Effect of drying on tartaric acid and malic acid in Shiraz and Merlot berries

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Abstract

Background and Aims: Grape berries are dried to concentrate sugar and aroma compounds to produce specific wine styles. This work aimed to characterise biochemical changes during drying of two different cultivars, considering in particular tartaric acid and malic acid evolution.

Methods and Results: Shiraz and Merlot grapes were dried at nine, 15, 21 and 27°C and berries were sampled every 2 to 3 days and sorted by density using NaCl solutions to account for berry heterogeneity. Mass loss of up to 45%, increase in sugar concentration of 71% and decrease in malic acid concentration of 64% were observed. The TA declined by up to 49%.

Conclusion: The decline in tartaric acid could be explained by enhanced potassium hydrogen tartrate precipitation inside the berry or during sampling.

Significance of the Study: The tartaric acid precipitation causes important analytical biases in physiological experiments. This study illustrates that common analytical methods are often inappropriate and are the reason for inconsistent tartaric acid values in many studies.

Keywords: *berry development, berry drying, grape ripening, potassium, tartaric acid*

Introduction

The surface temperature of the earth is likely to rise by between 2 and 4°C by the end of the century, depending on the rate of anthropogenic carbon dioxide and other greenhouse gas emissions (Intergovernmental Panel on Climate Change 2014). Grapes are a valuable horticultural fruit crop with a total annual production of around 7×10^9 kg (Food and Agriculture Organisation of the United Nations 2016) and are cultivated across a wide range of climates and are important economically in many regions of the world. Wine production has been significantly affected by global warming in many ways, and these effects will intensify as global mean temperature continues to increase (Fraga et al. 2012, 2013).

Although some effects on wine production may be beneficial for some regions, across most regions effects are expected to be deleterious to grape and wine production and composition. For instance, temperature increases advance vine phenology and consequently shift the ripening phase into earlier, warmer periods of the growing season (Duchêne and Schneider 2005).

Higher sugar accumulation rate, accelerated malic acid (MA) degradation (Ruffner and Kliewer 1975, Etienne et al. 2013, Sweetman et al. 2014), a decrease in anthocyanin in red berries because of degradation or inhibition of biosynthesis (Mori et al. 2007, Azuma and Yakushiji 2012, Salazar-Parra et al. 2015) with possible variation in acylation (Tarara et al. 2008) and changes in aromatic potential are among the most problematic consequences of elevated temperature (Buttrose et al. 1971, Schultz 2000, Keller 2010, Schultz and Stoll 2010, Rienth et al. 2014b, Kizildeniz et al. 2015). The greatest effects are likely to be seen in traditional growing regions where old, previously well adapted cultivars will continue to be grown (Mira de Orduña 2010). Most vine growing regions, however, will require agronomic and varietal adaptations (Hannah et al. 2013, Keller and Shrestha 2013, van Leeuwen et al. 2013, Schultz 2016). Hence, it is important to understand better berry physiology of different cultivars under abiotic stresses to provide physiological traits for breeders to enable the breeding of cultivars adapted better to future climatic conditions, for example high acidity and low sugar (Duchêne et al. 2014, Duchêne 2016).

The use of 'omic' tools in recent years together with the publication of the grapevine genome (Jaillon et al. 2007) have increased our understanding of some of the metabolic processes occurring during grape berry development under different abiotic and biotic conditions (Terrier et al. 2005, Lijavetzky et al. 2012, Rienth et al. 2014a). Many of the complex mechanisms and pathways, however, remain poorly understood.

Berry development can be divided into a green growth phase and a ripening phase separated by a lag phase. During the first phase, berries grow initially because of cell division and then cell expansion and accumulate tannins, tartaric acid (TA) and MA. During the lag phase growth ceases and organic acid accumulation stops. During the subsequent ripening phase the single berry softens within 24 h, anthocyanin accumulation begins, growth is resumed and sugar accumulation and acid degradation begin (Coombe and McCarthy 2000, Conde et al. 2007, Kuhn et al. 2014). In most fruit species the dominant organic acids are citric acid

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and MA. In *Vitis* and *Muscadina*, however, TA and MA comprise up to 90% of organic acids in pre-veraison grapes (Kliewer 1966, Kuhn et al. 2014). In ripening berries acid degradation is principally because of MA respiration which increases with temperature. The amount of TA per berry remains relatively stable but its concentration decreases because of dilution as berry volume increases (Coombe and McCarthy 2000, Conde et al. 2007)

Acidity plays a critical role in sensory perception of and the microbiological stability of wines. Research dealing with acid degradation has mainly focused on MA metabolism (Ruffner and Kliewer 1975, Terrier et al. 2001, Sweetman et al. 2009, Etienne et al. 2013) with few studies specifically investigating TA metabolism in relation to abiotic conditions. In grapes, TA synthesis results from L-ascorbic acid (vitamin C) breakdown via the conversion of L-idonate to 5-keto-D-gluconate under the action of L-idonate dehydrogenase (L-IdnDH), the only enzyme of this pathway known at the present time (DeBolt et al. 2006, 2008). Ascorbic acid is formed in plants by oxidation of sugars, such as glucose, mannose, and galactose or galacturonic acid derived from pectin by the Smirnoff–Wheeler pathway, which has been extensively studied, because of its importance in all plants and the intrinsic value for the human diet (Wheeler et al. 1998, Badejo et al. 2009, Melino et al. 2011).

In the ripening berry it is currently believed that little TA degradation takes place (DeBolt et al. 2006, Shangguan et al. 2015), which is why cultivars with a high natural content in TA are considered to be better adapted to high temperature and hence future climatic conditions. A naturally high TA content appears thus to represent an important selection criterion for new cultivars better adapted to higher temperature (Duchêne et al. 2014). Some older studies conducted with ^{14}C, however, show that there is a tartrate turnover or respiration in ripening berries (Drawert and Steffan 1966, Takimoto et al. 1976). Similarly, Cholet et al. (2016) observed an up to twofold variation in TA content per berry at harvest within the same vineyard (Ugni Blanc) in two climatically distinct years. Furthermore, a significant decrease in TA per berry was recently reported in Merlot berries during extended ripening of up to 34 days (Bondada et al. 2017).

Drying of grapes postharvest is used to produce particular wine styles in specialised regions (e.g., Passito Wines and Amarone wines). It improves 'ripeness' by concentrating sugar and flavour in northern regions such as Switzerland, where late ripening cultivars fail to reach optimum maturity in cool years. Several studies of grape berry drying have focused on molecular (Zamboni et al. 2008, Bonghi et al. 2012), biochemical and physiological (Tonutti and Bonghi 2013) aspects. Previous drying studies focused on the dynamics of bunch mass loss under different conditions, secondary metabolites and different volatile compounds (Costantini et al. 2006). Several early experiments using amplified fragment length polymorphism (AFLP) transcriptional profiling (Zamboni et al. 2008) and microarrays (Rizzini et al. 2009) showed that fruit tissues are metabolically active after detachment. A strong modulation of berry stilbene synthase (STS) (Versari et al. 2001) alcohol dehydrogenase (ADH) and lipoxygenase (LOX) enzyme activities with an increased content in ethanol and C6 compounds was observed (Costantini et al. 2006, 2008, Cirilli et al. 2012).

The most exhaustive survey of transcriptomic and metabolomic responses in drying berries of six genotypes subjected to identical conditions reported a modulation of phenylpropanoid metabolism and stilbene accumulation (Zenoni et al. 2016). The authors described a distinct metabolomic plasticity of genotypes, allowing the identification of candidate structural and regulatory genes but identified as well a core set of genes that was consistently modulated in all genotypes. Some of the latter genes are involved in oxidative and osmotic stress, defence responses, anaerobic respiration, and cell wall and carbohydrate metabolism. This indicates that compositional changes in the isolated berry are due not only to concentration effects but also to metabolic changes (Zenoni et al. 2016). This was shown in early experiments with carbonic maceration, where a general rise from aerobic to anaerobic metabolism as well as in ethanol production in the cut berry was observed (Flanzy 1978, Romieu et al. 1992).

It is surprising that a decline in TA content has been, hitherto, not reported. One simple explanation for this could be that winemakers and viticulturists often view TA on a concentration basis, thereby overlooking physiological changes inside the berry. A low sampling frequency in combination with intra-bunch berry heterogeneity in ripening berries and the consequent introduction of experimental noise could also have prevented the detection of the decrease in TA. A putative temperature dependent turnover of TA in ripening berries would raise important challenges for breeding strategies responsive to global warming.

First, the aim of this study was to confirm and characterise the kinetics of a TA decline during berry drying. Therefore, a series of grape berry drying experiments was conducted under controlled temperature in chambers with two distinct genotypes, strict berry sorting and a high frequency of sampling. Second, the study aimed to improve understanding of the development of primary metabolites during berry drying.

Materials and methods

Shiraz and Merlot were chosen as two cultivars that have been shown to have a distinct metabolic and transcriptomic pattern in previous drying experiments (Zenoni et al. 2016). Furthermore, both represent potential mid- to late-ripening cultivars that have not been grown in northern regions but could constitute important alternatives for local early ripening cultivars in response to global warming.

The Shiraz vineyard is located in the Vaud region in Switzerland in the community of Bursins (46°27′13.7″N 6°17′17.3″E). The vines were 15 years-old, planted on 3309C rootstock and cane pruned with shoots vertically positioned. The Merlot (RAC19) vineyard plot is located on the experimental vineyard at the agronomic research institution of Agroscope Changins (46°23′53.4″N 6°13′52.5″E). Vines were planted in 2008 on 3309 rootstock and cane pruned with shoots vertically positioned. Prior to the berry drying experiment, berry samples were taken from both experimental vineyards to monitor berry development and determine when to begin the experiment. Sampling of Shiraz and Merlot Controls began before veraison, on 18 August 2016.

Berry drying experiments were initiated when TSS reached 20.5°Brix (Merlot, 13 September 2016) or 18.7°Brix (Shiraz, 7 October 2017) and average berry mass was 1.2 g (Merlot) and 1.8 g (Shiraz) (Table 1). Approximately 140 kg of whole bunches were harvested and evenly distributed among 24 perforated plastic boxes (20 cm × 40 cm × 10 cm) in a single layer to permit optimal air circulation. Temperature

Table 1. Composition of Shiraz berries at commencement of sampling in the vineyard and at the start and end point of drying at 15, 21 and 27°C.

Composition	Control (vineyard) start	Control end (harvest)	15°C	21°C	27°C
Berry mass (g)	1.8 ± 0.1	1.9 ± 0.15	1.5 ± 0.12	1.2 ± 0.13	1.0 ± 0.1
TSS (°Brix)	18.7 ± 1.9	20.2 ± 1.2	23.2 ± 1.1	28.3 ± 1.7	32.0 ± 1.5
TA (mEq)	77 ± 4	99 ± 9	85 ± 8	74 ± 11	68 ± 6
TA (μEq/berry)	136 ± 17	185 ± 23	129 ± 25	89 ± 17	70 ± 11
K (mmol/berry)	47 ± 2.5	48 ± 6	38 ± 4.3	31 ± 2.8	26 ± 1.9
MA (mEq)	53 ± 3	62.1	62 ± 2	55 ± 6	34 ± 1
MA (μEq/berry)	95 ± 15	110 ± 17	93 ± 13	67 ± 8	35 ± 43
MA/TA	0.7 ± 0.04	0.63 ± 0.04	0.50 ± 0.05	0.74 ± 0.18	0.72 ± 0.07
Sugar (mg/berry)	325 ± 45	361 ± 45	326 ± 28	343 ± 32	332 ± 38
Sugar (mg/L)	185 ± 23.4	192 ± 24	219 ± 25	283 ± 26	317 ± 28

MA, malic acid; TA, tartaric acid.

in the four customised chambers (4 m × 3 m × 3 m) were adjusted to nine, 15, 21 and 27°C for Merlot and 15, 21 and 27°C for Shiraz for 30 days. Humidity was maintained between 74 and 77% and a ventilation system circulated air.

Berry sampling

At 2- to 3-day intervals, 300 berries were sampled from all drying treatments as well as from bunches in the vineyards (Control). All batches of berries were pressed using a custom-made hydraulic laboratory press and then centrifuged before analysis. Prior to pressing, berries were cut at the pedicel, weighed and sorted by density in NaCl solutions as described by Carbonell-Bejerano et al. (2013). To adjust the NaCl, prior to sorting, a subsample of 20 berries was drawn randomly out of the 300 berries. This subsample was pressed and analysed for TSS (°Brix) in order to adjust the NaCl gradients. Subsequently the remaining berries (around 280) were separated using three NaCl solutions into three batches. In this way at least 60 berries were allocated to a low, middle and high density batch for each sampling point. Each NaCl concentration used is specified in Table S1. Each batch was then processed separately.

Organic acids and sugar were analysed by HPLC, a 1260 Infinity Agilent HPLC system consisting of a G4225A degasser, a isocratic G1310 pump system, a GT329B autosample injector, a G1316A column oven, a G1314F UV-detector (Agilent Technologies, Santa Clara, CA, USA) that is connected to a Shodex RI-101 refractive index detector (Showa Denko, Kawasaki, Japan) maintained at 50°C. Samples were pre-treated by solid phase extraction using Waters Oasis HLB, 6 cm^3 (200 mg) cartridges (Waters Corporation, Milford, MA, USA), then filtered through 0.2 mm nylon filters (Millipore, Burlington, MA, USA) and 20 μL were directly injected onto an Aminex HPX-87H HPLC column 300 × 7.8 mm, 9 μm particle size (Bio-Rad Laboratories, Hercules, CA, USA). Separations were carried out under isocratic conditions at 80°C using a 0.65 mmol H_2SO_4 solution, mobile phase at a 0.5 mL/min flow rate. Organic acids were detected at 210 nm.

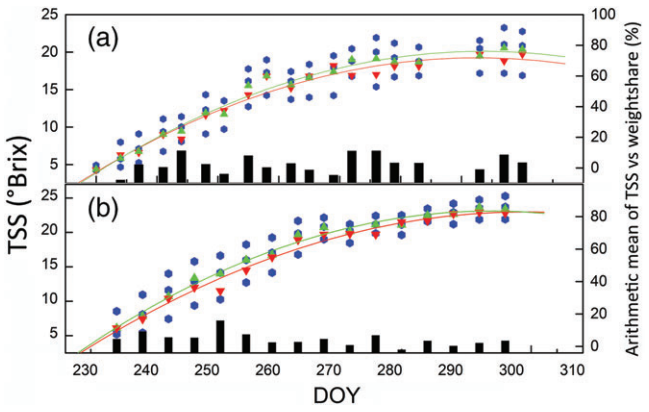

Figure 1. Average TSS of the 20 berry subsample (▼), weighted average sugar concentration (▲) and sugar concentration of single NaCl-sorted fractions (●) for (a) Shiraz and (b) Merlot as a function of the day of the year (DOY) of ripening of Controls from the vineyard. Black bars represent the under/overestimation between the 20-berry subsample and the weighted average.

Clarified samples were analysed as well by Fourier-transform infrared spectroscopy (WineScan, Foss, Hillerød, Denmark) for hexoses, TSS, acid and potassium.

All samples were analysed by HPLC and FTIR in parallel in order to validate the results of FTIR by HPLC. The correlation of MA to TA ratio, of FTIR and HPLC analysis is presented in Figure S1. A linear correlation ($y = 0.95x + 0.0096$, $R^2 = 0.88$) between FTIR and HPLC was obtained. Hexoses, TSS, acid and potassium data presented subsequently in the paper are values obtained by FTIR.

In order to avoid and/or re-solubilise potential TA precipitates, different protocols were tested, prior to berry pressing and analysis. Shiraz berries (540) were randomly sampled from the 27°C treatment. Berries were separated into six replicate groups (30 berries per group) and hand-crushed in a closed plastic bag and either heated in a water bath for 45 min at 80°C or autoclaved for 45 min or just hand-crushed at room temperature (Control) before pressing.

Temperature and precipitation in the control block were recorded during the growing season and are presented in Table S2. Origin Pro 7.0 and MS Excel (Microsoft, Richmond, WA, USA) were used for data analysis and curve fitting.

Results and discussion

Berry heterogeneity

Ripening of Controls in the vineyard is illustrated by TSS in Figure 1. Subsamples of 20 berries, collected before NaCl fractioning, are depicted together with the sugar concentration of the three separated fractions (Figure 1). The variation in TSS throughout ripening of up to 5°Brix in Shiraz and around 3°Brix in Merlot highlights the large variation of post-veraison berry ripening within a vineyard. Such heterogeneity of ripening berries even within one bunch can introduce considerable bias in molecular studies and other studies (Gouthu et al. 2014, Rienth et al. 2014b, 2016, Carbonell-Bejerano et al. 2016).

To account for this variability, weighted average was calculated using the proportion allocated to the respective fractions. Interestingly, it can be seen that the subsample generally underestimated the weighted average by up to 19% (Figure 1). Such variation in TSS, raises the question about which is the most representative sampling strategy for a vineyard plot. It also emphasises the necessity to use precise berry selection in physiological experiments. Finally, it challenges the usefulness of the commonly used term 'mid-veraison' in phenological studies defined as the moment when 50% of berries in one bunch has changed colour or softened, as discussed in previous studies (Rienth et al. 2014b, 2016).

Based on the results in this study it cannot be concluded if the observed differences in TSS are because of a phenological shift of berries passing through veraison at different times, or if each berry goes through the ripening period at a different speed. To our knowledge, only one study has examined this problem systematically using a transcriptomic approach (Gouthu et al. 2014). Their data led the authors to hypothesise that some berries lagged behind because of a phenological delay at veraison but that this was followed by a subsequent enhanced rate of ripening allowing these berries to 'catch up' with the others. As a result all other data (organic acids, sugars and ethanol) subsequently presented in this paper were calculated as weighted average.

Ripening of Shiraz and Merlot under cool conditions

Shiraz and Merlot as mid- to late-ripening cultivars have only recently been planted in cool viticulture regions such as the canton Vaud in western Switzerland. Thus, few studies have characterised and compared their berry development under these conditions.

Sampling of Control fruit started just after fruitset for Shiraz, whereas Merlot sampling commenced with the onset of sugar accumulation. For Shiraz, the main berry development phases were clearly distinguished with organic acid accumulation occurring simultaneously with an increase in berry mass until the lag phase was reached at around day of the year (DOY) 235 and lasted for approximately 10 days. Berry mass at the lag phase for Shiraz was 1 g which was approximately 50% of berry mass (1.9 g) at harvest and was similar to that in other published studies (Diakou-Verdin et al. 2002, Conde et al. 2007, Dai et al. 2011, Houel et al. 2013, Kuhn et al. 2014).

Although 'green development' in Merlot berries was not sampled it can be deduced from sugar and mass data that berry mass of Merlot berries at the lag phase was close to 0.6–0.7 g and, thus, also around 50% of the mass of berries at harvest (1.3 g). Accumulation of MA and TA plateaued at the lag phase at approximately 380 mEq/berry (250 MA and 130 TA) in Shiraz and 250 mEq/berry (170 MA and 80 TA) in Merlot, which corresponded to values previously reported (Rienth et al. 2014a).

During ripening TA on a per berry basis remained stable. This has been observed in most other studies where a TA turnover or respiration in the ripening berry was not reported (Coombe and McCarthy 2000, Conde et al. 2007). Malic acid declined exponentially in Shiraz by 3.5 μEq/(berry · day) until reaching a plateau at 110 μEq/berry for the last 15 days before harvest. Initially, in Merlot the average degradation was faster with 5.5 μEq/(berry · day) until around 60 μEq/berry. Then degradation slowed down with 0.47 μEq/(berry · day) over 30 days until harvest at 46 μEq/berry. Since MA respiration is temperature dependent

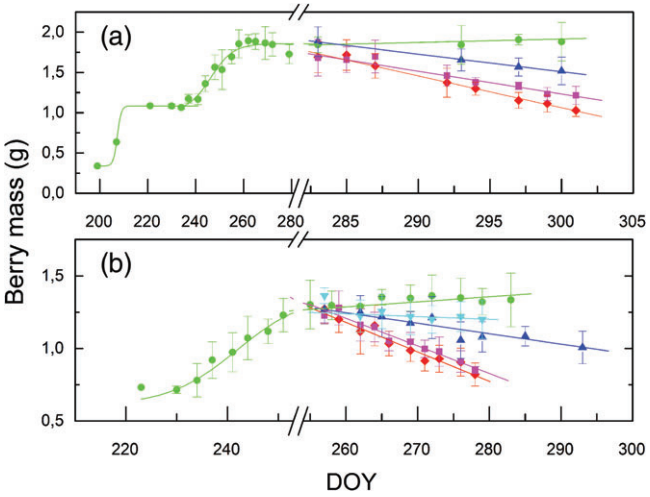

Figure 2. Development of mean berry mass of Control in the vineyard and during the berry drying experiment for (a) Shiraz and (b) Merlot Control (●), and at 9 (▼), 15 (▲), 21 (■) and 27°C (♦).

(Ruffner and Kliewer 1975), slower degradation was most likely because of cooler temperature at the end of the growing season, with monthly average temperature dropping from 20.7°C (August) to 18.2°C (September) and then to 10.1°C in October (Table S2).

Active sugar accumulation by phloem unloading in Shiraz ceased at around DOY 270 (Figures 2–3), when berry mass reached a maximum of 1.9 g in accordance with the mass during the lag phase, at around DOY 215–225. Hexose (glucose + fructose) concentration increased during ripening up to 185 g/L with an average of 4.1 g/(L · day) when drying was started. The subsequent increase in the vineyard until 192 g/L (harvest) was four times slower (0.1 g/day) and likely mostly because of a concentration related effect. In Merlot, hexose concentration increased at a rate of approximately 5.4 g/(L · day), reaching 190 g/L at the start of berry drying. The subsequent increase up to 230 g/L corresponds to a rate of approximately 1.55 g/day.

A phenological difference of around 10 days occurred between Shiraz and Merlot (Figures 1–3), which are generally classed as époque 2 – mid ripening cultivars reaching maturity 2.5 weeks after the reference cultivar Chasselas (plantgrape.org). In the current study, however, Shiraz was

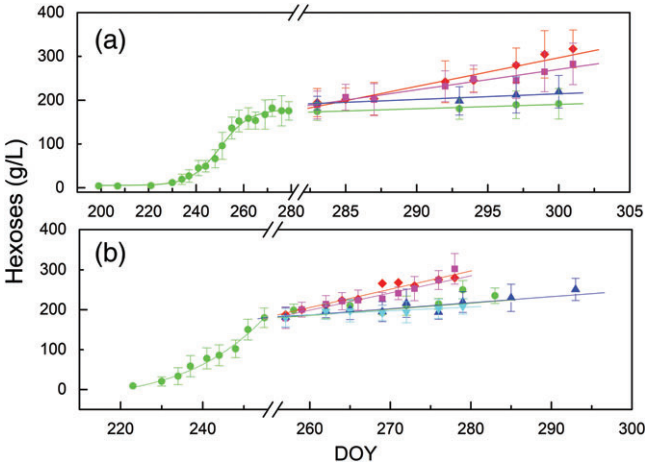

Figure 3. Hexose concentration (glucose + fructose, weighted average) of Control in the vineyard and during the berry drying experiment for (a) Shiraz and (B) Merlot Control (●), and at 9 (▼), 15 (▲), 21 (■) and 27°C (♦).

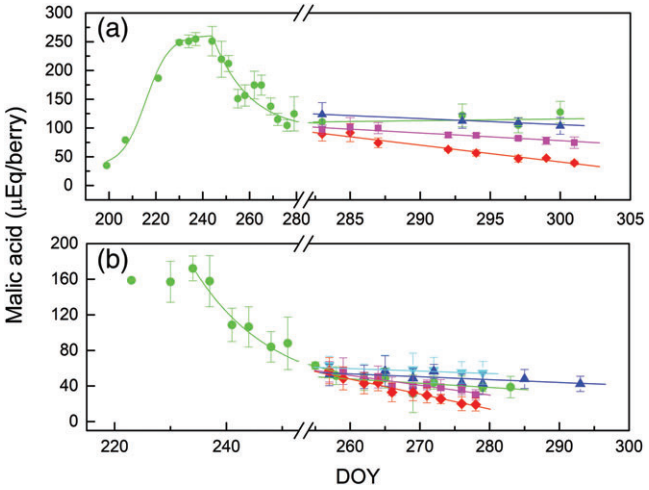

Figure 4. Malic acid content (weighted average) per berry of Control in the vineyard and during the berry drying experiment for (a) Shiraz and (B) Merlot Control (●), and at 9 (▼), 15 (▲), 21 (■) and 27°C (♦).

about 10 days behind Merlot, which confirms observations made by growers in northern regions. The ripening rates presented illustrate that it is difficult for the late ripening cultivars to reach an acceptable maturity, represented by a high sugar/acid ratio, even when harvest is delayed.

Grape berry drying

Current dehydration techniques last from 3 weeks to 4 months, causing a mass loss of 30–40% and yielding a final product that is richer in sugars, solutes and aroma compounds (Zenoni et al. 2016). In the present experiment, the maximum decrease in berry mass was, as expected, observed at 27°C for both cultivars. Interestingly, strong genotypic differences could be observed with a maximum loss in berry mass of 44.4% for Shiraz and only 31.7% for Merlot. Consequently, the increase in sugar concentration was highest at 27°C with an increase of 71.1% for Shiraz and 38.5% for Merlot (Figure 3, Tables S1, S2). The dehydration rate was linear at around 0.040 g/(berry · day) for Shiraz, which was twice as high as for Merlot (Table 3). Costantini et al. (2006) recorded a mass loss of 33 and 23% of the gain in sugar concentration at 15°C in drying experiments with Malvasia. The results of our study at the same temperature show that Shiraz lost only 16.7% of mass by gaining 24.1% of sugar whereas Merlot lost 10.0% of berry mass and gained 9.8% of sugar. These differences can be explained by a lower relative humidity (40%) in experiments with Malvasia but illustrates that there are important cultivar variations.

Even at a transcriptomic level, Shiraz during drying displayed the greatest change in expression of genes linked to the phenylpropanoid/stilbene biosynthesis pathway (Zenoni et al. 2016). The MA content per berry was strongly reduced during drying for both cultivars at 27°C being 63.2% for Shiraz (Tables 2, S3) and 62.0% for Merlot (Tables 3, S2, S4), which confirms the temperature dependent respiration of MA in the ripening berry (Ruffner and Kliewer 1975). Remarkably, genotypic differences are not as pronounced as for water loss and sugar accumulation, which emphasises MA respiration during ripening.

The observation of the drop in TA on a per berry basis of up to 48.5% in Shiraz and 35.5% in Merlot is somehow surprising and has, to our knowledge, not been observed before. Potassium content in dehydrating berries, however, decreased as in both cultivars indicating precipitation (Figure 6). The TA precipitates mainly with potassium as potassium hydrogen tartrate in must and wine, which could potentially occur inside the berry or during sample processing. A stoichiometric balance of both compounds, supposing that 1 mol K^+ precipitates 1 mol of tartrate indicates a systematic decrease of K^+ during ripening and during grape drying (Figure S2, Table S4).

To investigate a potential precipitation in the berry, several methods to resolve tartrate prior to pressing were tested and the results are presented in Table S5. Although no significant difference in TA concentration was observed, lower TA values indicate an eventual precipitation that occurs already inside the berry. This appears unlikely based on studies showing that tartrate does not form crystals with K^+ or Ca^{2+} in the intact berries and thus does normally not precipitate during ripening (Keller 2015). In contrast to widespread opinion, it has been shown that the crystals found in the fleshy pericarp of berries are because of the biomineralisation of calcium oxalate and not tartrate (DeBolt et al. 2004). Putatively, the dehydration of berries led to the disintegration of berry cells and thus to a loss of compartmentation leading to TA precipitation. Another hypothesis could be that compartmentation is lost early during berry drying and that the continuous loss of water led to enhanced precipitation. Previous studies showed that the rate at which TA, MA and K^+ are leached from flesh tissue increased as berries ripened, indicating a general increase in membrane permeability (Iland and Coombe 1988). In a study investigating cell death in grape berries it was hypothesised that different strategies exist during late berry development: either a programed cell death in the pericarp and loss of osmotically competent membranes or continued cell vitality and osmotically competent membranes that can allow high hydraulic conductance to the vine. In the latter experiment the cell death strategy was particularly

Table 3. Average speed of change for berry mass and principal compounds from the beginning to the end of grape drying (data from linear fits of Figures 2–5).

	Control		9°C	15°C		21°C		27°C	
Composition	**Shiraz**	**Merlot**	**Merlot**	**Shiraz**	**Merlot**	**Syrah**	**Merlot**	**Shiraz**	**Merlot**
Berry mass (g/day)	0.040	0.006	−0.008	−0.022	−0.007	−0.028	−0.019	−0.040	−0.020
Hexoses (g/(L · day)	0.10	1.55	0.97	1.48	1.51	4.77	4.30	6.54	4.59
MA berry (mEq/day)	0.29	−0.47	−0.27	−1.05	−0.32	−1.34	−1.10	−2.90	−1.68
TA berry (mEq/day)	2.00	0.09	0.00	−1.31	−0.79	−4.10	−2.34	−4.67	−3.35
K^+ berry (mmol/day)	0.10	0.09	−0.20	−0.56	−0.25	−0.74	−0.48	−1.00	−0.53

MA, malic acid; TA, tartaric acid.

Table 2. Composition of Merlot berries at commencement of sampling in the vineyard and at the start and end point of drying at 9, 15, 21 and 27°C.

Composition	Control (vineyard) start	Control end (harvest)	9°C	15°C	21°C	27°C
Berry mass (g)	1.2 ± 0.1	1.32 ± 0.9	1.20 ± 0.13	1.08 ± 0.12	0.85 ± 0.02	0.82 ± 0.08
TSS (°Brix)	20.5 ± 1.5	23.7 ± 1.1	21.9 ± 1.3	22.5 ± 1.2	28.1 ± 1.7	28.4 ± 1.2
TA (mEq)	89 ± 1.3	88 ± 1.3	94 ± 137	97 ± 6	96 ± 9.8	96 ± 9.8
TA (μEq/berry)	110 ± 10	116 ± 10	113 ± 16	105 ± 15	81 ± 10	71 ± 12
K (mmol/berry)	33 ± 2.5	34 ± 4.7	30 ± 3.0	24 ± 1.8	21 ± 0.6	14 ± 2.3
MA (mEq)	40 ± 5.9	29 ± 1.8	46 ± 5.8	40 ± 8.4	35 ± 6.1	23 ± 7.3
MA (μEq/berry)	50 ± 10.5	39 ± 4.8	55 ± 12.8	43 ± 12.2	31 ± 5.8	19 ± 7.2
MA/TA	0.4 ± 0.06	0.30 ± 0.1	0.43 ± 0.04	0.36 ± 0.06	0.33 ± 0.3	0.24 ± 0.07
Sugar (mg/berry)	245 ± 16.6	330.7 ± 18	250 ± 7	240	245	232
Sugar (mg/L)	199.6 ± 16.3	250 ± 23	208.3 ± 19	219 ± 26	290 ± 38	285 ± 35

MA, malic acid; tartaric acid.

dominant in the case of Shiraz berries (Tilbrook and Tyerman 2008).

Cholet et al. (2016) observed a high vintage effect on TA concentration and content under different climatic conditions in the same population of Ugni Blanc. In that study concentration and content of TA varied by up to twofold during two vintages, and differences in content per berry were mainly apparent after veraison. This could indicate a TA turnover during ripening affected by environmental conditions, or a higher potassium uptake and thus enhanced potassium tartrate precipitation. A similar phenomenon was observed in another recent experiment dealing with physiological effects during the delay of harvest of Merlot grapes (Bondada et al. 2017). The authors found TA per berry decreased when harvest was delayed, hypothesising that a possible conversion of TA into MA could occur, which is subsequently catabolised during ripening as observed to occur in grapevine leaves (Takimoto et al. 1977). A study performed on water relations and trophic competition with Grenache has shown that leaf surface and water deficit can impact berry composition, notably K^+ and TA. In the latter study water stress reduced TA (on a per berry basis) significantly in comparison to non-stressed vines in ripening berries (Etchebarne 2008).

From the data presented, it appears unlikely that TA turnover in ripening berries occurred, but rather that the decline and high variation observed here and in the literature are mainly because of precipitation after juice extraction from the berry. Published protocols for TA analysis in berries or juice/must normally do not include sufficient heating after thawing or other measures to prevent, re-solubilise or stabilise TA before analysis (Melino et al. 2009, Eyéghé-Bickong et al. 2012). Most physiological studies freeze or cool samples prior to analysis thereby enhancing TA precipitation as if processed directly (Cholet et al. 2016, Bondada et al. 2017).

Conclusion

This preliminary study showed that grape berry drying can provide a valuable tool to concentrate berries of mid- to late-ripening cultivars in northern winegrowing regions, where these cultivars represent a potential adaptation to

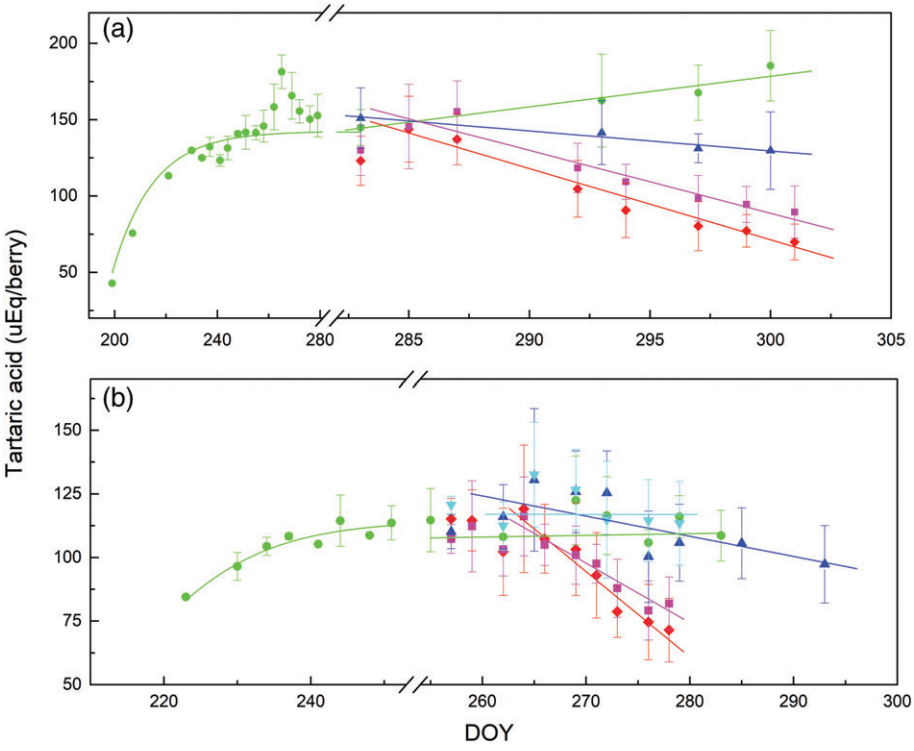

Figure 5. The tartaric acid content (weighted average) per berry of Control in the vineyard and during the berry drying experiment for (a) Shiraz and (b) Merlot Control (●), and at 9 (▼), 15 (▲), 21 (■) and 27°C (♦).

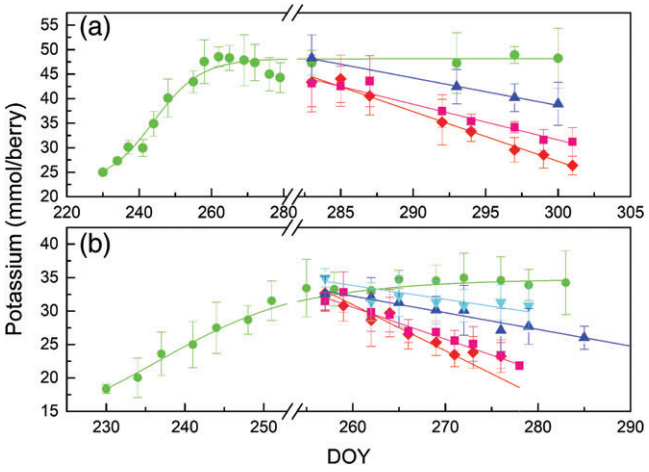

Figure 6. Potassium content (weighted average) per berry of Control in the vineyard and during the berry drying experiment for (a) Shiraz and (b) Merlot Control (●), and at 9 (▼), 15 (▲), 21 (■) and 27°C (♦).

increased global warming but may still struggle to reach optimum maturity in cool vintages. Drying differed with cultivar and this needs to be considered by growers who want to use this technique. The decline in TA is an important and a hitherto little reported phenomenon. It could be shown, however, that this decline is mainly because of crystallisation and precipitation of potassium hydrogen tartrate as the berry dries and during sample processing. Thus a turnover or respiration of TA can virtually be ruled out, which underlines the importance of continued use of TA as a breeding trait for future cultivars better adapted to global warming. The effect of sample processing, however, during the use of TA as a target for breeding programs needs to be considered to avoid analytical bias. The present study shows that the highly variable concentration of TA in published papers maybe due, in part, to precipitation problems during sample processing.

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References

Azuma, A. and Yakushiji, H. (2012) Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. Planta **236**, 1067–1080.

Badejo, A.A., Fujikawa, Y. and Esaka, M. (2009) Gene expression of ascorbic acid biosynthesis related enzymes of the Smirnoff-Wheeler pathway in acerola (*Malpighia glabra*). Journal of Plant Physiology **166**, 652–660.

Bondada, B., Harbertson, J., Shrestha, P.M. and Keller, M. (2017) Temporal extension of ripening beyond its physiological limits imposes physical and osmotic challenges perturbing metabolism in grape (*Vitis vinifera* L.) berries. Scientia Horticulturae **219**, 135–143.

Bonghi, C., Rizzini, F.M., Gambuti, A., Moio, L., Chkaiban, L. and Tonutti, P. (2012) Phenol compound metabolism and gene expression in the skin of wine grape (*Vitis vinifera* L.) berries subjected to partial postharvest dehydration. Postharvest Biology and Technology **67**, 102–109.

Buttrose, M.S., Hale, C.R. and Kliewer, W.M. (1971) Effect of temperature on the composition of "cabernet sauvignon" berries. American Journal of Enology and Viticulture **22**, 71–75.

Carbonell-Bejerano, P., Rodriguez, V., Hernáiz, S., Royo, C., Dal Santo, S., Pezzotti, M. and Martinez-Zapater, J.M. (2016) Reducing sampling bias in molecular studies of grapevine fruit ripening: transcriptomic assessment of the density sorting method. Theoretical and Experimental Plant Physiology **28**, 109–129.

Carbonell-Bejerano, P., Santa María, E., Torres-Pérez, R., Royo, C., Lijavetzky, D., Bravo, G., Aguirreolea, J., Sánchez-Díaz, M., Antolín, M.C., Martínez-Zapater, J.M., Santa Maria, E., Torres-Perez, R., Royo, C., Lijavetzky, D., Bravo, G., Aguirreolea, J., Sanchez-Diaz, M., Antolin, M.C. and Martinez-Zapater, J.M. (2013) Thermotolerance responses in ripening berries of *Vitis vinifera* L. cv Muscat Hamburg. Plant Cell Physiology **54**, 1200–1216.

Cholet, C., Claverol, S., Claisse, O., Rabot, A., Osowsky, A., Dumot, V., Ferrari, G. and Gény, L. (2016) Tartaric acid pathways in *Vitis vinifera* L. (cv. Ugni blanc): a comparative study of two vintages with contrasted climatic conditions. BMC Plant Biology **16**(144), 144.

Cirilli, M., Bellincontro, A., De Santis, D., Botondi, R., Colao, M.C., Muleo, R. and Mencarelli, F. (2012) Temperature and water loss affect ADH activity and gene expression in grape berry during postharvest dehydration. Food Chemistry **132**, 447–454.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. and Gerós, H. (2007) Biochemical changes throughout grape berry development and fruit and wine quality. Food **1**, 1–22.

Coombe, B.G. and McCarthy, M.G. (2000) Dynamics of grape berry growth and physiology of ripening. Australian Journal of Grape and Wine Research **6**, 131–135.

Costantini, L., Battilana, J., Lamaj, F., Fanizza, G. and Grando, M.S. (2008) Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. BMC Plant Biology **8**, 38.

Costantini, V., Bellincontro, A., De Santis, D., Botondi, R. and Mencarelli, F. (2006) Metabolic changes of Malvasia grapes for wine production during postharvest drying. Journal of Agricultural and Food Chemistry **54**, 3334–3340.

Dai, Z.W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., Van Leeuwen, C., Vivin, P. and Delrot, S. (2011) Ecophysiological, genetic, and molecular causes of variation in grape berry weight and composition: a review. American Journal of Enology and Viticulture **62**, 413–425.

DeBolt, S., Cook, D.R. and Ford, C.M. (2006) L-Tartaric acid synthesis from vitamin C in higher plants. Proceedings of the National Academy of Sciences of the United States of America **103**, 5608–5613.

DeBolt, S., Hardie, J., Tyerman, S. and Ford, C. (2004) Composition and synthesis of raphide crystals and druse crystals in berries of *Vitis vinifera* L. cv. Cabernet Sauvignon: ascorbic acid as precursor for both oxalic and tartaric acids as revealed by radiolabelling studies. Australian Journal of Grape and Wine Research **10**, 134–142.

DeBolt, S., Ristic, R., Hand, P.G. and Ford, C.M. (2008) Altered light interception reduces grape berry weight and modulates organic acid biosynthesis during development. Horticultural Science **43**, 957–961.

Diakou-Verdin, P., Carde, J.-P., Gaudillère, J.-P., Barrieu, F., Ollat, N. and Moing, A. (2002) Grape berry development: a review. OENO One **36**, 109–131.

Drawert, F. and Steffan, H. (1966) Biochemische physiologische Untersuchungen an Traubenbeeren. Vitis **5**, 377–384.

Duchêne, E. (2016) How can grapevine genetics contribute to the adaptation to climate change? OENO One **50**(3), 113–124.

Duchêne, E. and Schneider, C. (2005) Grapevine and climatic changes: a glance at the situation in Alsace. Agronomy for Sustainable Development **25**, 93–99.

Duchêne, E., Dumas, V., Jaegli, N. and Merdinoglu, D. (2014) Genetic variability of descriptors for grapevine berry acidity in Riesling, Gewürztraminer and their progeny. Australian Journal of Grape and Wine Research **20**, 91–99.

Etchebarne, F. (2008) Influence du régime hydrique de la vigne et du rapport feuilles-fruits sur la composition minérale, et sur le bilan en sucres et en eau de la baie cv. Grenache Noir (Vitis vinifera L.) (SupAgro Montpellier – École doctorale : Systèmes Intégrés en Biologie, Agronomie, Géosciences, HydroSciences et Environnement: Montpellier, France).

Etienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D. and Bugaud, C. (2013) What controls fleshy fruit acidity? A review of

malate and citrate accumulation in fruit cells. Journal of Experimental Botany **64**, 1451–1469.
Eyéghé-Bickong, H.A., Alexandersson, E., Gouws, L., Young, P. and Vivier, M. (2012) Optimisation of an HPLC method for the simultaneous quantification of the major sugars and organic acids in grapevine berries. Journal of Chromatography **885**, 43–49.
Food and Agriculture Organisation of the United Nations (2016) FAOSTAT Statistics Database (Food and Agriculture Organisation of the United Nations: Rome, Italy).
Flanzy, C. (1978) Etude sur le metabolisme anaerobie du raisin. PhD Thesis, Faculté des Sciences, Marseille-Luminy, France.
Fraga, H., Malheiro, C., Moutinho-Pereira, J. and Santos, J. (2012) An overview of climate change impacts on European viticulture. Food and Energy Security **1**, 94–110.
Fraga, H., Malheiro, C., Moutinho-Pereira, J. and Santos, J. (2013) Future scenarios for viticultural zoning in Europe: ensemble projections and uncertainties. International Journal of Biometeorology **57**, 909–925.
Gouthu, S., O'Neil, S.T., Di, Y., Ansarolia, M., Megraw, M. and Deluc, L.G. (2014) A comparative study of ripening among berries of the grape cluster reveals an altered transcriptional programme and enhanced ripening rate in delayed berries. Journal of Experimental Botany **65**, 5889–5902.
Hannah, L., Roehrdanz, P.R., Ikegami, M., Shepard, V., Shaw, M.R., Tabor, G., Zhi, L., Marquet, P. and Hijmans, R.J. (2013) Climate change, wine, and conservation. Proceedings of the National Academy of Sciences of the United States of America **110**, 6907–6912.
Houel, C., Martin-Magniette, M.L., Nicolas, S.D., Lacombe, T., Le Cunff, L., Franck, D., Torregrosa, L., Conéjéro, G., Lalet, S., This, P. and Adam-Blondon, A.F. (2013) Genetic variability of berry size in the grapevine (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research **19**, 208–220.
Iland, P.G. and Coombe, B.G. (1988) Malate, tartrate, potassium, and sodium in flesh and skin of shiraz grapes during ripening – concentration and compartmentation. American Journal of Enology and Viticulture **39**, 71–76.
Intergovernmental Panel on Climate Change (2014) Summary for policymakers. Climate change (2014) impacts, adaptation, vulnerabilityContribution of working group II to the fifth assessment report of the intergovernmental panel on climate change (Cambridge University Press: Cambridge, England) pp. 1–32.
Jaillon, O., Aury, J.-M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyère, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lecharny, A., Scarpelli, C., Artiguenave, F., Pè, M.E., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A.-F., Weissenbach, J., Quétier, F. and Wincker, P. (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature **449**, 463–467.
Keller, M. (2010) Managing grapevines to optimise fruit development in a challenging environment: a climate change primer for viticulturists. Australian Journal of Grape and Wine Research **16**, 56–69.
Keller, M. (2015) Grape composition and fruit quality. The science of grapevines; anatomy and physiology, 2nd edn (Elsevier: Amsterdam, The Netherlands) pp. 216–219.
Keller, M. and Shrestha, P.M. (2013) Solute accumulation differs in the vacuoles and apoplast of ripening grape berries. Planta **239**, 1–10.
Kizildeniz, T., Mekni, I., Santesteban, H., Pascual, I., Morales, F. and Irigoyen, J.J. (2015) Effects of climate change including elevated CO_2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (*Vitis vinifera* L.) cultivars. Agricultural Water Management **159**, 155–164.
Kliewer, W.M. (1966) Sugars and organic acids of *Vitis vinifera*. Plant Physiology **41**, 923–931.
Kuhn, N., Guan, L., Dai, Z.W., Wu, B.H., Lauvergeat, V., Gomès, E., Li, S.H., Godoy, F., Arce-Johnson, P. and Delrot, S. (2014) Berry ripening: recently heard through the grapevine. Journal of Experimental Botany **65**, 4543–4559.
Lijavetzky, D., Carbonell-Bejerano, P., Grimplet, J., Bravo, G., Flores, P., Fenoll, J., Hellín, P., Oliveros, J.C. and Martínez-Zapater, J.M. (2012) Berry flesh and skin ripening features in *Vitis vinifera* L. as assessed by transcriptional profiling. PLoS One **7**(6), e39547.
Melino, V.J., Soole, K.L. and Ford, C.M. (2009) A method for determination of fruit-derived ascorbic, tartaric, oxalic and malic acids, and its application to the study of ascorbic acid catabolism in grapevines. Australian Journal of Grape and Wine Research **15**, 293–302.
Melino, V.J., Hayes, M.A., Soole, K.L. and Ford, C.M. (2011) The role of light in the regulation of ascorbate metabolism during berry development in the cultivated grapevine *Vitis vinifera* L. Journal of the Science of Food and Agriculture **91**, 1712–1721.
Mira de Orduña, R. (2010) Climate change associated effects on grape and wine quality and production. Food Research International **43**, 1844–1855.
Mori, K., Goto-Yamamoto, N., Kitayama, M. and Hashizume, K. (2007) Loss of anthocyanins in red-wine grape under high temperature. Journal of Experimental Botany **58**, 1935–1945.
Rienth, M., Torregrosa, L., Gautier, S., Ardisson, M., Brillouet, J.-M. and Romieu, C. (2016) Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels its transcriptome. BMC Plant Biology **16**, 164.
Rienth, M., Torregrosa, L., Kelly, M.T., Luchaire, N., Pellegrino, A., Grimplet, J. and Romieu, C. (2014a) Is transcriptomic regulation of berry development more important at night than during the day? PLoS One **9**, e88844.
Rienth, M., Torregrosa, L., Luchaire, N., Chatbanyong, R., Lecourieux, D., Kelly, M.T. and Romieu, C. (2014b) Day and night heat stress trigger different transcriptomic responses in green and ripening grapevine (*Vitis vinifera*) fruit. BMC Plant Biology **14**, 108.
Rizzini, F.M., Bonghi, C. and Tonutti, P. (2009) Postharvest water loss induces marked changes in transcript profiling in skins of wine grape berries. Postharvest Biology and Technology **52**, 247–253.
Romieu, C., Tesniere, C., Than-Ham, L., Flanzy, C. and Robin, J.-P. (1992) An examination of the importance of anaerobiosis and ethanol in causing injury to grape mitochondria. American Journal of Enology and Viticulture **43**, 129–133.
Ruffner, H.P. and Kliewer, W.M. (1975) Phosphoenolpyruvate carboxykinase activity in grape berries. Plant Physiology **56**, 67–71.
Salazar-Parra, C., Aranjuelo, I., Pascual, I., Erice, G., Sanz-Sáez, Á., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Araus, J.L. and Morales, F. (2015) Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient. Journal of Plant Physiology **174**, 97–109.
Schultz, H.R. (2000) Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. Australian Journal of Grape and Wine Research **6**, 2–12.
Schultz, H.R. (2016) Global climate change, sustainability, and some challenges for grape and wine production. Journal of Wine Economics **11**, 181–200.
Schultz, H.R. and Stoll, M. (2010) Some critical issues in environmental physiology of grapevines. Society **16**, 4–24.
Shangguan, L., Sun, X., Zhang, C., Mu, Q., Leng, X. and Fang, J. (2015) Genome identification and analysis of genes encoding the key enzymes involved in organic acid biosynthesis pathway in apple, grape, and sweet orange. Scientia Horticulturae **185**, 22–28.
Sweetman, C., Deluc, L.G., Cramer, G.R., Ford, C.M. and Soole, K. L. (2009) Regulation of malate metabolism in grape berry and other developing fruits. Phytochemistry **70**, 1329–1344.
Sweetman, C., Sadras, V.O., Hancock, R.D., Soole, K.L. and Ford, C. M. (2014) Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. Journal of Experimental Botany **65**, 5975–5988.
Takimoto, K., Saito, K. and Kasai, Z. (1976) Diurnal change of tartrate dissimilation during the ripening of grapes. Phytochemistry **15**, 927–930.
Takimoto, K., Saito, K. and Kasai, Z. (1977) Conversion of tartrate to malate and monoethyl tartrate in grape leaves. Phytochemistry **16**, 1641–1645.

Tarara, J.M., Lee, J., Spayd, S.E. and Scagel, C.F. (2008) Berry temperature and solar radiation alter acylation , proportion, and concentration of anthocyanin in Merlot grapes. American Journal of Enology and Viticulture **59**, 235–247.
Terrier, N., Sauvage, F.X., Ageorges, A. and Romieu, C. (2001) Changes in acidity and in proton transport at the tonoplast of grape berries during development. Planta **213**, 20–28.
Terrier, N., Glissant, D., Grimplet, J., Barrieu, F., Abbal, P., Couture, C., Ageorges, A., Atanassova, R., Léon, C., Renaudin, J. P., Dédaldéchamp, F., Romieu, C., Delrot, S. and Hamdi, S. (2005) Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. Planta **222**, 832–847.
Tilbrook, J. and Tyerman, S.D. (2008) Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. Functional Plant Biology **35**, 173–184.
Tonutti, P. and Bonghi, C. (2013) Biochemistry and physiology of dehydrating berriesSweet, reinforced and fortified wines (John Wiley & Sons: Hoboken, NJ, USA) pp. 77–90.
van, Leeuwen, C., Schultz, H.R., Garcia de Cortazar-Atauri, I., Duchêne, E., Ollat, N., Pieri, P., Bois, B., Goutouly, J.-P., Quénol, H., Touzard, J.-M., Malheiro, A.C., Bavaresco, L. and Delrot, S. (2013) Why climate change will not dramatically decrease viticultural suitability in main wine-producing areas by 2050. Proceedings of the National Academy of Sciences of the United States of America **110**, E3051–E3052.
Versari, A., Parpinello, G.P., Tornielli, G.B., Ferrarini, R. and Giulivo, C. (2001) Stilbene compounds and stilbene synthase expression during ripening, wilting, and UV treatment in grape cv Corvina. Journal of Agricultural and Food Chemistry **49**, 5531–5536.
Wheeler, L.G., Jones, A.M. and Smirnoff, N. (1998) The biosynthetic pathway of vitamin C in higher plants. Nature **393**, 365–368.
Zamboni, A., Minoia, L., Ferrarini, A., Tornielli, G.B., Zago, E., Delledonne, M. and Pezzotti, M. (2008) Molecular analysis of post-harvest withering in grape by AFLP transcriptional profiling. Journal of Experimental Botany **59**, 4145–4159.
Zenoni, S., Fasoli, M., Guzzo, F., Santo, S.D., Amato, A., Anesi, A., Commisso, M., Herderich, M., Ceoldo, S., Avesani, L., Pezzotti, M. and Tornielli, G.B. (2016) Disclosing the molecular basis of the postharvest life of berry in different grapevine genotypes. Plant Physiology **172**, 1821–1843.

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Supporting information

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Table S1. Effect of NaCl concentration, which has been used for berry separation, on the TSS of the separated batches of berries.

Table S2. Monthly average temperature and precipitation during the 2017 growing season.

Table S3. Relative evolution of major berry compounds in Shiraz at the end of the drying experiment based on the composition at the start.

Table S4. Relative evolution of major compounds in Merlot berries at the end of the drying experiment based on the composition at the start.

Table S5. Effect of extraction method of berries prior to pressing and analysis.

Figure S1. Correlation of the malic acid to TA ratio of all samples (each batch) of Merlot (♦) and Shiraz (▲) analysed by HPLC and FTIR ($y = 0.95x + 0.0096$, $R^2 = 0.88$).
Figure S2. Theoretical stoichiometric balance between potassium (K^+) and potassium hydrogen tartrate ($KC_4H_5O_6$) for (a) Shiraz and (b) Merlot Control (●), and drying at nine (▼), 15 (▲), 21 (■) and 27°C (♦).
Figure S3. pH of all analysed batches of Merlot (♦) and Shiraz (▲).