

Berry Shriveling Significantly Alters Shiraz (*Vitis vinifera* L.) Grape and Wine Chemical Composition

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S Supporting Information

ABSTRACT: Berry shriveling is an often reported occurrence in the Shiraz (*Vitis vinifera* L.) cultivar. This study investigated the effect of berry shriveling occurring in a high yielding (18.6 ± 1.6 kg/vine) Shiraz vineyard in relation to a temporal investigation of grape and wine composition using three harvest dates. Berry shriveling resulted in delayed total soluble solids and amino acid accumulation into the berry, however differences between treatments diminished or became smaller by the third harvest date. Similarly, ethyl esters of fatty acids and higher alcohol acetates were lower in wines from shriveled berries from the first two harvests; anthocyanins were reduced in wines from shriveled berries at all harvest dates, whereas terpenes were unaltered. Wines made from shriveled berries had higher γ -nonalactone and β -damascenone concentrations. This study provides novel information on the chemical alterations of grapes and wines made from grapes affected by shriveling.

KEYWORDS: maturity, fermentation, wine aroma, ANOVA-PCA, vineyard

INTRODUCTION

Berry shriveling is an important phenomenon significantly changing grape morphological and histological traits.^{1–3} It occurs through grape berry water loss due to the alteration of the fruit water budget when transpiration and potential water back flow is exceeding the import of water into the berry through the phloem and xylem.⁴ Berry shriveling can have a significant economical impact, reducing yields by up to 25%.⁵ Its effect is expected to increase due to predicted climate warming, shifting grape development and ripening into warmer periods (i.e., heat waves).^{6,1} Berry fresh mass loss is variable between seasons, sites, and vineyards and it seems to be accelerated by higher temperatures, water constraints and/or stress and excessive bunch sun exposure.^{7,8} Previous reports on Shiraz demonstrated a good correlation between the beginning of berry weight loss and the onset of cell death.^{9,10} Berry shriveling can occur, before (already at bloom affecting the ovaries) or after veraison in red and white varieties such as Cabernet Sauvignon, Zweigelt, Barbera, Grenache, Semillon, Sauvignon Blanc, Shiraz, and other cultivars.^{10–12}

Four types of berry shriveling are reported in the literature, however the etiologies are not well characterized; (i) sun burn either before or after veraison, resulting in poor color development in red varieties and raisin formation in severe occasions,¹³ (ii) late season fruit dehydration, characterized by an increase in total soluble solids (TSS) concentration,^{11,13} (iii) bunchstem necrosis characterized by necrotic rachis tissue affecting entire clusters or often only bunch shoulders and tips,^{13,14} and (iv) sugar accumulation disorder (SAD) resulting in soft, irregular-shaped berries, with low fresh weight, reduced anthocyanins and sugar accumulation.^{2,13,15–17} Early bunch stem necrosis can occur at bloom, whereas late season bunch

stem necrosis appears after veraison and, depending on the occurrence, grape composition is differentially impacted.¹³

Shiraz (*Vitis vinifera* L.) is particularly known to be prone to berry fresh weight loss in late ripening.^{7,18,19} This late season fruit dehydration is a result of increased berry transpiration¹⁹ and decreased phloem²⁰ resulting in increased TSS concentrations.^{11,13} Recently carried out work suggested that Shiraz berry shriveling negatively impacted grape anthocyanin concentrations, total terpenoids, alcohols, hydrocarbons, and radical scavenging capacity of grapes.¹¹ Another study demonstrated an increase in basic juice constituents such as TSS and total acidity in Cabernet Sauvignon shriveled berries, whereas severely shriveled berries had the lowest antioxidant activity, total monomeric anthocyanins and total flavanols.¹²

To the authors' knowledge, there are no reports in the literature that describe the impact of berry shriveling on Shiraz wine composition. The purpose of this investigation was to characterize the changes in chemical composition in grapes and wines arising from shriveled berries harvested at three different dates. Due to several types of berry shriveling occurring in the vineyard at the same time which are sometimes difficult to differentiate, all shriveled berries (regardless the type of shriveling) were compared to nonshriveled fruit used as a control at three different harvest times. However, according to visual inspections of the vineyard, bunch stem necrosis was the most frequently occurring type of shriveling in the experimental plot. Small scale vinifications were carried out and the effect of

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grape berry shriveling on Shiraz wine volatile and nonvolatile composition was analyzed and reported herein.

MATERIALS AND METHODS

Vineyard and Harvest. Shiraz (*Vitis vinifera* L.) grapes were sourced from a commercial vineyard located in Griffith (Riverina, New South Wales, Australia; 34°14'14"; 146°06'00"). This vine growing region is classified as very warm according to the Huglin index, which was determined as 3122 units for the season 2014–2015, inferring that the experimental site is in a very warm climate.²¹ Shiraz grapevines, clone BVRC12 were planted in 2008 with the row orientation North-East to South-West. Grapevines were own-rooted, drip irrigated, trellised to a bilateral cordon, and mechanically pruned to an open sprawling canopy. Mesoclimatic temperature data were collected from TinyTag TGP 4500 (Gemini data loggers Ltd., Chichester, U.K.) hosted in white weather screen, positioned two meters above the ground. Average hourly temperature evolution is presented in Figure S1. Rain data for the growing season 2014–2015 were collected from a Nericon weather station (http://www.awri.com.au/industry_support/weather-nsw/?location_id=6&view) located approximately 5 km from the experimental plot (Figure S2). Soil moisture probes (Gbugs, MEA, Australia) installed at three depths and stem water potential measurement conducted with Schollander pressure chamber^{22,23} indicated that vines were well watered during ripening. The vineyard was high yielding, with 92 ± 8 primary shoots per vine ($n = 23$ vines) and the average yield per vine was 18.6 ± 1.6 kg recorded on 6 vines at the first harvest date. Grapes were harvested sequentially on 3 occasions according to the evolution of berry sugar accumulation.²⁴ The first harvest was conducted on 17 February 2015 (H1), second harvest on 1 March 2015 (H2) and the third harvest on 6 March 2015 (H3), corresponding to 12, 24, and 30 days after the slowdown of sugar accumulation per berry,²⁴ respectively. The H3 preceded the commercial harvest date by 3 days. For each harvest date approximately 8 kg of bunches were randomly collected across 400 vines and transported to the laboratory and stored at +4 °C. To prevent damage and oxidation, berries were carefully excised from bunches and classified in two classes, i.e., shriveled (S) and nonshriveled (NS) based on visual assessment. Berries which appeared turgid were further evaluated for plasticity where hard berries, with no visual deformation by finger pressure were classified as NS, and berries with visual cavities (deformations) were classified as S. In this study, berry shriveling is defined as irreversible berry water loss and does not refer to a specific berry shriveling type. After the initial classification berries were kept frozen at –20 °C for a month for further analysis.

Small Scale Vinifications. A plastic bag containing 1500 g of previously sorted and frozen berries was defrosted at room temperature. Berries were crushed by hand in a plastic resealable bag with the addition of potassium metabisulfite to yield a concentration of 40 mg/L of sulfur dioxide. The grape slurry was divided into three fermenters ensuring that juice/pulp ratio was constant for the three replicates of each treatment. Before fermentation 2 mL of juice was collected and frozen at –20 °C for further analyses. Fermentations in triplicate were conducted in 1 L French Press coffee plungers wrapped in aluminum foil to avoid light penetration. The acidity of each fermenter was adjusted to approximately pH 3.5 by adding tartaric acid. The grape slurry was inoculated with 30 g/hL *Sacharomyces cerevisiae* EC1118 (Lallemand, Edwardstown, Australia) and fermentations conducted in a temperature-controlled room at +24 °C. Punch downs were performed twice daily and fermentation progress was monitored at around 9 am and 5 pm by measuring TSS expressed as °Baume and temperature, using a portable density meter (DMA35N, Anton Paar, Gladestown, Australia). After 6 days of fermentation on skins all the ferments were pressed off and the wine left for two additional days on gross lees at +24 °C to conclude fermentation. Wines were pressed off manually, by forcing juice solids with plunger toward the bottom of French press and draining the juice simultaneously. All samples fermented to below 3 g/L of residual sugar, which was confirmed by high performance liquid chromatography (HPLC)²⁵ analyses. When residual sugars concen-

tration was confirmed to be below 3 g/L potassium metabisulfite was added to final concentration of 80 mg/L sulfur dioxide. Samples were centrifuged (Hettich Universal 16, Tuttlingen, Germany) at 3000 rpm for 10 min to remove lees and centrifuged wine was bottled into 100 mL bottles which were beforehand purged with nitrogen. Bottles were filled so that the remaining headspace was approximately 3 mL. The headspace was purged with nitrogen and bottles were capped and stored at +4 °C for further analysis. No malolactic fermentation was conducted in this study.

Berry Weight and Prefermentative Measurements. A 50 berry subsample ($n = 3$ per treatment and harvest date) of previously classified berries was defrosted, dried with paper towel and berry fresh mass was measured. Berry pH was measured using an UltraBasic Benchtop Meters (Denver Instrument, New York, U.S.A.); ammonia and α -amino acids concentrations were determined using commercially available enzymatic tests purposely designed and developed for an Arena discrete analyzer (ThermoFisher, Scoresby, Australia). Yeast assimilable nitrogen (YAN) was calculated from ammonia and free amino nitrogen according to published methods.²⁶

Organic Acids, Carbohydrates, and Ethanol. Grape juice was analyzed for organic acids whereas wines were also analyzed for the content of residual sugars and ethanol by HPLC according to the method previously published.²⁵ Juice samples were diluted 1:10 (v:v) and filtered through 0.45 μ m filter (Merck, Frenchs Forest, Australia) prior to injection; wine samples were filtered (0.45 μ m) and injected directly.²⁵ Quantification of organic acids and sugars was performed on two 300 $\times$ 7.8 mm² Aminex HPX-87H ion exclusion columns (Bio-Rad Laboratories, Berkeley, U.S.A.) fitted with micro guard cation H⁺ column (Bio-Rad Laboratories, Berkeley, U.S.A.) at +65 °C using a Waters 600 HPLC controller (Milford, U.S.A.) connected to Waters 717 plus autosampler coupled to PDA and RI detectors (Waters, U.S.A.).²⁵

Amino Acids in Grape Juice. Frozen juice stored at –20 °C was thawed and centrifuged (Beckman Coulter, Microfuge 20 Series, Brea, U.S.A.) at 13 000 rpm for 10.5 min. The supernatant was collected and used for amino acids determination according to the previously published method.²⁷ Briefly samples were diluted 1:24 (v/v) with 0.25 M borate buffer (pH 8.5) and L-hydroxyproline as internal standard at 13.1 mg/L was added. The amino group was derivatized with 9-fluorenylmethyl chloroformate and samples analyzed by HPLC using a Waters 600 controller (Milford, U.S.A.) connected to an autosampler (Waters 717 plus, U.S.A.), and quantified by a fluorescence detector (Waters 2475, U.S.A.). Amino acids separation was performed on a reverse phase column (Zorbax Eclipse Plus, Agilent Technologies, Mulgrave, Australia) at +55 °C. Quantification was performed as previously reported.²⁷

Wine Total Anthocyanins, Color, Polyphenols, and Tannin Analyses. Wine total anthocyanins, color parameters, and polyphenols were analyzed as outlined previously.²⁶ Prior to wine color and polyphenols measurements, wine pH was adjusted to 3.5 and measurements were conducted using a UV-1700 Shimadzu spectrophotometer (Kyoto, Japan). Wine total tannins were analyzed by methyl cellulose perceptible tannin assay as described by Illand and co-workers and expressed as equivalents of epicatechin.²⁶

General Volatiles Analysis. Esters, higher alcohols, C6 compounds, and lactones, were analyzed by gas chromatography–mass spectrometry (GC-MS) according to previously outlined methods.^{28,29} A mixture of isotopically labeled esters from CDN isotopes (Pointe-Chaire, Canada) was used as internal standard to quantify esters whereas octan-2-ol (Fluka, Castle Hill, Australia) was used as internal standard for C6 compounds, higher alcohols and lactones. Samples were spiked with the internal standard solution mix containing 20 mg/L [²H₅]-ethyl butyrate, 20 mg/L [²H₅]-ethyl hexanoate, [²H₁₅]-ethyl octanoate, 4 mg/L [²H₂₃]-ethyl decanoate, 5 mg/L [²H₅]-ethyl cinnamate and 5 mg/L 2-octanol. In this study, 5 mL wine sample was added to a 20 mL SPME vial with 3 g NaCl and 5 mL deionized water, and spiked with 10 μ L of internal standard solution. Vials were immediately capped, vortexed, and analyzed by head space solid-phase micro extraction (HS-SPME) with a PDMS-CAR-DVB fiber (Supelco, Bellefonte, U.S.A.). Esters absorbed by the

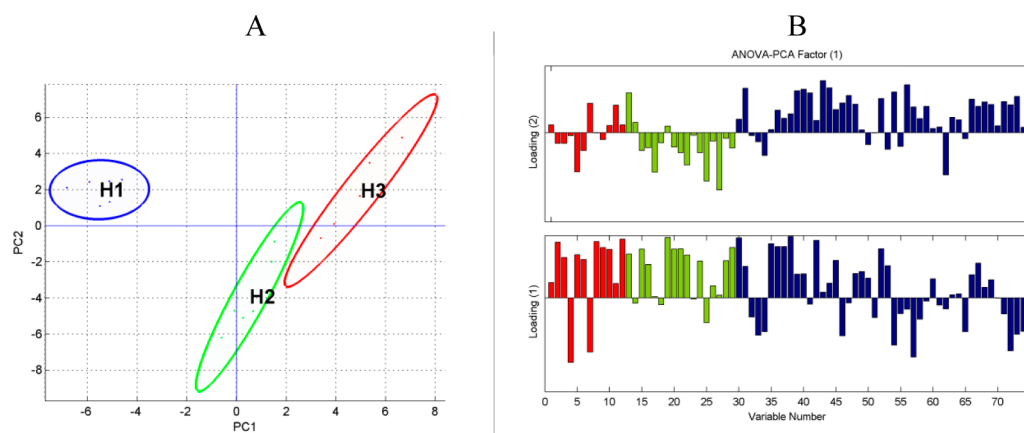


Figure 1. ANOVA-PCA conducted on the parameters measured on grapes, grape juice, and wine as listed in Tables 1–3. (A) Scores of ANOVA-PCA for the first two principal components for variable "harvest time" and (B) loadings for the first two principal components for grape, grape juice, and wine general parameters (red, Table 1), juice amino acids (green, Table 2), and wine volatile chemical composition (blue, Table 3). Each number was assigned to a chemical parameter measured and noted in Tables 1–3. H1, first harvest date; H2, second harvest date; and H3, third harvest date. Ellipses represent the 95% confidence intervals for sample groups.

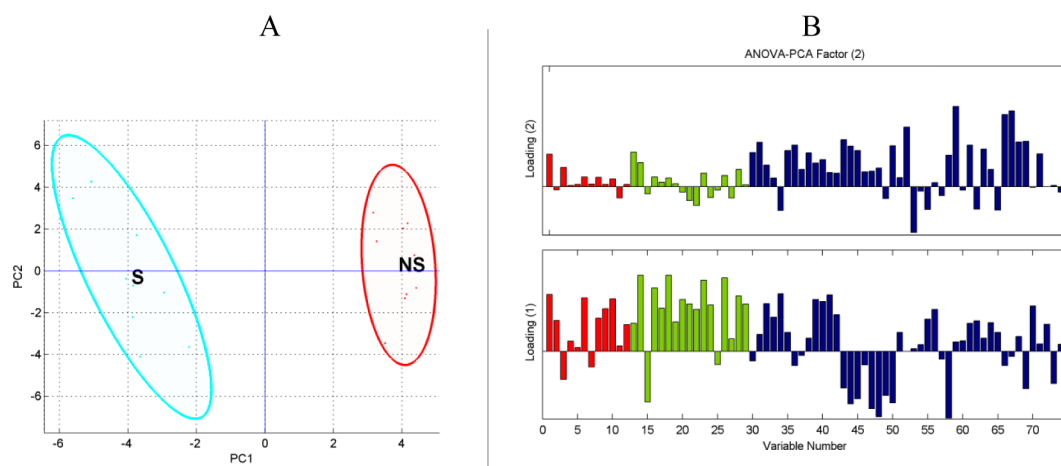


Figure 2. ANOVA-PCA conducted on the parameters measured on grapes, grape juice and wine as listed in Tables 1–3. (A) Scores of ANOVA-PCA for the first two principal components for variable "Treatment" and (B) loadings for the first two principal components for grape, grape juice and wine general parameters (red, Table 1), juice amino acids (green, Table 2) and wine volatile chemical composition (blue, Table 3). Each number was assigned to a chemical parameter measured and noted in Tables 1–3. S, shriveled berries treatment; NS, nonshriveled berries treatment. Ellipses represent the 95% confidence intervals for sample groups.

fiber were released into an Agilent 7890 gas chromatography equipped with a DB-WAXetr capillary column (60 m, 0.25 mm, 0.25 μ m film thickness, J&W Scientific, Folsom, CA) and coupled with a Gerstel MPX autosampler with a Peltier tray cooler set at +4 $^{\circ}$ C. The GC was connected to a 5975C mass spectrometer (Agilent Technologies) that can perform electron ionization mode by SIM (selected ion monitoring) and scan modes simultaneously. The ions used for ester quantification and higher alcohol peak area ratios are reported elsewhere.^{30,29} γ -lactones, hexanol, *trans*-2-hexenol, *trans*-3-hexenol, and *cis*-3-hexenol were quantified using ions 85, 56, 57, and 67 respectively and ion 45 was used for octan-2-ol. Validation of the method for lactones and C6-compounds analysis was performed (Table S1).

Terpenoids and Norisoprenoids Analyses. A group of 13 terpenoids, including 10 monoterpenes and three C13-norisoprenoids were analyzed in wines by HS-SPME-GC-MS according to an adapted version of a previously published method³¹ using the same instrumentation as for general volatile analyses. Twenty μ L of a stock solution of octan-2-ol, [2 H₃]-linalool and [2 H₅]-ethyl cinnamate (internal standards) at 5 mg/L in absolute methanol was added to 10 mL of wine. To a 20 mL headspace vial 3 g of NaCl, 5 mL of wine spiked with internal standards, and 5 mL of deionized water were

added. The vial was then vortexed and tightly sealed with a PTFE-lined cap. The extraction consisted of preincubating the vial with swirling (at 500 rpm) for 10 min at 40 $^{\circ}$ C, then inserting a 1 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μ m fiber (Supelco, Bellefonte, PA, U.S.A.) into the headspace for 30 min at 40 $^{\circ}$ C as the solution was swirled again. The fiber was then transferred to the injector for desorption at 250 $^{\circ}$ C for 1 min, withdrawn and injected into a second injector set at 270 $^{\circ}$ C with a 50:1 split for 10 min with a 10 mL/min purge flow to clean the fiber, prior to the next sample analysis. A DB-WAXetr capillary column (60 m, 0.25 mm, 0.25 μ m film thickness, J&W Scientific, Folsom, CA) was used for compound separation by gas-chromatography. The injector block was fitted with a 1 mm internal diameter borosilicate liner (SGE) and the injector temperature set to 250 $^{\circ}$ C in splitless mode. The oven temperature program commenced at 40 $^{\circ}$ C for 5 min; increased to 200 $^{\circ}$ C at a rate of 3 $^{\circ}$ C/min; with a final increase to 240 $^{\circ}$ C at a rate of 10 $^{\circ}$ C/min and held for 1 min. The total run time was 72.3 min. The flow rate of ultra high purity helium gas was constant at 3 mL/min. The MS source, quadrupole and transfer line temperatures were set to 230, 150, and 260 $^{\circ}$ C respectively. Ions 45, 74, and 181 were used for octan-2-ol, [2 H₃]-linalool and [2 H₅]-ethyl cinnamate, respectively. The ions and the internal standards used to quantify

Table 1. Grape, Juice, and Wine General Parameters

ID		concentration ^a						p values ^b		
		H1S	H1NS	H2S	H2NS	H3S	H3NS	H	T	H*T
1	grape berry fresh mass (g) juice	1.21 ± 0.31c	1.43 ± 0.03a	1.24 ± 0.02b	1.35 ± 0.07ab	1.29 ± 0.08b	1.35 ± 0.12ab	ns	**	*
2	TSS (°Brix)	21.0 ± 0.1d	23.0 ± 0.10c	23.1 ± 0.21c	24.1 ± 0.27b	24.6 ± 0.27a	24.4 ± 0.10ab	***	***	***
3	tartaric acid (g/L)	3.22 ± 1.09ab	2.50 ± 0.20b	3.70 ± 0.25a	3.50 ± 0.18a	3.78 ± 0.51a	3.52 ± 0.15a	*	ns	ns
4	malic acid (g/L)	3.21 ± 0.05b	3.40 ± 0.01a	2.83 ± 0.12d	3.00 ± 0.03c	2.46 ± 0.14e	2.39 ± 0.04e	***	*	*
5	pH	4.35 ± 0.02d	4.46 ± 0.02c	4.59 ± 0.02a	4.58 ± 0.01a	4.56 ± 0.01a	4.49 ± 0.02b	***	ns	***
6	YAN (mg N/L)	221.6 ± 1.5e	274.2 ± 1.5c	263.1 ± 2.1d	293.9 ± 2.3a	274.5 ± 3.4c	281.8 ± 4.5b	***	***	***
7	ammonia (mg N/L)	30.7 ± 0.6a	29.3 ± 0.6b	26.0 ± 0.0d	27.0 ± 0.0c	27.3 ± 0.6c	25.7 ± 0.6d	***	*	***
8	wine ethanol (% v/v)	10.24 ± 0.18d	11.64 ± 0.12c	11.44 ± 0.06c	12.36 ± 0.19b	12.79 ± 0.04a	12.68 ± 0.04a	***	***	***
9	glycerol (g/L)	6.93 ± 0.10e	8.63 ± 0.07c	8.35 ± 0.18d	8.96 ± 0.010b	9.15 ± 0.07a	9.31 ± 0.011a	***	***	***
10	total anthocyanins (mg/L)	207 ± 9e	318 ± 6d	260 ± 5c	353 ± 10b	355 ± 5b	416 ± 20a	***	***	**
11	total tannins (g/L)	2.6 ± 0.3b	2.7 ± 0.1b	2.9 ± 0.3b	2.9 ± 0.3b	3.3 ± 0.3a	3.6 ± 0.2a	***	ns	ns
12	total phenolics (au)	22.5 ± 0.9d	24.8 ± 0.9c	25.1 ± 0.1c	27.0 ± 0.6b	29.3 ± 0.5a	30.9 ± 1.9a	***	**	ns

^aANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). All stated uncertainty is the standard deviation of three replicates per treatment. ^bSignificance of two way ANOVA for H, harvest date; T, treatment and; interaction H*T, harvest date*treatment. Stars indicate level of significance * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, whereas ns indicates no significant differences.

Table 2. Amino Acids Composition in Grape Juice (mg/L)

	ID	concentration ^a						p values ^b		
		H1S	H1NS	H2S	H2NS	H3S	H3NS	H	T	H*T
yeast preferred amino acids										
aspartic acid	13	14.1 ± 2.1cd	16.1 ± 1.9bc	11.8 ± 1.5d	15.5 ± 2.8cd	19.7 ± 2.6b	24.7 ± 2.3a	***	**	ns
glutamic acid	14	13.6 ± 1.0c	25.3 ± 1.2a	12.3 ± 0.6c	23.1 ± 2.5ab	14.2 ± 1.0c	22.3 ± 1.5b	ns	***	ns
asparagine	15	7.23 ± 0.36d	5.11 ± 0.50e	13.10 ± 0.27b	8.49 ± 0.22c	13.93 ± 0.2a	9.11 ± 0.52c	***	***	***
serine	16	40.3 ± 1.7e	70.2 ± 4.3c	57.7 ± 0.6d	83.3 ± 3.9a	66.1 ± 1.0c	76.5 ± 3.6ab	***	***	***
arginine	17	1195 ± 63d	1488 ± 44b	1456 ± 9b	1536 ± 55a	1326 ± 5c	1330 ± 24c	***	***	***
alanine	18	58.9 ± 3.8d	104.7 ± 1.0a	66.6 ± 0.5c	103.7 ± 2.1a	67.7 ± 2.4c	85.8 ± 2.2b	***	***	***
glutamine	19	104 ± 4e	137 ± 5d	133 ± 1d	199 ± 6c	232 ± 2b	252 ± 7a	***	***	***
branched amino acids										
valine	20	34.0 ± 1.8d	51.2 ± 0.8c	48.6 ± 3.0c	63.1 ± 4.4a	57.8 ± 1.8b	65.9 ± 0.3a	***	***	*
leucine	21	33.0 ± 1.9e	45.2 ± 2.5d	46.1 ± 3.2cd	55.0 ± 5.4ab	51.5 ± 2.8bc	57.6 ± 0.9a	***	***	ns
isoleucine	22	14.5 ± 0.5c	16.5 ± 3.0bc	19.8 ± 2.5b	24.8 ± 2.8a	20.2 ± 3.3b	24.5 ± 0.1a	***	**	ns
phenylalanine	23	14.2 ± 1.7b	22.5 ± 3.4a	15.8 ± 2.1b	21.0 ± 2.9a	15.2 ± 0.6b	21.0 ± 1.7a	ns	***	ns
other amino acids										
histidine	24	28.4 ± 2.6d	55.8 ± 6.4bc	48.9 ± 0.7c	66.5 ± 10.1a	61.0 ± 3.6ab	58.9 ± 4.3ab	***	***	**
glycine	25	18.2 ± 1.0bc	18.5 ± 0.7b	20.3 ± 0.2a	19.92 ± 0.7ab	17.4 ± 0.2c	15.9 ± 0.3d	***	ns	*
threonine	26	66.7 ± 3.6e	103.8 ± 3.1a	77.1 ± 2.6d	107.6 ± 3.9a	85.1 ± 0.8c	97.3 ± 1.9b	**	***	***
γ-amino butyric acid	27	215 ± 8c	254 ± 8b	289 ± 10a	288 ± 10a	241 ± 5b	226 ± 4c	***	ns	***
tyrosine	28	8.9 ± 0.9d	11.8 ± 2.2bc	9.5 ± 0.5cd	16.6 ± 1.8a	12.9 ± 1.0b	15.8 ± 1.8a	**	***	*
proline	29	404 ± 18d	789 ± 16c	748 ± 25c	1153 ± 60a	1012 ± 20b	1108 ± 13a	***	***	***

^aANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). All stated uncertainty is the standard deviation of three replicates per treatment. ^bSignificance of two way ANOVA for H, harvest date; T, treatment and; interaction H*T, harvest date*treatment. Starts indicate level of significance * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, whereas ns indicates no significant differences.

terpenoids and the validation parameters of the method are displayed in Table S2).

Statistical Analyses. One-way and two-way analyses of variance (ANOVA) for variables shriveling and harvest date were performed on the chemical data using Statistica, Version 12 (StatSoft, Tulsa, OK, U.S.A.), and the means were separated using Stats-Fisher's LSD test (different letters account for significant differences at $p \leq 0.05$). All stated uncertainty is the standard deviation of three replicates of one treatment. ANOVA-principal component analyses (ANOVA-PCA) was conducted on a concatenated data block consisting of all measured variables 1–74; grape, grape juice and wine general parameters 1–12; juice amino acids 13–29; and wine volatile chemical composition 30–74. This approach partitions variances according to experimental factor levels and interactions followed by PCA of the corresponding data matrices with the residuals added back to each factor matrix immediately prior to PCA.^{32,33} Hotelling T^2 metrics can be used to inspect the confidence interval ellipse for each group of samples thus enabling a direct measure of the importance of sample groupings according to the scores and contribution of variables from loading plots. Prior to ANOVA-PCA the data block was mean centered and standardized to unit variance. ANOVA-PCA was conducted using Matlab version 7.14.0.739 (The Mathworks, Natick, MA, U.S.A.).

RESULTS AND DISCUSSION

In order to clarify the overall impact of harvest dates and berry shriveling on grape and wine chemical composition, ANOVA-PCA was conducted on all the measured parameters in this study (grape, grape juice, and wine general parameters; juice amino acids; and wine volatile chemical composition). When considering the data set as a whole, both harvest date and berry shriveling had a significant influence on the studied parameters (Figures 1, 2). All the samples (3 different harvest dates and 2 treatments, i.e., shriveled berries and nonshriveled berries) could be classified into 3 groups according to the “harvest date” (Figure 1), corresponding to H1, H2, and H3, irrespective of shriveling. Later harvest dates were positively loaded in the first dimension of principal component (PC) 1 together with grape,

grape juice, and wine general parameters (TSS, ethanol, glycerol, total anthocyanins, and others) and negatively correlated with malic acid and ammonia concentrations of grape juice (Figure 1B). Furthermore, later harvests were also associated with higher amino acids concentrations, with exception of glutamic acid, alanine and glycine (Figure 1B), whereas more dynamic trend was observed for wine volatiles (Figure 1B). Samples could also be grouped into two clusters, representing shriveled and nonshriveled treatment, irrespective of the harvest date (Figure 2). Nonshriveled samples were positively associated with the majority of general grape and juice parameters, whereas S berries were associated in negative dimension with lower TSS and YAN values. Grape amino acids were positively related to the NS treatment, similarly also wine ethyl esters of fatty acids (EEFAs) and majority of higher alcohol acetates (HAAs), Figure 2B. These results suggest that both harvest date and berry shriveling could independently influence grape and wine chemical composition to the extent that significant differences between treatments and harvest dates are apparent. Detailed discussion on the effect of berry shriveling and sequential harvest on grape and wine composition will be provided in further paragraphs.

General Grape and Grape Juice Parameters. As expected, berry fresh mass was significantly influenced by shriveling, however differences between S and NS berries were minor for H2 and H3 (regardless of visual and tactile shape deformation), Table 1. This observation may arise from larger berries undergoing faster water loss compared to smaller counterparts and often remain heavier at harvest.⁷ Moreover, cell membrane degradation, as occurs in SAD,¹⁵ may result in accelerated back flow of water and rapid fruit fresh mass loss. Berries from well watered plants are able to maintain water content regardless of the occurrence of cell death for a short period of time.³⁴ The increase in TSS concentration was continuous from H1 to H3 for S berries, and only from H1 to

Table 3. Wine Volatiles

compound	ID	concentration ^a						p values ^b		
		H1S	H1NS	H2S	H2NS	H3S	H3NS	H	T	H*T
ethyl esters of fatty acids ($\mu\text{g/L}$)										
ethyl butyrate	30	124 $\pm$ 3c	129 $\pm$ 9c	140 $\pm$ 10bc	151 $\pm$ 11b	176 $\pm$ 17 a	154 $\pm$ 11b	***	ns	*
ethyl hexanoate	31	280 $\pm$ 8ab	289 $\pm$ 5ab	275 $\pm$ 7b	282 $\pm$ 8ab	297 $\pm$ 5a	297 $\pm$ 19a	*	ns	ns
ethyl octanoate	32	267 $\pm$ 34b	343 $\pm$ 28a	296 $\pm$ 8b	312 $\pm$ 20ab	271 $\pm$ 5b	292 $\pm$ 38b	ns	**	ns
ethyl decanoate	33	62.2 $\pm$ 9.0bc	80.0 $\pm$ 13.2a	66.8 $\pm$ 2.0abc	70.6 $\pm$ 8.0ab	53.6 $\pm$ 2.4c	58.6 $\pm$ 11.9bc	*	ns	ns
ethyl dodecanoate	34	4.17 $\pm$ 0.35bc	5.8 $\pm$ 0.71a	4.48 $\pm$ 0.69abc	5.29 $\pm$ 0.47ab	3.07 $\pm$ 1.43c	4.62 $\pm$ 0.53ab	*	**	ns
SUM		738 $\pm$ 47b	847 $\pm$ 32a	782 $\pm$ 16ab	821 $\pm$ 44a	801 $\pm$ 123ab	806 $\pm$ 78ab	ns	*	ns
higher alcohol acetates ($\mu\text{g/L}$)										
propyl acetate	35	49.4 $\pm$ 4.1c	62.6 $\pm$ 5.9b	64.1 $\pm$ 6.2b	74.0 $\pm$ 9.0ab	76.6 $\pm$ 7.5a	72.8 $\pm$ 7.8ab	**	ns	ns
isobutyl acetate	36	47.7 $\pm$ 3.3b	49.4 $\pm$ 5.3b	51.8 $\pm$ 5.9b	54.9 $\pm$ 8.8ab	66.6 $\pm$ 10.0a	57.3 $\pm$ 5.2ab	*	ns	ns
butyl acetate	37	2.2 $\pm$ 0.4b	2.8 $\pm$ 0.6ab	2.8 $\pm$ 0.4bc	3.6 $\pm$ 0.3b	5.2 $\pm$ 1.5a	3.8 $\pm$ 0.5b	**	ns	*
isoamyl acetate	38	1199 $\pm$ 42d	1472 $\pm$ 108c	1460 $\pm$ 194c	1736 $\pm$ 182bc	2110 $\pm$ 223ab	2005 $\pm$ 165a	*	ns	ns
hexyl acetate	39	28.6 $\pm$ 1.3d	43.3 $\pm$ 1.5b	20.3 $\pm$ 3.0e	34.2 $\pm$ 3.5c	39.5 $\pm$ 1.5b	51.2 $\pm$ 4.5a	***	***	ns
(E)-2-hexenyl acetate	40	0.57 $\pm$ 0.02c	0.85 $\pm$ 0.03b	0.41 $\pm$ 0.05d	0.66 $\pm$ 0.07c	0.78 $\pm$ 0.05b	1.00 $\pm$ 0.1a	***	***	ns
(Z)-3-hexenyl acetate	41	0.7 $\pm$ 0.03c	1.25 $\pm$ 0.04a	0.52 $\pm$ 0.02e	0.69 $\pm$ 0.05d	0.75 $\pm$ 0.05c	1.13 $\pm$ 0.1b	***	***	***
phenylethyl acetate	42	79.4 $\pm$ 1.0f	139 $\pm$ 8d	117 $\pm$ 9e	176 $\pm$ 8c	205 $\pm$ 17b	238 $\pm$ 12a	***	***	ns
SUM		1408 $\pm$ 51d	1772 $\pm$ 129bc	1718 $\pm$ 218 cd	2080 $\pm$ 207b	2505 $\pm$ 255a	2431 $\pm$ 185a	***	*	ns
ethyl esters of branched acids ($\mu\text{g/L}$)										
ethyl isobutyrate	43	14.9 $\pm$ 0.6a	12.6 $\pm$ 0.1ab	11.6 $\pm$ 1.5b	11.7 $\pm$ 1.1b	14.6 $\pm$ 3.4a	13.0 $\pm$ 0.7ab	ns	ns	ns
ethyl-2-methyl butyrate	44	2.27 $\pm$ 0.06a	1.71 $\pm$ 0.06b	1.81 $\pm$ 0.26b	1.74 $\pm$ 0.14b	2.26 $\pm$ 0.37a	1.95 $\pm$ 0.08ab	*	**	ns
ethyl isovalerate	45	2.8 $\pm$ 0.25ab	2.4 $\pm$ 0.1b	2.89 $\pm$ 0.28ab	2.6 $\pm$ 0.13b	3.4 $\pm$ 0.83a	2.8 $\pm$ 0.18ab	ns	*	ns
ethyl leucate	46	13.1 $\pm$ 0.6a	11.5 $\pm$ 0.4b	10.5 $\pm$ 0.6b	11.0 $\pm$ 0.9b	10.7 $\pm$ 0.7b	11.0 $\pm$ 0.9b	**	ns	*
ethyl phenylacetate	47	2.78 $\pm$ 0.19a	1.84 $\pm$ 0.19 cd	2.08 $\pm$ 0.19bc	1.71 $\pm$ 0.17d	2.31 $\pm$ 0.17b	2.17 $\pm$ 0.03b	**	***	**
SUM		35.8 $\pm$ 0.9a	30.0 $\pm$ 0.8b	28.8 $\pm$ 2.6b	28.8 $\pm$ 1.6b	33.3 $\pm$ 5.4ab	30.9 $\pm$ 1.8b	*	ns	ns
other esters ($\mu\text{g/L}$)										
ethyl propionate	48	137 $\pm$ 7ab	109 $\pm$ 7c	129 $\pm$ 9ab	108 $\pm$ 12c	148 $\pm$ 16a	124 $\pm$ 6bc	*	**	ns
ethyl cinnamate	49	0.063 $\pm$ 0.013b	0.059 $\pm$ 0.009b	0.075 $\pm$ 0.006ab	0.057 $\pm$ 0.009b	0.088 $\pm$ 0.017a	0.063 $\pm$ 0.007b	ns	**	ns
ethyl dihydrocinnamate	50	0.078 $\pm$ 0.005abc	0.07 $\pm$ 0.003c	0.088 $\pm$ 0.006a	0.078 $\pm$ 0.007abc	0.081 $\pm$ 0.008ab	0.073 $\pm$ 0.006bc	ns	*	ns
higher alcohols										
isobutanol ^f	51	1.10 $\pm$ 0.046a	1.00 $\pm$ 0.056a	1.00 $\pm$ 0.082a	1.03 $\pm$ 0.11a	0.862 $\pm$ 0.21a	1.07 $\pm$ 0.071a	ns	ns	ns
isoamyl alcohol ^e	52	28.0 $\pm$ 1.5a	27.2 $\pm$ 1.5a	27.6 $\pm$ 2.2a	28.9 $\pm$ 2.5a	30.8 $\pm$ 2.0a	31.4 $\pm$ 1.5a	*	ns	ns
phenylethanol ^e	53	2315 $\pm$ 54a	2271 $\pm$ 147 a	2496 $\pm$ 229a	2357 $\pm$ 196 a	2449 $\pm$ 149a	2611 $\pm$ 191a	ns	ns	ns
C6 alcohols ($\mu\text{g/L}$)										
hexanol	54	2314 $\pm$ 31a	2171 $\pm$ 53b	1613 $\pm$ 47e	1711 $\pm$ 77d	1755 $\pm$ 28d	1930 $\pm$ 59c	***	ns	***
(E)-2-hexenol	55	4 $\pm$ 0.25a	5.12 $\pm$ 0.76a	4.61 $\pm$ 0.53a	4.5 $\pm$ 1.69a	3.48 $\pm$ 0.7a	4.73 $\pm$ 1.51a	ns	ns	ns
(E)-3-hexenol	56	40.5 $\pm$ 0.1b	46.1 $\pm$ 1.2a	27.8 $\pm$ 0.8e	32.6 $\pm$ 0.4d	36.0 $\pm$ 1.7c	47.5 $\pm$ 1.6a	***	***	***
(Z)-3-hexenol	57	215 $\pm$ 5a	200 $\pm$ 9b	141 $\pm$ 5c	111 $\pm$ 4d	116 $\pm$ 11d	134 $\pm$ 6c	***	*	***
SUM		2574 $\pm$ 36a	2423 $\pm$ 63b	1786 $\pm$ 52e	1859 $\pm$ 83de	1910 $\pm$ 31d	2117 $\pm$ 66c	***	ns	***
lactones ($\mu\text{g/L}$)										
γ -nonalactone	58	5.05 $\pm$ 0.14a	3.95 $\pm$ 0.27bcd	4.78 $\pm$ 1.16ab	3.52 $\pm$ 0.3 cd	4.41 $\pm$ 0.26abc	3.13 $\pm$ 0.23d	ns	***	ns
γ -decalactone	59	0.1 $\pm$ 0.01a	0.09 $\pm$ 0.02a	0.08 $\pm$ 0.03a	0.09 $\pm$ 0.02a	0.08 $\pm$ 0.01a	0.09 $\pm$ 0.02a	ns	ns	ns
γ -undecalactone	60	0.03 $\pm$ 0.002a	0.03 $\pm$ 0.01a	0.03 $\pm$ 0.004a	0.03 $\pm$ 0.01a	0.03 $\pm$ 0.006a	0.03 $\pm$ 0.004a	ns	ns	ns

Table 3. continued

compound	ID	concentration ^a						p values ^b		
		H1S	H1NS	H2S	H2NS	H3S	H3NS	H	T	H*T
lactones (μg/L)										
γ-dodecalactone	61	0.018 ± 0.003a	0.017 ± 0.002a	0.016 ± 0.002a	0.019 ± 0.003a	0.015 ± 0.003a	0.019 ± 0.004a	ns	ns	ns
SUM		5.2 ± 0.1a	4.1 ± 0.3bcd	4.9 ± 1.2ab	3.7 ± 0.3 cd	4.5 ± 0.2abc	3.3 ± 0.2d	ns	***	ns
terpenes (μg/L)										
(E)- geraniol	62	6.4 ± 0.59b	7.03 ± 0.39ab	7.02 ± 0.22ab	7.54 ± 0.68a	6.58 ± 0.47b	6.44 ± 0.28b	*	ns	ns
1,8-cineole	63	0.119 ± 0.025a	0.14 ± 0.023a	0.13 ± 0.01a	0.127 ± 0.008a	0.134 ± 0.007a	0.128 ± 0.008a	ns	ns	ns
1,4-cineole	64	0.015 ± 0.003a	0.018 ± 0.001a	0.015 ± 0.001a	0.017 ± 0.001a	0.017 ± 0.002a	0.016 ± 0.001a	ns	ns	ns
4-terpineol	65	2.16 ± 0.19b	3.03 ± 0.26a	2.03 ± 0.43b	3.48 ± 0.11a	3.02 ± 0.49a	3.22 ± 0.48a	ns	***	*
α- terpinene	66	0.009 ± 0.002a	0.01 ± 0.001a	0.009 ± 0.003a	0.01 ± 0.0003a	0.012 ± 0.002a	0.01 ± 0.001a	ns	ns	ns
γ- terpinene	67	0.007 ± 0.001a	0.007 ± 0.001a	0.007 ± 0.002a	0.009 ± 0.001a	0.009 ± 0.002a	0.008 ± 0.002a	ns	ns	ns
terpinolene	68	0.039 ± 0.007a	0.058 ± 0.017a	0.041 ± 0.013a	0.045 ± 0.004a	0.054 ± 0.01a	0.047 ± 0.007a	ns	ns	ns
linalool	69	2.55 ± 0.29ab	2.33 ± 0.18ab	2.64 ± 0.45a	2.14 ± 0.17b	2.6 ± 0.19ab	2.59 ± 0.14ab	ns	ns	ns
α- terpineol	70	0.53 ± 0.03b	0.66 ± 0.09a	0.55 ± 0.07b	0.61 ± 0.07ab	0.6 ± 0.03ab	0.61 ± 0.03ab	ns	*	ns
(E)- geranyl acetone ^c	71	0.38 ± 0.05a	0.33 ± 0.13a	0.28 ± 0.07a	0.28 ± 0.08a	0.28 ± 0.02a	0.36 ± 0.01a	ns	ns	ns
SUM		10.1 ± 0.2a	10.6 ± 0.7a	10.7 ± 0.8a	10.8 ± 0.9a	10.3 ± 0.3a	10.2 ± 0.4a	ns	ns	ns
C13 norisoprenoids (μg/L)										
β- ionone	72	0.37 ± 0.01b	0.42 ± 0.02a	0.29 ± 0.02d	0.31 ± 0.03 cd	0.28 ± 0.004d	0.32 ± 0.01c	***	**	ns
β-damascenone	73	2.77 ± 0.1a	2.51 ± 0.09b	2.31 ± 0.15bc	1.49 ± 0.15e	2.09 ± 0.02d	2.19 ± 0.13 cd	***	***	***
α-ionone	74	0.022 ± 0.007a	0.026 ± 0.012a	0.019 ± 0.002a	0.019 ± 0.008a	0.019 ± 0.001a	0.017 ± 0.001a	ns	ns	ns
SUM		3.2 ± 0.1a	3 ± 0.1a	2.6 ± 0.1b	1.8 ± 0.2b	2.4 ± 0.01c	2.5 ± 0.1b	***	***	***

^aANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). All stated uncertainty is the standard deviation of three replicates per treatment. ^bSignificance of two way ANOVA for H, harvest date; T, treatment and; interaction H*T, harvest date*treatment. Starts indicate level of significance * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, whereas ns indicates no significant differences. ^cIndicates peak area ratio.

H2 for NS treatment (Table 1). The TSS concentrations were significantly lower in S berries compared to NS at H1 and H2, but the results suggest no significant differences at H3 (Table 1). Sugar per berry for NS treatment was 329.9, 326.3, and 330.4 mg/berry, at H1, H2, and H3, respectively, indicating that there was no further significant sugar loading into the berry. Indeed, this confirms results from previous studies, which reported slowdown of sugar accumulation into the berry in late ripening.^{7,24} Opposite, in the S berries continuous sugar accumulation from H1 to H3 was noticed, i.e., 254.8 at H1 to 330.4 mg/berry at H3. Therefore, it seems plausible that shriveling in this study slowed down accumulation of sugars by affecting phloem and xylem unloading. Slow down of sugar accumulation was reported to occur in berries affected by bunch stem necrosis and SAD.^{2,35} The later authors observed no significant increase in TSS accumulation in first 2 weeks after veraison in berries affected by bunch stem necrosis, followed by gradual increase in later ripening.³⁵ It cannot be excluded, that other berry shriveling types from our berry population, such as late season berry dehydration resulted in the increase in TSS concentration of S berries at later ripening stages.¹³ Indeed, late season berry dehydration is well reported to occur in Shiraz vineyards in late ripening, resulting in increased TSS concentrations and yield losses.^{9,11,13} Furthermore, also bunch stem necrosis, occurring late in the season can result in TSS concentrations comparable with unaffected controls, and subsequent shriveling can result in higher TSS concentrations.¹³ Tartaric and malic acid concentrations and pH were influenced by harvest date and to a lesser extent by berry shriveling. Concentrations of malic acid were higher in the grape juice from NS berries, with the exception at H3, where no differences between treatments were noticed. Modifications of organic acid concentrations and pH values may have occurred by berry freezing and subsequently thawing. An increase in the titratable acidity with increasing degree of berry shriveling was previously reported for Cabernet Sauvignon grapes.¹²

Grape Juice Nitrogen Composition. Harvest time, shriveling and their interaction significantly impacted YAN concentrations in juice. As observed for TSS, juice from S berries had lower YAN concentrations compared to its NS counterpart; however the differences at H3 between treatments became less apparent (Table 1). Small variations between harvest dates and treatments for ammonia were noticed, with concentrations ranging between 25.7 and 30.7 mg N/L (Table 1). Contrary, authors have previously reported that berries affected by SAD were characterized with significantly higher ammonia concentrations compared to unaffected berries.¹⁸ In the present study, the variations observed for YAN were mainly related to the modifications of yeast assimilable amino acid composition. Therefore, it is not surprising to see a similar trend also in variation of individual amino acids (Table 2).

Grape juice amino acids are important source of nitrogen for yeast during fermentation and strongly influence the production of aromatic compounds such as higher alcohols and esters.^{36,29,37} Grape juice amino acids were divided into groups according to their importance for yeast metabolism during fermentation.³⁸ All amino acids were influenced either by the harvest date or shriveling and some also by their interaction (Table 2). Amino acid concentrations are known to increase during grape maturation, however these compounds can also reach a plateau or even decrease at late ripening stage.³⁹ A trend was noticed for lower amino acid

concentrations in juice from S berries compared to NS, excluding glycine, asparagine and γ -aminobutyric acid (Table 2). These results are concordant with a reported decrease of most amino acids in Zweigelt grapes⁴⁰ and nitrogen containing compounds in Cabernet Sauvignon grapes affected by SAD.¹³ Nonfunctional phloem could affect amino acids transportation to the berry (similar to TSS) which are partly transported from the leaf to the heterotrophic sinks by phloem.⁴¹ Indeed, concentrations of glutamine, which is known to be transported by phloem into the berry,⁴¹ were significantly lower in S compared to NS berries at all harvest dates (Table 2). Berry shriveling could also affect enzymes such as glutamine synthetase and glutamate dehydrogenase responsible for conversion of glutamine into proline and arginine, whose concentrations were also significantly lower in S berries compared to NS counterparts (with the exception of arginine at H3).

General Wine Compositional Analyses. Ethanol and glycerol concentrations increased from H1 to H3 in wines from both S and NS treatments. Wines from S berries resulted in significantly lower glycerol and ethanol concentrations compared to NS treatments, with the exception at H3 when differences between the two treatments diminished (Table 1). Ethanol and glycerol are the main products of anaerobic yeast sugar metabolism during the fermentation.³⁶ Therefore, it is not surprising that these two compounds exhibited a similar trend as observed previously for TSS concentrations (Table 1). Glycerol is mainly produced at the early stage of fermentation, as an osmoregulator.⁴²

Spectrophotometric analyses revealed significant differences in total wine anthocyanins, which were reduced in wines from S berries. Reduction in total grape anthocyanins in Shiraz, Cabernet Sauvignon, and Zweigelt with berry shriveling at harvest was previously observed.^{11,12,40} The latter authors⁴⁰ have shown the reduction of anthocyanin accumulation in grapes affected by SAD when compared to unaffected grapes. Several studies have reported close, but indirect, correlation between anthocyanins and sugar accumulation during the phase of rapid sugar accumulation into the berry.^{7,43} Anthocyanin synthesis is regulated by the phenylpropanoid pathway⁴⁴ and environmental factors, such as high temperature and water stress.^{45,46} Interestingly, it was recently suggested that concentrations of aromatic amino acids, such as phenylalanine do not influence anthocyanin synthesis.⁴⁷ Further investigation is needed to provide adequate answers on anthocyanin accumulation in grapes affected by berry shriveling, depending on type of berry shriveling and time occurring during the ripening process.

Berry shriveling resulted in significantly lower total polyphenol concentrations in wines, and trends to lower tannin concentrations in wines (Table 1). Lower total polyphenols in Shiraz and Cabernet Sauvignon grapes affected by berry shriveling have been reported, however the trend for total oligomeric proanthocyanidins was not so clear.^{12,11}

Wine Volatiles. Ethyl Esters of Fatty Acids. Concentrations EEFA did not significantly change with harvest date as a group whereas it was altered by berry shriveling (Table 3). An increase in EEFA with increased TSS concentration and/or delay of harvest time was noticed in Cabernet Sauvignon.⁴⁸ However, in other studies conducted on Shiraz, Cabernet Sauvignon,²⁹ and Riesling,⁴⁹ no specific trend for EEFA related to harvest date and sugar concentration was reported. Interestingly, ethyl octanoate and dodecanoate were the only

EEFAs which showed a significant decrease linked to berry shriveling at the first harvest date (Table 3). Similarly, berry shriveling also resulted in lower ethyl decanoate concentrations, however it was not significant at all harvest dates. These esters are known to be the most affected by hydrolysis during the first months of wine aging.^{50,51} Phenolic compounds with antioxidant activity can limit the rate of ester hydrolysis in a wine-like medium.^{52,53} Lower antioxidant activity has also been reported in shriveled berries,¹² therefore, a faster hydrolysis of long carbon chain ethyl esters in wines made with S berries is also possible.

Higher Alcohol Acetates. In our study HAAs could be divided into two groups according to its origin and intergroup behavior. The first group of HAAs, produced from yeast sugar and nitrogen metabolism (propyl acetate, isobutyl acetate, butyl acetate and isoamyl acetate) exhibited similar trend as previously noticed for TSS and nitrogen containing compounds in grapes (Tables 1–3). Concentrations of these esters, with the exception of isobutyl acetate were lower in wines from S berries, compared to NS counterparts at H1, and the differences between treatments diminished by H3. Acetates derived from yeast sugar and nitrogen metabolism increased with delayed harvest time, whereas concentrations of higher alcohols did not increase (Table 3). Yeast synthesis of HAAs is regulated by alcohol acetyltransferases from a corresponding alcohol and acetyl Co-A, and substrate availability is not the limiting factor for HAAs production.³⁶ The variations observed here are likely related to modifications of grape composition over ripening favoring the expression of yeast acetyltransferase activities. The second group of HAAs in this study are acetates derived from grape lipid degradation, such as (*E*)-2-hexenyl acetate, (*Z*)-3-hexenyl acetate and hexyl acetate. Concentrations of these esters were significantly reduced in wines from S treatments compared to NS counterpart. The link between C6 acetates and the corresponding C6 alcohols in our study was not as clear as previously reported.⁵⁴ Hexanol, (*Z*)-3-hexenol, and (*Z*)-3-hexenal, a precursor of (*Z*)-3-hexenol, have been reported to be markers of early maturity stage Shiraz grapes from the same region using the same physiological indicator to determine harvest dates as in the present study.²⁹ Delay in ripening in S berries compared to NS could be responsible for higher (*Z*)-3-hexenol at H1 and H2 and hexanol concentrations measured in wines from S berries at the first harvest date (Table 3). As mentioned previously, modifications of juice parameters significantly affecting alcohol acetyltransferases activity could be responsible for the lack of correlation between hexyl esters and corresponding alcohols.

Ethyl Esters of Branched Acids. Considered as a group, ethyl esters of branched acids (EEBA) were altered by harvest date, whereas berry shriveling influenced EEBA concentrations only at H1 (Table 3). Ethyl propionate can be added to this group as its occurrence in wine is similar to EEBA.⁵² Higher EEBA concentrations in wines made with S berries were measured at H1. This trend was steady over ripening for ethyl phenylacetate at H1 and H2, and ethyl propionate for all the harvest dates. It is known that EEBA are synthesized only in small amounts during the fermentation from branched amino acids, whereas concentrations increase during wine aging by esterification of the corresponding branched acids originating from Ehrlich pathway as higher alcohols.⁵² However, no relationship between branched amino acids (Table 2) and branched esters and higher alcohols (Table 3) were apparent in our study. The synthesis of branched acids and EEBA is also related to yeast

redox metabolism.⁵² A slight modification of Ehrlich pathway and/or yeast redox metabolism due to different grape composition between S and NS berries is therefore possible.

Wine Lactones, Terpenes, Norisoprenoids. Terpenes, considered as a group were not significantly influenced either by the harvest date or berry shriveling (Table 3). Two terpenes, 4-terpineol and α -terpineol exhibited higher concentrations in wines from NS fruits. Similarly, lower concentrations of the same terpenoids were found in Shiraz grapes in very shriveled berries compared to less shriveled fruit¹¹ whereas alteration of terpenoid and norisoprenoid pathway in response to stress was recently investigated.⁵⁵ More interesting observations were made for γ -nonalactone and norisoprenoids. In particular, concentrations of γ -nonalactone and β -damascenone, with the exception at H3 for β -damascenone, were present in higher concentrations in wines from S berries. Interestingly, these two compounds were reported to possess strong prune aroma in premature aged red wines,⁵⁶ and γ -nonalactone was suggested to be a possible marker of Merlot wines made with shriveled berries at their late ripening stage.⁵⁷ Contrary to β -damascenone, β -ionone was found in higher concentrations in wines from NS berries.

This study demonstrated that berry shriveling in combination with the selected harvest dates resulted in significant alterations in grape and wine volatile and nonvolatile chemical composition. Contrary to the most common type of berry shriveling in Shiraz, i.e., late season berry dehydration (increasing TSS content), a significant delay of ripening due to shriveling in our study was noticed. Importantly, berry shriveling resulted in wines with significant decreases in anthocyanins, ethanol and esters concentrations. However, the differences in some examined compounds between shriveled and nonshriveled berries were less pronounced with the later harvest date. This study did not determine the exact type of berry shriveling occurring in the vineyard. From visual bunch inspections, bunch stem necrosis was a frequent shriveling type, however other types of shriveling cannot be excluded. Therefore, further studies providing information on a single type of berry shriveling (i.e., SAD, bunch stem necrosis, sun burn, or berry dehydration) and its consequence for grape and wine chemical composition are warranted. This study suggested new perspectives on the potential influence of berry shriveling and harvest times on wine composition but requires further investigation.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b05158.

Table S1: Validation parameters of the method for quantification of C6 compounds and lactones in wines; Table S2: Validation parameters of the method for quantification of terpenes and norisoprenoids in wines; Figure S1: Hourly average temperature data for two phenological stages, i.e., 1.10.2014–31.12.2014 period from approximately 2 weeks before flowering until 30% of berries were colored. Period from 1.1.2015–6.3.2015 represents a period from 30% colored berries until harvest; Figure S2: Monthly rain summation (mm) for season 2014–2015 (PDF)

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Notes

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