Classification of Grape Berries According to Diameter and Total Soluble Solids To Study the Effect of Light and Temperature on Methoxypyrazine, Glutathione, and Hydroxycinnamate Evolution during Ripening of Sauvignon blanc (*Vitis vinifera* L.)

Katja Šuklje,[†] Klemen Lisjak,[†] Helena Baša Česnik,[†] Lucija Janeš,[†] Wessel Du Toit,[§] Zelmari Coetzee,[§] Andreja Vanzo,[†] and Alain Deloire*,[§]

[†]Central Laboratories, Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia
[§]Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

Ⓢ *Supporting Information*

ABSTRACT: Grape berries were classified according to diameter and total soluble solids (TSS) to study the effect of light and temperature on methoxypyrazines (MPs), glutathione (GSH), and hydroxycinnamates (HCAs) during the ripening of Sauvignon blanc. The light exposure of the fruiting zone was modified within leaf and lateral removal at the phenological stage berry of peppercorn size and no removal (control). In comparison to the control, the concentration of 3-isobutyl-2-methoxypyrazine (IBMP) was below the limit of detection in leaf removal 2 weeks before harvest. Leaf removal had no significant influence on GSH and HCAs in the grape juice at harvest. Berry diameter significantly influenced the concentration of IBMP in the grape juice and did not influence the concentration of GSH and HCAs. At harvest, the concentrations of IBMP in grape juices of similar TSS in the control were 12.6 and 5.2 ng/L in 15.5 and 13.5 mm berry diameter classes, respectively. Furthermore, the study showed that berries of the same diameter were not at the same physiological ripening level (not the same TSS).

KEYWORDS: *Sauvignon blanc, grape berry diameter, total soluble solids, light, temperature, methoxypyrazines, glutathione, hydroxycinnamates*

■ INTRODUCTION

Grape berry growth and maturity are characterized by asynchrony between the berries within a bunch and between bunches within the vine. Therefore, fruit classification methods are implemented to minimize berry heterogeneity and provide possible trends in the metabolism of major berry compounds.[1−4] Fruit classification according to the diameter and total soluble solids (TTS) has already been utilized in several studies, although mainly in relation to red cultivars. It has been used to enhance the understanding of grapevine fruit growth and the associated biochemical composition.[4−6]

The green aroma descriptors of Sauvignon blanc wines originate from 3-alkyl-2-methoxypyrazines (MPs), whereas volatile thiols are responsible for the tropical characteristics of the wines.[7−9] The most important MPs found in grapes and wines are 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP). IBMP contributes to the green pepper and asparagus aromas, whereas IPMP imparts earthier aromas.[10−13] The sensory detection threshold for IBMP was found to be very low, around 2 ng/L in water, 8 ng/L in Sauvignon blanc wines, and 15 ng/L in red Bordeaux wines.[10,13,14] High IBMP concentrations in grapes may have a negative impact on the quality of the wine aroma.[13]

Abiotic factors such as light and temperature at the bunch level, vine water status, and various viticulture practices can influence the concentration of MPs in the berry and wine.[15−17] It has been shown that grapes and wines from cooler climatic regions contain higher concentrations of IBMP than grapes produced in warm regions.[18] In addition, pre-véraison bunch exposure to sunlight can reduce IBMP concentrations in grapes at harvest. However, bunch exposure after véraison is reported to have little effect.[16,17]

Glutathione (GSH) and hydroxycinnamates (HCAs) are important antioxidants that preserve freshness in white wines.[19] GSH is a tripeptide composed of glutamic acid, cysteine, and glycine, which exists in a reduced or oxidized form. Its concentration ranges from 14 to 102 mg/L in grapes and up to 35 mg/L in wines.[20,21]

In grape berries, GSH synthesis starts with sugar accumulation in the berry, whereas HCAs are synthesized as early as berry formation begins. Adams and Liyanage have shown that there is a close correlation between GSH and TSS concentration until the berries reach 16 °Brix and that GSH concentration increases on a per berry basis.[22] During the oxidation of white must, the caftaric acid *O*-quinone, included in the browning of white wines, can be reduced by GSH (if present), resulting in the production of colorless 2-*S*-glutathionyl caftaric acid, also called grape reaction product (GRP).[23] Furthermore, GSH is required for the synthesis of glutathione-3-mercaptohexan-1-ol, one of the precursors of the prominent varietal thiol 3-mercaptohexan-1-ol (3MH). This

compound imparts passion fruit aroma and plays a central role in the aromatic typicity of Sauvignon blanc wines.[24,25] GSH preserves the aromatic potential of white wines, especially varietal thiols and esters, and participates in the reversible redox reaction of the thiol group.[26,27]

HCAs found in grapes are *cis-* and *trans*-forms of caftaric, coutaric, and fertaric acids, which are tartaric esters of hydroxycinnamic acids: caffeic, *p*-coumaric, and ferulic acids, respectively. They are the major class of nonflavonoid phenolics in white wines. The free forms of HCAs appear in wine due to the hydrolytic activity of yeasts and/or grape enzymes or due to acid hydrolysis in the wine.[28] The concentration of HCAs in wines ranges from 80 to 166 mg/L.[28] Cultivars with high HCAs but low GSH concentrations have increased browning potential when exposed to oxygen.[29,30]

Internationally, including South Africa, Sauvignon blanc is an important cultivar. Although numerous studies have been conducted on this cultivar,[13,24,31−33] there are still pending questions regarding the physiology of ripening and the metabolism of aromatic precursors and their preservatives.

The aim of this work was to study the influence of bunch microclimate (light and temperature) on the evolution of MPs, GSH, and HCAs in Sauvignon blanc grapes during ripening. To understand the differences in grape berry quality within the vineyard, grape berries were classified according to their diameter and thereafter according to their TSS concentration. Such classifications provided a novel approach for studying the dynamics of MPs, GSH, and HCAs during the ripening of *Vitis vinifera* L. Sauvignon blanc grape berries.

MATERIALS AND METHODS

Experimental Vineyard. A commercial vineyard located in the Overberg region of the western coastal area, South Africa (E 19° 1′ 68″, S 34° 9′ 52.76″), was used in this study. The experiment was performed on Sauvignon blanc vines (*V. vinifera* L.), clone 316, grafted onto rootstock 101.14. The row orientation was northwest−southeast (2.5 m × 1.8 m) and the training system was vertical shoot positioning, pruned within a double cordon with two buds per spur. Canopy management was done by hedging the vines at a height of 1.4 m and lateral shoot cutting to maintain the width of the canopy at 40 cm. Irrigation was managed to avoid water constraints and was monitored by using a pressure chamber and measuring the stem water potential.[34,35]

The light exposure of the fruiting zone was modified within leaf and lateral removal (leaf removal) and no removal (control). The experimental design consisted of four rows with four replications of leaf removal and four replications of control per row. Each replicate consisted of four contiguous vines. Replicates were randomized in a block layout. In the leaf removal treatment leaves and laterals were completely removed from the bunch zone at a height of 40 cm from the cordon on the morning (eastern) side of the canopy, which resulted in 100% exposed bunches from the eastern side. Leaf and lateral removal was performed on December 17, 2010, at berry peppercorn size (E-L 29).[36] Bunches in the leaf removal treatment were 100% shaded from the afternoon (western) side of the canopy. The control consisted of 100% shaded bunches from both sides of the canopy, which was possible to realize due to the thickness of the canopy at the bunch zone.

Sampling Protocol. For the control, one bunch per vine was sampled randomly from the inside of the canopy. For the leaf removal treatment, only fully exposed bunches were sampled. For each sampling date, 40 bunches were collected per treatment. Three developmental stages were analyzed, at véraison on January 25 (E-L 35), 4 weeks after véraison on February 21 (E-L 37), and at harvest on March 1. Véraison was determined at the time when 50% of the berries were soft.

Bunch samples were kept in a cooling box and transported to the laboratory. To prevent oxidation, all berries from sampled bunches were carefully cut at the torus with a pair of scissors. The total number of berries in a sample of 40 bunches was counted, and it ranged between 2033 and 2935. Berries were classified according to their diameter using special Perspex plates. Each classification plate contained holes of different diameters from 10.5 to 16.5 mm, increasing at 1 mm intervals. Berry classification started with the classification of the largest diameter and continued to the smallest to obtain different berry size classes. Berries in each diameter class were counted, and the distribution percentage was established.

For the second classification, according to TSS concentration, two of the most representative diameter classes with at least 2 mm difference were used. Berry TSS concentration was estimated by flotation in sucrose solutions of different concentrations (from 80 to 260 g/L $C_{12}H_{22}O_{11}$).[37] The difference in density of two consecutive sucrose solutions was 10 g/L. Berries were classified, depending on the sampling date, in five to eight TSS classes. Berries of the same diameter were floated in the sucrose solution, starting with the least dense. The floated berries were considered to have the same TSS concentration as the solution. These berries were separated from the others, rinsed with water, dried, and counted. The sunken berries were collected and placed into the following, denser solution. The same procedure was repeated for all sucrose solutions. For each of the most representative berry diameter class, two classes of berries were selected according to TSS classification, with a TSS concentration difference of at least 2 °Brix. Grape berries belonging to each TSS class were counted, and their distribution percentage was established. All of the berries were inspected visually before analyses to exclude oxidation and thereby influence GSH and HCAs concentrations. These sorting methods, which were also used by other authors,[1,2,4,37,38] strongly reduce the biological heterogeneity between berry classes, which leads to replicates not being obtained.

Photosynthetic Active Radiation, Vine Water Status, and Temperature Measurements. Photosynthetic active radiation (PAR) (μmol m^{-2} s^{-1}) was measured with the Accupar PAR/LAI ceptometer, model LP-80 (Decagon Devices Inc., Pullman, WA, USA). The light sensor rod was placed parallel to the cordon to mimic the light interception of the bunches. The vine water status (Ψ_{SWP}) was measured using the stem water potential method.[34,35] Light intensity measurements were performed at 10 a.m., whereas stem water potential was measured between 12 and 2 p.m., parallel with the sampling dates. The temperature was monitored continuously inside the canopy and at the bunch-berry levels using Gemini data loggers TGP-4500 and TGP-4520, respectively (Chichester, UK).

The total soluble solids and L-malic acid concentration were determined according to standard methods.[39] A subsample of 200 berries was taken per class. Berries were weighed, transferred into a plastic bag, and crushed by hand, and the juice was collected for analyses. TSS was measured using a digital refractometer (Atago PAL-1, Tokyo, Japan) with temperature correction. The L-malic content was determined spectrophotometrically (Agilent 8453, Palo Alto, CA, USA) using enzymatic kits (Megazyme, Ireland).

Determination of Methoxypyrazines. *Preparation of Standards and Solvents.* IBMP (Sigma-Aldrich, St. Louis, MO, USA) with a purity of 99%, 2-isobutyl-3-methoxy-d_3-pyrazine ([^{2}H$_3$]-IBMP) (C/D/N/Isotopes, Quebec, Canada) with a purity of 99%, and IPMP (Sigma-Aldrich) with a purity of 99% were used for the preparation of standards in solvent. Stock solutions of IBMP (250 mg/L), [^{2}H$_3$]-IBMP (500 mg/L), and IPMP (280 mg/L) were prepared in methanol (Sigma-Aldrich). Intermediate solutions (IBMP = 2.5 mg/L, [^{2}H$_3$]-IBMP = 5.0 mg/L, and IPMP =2.8 mg/L) and working solutions (IBMP = 2.5 μg/L, [^{2}H$_3$]-IBMP = 5.0 μg/L, and IPMP = 2.8 μg/L) were prepared in methanol as well.

Preparation of Sugar Solution. Five hundred milliliters of water purified by a Milli-Q system (Bedford, MA, USA) was placed in a 1000 mL volumetric flask. Ninety grams of fructose (Sigma-Aldrich), 90 g of glucose (Sigma-Aldrich), and 1 g of tartaric acid (Merck, Darmstadt, Germany) were added and dissolved. The volumetric flask was made

Table 1. Standard Deviation and Measurement Uncertainty of the Method for Determining Methoxypyrazines (ng/L)

	dearomatized must		Sauvignon blanc from Slovenia			Sauvignon blanc from New Zealand		
	IBMP	IPMP	IBMP	IBMP	IPMP	IBMP	IBMP	IPMP
spiking level	9.8	10.0		25.0	25.0		25.0	25.0
means of the levels	10.9	10.3	1.9	20.3	21.0	6.9	23.3	20.2
standard deviation of repeatability (s_r)	0.6	0.4	0.1	0.5	0.5	0.3	0.6	0.7
relative standard deviation of repeatability (RSD$_r$) (%)	5.6	3.5	6.6	2.5	2.4	4.3	2.7	3.5
standard deviation of reproducibility (s_R)	1.5	0.7	0.5	1.3	1.1	1.0	1.6	1.7
relative standard deviation of reproducibility (RSD$_R$) (%)	14.1	6.9	25.9	6.5	5.4	15.0	7.0	8.6
uncertainty of repeatability (U_r)	1.4	0.8	0.3	1.2	1.1	0.7	1.4	1.6
relative uncertainty of repeatability (%)	14.2	8.3	15.0	4.7	4.6	9.6	5.8	6.5
uncertainty of reproducibility (U_R)	3.5	1.6	1.1	3.0	2.6	2.3	3.7	3.9
relative uncertainty of reproducibility (%)	35.5	16.2	58.7	11.9	10.3	33.9	14.7	15.6

up to volume with purified water, and the pH was adjusted to 3.2 with NaOH.

Dearomatization of Grape Juice. Forty-five milliliters of Sauvignon blanc juice was placed in the 50 mL tube and centrifuged for 5 minutes at 5000 min^{-1}. The liquid was then transferred to a 5 L flask; 3 L of previously centrifuged Sauvignon blanc juice was evaporated under reduced pressure to approximately 90% of the initial volume. The evaporated liquid was replaced by purified water. Afterward, the juice was transferred to a beaker and heated until it reached 80 °C to evaporate or decompose the MPs still present in the juice.

Preparation of Alcoholic Solution. Five hundred milliliters of purified water, 120 mL of absolute ethanol (Sigma-Aldrich), and 1 g of tartaric acid were added to a 1000 mL volumetric flask. The volumetric flask was then made up to volume with purified water, and the pH was adjusted to 3.2 with NaOH.

Preparation of Calibration Standards. Calibration standards were prepared in a sugar solution, an alcoholic solution, and a dearomatized must using working solutions of IBMP, [^{2}H$_3$]-IBMP, and IPMP. Some sugar solution, alcoholic solution, or dearomatized must was transferred to a 25 mL volumetric flask, [^{2}H$_3$]-IBMP, IBMP, and IPMP were added, and then the flask was made up to the volume to reach the final concentration of 25 ng/L of [2H3]-IBMP and IBMP and 28 ng/L of IPMP. NaCl was placed into a 20 mL SPME vial along with a stir bar, followed by 1.6 mL of the prepared solution, 6.4 mL of purified water, and 2 mL of 4 M NaOH. The vial was closed and placed onto a magnetic stir plate to dissolve the NaCl.

Preparation of Sample. The grape juice sample was prepared by hand-crushing undamaged berries in a plastic bag for 2 min. Some strained grape juice was transferred to a 25 mL volumetric flask, 125 μL of [^{2}H$_3$]-IBMP (internal standard) was added with a concentration of 5 μg/L, to reach the final concentration 25 ng/L of [^{2}H$_3$]-IBMP, and the flask was made up to the volume with grape juice. NaCl was placed into a 20 mL SPME vial along with a stir bar, followed by 1.6 mL of the prepared sample, 6.4 mL of purified water, and 2 mL of 4 M NaOH. The vial was closed and placed onto a magnetic stir plate to dissolve the NaCl.

Apparatus and Determination Procedure. The samples were analyzed using a gas chromatograph (Agilent Technologies 7890A, Shanghai, China) equipped with a Gerstel MPS2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) and two successively connected columns, an HP 1 MS (Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 μm film thickness) and an HP INNOWAX (Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 μm film thickness), with a constant flow of helium at 1.5 mL/min. The vial was incubated for 5 min at 40 °C. The extraction on fiber DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) was performed for 40 min at 40 °C with constant stirring at 250 min^{-1}. The injector was held at 250 °C for 3 min for the analytes to desorb from the fiber.

The GC oven was programmed as follows: 60 °C for 10 min, from 60 to 100 °C at 7 °C/min, held at 100 °C for 10 min, from 100 to 170 °C at 7 °C/min, from 170 to 230 °C at 40 °C/min, held at 230 °C for 20 min, from 230 to 60 °C at 40 °C/min, and held at 60 °C for 3 min.

For the determination of analytes, a mass spectrometer (Agilent Technologies 5975C, upgraded with a triple-axis detector, Palo Alto, CA, USA) was used. The temperature of the ion source was 230 °C, the auxiliary temperature was 250 °C, and the quadrupole temperature was 150 °C. For qualitative determination, retention time and mass spectrum in selective ion monitoring mode (SIM) were used.

The mass channel was m/z 137 and 152 for IPMP, m/z 124 and 151 for IBMP, and m/z 127 and 154 for [^{2}H$_3$]-IBMP. Ions 137, 124, and 127 were the target ions used for quantification, whereas 152, 151, and 154 were used as qualifier ions. Calibration was performed with calibration standards in sugar solution for must and in alcoholic solution for wine. Linearity was verified by using spiked samples of dearomatized must and alcoholic solutions for wine (four repetitions for one concentration level, nine concentration levels for the calibration curve). Linearity and range were determined by multiple linear regressions, using the F test.

Calibration curves were derived using increasing amounts of IBMP (1−196 ng/L) and IPMP (1−200 ng/L) spiked in a dearomatized must, a sugar solution, and an alcohol solution. Good linearity was obtained for both analytes: IBMP (R^2 for dearomatized must was 0.9996; for sugar solution, 0.9991; and for alcohol solution, 0.9986) and IPMP (R^2 for dearomatized must was 0.9992; for sugar solution, 0.9981; and for alcohol solution, 0.9985).

The limit of detection (LD) and the limit of quantification (LQ) were calculated from the calibration curve. For IBMP, the LD of the dearomatized must was 0.6 ng/L, and for the alcohol solution it was 0.4 ng/L. The LQ for IBMP was 2.0 ng/L for the dearomatized must and 1.2 ng/L for the alcohol solution. For IPMP the LD of the dearomatized must was 0.6 ng/L, and for the alcohol solution it was 0.5 ng/L. The LQ for IPMP was 2.1 ng/L for the dearomatized must and 1.6 ng/L for the alcohol solution.

For the determination of precision,[40] that is, repeatability and reproducibility, a spiked sample of dearomatized must and two unspiked and two spiked samples of wine (Sauvignon blanc from Slovenia and Sauvignon blanc from New Zealand) were analyzed. Within a period of 10 days, two parallel samples of must and three of wine were analyzed each day. The standard deviation of repeatability (r) of the level and the standard deviation of reproducibility (R) of the level were both calculated. The results are given in Table 1.

The uncertainty of repeatability and uncertainty of reproducibility were calculated by multiplying the standard deviation of repeatability and standard deviation of reproducibility by Student's t factor for 9 degrees of freedom and a 95% confidence level ($t_{95;9} = 2.262$). The results are presented in Table 1.

Trueness was verified by checking the recoveries. Recoveries were calculated from concentrations of samples used for the precision and uncertainty evaluation. The average of the recoveries was calculated. The results are given in Table 2.

Determination of Glutathione. Intact grape berries (200 berries) were carefully cut at the torus with scissors, transferred to a bag, purged with nitrogen for 5 min to reduce oxidation, and crushed manually. After crushing, the grape juice was immediately placed in

Table 2. Recoveries of the Method for the Determination of Methoxypyrazines

	dearomatized must		Sauvignon blanc from Slovenia		Sauvignon blanc from New Zealand	
	IBMP	IPMP	IBMP	IPMP	IBMP	IPMP
spiking level (ng/L)	9.8	10.0	25.0	25.0	25.0	25.0
recovery (%)	111.3	103.2	81.2	84.2	93.3	80.7
RSD (%)	13.8	6.8	6.3	5.3	6.8	8.3

methanol (1:10), with *N*-acetyl-L-cysteine as the internal standard, filtered through 0.45 μm Minisart RC 25 filters Sartorious (Goettingen, Germany), diluted 1:1 with a 5 mM sodium acetate buffer containing 0.1 mM EDTA, and immediately analyzed as previously described.[21] The concentration of GSH was determined by an Agilent Technologies 1200 HPLC with fluorescence detection with online precolumn derivatization, controlled by Agilent Chemstation Rev. B.03.01 from Agilent Technologies (Palo Alto, CA, USA) as previously described.[21] Briefly, separation was performed at 25 °C using a Synergi Fusion-RP 80A column (4 μm, 150 mm × 2.0 mm i.d.) from Phenomenex (Torrance, CA, USA).

The mobile phase consisted of (A) 50 mM sodium acetate buffer, pH 5.7, and (B) methanol, and the injection volume was 9 μL. The wavelength for the excitation was 340 and 450 nm for the emission. A nine-point calibration curve for standard GSH was linear over the injected range (0.2−60 mg/L) with a correlation coefficient of 0.9984.

Determination of Hydroxycinnamates. Undamaged fresh berries were cooled to 5 °C and crushed in the inert atmosphere, flushed with nitrogen for 5 min. After hand pressing in an inert atmosphere, the juice was collected, and 1000 ppm SO_2 was added to inhibit enzymatic activity. Grape juice was filtered through a 0.45 μm Millipore PVDF filter (Bedford, MA, USA) into a HPLC vial and directly injected. An Agilent Technologies 1100 HPLC with DAD connected to an Agilent NDS ChemStation was used for the detection and quantification of HCAs in grape juice as described previously.[28] The method was developed for monitoring *cis-* and *trans*-caftaric acid, coutaric acid, and fertaric acid, respectively, together with caffeic, *p*-coumaric, and ferulic acid and also a glutathione derivative of caftaric acid (GRP). Briefly, separation was performed on a 250 × 2.1 mm, 5 mm, ODS Hypersil C18 column connected to a 20 × 2.1 mm, 5 mm, ODS Hypersil guard column (Thermo Scientific). The mobile phase consisted of (A) 0.5% formic acid in water and (B) 2% formic acid in methanol, and gradient was carried out as described, [28] only here the injection volume was 10 μL. Compounds were identified by their UV−vis spectra and retention times. The quantification of compounds was based on peak areas at λ = 320 nm, and the respective concentrations in samples were expressed as *trans*-caftaric acid equivalents. A calibration curve was prepared by injecting a standard of *trans*-caftaric acid in the range from 1.05 to 500 mg/L. It was linear over the injected range with a correlation coefficient of 0.9999. The LD of *trans*-caftaric acid was 0.05 mg/L, whereas the LQ was 0.17 mg/L. To assess the repeatability of the method, 121 mg/L of standard *trans*-caftaric acid solution and a sample of grape juice were sequentially injected (both N = 10), and the relative standard deviations of repeatability RSD_r were 0.19 and 3.7%, respectively.

Data Analysis and Statistical Methods. Physiological and morphological heterogeneity in the sample was largely diminished by berry classification according to diameter and a second classification according to TSS concentration. The measured sample was homogeneous, containing berries of the same diameter and same maturation level in terms of TSS concentration. Regressions and correlations were performed with Origin 6.1 (OriginLab Corp., Northampton, MA, USA). The linearity and range of the method were determined by linear regression, using the F test. Student's t test was used for the calculation of standard deviation of the reproducibility and repeatability of the method, using Statgraphics Centurion XVI (StatPoint Technologies, Warrenton, VA, USA).

RESULTS AND DISCUSSION

Photosynthetic Active Radiation, Vine Water Status, and Temperature Measurements. The vine water measurements showed that there were no water constraints during the growing season, including during the ripening period. The stem water potential values were invariably between −0.4 and −0.45 MPa.[35,41] The mean PAR for the leaf removal was around 150 μmol m^{-2} s^{-1}, and for the control it was around 50 μmol m^{-2} s^{-1}. Therefore, the PAR at the bunch level in the leaf removal was 3 times higher than in the control, with a mean ambient PAR of 1000 μmol m^{-2} s^{-1}. The evolution of daily mean temperature during the ripening period (from January to March 2011) showed the highest temperature for bunches in the leaf removal from 10 a.m. to 2 p.m. On a daily basis, from mid-day onward the cooler wind from the Atlantic Ocean decreased the temperature of the bunches in leaf removal through the sea breeze effect (Figure 1).[42] For both treatments the mean
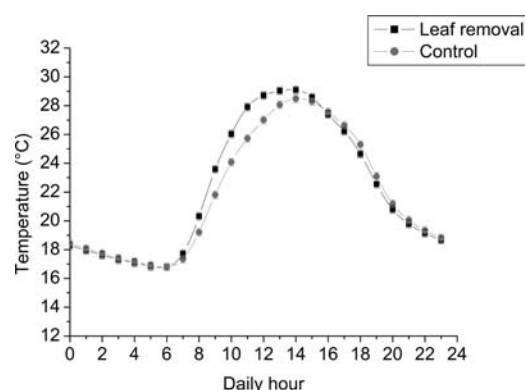

Figure 1. Mean hour temperature (°C) over the ripening period from January to March, $p < 0.05$.

temperature never exceeded 30 °C, which is the upper limit of the optimal physiological response threshold.[43] From 2 p.m. until 7 a.m. the following day, the bunch temperature of the leaf removal was slightly lower than that of the control. The coolest temperatures for both treatments were seen at 6 a.m., around sunrise, whereas the highest temperature was observed at 1 p.m.

Berry Classification. The distribution percentages of grape berries in different diameter classes during maturation are presented in Figure 2. The figure shows that the grape berries
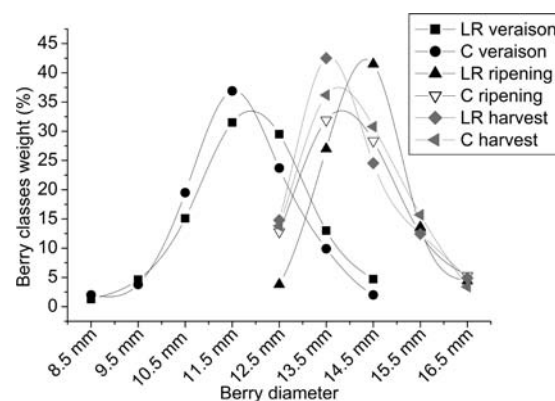

Figure 2. Distribution of Sauvignon blanc grape berries (%) in different diameter classes for all sampling dates, for leaf removal (LR) and control (C).

were distributed evenly along a Gaussian bell-shape curve for all three sampling dates, which confirmed the homogeneous distribution of the berries across the three major berry classes. These three major classes of berry diameter represented 80–85% of the berry population. This indication of low berry heterogeneity suggests that the vines did not experience any abiotic or biotic constraints.

Total Soluble Solids. The average TSS concentrations at harvest for the leaf removal and control treatments were 23.2 and 22.3 °Brix, respectively. A strong positive correlation was found between berry diameter and TSS accumulation on a per berry basis ($R^2 = 0.96$), irrespective of sun exposure and berry temperature (Figure 3). TSS accumulation per berry, in parallel
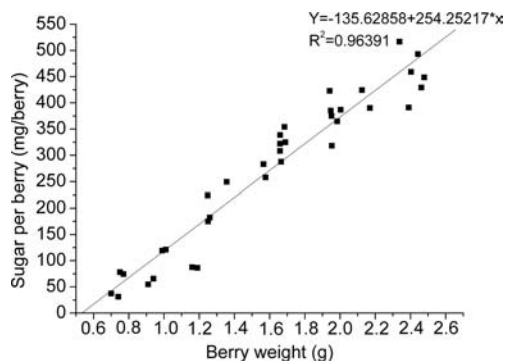
Figure 3. Relationship between sugar per berry (mg/berry) and average berry fresh mass (g) for each maturity class. The regression coefficient is calculated for all maturity classes. Regression coefficient $R^2 = 0.96$, $p < 0.05$.

with an increase in berry diameter, was continuous during the ripening period, which is related to the absence of vine water or other abiotic constraints.[41,44,45] Previous works have shown that there is a positive relationship between berry dry mass accumulation and °Brix increase up to a value of around 24 °Brix, varying on the basis of the cultivar and the production region.[1] In our study, a close correlation between berry diameter and TSS concentration was observed until 20 °Brix (data not shown). Berries of the same diameter had different TSS concentrations, which was in concurrence with previous results.[35] This is consistent with the functional link between berry sugar accumulation, fruit transpiration, and berry water accumulation, in the context of the position of a specific berry in a bunch and the related evaporative demand.[3,46] Berry sugar accumulation could also be controlled at the fruit level by the functioning of sucrose and hexose transporters.[47]

Methoxypyrazines. The concentrations of IBMP and IPMP were analyzed in grape juice from berries classified according to diameter and TSS concentration. In the leaf removal, the IPMP concentration was already below the LD at véraison, whereas it ranged from 4.1 to 2.3 ng/L in the control (data not shown). For the second and final sampling date, IPMP was not detected in either treatment (LD = 0.6 ng/L). IBMP was found in all of the berry diameter classes at véraison, irrespective of the grape light exposure, with concentrations ranging from 4.0 to 72.4 ng/L (data not shown). For the second sampling date (4 weeks after véraison) in all of the berry diameter classes, IBMP levels were under the LD in the berries from the leaf removal treatment. IBMP concentrations in grape juice from both treatments during ripening in the two most representative diameter classes are shown in Figure 4. In
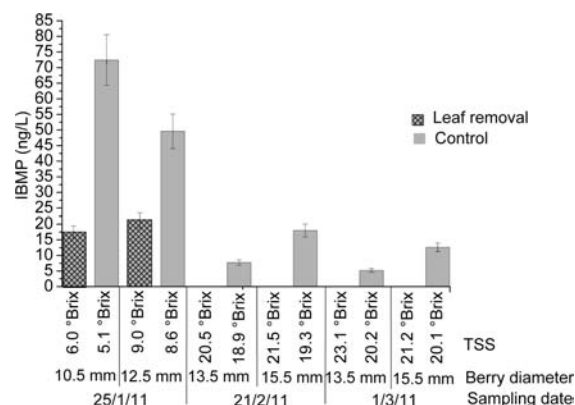

Figure 4. Effect of berry diameter (mm) and light exposure on 3-isobutyl-2-methoxypyrazine (IBMP) concentration (ng/L) in grape juice for the sampling dates January 25 (véraison), February 21 (4 weeks after véraison), and March 1 (harvest). Where no bars are shown, IBMP concentrations were below the LD. Error bars represent tolerance values for IBMP $\pm$ 2RSD$_r$ (%). At each sampling date two of the most representative diameter classes with 2 mm difference are shown.

agreement with other studies, our results indicate that bunch light exposure has a significant impact on IBMP concentrations in berries.[10,15,16] Berry diameter significantly influenced the concentration of IBMP in grape juice. At harvest, the concentrations of IBMP in grape juice of similar TSS in the control were 12.6 and 5.2 ng/L in 15.5 and 13.5 mm berries, respectively (Figure 4). Interestingly, at véraison the highest concentration of IBMP (72.4 ng/L) was found in the grape juice from berries of smaller diameter (10.5 mm) (Figure 4). However, at véraison TSS concentrations for berries of 10.5 and 12.5 mm diameter were not the same, that is, 5.1 °Brix compared to 8.6 °Brix. The IBMP concentration in grape juice of the control was significantly influenced by TSS concentration (Figure 5). At harvest the IBMP concentration in grape juice was below the limit of detection in berries with higher TSS concentration, whereas IBMP was still present in berries with lower TSS concentration, at the same berry diameter (Figure
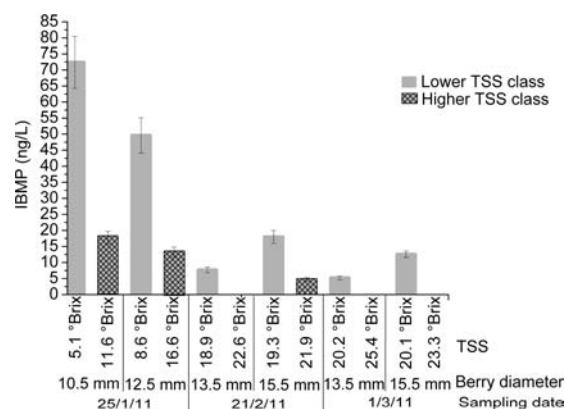

Figure 5. Effect of total soluble solids (TSS) (°Brix) on 3-isobutyl-2-methoxypyrazine (IBMP) concentration (ng/L) in grape juice for the control during the course of the ripening: January 25 (véraison), February 21 (4 weeks after véraison), and March 1 (harvest). Where no bars are shown, IBMP concentrations were below the LD. Error bars represent tolerance values for IBMP $\pm$ 2RSD$_r$ (%). At each sampling date two of the most representative diameter classes with 2 mm difference are shown.

5). This indicates that the °Brix level in unison with berry diameter strongly influences the IBMP concentration in grape juice. In our study the IBMP concentration was below LD (0.6 ng/L) when berries reached 20.2 °Brix, irrespective of the treatment.

Methoxypyrazines and L-Malic Acid. A good correlation ($R^2 = 0.74$) was observed between the breakdown of IBMP and L-malic acid during ripening (data not shown), which is in agreement with results found in Cabernet Sauvignon and Merlot.[10] The IBMP and L-malic acid concentrations in our study were determined in grape juice, which can explain the lower correlation compared to that of Roujou de Boubée et al., who determined IBMP concentration in whole berries.[10] These results clearly demonstrate that IBMP is quickly extracted from the skins into the grape juice.[48] The evolution and response to light and temperature exposure of IBMP and L-malic acid are distinct.[15] The IBMP concentration in grapes is related to light and temperature,[11] whereas the concentration of L-malic acid is more related to temperature.[49] The correlation between the concentrations of L-malic acid and IBMP needs further investigation; therefore, at this stage one compound could not be used to predict the degradation of the other.

Glutathione. Leaf removal had no significant effect on GSH concentration during ripening; as well, no significant effect of berry diameter was found (Supporting Information, Figure S1). A clear increase in GSH concentration in parallel with an increase in TSS was observed at véraison, whereas the concentration of GSH did not differ significantly between the different berry TSS classes at harvest for most representative diameters (Supporting Information, Figure S2). Studies of gene expression at the beginning of grape maturation showed that glutathione-S-transferase exhibits the same expression profile as the enzymes responsible for anthocyanin accumulation, which are strongly related to sugar accumulation.[50] This might explain the sudden increase in GSH concentration after véraison. A strong positive correlation ($R^2 = 0.89$) was observed between GSH and °Brix from 5.1 to 25.4 °Brix (Figure 6), whereas other
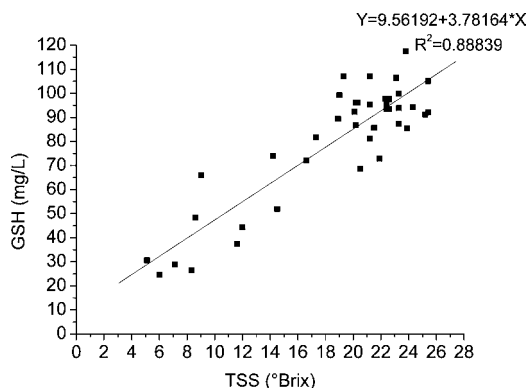

Figure 7. Relationship between glutathione (GSH) (mg/berry) and sugar per berry (mg/berry). The regression coefficient is calculated for all maturity classes. Regression coefficient $R^2 = 0.95$, $p < 0.05$.

Hydroxycinnamates. The HCAs concentration decreased with increasing °Brix, as seen in Figure 8. A decrease in HCAs

Figure 8. Relationship between concentration of total soluble solids (TSS) (°Brix), hydroxycinnamates (HCAs) (mg/L of caftaric acid), and glutathione (GSH) (mg/L) in grape juice, respectively.

Figure 6. Relationship between glutathione (GSH) concentration in grape juice (mg/L) and total soluble solids (TSS) concentration (°Brix). The regression coefficient is calculated for all maturity classes. Regression coefficient $R^2 = 0.89$, $p < 0.05$.

studies observed a correlation between the concentration of GSH and TSS, up to 16 °Brix.[22] It should be noted that there was a strong correlation ($R^2 = 0.95$) between GSH content and TSS on a per berry basis (Figure 7), which shows that the increase in GSH on a per berry basis follows that of TSS.
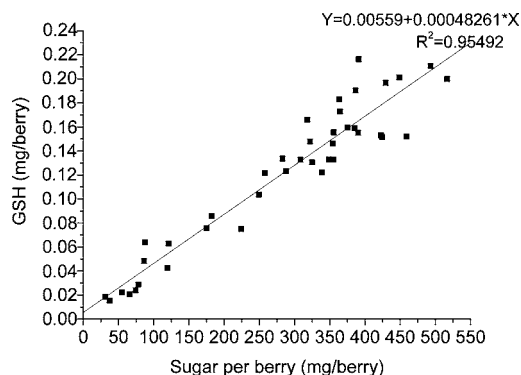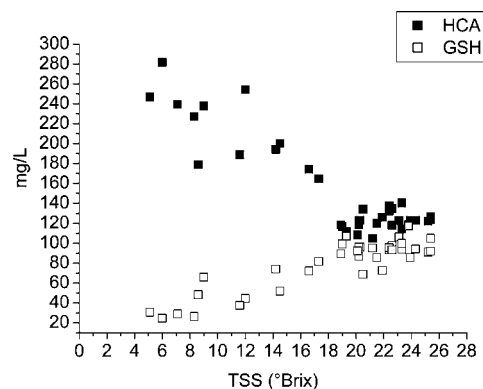
concentration in grape juice occurred mainly due to the decrease in the concentrations of caftaric and coutaric acid (data not shown). A significant decrease in HCAs concentration occurred between véraison and the second sampling date (4 weeks after véraison) in both leaf removal and control, whereas the changes were not significant later (Supporting Information, Figure S3). This is in accordance with Singleton et al., who showed that HCAs concentration did not change significantly with grape maturation.[51] With the exception of sampling at véraison, later leaf removal had no significant effect on HCAs concentration in grape juice; as well, no significant effect of berry diameter was found in both treatments (Supporting Information, Figure S3). It could be that leaf removal in our study was not applied early enough to influence the concentration of HCAs at the harvest. The obtained results are in accordance with a study conducted on Pinot noir, in which it was demonstrated that leaf removal at berry set was very effective in enhancing the concentrations of caftaric and coutaric acids throughout the maturation, whereas leaf removal at véraison had no significant effect.[52] Furthermore, with the exception of sampling at véraison, there was as well no significant effect of TSS on the HCAs concentration in grape berry juice in both treatments (Supporting Information, Figure S4). The HCAs concentration decreased with TSS accumulation, whereas GSH concentration increased with increasing

TSS (Figure 8). The ratio between HCAs and GSH concentration varied from 2.0 to 11.4 at véraison and from 1.1 to 1.4 at harvest. A higher ratio at véraison could occur due to the different timing of HCAs and GSH syntheses.

On the basis of these results, it appears that classifications upon berry diameter and TSS allowed the study of the asynchronous nature of grapevine fruit maturation. These two types of successive classification provided a novel approach to study the dynamics of secondary metabolites in *V. vinifera* L. Sauvignon blanc grape berries during ripening. The classification reduced berry heterogeneity and showed relevant trends in the evolution of MPs, GSH, and HCAs. Berries of the same diameter were classified further to numerous TSS classes. It was shown in the study that berries having the same diameter can have different TSS concentrations, meaning that these berries are not at the same level of physiological ripening, which could have an impact on secondary metabolite concentrations. MPs concentrations were the most influenced by berry heterogeneity. Both diameter and TSS concentration significantly influenced MPs during ripening and did not significantly influence GSH and HCAs concentrations.

It seems that at a certain TSS concentration, berry metabolism shifts toward an aging process. Berry sugar loading seemed to be erratic from around 20 °Brix onward, meaning that there is no longer a relationship between berry volume and TSS concentration, although Garcia de Cortazar-Atauri et al. showed that a relationship exists up to 24 °Brix.[1] The most significant effect of leaf removal in this experiment was observed in the concentration of MPs, confirming that bunch light exposure drastically decreases the concentration of MPs. Leaf removal had no effect on GSH and HCAs concentrations. Separating the effects of sunlight and temperature on grape berry composition is complex and difficult, as many of the biochemical pathways are affected by light and temperature. The concentration of MPs is a relevant indicator of bunch light exposure, due to a photochemical degradation reaction that could be affected secondarily by the increase in temperature related to sun exposure. The concentrations of HCAs were negatively correlated with an increase in the TSS concentration, whereas GSH was positively correlated. A correlation was observed between GSH synthesis and TSS accumulation in the berry as well as between the degradation of HCAs concentration and an increase in °Brix. After the berries reached a certain maturation level, there were no significant changes in GSH and HCAs concentrations. When the grapes reached 20.2 °Brix, MP concentration had already decreased below the LD. Concentrations of GSH and HCAs were in the same range as already found in the South African and Slovenian grape juices and wines, which could have a potential positive effect on the sensory characteristics of Sauvignon blanc wines.[20,28] Further investigations of the effect of berry diameter and berry sugar content on the aromatic expression of Sauvignon blanc grapes and the resulting wines are needed.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +27 (0) 218082747. Fax: +27 (0)218084871. E-mail: deloire@sun.ac.za.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Garcia de Cortazar-Atauri, I.; Brisson, N.; Ollat, N.; Jacquet, O.; Payan, J.-Ch. Asynchronous dynamic of grapevine (*Vitis vinifera*) maturation: experimental study for a modelling approach. *J. Int. Sci. Vigne Vin* **2009**, *43*, 83−97.

(2) Rolle, L.; Río Segade, S.; Torchio, F.; Giacosa, S.; Cagnasso, E.; Marengo, F.; Gerbi, V. Influence of grape density and harvest date on the changes in phenolic composition, phenol extractability indices and instrumental texture properties during ripening. *J. Agric. Food. Chem.* **2011**, *59*, 8796−8805.

(3) Sadras, V. O.; Petrie, P. R. Quantifying the onset, rate and duration of sugar accumulation in berries from commercial vineyards in contrasting climates of Australia. *Aust. J. Grape Wine Res.* **2011**, *17*, 190−198.

(4) Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of a pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002**, *53*, 261−267.

(5) Ollé, D.; Guiraud, J. L.; Souquet, J. M.; Terrier, N.; Ageorges, A.; Cheynier, V.; Verries, C. Effect of pre- and post-veraison water deficit on proanthocyanidin accumulation during Shiraz berry development. *Aust. J. Grape Wine Res.* **2011**, *17*, 90−100.

(6) Rolle, L.; Torchio, F.; Zeppa, G.; Gerbi, V. Relationship between skin break force and anthocyanin extractability at different ripening stages. *Am. J. Enol. Vitic.* **2009**, *60*, 93−97.

(7) Swiegers, J. H.; Kievit, R. L.; Siebert, T.; Lattey, K. A.; Bramley, B. R.; Francis, I. L.; King, E. S.; Pretorius, I. S. The influence of yeast on the aroma of Sauvignon Blanc wine. *Food Microbiol.* **2009**, *26*, 204−211.

(8) Maga, J. A. Pyrazine update. *Food Rev. Int.* **1992**, *8*, 479−558.

(9) Murray, K. E.; Withfield, F. B. Occurrence of 3-alkyl-2-methoxypyrazine in raw vegetables. *J. Sci. Food Agric.* **1975**, *26*, 973−986.

(10) Roujou de Boubée, D.; Van Leeuwen, C.; Doubourdieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830−4834.

(11) Allen, M. S.; Lacey, M. J. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Vitic. Enol. Sci.* **1993**, *48*, 211−213.

(12) Allen, M. S.; Lacey, M. J.; Boyd, S. Determination of methoxypyrazines in red wines by stable-isotope dilution gas-chromatography mass-spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 1734−1738.

(13) Allen, M. S.; Lacey, M. J.; Hars, R. L. N.; Brown, W. V. Contribution of methoxypyrazines to Sauvignon Blanc wine aroma. *Am. J. Enol. Vitic.* **1991**, *42*, 109−112.

(14) Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969**, *17*, 1322−1327.

(15) Sala, C.; Busto, O.; Guasch, J.; Zamora, F. Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety

Cabernet sauvignon: influence of irrigation and plantation density. *J. Sci. Food Agric.* **2005**, *85*, 1131–1136.

(16) Scheiner, J. J.; Sacks, G. L.; Pan, B.; Ennahli, S.; Tarlton, L.; Wise, A.; Lerch, S. D.; Vanden Heuvel, J. E. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red wine grapes. *Am. J. Enol. Vitic.* **2010**, *61*, 358–364.

(17) Ryona, I.; Pan, B. S.; Intrigliolo, D. S.; Lakso, A. N.; Sacks, G. L. Effect of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *J. Agric. Food Chem.* **2008**, *56*, 10838–10846.

(18) Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V. Methoxypyrazines in Sauvignon Blanc grapes and wine. *Am. J. Enol. Vitic.* **1991**, *42*, 103–108.

(19) Roussis, I. G.; Lambropoulos, I.; Tzimas, P. Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *Am. J. Enol. Vitic.* **2007**, *58*, 274–278.

(20) Du Toit, W. J.; Lisjak, K.; Stander, M.; Prevoo, D. Using LC-MSMS to asses glutathione levels in South African white grape juices and wines made with different levels of oxygen. *J. Agric. Food Chem.* **2007**, *55*, 2765–2769.

(21) Janeš, L.; Lisjak, K.; Vanzo, A. Determination of glutathione content in grape juice and wine by high-performance liquid chromatography with fluorescence detection. *Anal. Chim. Acta* **2010**, *674*, 239–242.

(22) Adams, D. O.; Liyanage, C. Glutathione increases in grape berries at the onset of ripening. *Am. J. Enol. Vitic.* **1993**, *44*, 333–338.

(23) Singleton, V. L.; Salgues, M.; Zaya, J.; Trousdale, E. Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. *Am. J. Enol. Vitic.* **1985**, *36*, 50–56.

(24) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Sulfur aroma precursors present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* **2000**, *50*, 4076–4079.

(25) Coetzee, C.; Du Toit, W. A comprehensive review on Sauvignon Blanc aroma with a focus on certain positive volatile thiols. *Food Res. Int.* **2012**, *45*, 287–298.

(26) Dubourdieu, D.; Moine-Ledoux, V.; Lavigne Cruège, V.; Blanchard, L.; Tominaga, T. Recent advances in white wine aging: the key role of the lees. In *Proceedings, ASEV 50th Anniversary Annual Meeting*, American Society for Enology and Viticulture: Davis, CA, 2001; pp 345–352.

(27) Massela, R.; Di Benedetto, R.; Vari, R.; Filesi, C.; Giovanini, C. Novel mechanisms of natural antioxidant compounds in biological system: involvement of glutathione and glutathione-related enzymes. *J. Nutr. Biochem.* **2005**, *16*, 577–586.

(28) Vanzo, A.; Cecotti, R.; Vrhovšek, U.; Torres, A. M.; Mattivi, F.; Passamonti, S. The fate of *trans*-caftaric acid administered into the rat stomach. *J. Agric. Food Chem.* **2007**, *55*, 1604–1611.

(29) Mattivi, F.; Fedrizzi, B.; Zenato, A.; Tiefenthaler, P.; Tempesta, S.; Perenzoni, D.; Cantarella, P.; Simeoni, F.; Vrhovsek, U. Development of reliable analytical tools for evaluating the influence of reductive winemaking on the quality of Lugana wines. *Anal. Chim. Acta* **2012**, *732*, 194–202.

(30) Cheynier, V.; Riguad, J.; Souquet, J. M.; Barillere, J. M.; Moutounet, M. Effect of pomace content and hyperoxidation on the phenolic composition and quality of Grenache and Chardonnay wines. *Am. J. Enol. Vitic.* **1989**, *40*, 36–42.

(31) King, E. S.; Kievit, R. L.; Curtin, C.; Swiegers, J. H.; Pretorius, I. S.; Bastian, S. E. P.; Francis, L. I. The effect of multiple yeast co-inoculations on Sauvignon Blanc wine aroma composition, sensory properties and consumer preference. *Food Chem.* **2010**, *122*, 618–626.

(32) Masneuf-Pomaréde, I.; Mansour, C.; Murat, M.; Tominaga, T.; Dubourdieu, D. Influence of fermentation temperature on volatile thiols concentrations in Sauvignon Blanc wines. *Int. J. Food Microbiol.* **2006**, *108*, 385–390.

(33) Lund, C. M.; Nicolau, L.; Gardner, R. C.; Kilmartin, P. A. Effect of polyphenols on the perception of key aroma compounds from Savignon blanc wines. *Aust. J. Grape Wine Res.* **2009**, *15*, 18–26.

(34) Choné, X.; Van Leeuwen, C.; Dubourdieu, D.; Gaudillère, J. P. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* **2001**, *87*, 477–483.

(35) Deloire, A.; Carbonneau, A.; Wang, Z.; Ojeda, H. Vine and water: a short review. *J. Int. Sci. Vigne Vin* **2004**, *38*, 1–13.

(36) Eichorn, K. W.; Lorenz, D. H. Phänologische Entwicklung der Rebe. *Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschweig, Ger.)* **1977**, *21*, 119–120.

(37) Ojeda, H.; Deloire, A.; Carbonneau, A. Influence of water deficit on grape berry growth. *Vitis* **2001**, *40*, 141–145.

(38) Rolle, L.; Torchio, F.; Giacosa, S.; Segade, S. R.; Cagnasso, E.; Gerbi, V. Assesment of physiochemical differences in Nebbiolo grape berries from different production areas and sorted by flotation. *Am. J. Enol. Vitic.* **2012**, *63*, 195–204.

(39) Commision Regulation (EEC) N. 2676/90 of 17 September 1990 determining community methods for the analysis of wines. *Off. J. Eur. Communities* **1990**, *33* (L272), 1–192.

(40) ISO 5725: Accuracy (trueness and precision) of measurement methods and results – part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method, 1994; pp 1–42

(41) Deloire, A.; Heyms, D. The leaf water potentials: principles, method and thresholds. *Wineland Mag., S. Afr.* **2011**, 119–121.

(42) Bonnardot, V.; Planchon, O.; Cautenet, S. Sea breeze development under an offshore synoptic wind in the South-Western Cape and implications for the Stellenbosch wine-producing area. *Theor. Appl. Clim.* **2005**, *81*, 203–218.

(43) Winkler, A. J. Effects of over cropping. *Am. J. Enol. Vitic.* **1954**, *5*, 4–12.

(44) May, P. From bud to berry, with special reference to inflorescence and bunch morphology in *Vitis vinifera* L. *Aust. J. Grape Wine Res.* **2000**, *6*, 82–98.

(45) Wang, Z.; Deloire, A.; Carbonneau, A.; Federspiel, B.; Lopez, F. Study of sugar phloem unloading in ripening grape berry under water stress conditions. *J. Int. Sci. Vigne Vin* **2003**, *37*, 213–222.

(46) Rebucci, B.; Poni, S.; Intrieri, C.; Magnanini, E.; Lakso, A. N. Effects of manipulated grape berry transpiration on post-veraison sugar accumulation. *Aust. J. Grape Wine Res.* **1997**, *3*, 57–65.

(47) Ageorges, A.; Issly, N.; Picaud, S.; Delrot, S.; Romieu, C. Indentification and functional expression in yeast of a grape berry sucrose carrier. *Plant Physiol. Biochem.* **2000**, *38*, 177–185.

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

(49) Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J. C. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* **2002**, *53*, 171–182.

(50) Terrier, N.; Gllisant, D.; Grimplet, J.; Barrieu, F.; Abbal, P.; Couture, C.; Ageorges, A.; Antanassova, R.; Leon, C.; Ranaudin, J. P.; Dedaldechamp, F.; Romieu, C.; Delrot, S.; Hamdi, S. Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. *Planta* **2005**, *222*, 832–847.

(51) Singleton, V. L.; Zaya, J.; Trousdale, E. K. Compositional changes in ripening grapes: caftaric and coutaric acids. *Vitis* **1986**, *25*, 107–117.

(52) Sternad Lemut, M.; Trost, K.; Sivilotti, P.; Vrhovsek, U. Pinot Noir grape colour related phenolics as affected by leaf removal treatments in the Vipava Vally. *J. Food Compos. Anal.* **2011**, *24*, 777–784.