The grapes at approximately 22 °Brix and 9.5 g/L TA were harvested by hand into rectangular flat bins, which held 15–20 kg of fruit. The grapes were transported in the shade in an air-conditioned vehicle and stored at room temperature in the laboratory until they were processed the next morning. About 20 kg of fruit was selected randomly and put through a crusher/destemmer. The grape mass collected was gently and uniformly macerated by hand to which 50 mg/L of potassium metabisulfite was added. Five different lots of around 20 kg of crushed and destemmed grape masses were collected. The control without skin contact was pressed immediately at 0.4 and 2 atm using a locally made small pneumatic press (22 cm diameter and 7 cm deep basket of approximately 2.5 kg capacity). The basket was lined with a muslin cloth, and the fruit was compressed using a bladder inflated using compressed air. Pressing was controlled using a pressure valve, and juice was collected from the base of the press. From 1 kg of grapes, around 600 mL of juice was recuperated (around 1/2 free run juice, 1/4 after pressing at 0.4 atm, and 1/4 after pressing at 2 atm). The remaining fruit underwent a skin contact period of 4, 16, or 32 h at room temperature (around 20 °C) and was then processed as above. The free run juice and each pressing fraction were collected separately and frozen at −20 °C until analysis. The experiment was performed once, without replication.

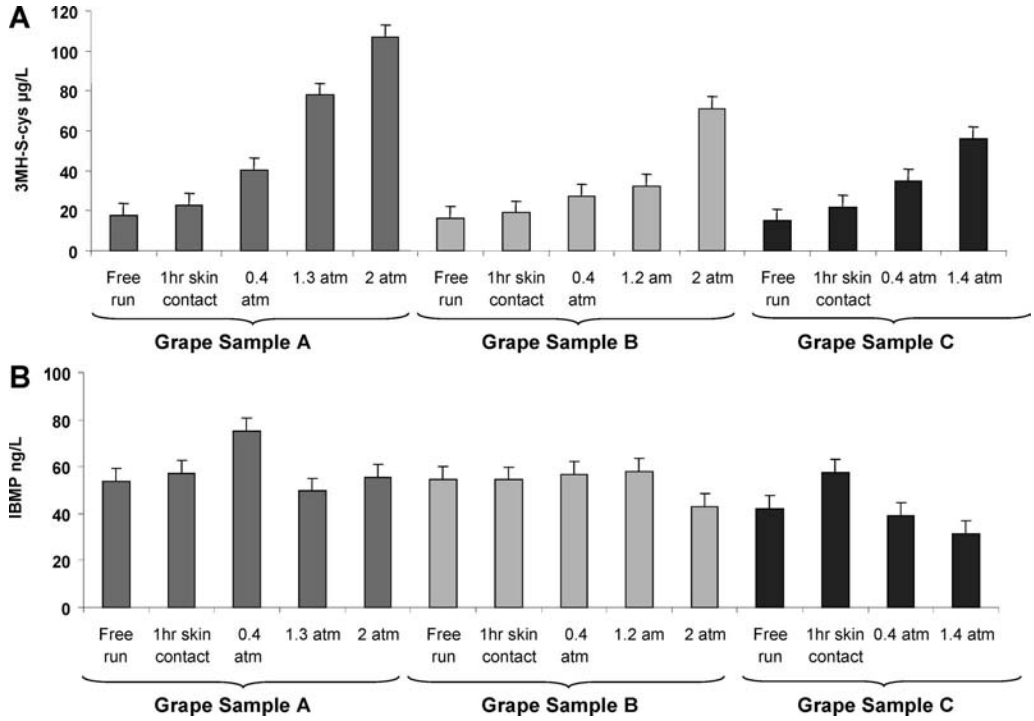

Figure 1. Average concentration of 3MH-*S*-cys (**A**) and IBMP (**B**) in grape juice samples at different stages in the pressing of three Sauvignon Blanc musts. The Fisher's $LSD_{0.05}$ is represented by the *Y* error bars. If the difference between two averages is higher than the Fisher's LSD value, then these averages are statistically different.

Synthesis of 3MH-*S*-cys. 3MH-*S*-cys was synthesized using the method described by Wakabayashi et al. (*21*). The method was modified to use pyridine as the base and instrument grade nitrogen to provide the inert atmosphere, instead of triethylamine and argon. The purity and the concentration of the synthesized precursor were assessed by 400 MHz NMR.

3MH-*S*-cys Analysis. The quantification of 3MH-*S*-cys was carried out according to the method described by Murat et al. (*22*) involving the purification of very small volumes of juice containing 3MH-*S*-cys by affinity chromatography (Chelating Sepharose containing immobilized copper) and gas chromatography coupled with mass spectrometry detection of the purified precursor as a trimethylsilyl derivative. The initial method was modified by using 1 mL of juice instead of 0.5 mL and by using 40 μL of a 1 mg/L *S*-benzyl cysteine as the internal standard instead of *S*-3-(hexan-1-ol)-L-cysteine $^{15}N_2$. 3MH-*S*-cys and the internal standard were detected in selected ion monitoring mode. The quantification ions were $m/z = 320$ for 3MH-*S*-cys and $m/z = 238$ for the internal standard, while the ion at $m/z = 218$ was used as a qualifier for both 3MH-*S*-cys and the internal standard.

The standard curve was prepared by adding increasing quantities of 3MH-*S*-cys to a Sauvignon Blanc must (Marlborough, 2004 vintage), 4−145 μg/L, to obtain six different concentrations. The regression equation obtained was $y = 73.333x + 3.8864$ with $R^2 = 0.996$. A relative standard deviation of 7.8% was obtained by assessing five samples of the same must.

IBMP Analysis. The quantification of IBMP was carried out according to the method described by Kotseridis et al. (*23*). In brief, the organic phase of a triple extraction of 200 mL of wine (pH 8) with 1:1 diethyl ether:hexane was concentrated down to 100 μL, and 2 μL was analyzed by gas chromatography coupled with mass spectrometry using a capillary column BP20 (50 m × 220 μm × 0.25 μm) (polyethylene glycol stationary phase). The only modification made to the initial method was the utilization of methoxy-3-([$^2H_3$]isobutyl) pyrazine as the internal standard instead of methoxy-3-([$^2H_2$]isobutyl) pyrazine. The quantification ion for the internal standard was at $m/z = 127$, while ions with $m/z = 154$ and 169 were used as qualifiers. The standard curve was prepared by adding increasing quantities of IBMP to a Sauvignon Blanc must (Marlborough, 2004 vintage), 2.8−98 ng/L, to obtain eight different concentrations. The regression equation obtained was $y = 1077x - 1.3699$ with $R^2 = 0.9957$. A relative standard deviation of 4.8% was obtained by assessing 10 samples of the same must.

Polyphenols and Glutathione Analysis. Monomeric wine polyphenols were determined using an HPLC method described previously (*24*) and adapted for white wine analysis with a shorter run time of 70 min. In addition to this method, a Coulochem III electrochemical detector with model 5010 analytical cell (ESA Laboratories, Chelmsford, MA) was added in series after the diode array detector, permitting the quantification of glutathione in the same run as the phenolic compounds. In brief, about 1 mL of wine was filtered through a 0.45 μm cellulose filter (Ministart RC-4), of which 20 μL was injected onto a Phenomenex Luna C18 column (4.6 mm × 250 mm, 5 μm particle size) (Torrence, CA) on an Agilent 1100 series instrument (Waldbronn, Germany). The diode array detector was set at 280 (for flavan-3-ols), 320 (for hydroxycinnamic acids), and 365 nm (for flavonols), and the electrochemical detector was set at 0.75 V. 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. Beginning with 45% A and 55% B, the gradient shifted to 100% B after 10 min, while from 15 to 40 min, the gradient moved from 100% B to 80% B and 20% C, on to 55% B and 45% C after 50 min, then back to 45% A and 55% B after 60 min. External standard calibration was made using 1−100 mg/L standards of glutathione, caffeic acid (for the hydroxycinnamic acids), and quercetin (for the flavonols) at eight different concentrations. The linearities obtained were excellent, and the R^2 values were greater than 0.9995 in each case.

Statistical Analysis. All of the samples were analyzed in triplicate. The triplicate analysis data were subjected to single factor analysis of variance (ANOVA), to calculate Fisher's least significant difference (LSD) with a 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. The laboratory trial data were subjected to a multivariante ANOVA; GENSTAT 10.1 software was used for this statistical analysis.

RESULTS AND DISCUSSION

The 3MH-*S*-cys concentration of all of the juices (**Figure 1A**) showed a clear increase during the winery pressing cycle.

While the changes in concentration in the juice were small after 1 h of skin contact, they increased markedly as the amount of pressure was increased. Although only free run and lightly pressed juices are usually used by Marlborough winemakers for premium Sauvignon Blanc wines, the passion fruit aroma potential, that is, the 3MH-*S*-cys concentration, of juice pressed at higher pressures is also important. Wines derived from hard-pressed juice usually require a higher level of bentonite fining to ensure protein stability.

During the pressing cycle of grape sample A, the level of 3MH-cys precursor of the passion fruit aroma increased 4.4 times in the juice obtained at 1.3 atm and 6.2 times in the juice obtained at 2 atm, as compared with the free run juice. The same trend but with a lower increase was obtained for the grape sample B with 2 and 4.4 times more 3MH-cys extracted in the juices obtained at 1.2 and 2 atm, respectively, when compared to the free run juice. Unfortunately, the sample at 2 atm is missing for grape sample C, and the press cycle in this case stopped at 1.4 atm when 3.8 times more 3MH-cys had been extracted as compared with the free run juice.

The effect of the winery pressing cycle on the IBMP concentration (**Figure 1B**) was less evident. The IBMP levels were already high in the free run juice (54, 55, and 42 ng/L for grape sample A, B, and C, respectively) when compared with the average level of 22 ng/L found in a previous study of Marlborough Sauvignon Blanc wines (*12*). Although the concentration increased in some of the later pressings, the amount of increase was not consistent. A statistically significant increase in the IBMP level was observed in the 0.4 atm pressing and 1 h of skin contact juice fractions for grape samples A and C. These increases were followed by a statistically significant decrease in the IBMP levels in the 1.3 and 2 atm juice fractions for grape sample A and in the 0.4 and 1.4 atm juice fractions of grape sample C. However, during the pressing of grape sample B, the level of IBMP did not increase significantly and a significant decrease was only observed in the 2 atm juice fraction. Similar trends in the extractability of IBMP during commercial pressing of Sauvignon Blanc grapes were observed in 2002 by Roujou de Boubee et al. (*5*), suggesting a high solubility of the skin situated IBMP in the juice aqueous solution.

Several polyphenol compounds were monitored in the 70 min HPLC run alongside glutathione, which appeared after 5.4 min and was detected using an electrochemical detector set at 0.75 V (**Figure 2A**). At a concentration of greater than 2 mg/L, glutathione levels were obtained using the peak at this point, although the presence of further oxidizable compounds, seen particularly with the 2.0 atm pressed juice, prevented a reliable determination of glutathione at lower concentrations. Several hydroxycinnamic acids eluted between 20 and 35 min, and these were identified using their UV maximum absorbances (λ_{max}) (**Table 1**) and in comparison with previously published elution orders (*25*, *26*). The major hydroxycinnamic acid in the free run juice, which eluted after 20.2 min, was ascribed to *trans*-caftaric acid, which was followed at 22.1 min by *S*-glutathionyl caftaric acid (**Figure 2B**), both of which showed a λ_{max} of 327 nm. Two further peaks at 27.7 and 28.6 min were ascribed to *cis*- and *trans*-coutaric acids, with λ_{max} in the 310−312 nm range. No peak was determined that could be ascribed to fertaric acid, which was previously shown to be present only at very low levels in Sauvignon Blanc grape juice (*27*). Standards of caffeic acid and *p*-coumaric acid were found to elute after 33.5 and 41.3 min, respectively, but peaks at these times were largely absent except for the highest (2.0 atm) pressing where a level

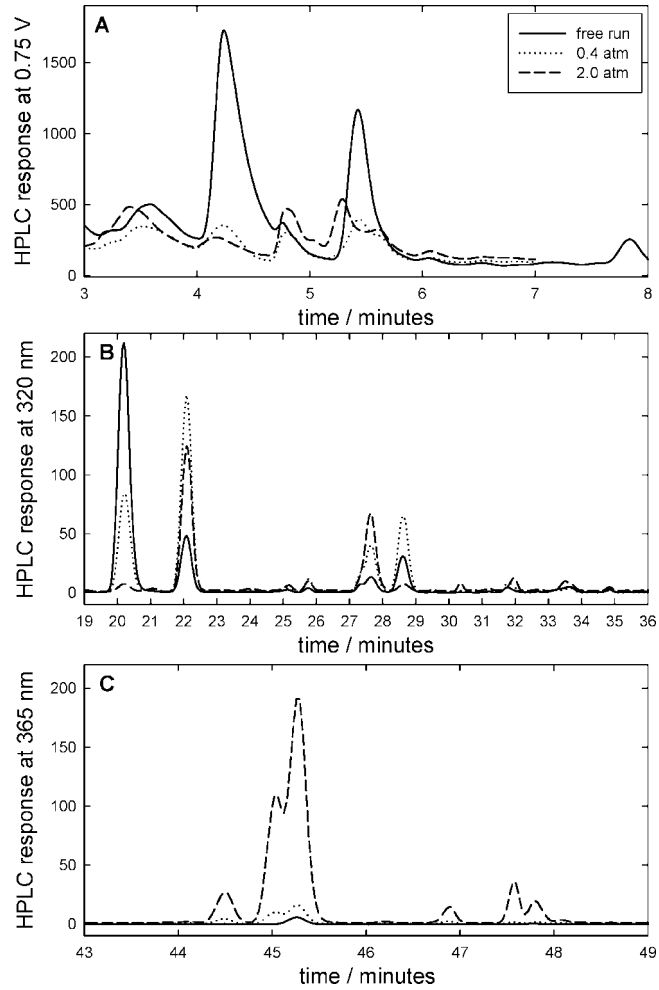

Figure 2. HPLC chromatograms recorded at (**A**) 0.75 V, (**B**) 320 nm, and (**C**) 365 nm for Sauvignon Blanc juice (grape sample B) at different stages in the pressing (free run, 0.4 atm, and 2 atm) (atm = atmosphere).

of free caffeic acid of at most 1 mg/L was obtained. The level of flavonols was very low in the free run juices, but in the higher pressings, a number of peaks were seen in the chromatograms with a λ_{max} in the 350−365 nm range (**Figure 2C**). A quercetin-3-glucoside standard was found to elute at the same time as the largest of the peaks at 45.3 min, while the other flavonol peaks could be due to quercetin-3-glucuronide or various kaempferol glycosides (*25*, *28*). On the other hand, significant peaks due to the flavan-3-ols catechin and epicatechin were not seen in the chromatogram recorded at 280 nm at the times given by the injection of the respective standards; the area of a shoulder seen in some of the chromatograms would indicate the presence of at most 1 mg/L of catechin.

The free run juice from all of the three grape samples contained high levels of both glutathione (36−40 mg/L) and caftaric acid (32−44 mg/L) (**Table 1**). Despite the inevitable oxygen exposure that the grapes undergo during machine harvesting, the glutathione levels of the free run juices were very close to the glutathione level (47 mg/L) previously reported by Cheynier et al. (*29*) in white grapes analyzed under anaerobic conditions. However, the presence of moderate amounts of *S*-glutathionyl caftaric acid (9−15 mg/L) (**Table 1**) indicates that oxidation of grape components had occurred as a result of the machine harvesting.

After 1 h of skin contact, the levels of glutathione had fallen by up to half and for the 0.4 atm pressing were only present in

Table 1. Concentration of Glutathione and Monomeric Polyphenols by HPLC in the Three Juices from the Winery Trial ($n = 3$)[a]

	glutathione (mg/L)	caftaric acid (mg/L CAE)	*S*-glutathionyl caftaric acid (mg/L CAE)	*cis*-coutaric acid (mg/L CAE)	*trans*-coutaric acid (mg/L CAE)	quercetin-3-glucoside (mg/L QE)
retention time (min)	5.4	20.2	22.1	27.7	28.6	45.3
λ_{max}		327	327	310	312	354
			grape sample A			
free run	39.6 (±0.8) a	36.8 (±2.4) a	12.0 (±0.1) a	1.51 (±0.06) a	3.85 (±0.01) a	0.15 (±0.01) a
1 h of skin contact	23.3 (±0.6) b	27.3 (±0.1) b	11.1 (±0.1) b	2.47 (±0.01) a	4.94 (±0.03) b	0.07 (±0.01) a
0.4 atm	ND[b]	18.5 (±0.1) c	30.8 (±0.1) c	11.5 (±0.1) b	14.4 (±0.1) c	1.65 (±0.02) b
1.3 atm	ND	1.9 (±0.2) d	20.5 (±0.2) d	13.6 (±0.1) b,c	2.38 (±0.04) d	7.73 (±0.01) c
2.0 atm	ND	2.1 (±0.1) d	19.7 (±0.1) e	14.5 (±0.1) c	2.82 (±0.07) e	11.44 (±0.03) d
			grape sample B			
free run	36.5 (±1.3) a	44.4 (±0.1) a	9.2 (±0.02) a	3.1 (±0.4) a	5.88 (±0.03) a	0.34 (±0.01) a
1 h of skin contact	23.1 (±0.4) b	37.5 (±0.1) b	11.0 (±0.1) b	4.37 (±0.05) a	7.41 (±0.03) b	0.13 (±0.03) b
0.4 atm	8.9 (±0.3) c	17.6 (±0.1) c	31.5 (±0.2) c	9.3 (±0.1) b	12.4 (±0.1) c	0.96 (±0.01) c
1.2 atm	ND	7.4 (±0.1) d	25.4 (±0.1) d	12.3 (±0.1) c	7.03 (±0.03) d	3.02 (±0.01) d
2.0 atm	ND	1.4 (±0.1) e	22.9 (±0.1) e	13.6 (±0.1) c	1.09 (±0.08) e	11.55 (±0.01) e
			grape sample C			
free run	37.5 (±0.7) a	32.5 (±0.1) a	15.1 (±0.1) a	2.01 (±0.02) a	4.59 (±0.01) a	0.05 (±0.01) a
1 h of skin contact	16.7 (±0.2) b	30.3 (±0.1) b	15.9 (±0.2) b	5.11 (±0.06) b	7.0 (±0.1) b	0.40 (±0.01) b
0.4 atm	ND	5.9 (±0.1) c	27.3 (±0.3) c	10.0 (±0.1) c	5.0 (±0.1) c	2.37 (±0.06) c
1.2 atm	ND	3.80 (±0.02) d	23.3 (±0.1) d	6.8 (±5.1) b	3.38 (±0.03) d	5.4 (±0.1) d

[a] Standard deviations are given in parentheses after each value. [b] ND, not detected. For each individual must, samples marked by the same letter are not significantly different by Fischer $LSD_{0.05}$ of the triplicate analysis.

a detectable amount in grape sample B. Likewise, levels of caftaric acid declined with the higher pressings to a concentration of less than 4 mg/L in the 2.0 atm pressings. While the juice was held in contact with the skins, particularly after the removal of the free run juice, oxidation processes involving polyphenol oxidase enzymes were likely responsible for these declines. On the other hand, an increase in the concentrations of *S*-glutathionyl caftaric acid was seen in the 0.4 atm pressings, being the likely initial product of the oxidized caftaric acid quinone reacting with available glutathione. At higher pressings, and with the supply of glutathione already exhausted, some decline in the concentrations of *S*-glutathionyl caftaric acid was observed. Similar trends in the development of caftaric acids have previously been seen in controlled oxygenation studies (*15*).

In the free run juice, the concentration of *trans*-coutaric acid (4−6 mg/L) was higher than that of *cis*-coutaric acid (1.5−3 mg/L) (**Table 1**). Increased concentrations of both coutaric acids were seen in the intermediate pressings, particularly at 0.4 atm in each case, while for the highest pressings the order was reversed with higher concentrations of *cis*-coutaric acid (7−15 mg/L) as compared with *trans*-coutaric acid (1−3.5 mg/L). It has previously been reported that the coutaric acids are largely located in the skins (*27*), meaning that more time is required for their extraction, while *trans*-coutaric acid can be oxidized by grape polyphenol oxidases (*15*) or converted to *cis*-coutaric acid under the influence of UV light (*30*). *cis*-Coutaric acid has also been observed to be stable against certain enzymatic esterase treatments, which act upon *trans*-coutaric acid (*26*).

By contrast, flavonols were largely absent from the free run juices, but a marked increase in concentrations was seen with the higher juice pressings (**Table 1**). The concentration of the main peak, ascribed to quercetin-3-glucoside, reached 11.5 mg/L for the 2.0 atm pressing in both grape samples A and B, and a total flavonol level of around 20 mg/L (quercetin equivalents) was reached. An increase in flavonol content with several hours of skin contact has been previously reported (*16*, *31*–*33*). The phenolic composition of the 2.0 atm pressed juice was thus markedly different from that of the free run juice. The higher concentrations of flavonols coupled to the absence of protective glutathione in the higher pressings led to an increase in the oxidative potential of the juice during the winery pressing cycle, in addition to the higher level of oxidized polyphenols expected in the higher press fractions.

During pressing within the winery, the juice composition was affected by a cumulative effect of pressing and skin contact. To specifically assess the role of these two winemaking procedures, a laboratory trial was set up by pressing the grapes after different skin contact times (0, 4, 16, and 32 h) at three different pressures (0 or free run, 0.4, and 2 atm). The effects of skin contact and pressure on the level of 3MH-*S*-cys and IBMP are presented in **Figure 3**. Unlike the winery scale trial, there was not a significant difference in the level of 3MH-*S*-cys (**Figure 3A**) between the no skin contact and the 4 h of skin contact free run juices and 0.4 atm juices. For those skin contact times, only the juices pressed at 2 atm showed an increase in 3MH-*S*-cys concentration. While potassium metabisulfite had been added to the must after initial fruit processing, it is possible that yeast activity was not completely suppressed and some initial fermentation processes and utilization of precursors had already started. Despite this, the extraction of 3MH-*S*-cys was more significant after 16 h of skin contact and increased again after 32 h. At this time, pressing at 0.4 and 2 atm released more 3MH-*S*-cys and resulted in the highest juice concentrations. However, this release was not significantly higher in the juices obtained at 2 atm when compared with the one obtained at 0.4 atm. Moreover, there was a significant decrease when comparing juices obtained at 2 atm and at 0.4 atm pressing pressures for the grapes with 16 h of skin contact. These results are in general agreement with a previous report (*11*) where an increase in 3MH-*S*-cys content was observed after 19 h of skin contact under laboratory conditions.

The level of IBMP (**Figure 3B**) showed a more consistent increase. This increase began to be statistically significant from the 2 atm juice obtained after 4 h of skin contact as compared with the juices obtained from grapes pressed without skin contact. When the free run juices for each of the skin contact times of 4, 16, and 32 h were compared with the corresponding pressed juices, only the juice obtained at 2 atm was significantly different. An increase in IBMP content with several hours of skin contact under laboratory conditions has been previously reported (*16*). Overall, the skin contact time seemed to have a

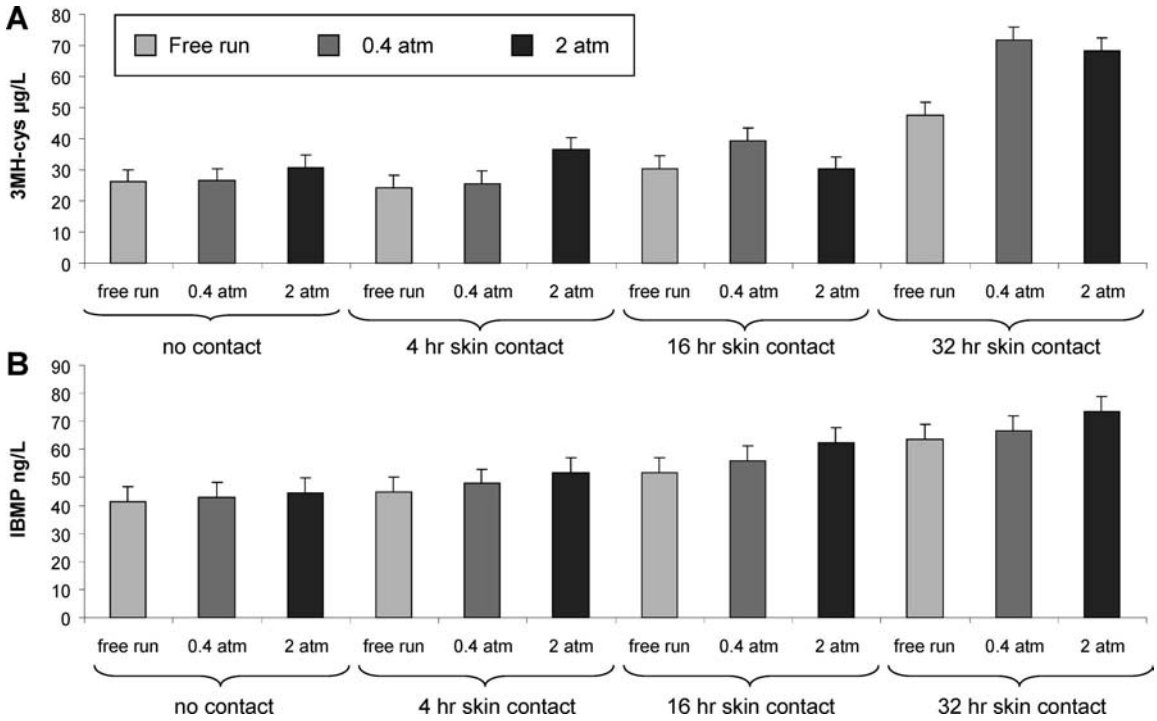

Figure 3. Average concentration of 3MH-*S*-cys (**A**) and IBMP (**B**) in Sauvignon Blanc grape juices obtained in a laboratory trial by pressing the grapes after different skins contact times (0, 4, 16, and 32 h) at three different pressures (0 or free run, 0.4, and 2 atm) (atm = atmosphere). The Fisher's $LSD_{0.05}$ is represented by the *Y* error bars. If the difference between two averages is higher than the Fisher's LSD value, then those averages are statistically different.

Table 2. Concentration of Glutathione and Monomeric Polyphenols by HPLC in the Juices from the Laboratory-Scale Trial ($n = 3$)[a]

	glutathione (mg/L)	caftaric acid (mg/L CAE)	*S*-glutathionyl caftaric acid (mg/L CAE)	*cis*-coutaric acid (mg/L CAE)	*trans*-coutaric acid (mg/L CAE)	quercetin-3-glucoside (mg/L QE)
			no skin contact			
free run	38.2 (±1.4) a	38.3 (±0.6) a	7.70 (±0.2) a	1.31 (±0.05) a	3.70 (±0.01) a	<0.05
0.4 atm	37.9 (±1.3) a	37.3 (±0.1) b	6.15 (±0.02) b	1.47 (±0.02) b	3.82 (±0.04) b	<0.05
2.0 atm	33.5 (±0.9) b	40.8 (±0.1) c	5.22 (±0.01) c	1.51 (±0.07) b	4.14 (±0.01) c	<0.05
			4 h of skin contact			
free run	24.5 (±0.9) c	30.1 (±0.2) d	8.37 (±0.02) d	1.35 (±0.03) a	3.51 (±0.01) d	ND
0.4 atm	ND[b]	0.74 (±0.09) e,h	8.74 (±0.02) e	0.78 (±0.03) c	ND	<0.05
2.0 atm	ND	0.21 (±0.01) f	2.73 (±0.01) f	0.28 (±0.02) d	ND	0.08 (±0.1) a
			16 h of skin contact			
free run	ND	1.34 (±0.03) g	6.95 (±0.02) g	1.14 (±0.05) e	ND	0.31 (±0.01) b
0.4 atm	ND	0.19 (±0.01) f	3.03 (±0.04) h	0.40 (±0.07) f	ND	0.51 (±0.01) c
2.0 atm	ND	0.21 (±0.02) f	3.05 (±0.01) h	0.32 (±0.02) d	ND	0.56 (±0.01) d
			32 h of skin contact			
free run	ND	0.45 (±0.01) f,h	3.96 (±0.06) i	1.07 (±0.01) e	ND	0.38 (±0.01) e
0.4 atm	ND	0.24 (±0.02) f	3.80 (±0.01) j	0.46 (±0.03) f	ND	0.45 (±0.01) f
2.0 atm	ND	0.21 (±0.01) f	1.06 (±0.04) k	0.83 (±0.01) c	ND	0.62 (±0.01) g

[a] Standard deviations are given in parentheses after each value. [b] ND, not detected. Samples marked by the same letter are not significantly different by Fischer $LSD_{0.05}$ of the triplicate analysis.

greater impact on the concentration of 3MH-*S*-cys and IBMP than the amount of pressure applied. An increase of 1.8-fold in the level of 3MH-*S*-cys was observed between the free run juices with no skin contact and 32 h of skin contact time. The level of 3MH-*S*-cys was further increased with continued pressing, and an increase of 2.2-fold was observed between the 2 atm juices with no skin contact and 32 h of skin contact. However, this increase was less important, only 1.4-fold, for 32 h of skin contact, when free run and 2 atm juices were compared. By contrast, an increase of only 1.54-fold in the level of IBMP was observed between the free run juices with no skin contact and 32 h of skin contact. The level of IBMP increased less with pressing than the 3MH-*S*-cys levels, and only a 1.6-fold increase was observed between the 2 atm juices with no skin contact and 32 h of skin contact, and only a 1.15-fold increase in the IBMP level was observed when the 32 h skin contact free run juices and 2 atm juices were compared.

Supporting information regarding the comportment of these two compounds during the winemaking procedures was added by a multivariate ANOVA looking at the interaction between the four skin contact times and the different applied pressures. As expected, the main effects were significant ($P < 0.001$) for both compounds, confirming that skin contact influenced the concentration of the compounds, as did the different amounts of pressure during pressing. Furthermore, an interaction effect ($P < 0.001$) between these main effects was obtained for 3MH-*S*-cys, showing that the influence of skin contact was different for different applied pressures. However, the skin contact time

did not affect the extraction of IBMP during pressing, as no interaction effect was obtained between these two main effects. This can help explain differences observed between the extraction of IBMP under commercial and laboratory conditions during our study and in past studies (*5*, *16*). When compared with laboratory conditions, the increase in the level of IBMP could not be reliably demonstrated during a commercial pressing, despite the cumulative effect of skin contact time and along with increased pressure under these conditions.

The juices pressed off without skin contact showed a high concentration of glutathione and caftaric acid (**Table 2**), similar to the levels seen with free run juices in the winery trials. However, once a period of 4 h of skin contact was included, any glutathione, and caftaric acid at more than 2 mg/L, was only seen in the free run juice. For 16−32 h of skin contact, these two compounds were largely absent, as was *trans*-coutaric acid. For longer skin contact times, the major polyphenols detected were *S*-glutathionyl caftaric acid (up to 3 mg/L), along with some *cis*-coutaric acid and quercetin-3-glucoside but in both cases at concentrations generally less than 1 mg/L. However, the decrease in caftaric acid, glutathione, and *trans*-coutaric acid was more rapid than that observed during the winery scale trial, probably reflecting the small sample size used in the laboratory trial and the greater susceptibility of grapes and juice to oxidation. Increases in quercetin-3-glucoside, *trans*, and *cis*-coutaric acid concentrations were also lower in the laboratory trial, possibly reflecting a less efficient pressing system. Overall, a dramatic impact of skin contact time upon the concentration of glutathione and phenolic compounds was observed, and with higher pressures, the concentrations were further affected. This was confirmed by a multivariate ANOVA. The main effects were significant ($P < 0.001$) for all of the compounds, confirming the effect of these two winemaking procedures on the concentration of these compounds, and furthermore, an interaction effect ($P < 0.001$) between these two main effects was obtained for all of the compounds showing that the influence of applied pressure was different for different skin contact times.

The duration of skin contact and the amount of pressure during pressing are important parameters determining the final composition of juice for winemaking. In general, 3MH-*S*-cysteine concentrations increased as pressing time and the amount of pressures applied during pressing increased. By contrast, water-soluble IBMP was less affected under these commercial conditions. However, the beneficial extraction of varietal aroma resulting from longer skin contact and pressing pressures was clearly offset by an increase in juice oxidative potential, observed as a decline in the protective glutathione content and increase in particular oxidizable polyphenol compounds. A decrease in wine sensorial quality due to longer skin contact has already been shown in the past (*16*). Winemakers also empirically appreciate that juice quality declines during the pressing cycle, and they generally choose only free run and lightly pressed juices for premium wine production, an observation supported by the results of the present study.

A better understanding of the balance between skin contact time, amount of pressure during pressing, varietal aroma release, and oxidative balance will enable winemakers to maximize the recovery of quality juice for premium wine production.

ABBREVIATIONS USED

3MH-*S*-cys, *S*-(3-hexan-l-ol)cysteine; IBMP, 2-methoxy-3-isobutylpyrazine; CE, catechin equivalents; CAE, caffeic acid equivalents; QE, quercetin equivalents.

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