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Original article

Phenolic composition and sensory characteristics of Cabernet Sauvignon wines: effect of water stress and harvest date

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Summary The effect of three water status level and two harvest date 55 and 64 days after *veraison* (DAV) on phenolic and sensory composition of Cabernet Sauvignon wines were investigated. The later harvest date led to wine with a higher alcohol content. Total phenols varied from 1439.66 to 1643.08 mg L^{-1} with higher values at 64 DAV. No differences were observed between irrigation treatments. For total tannins and anthocyanins, no differences were found between harvest date. Separation of proanthocyanidins by Sep-Pak Plus tC_{18} cartridges showed only differences in concentration but not in the proportion of proanthocyanidin fractions. The wines from the most restricted treatment had a better colour and the same aroma of red fruits, persistence, astringency, fullness and bitterness, as the wine from the treatment with highest irrigation. Under the assay conditions, it was possible to obtain wines with a similar chemical and sensory composition earlier and using less irrigation water.

Keywords Anthocyanins, Cabernet Sauvignon, controlled water deficit, phenolic compounds, ripening, sensory evaluation, wine.

Introduction

Controlled water deficit is a common practice in global viticulture, particularly in areas with warm climates. It involves supplying an amount of water below the level of total evapotranspiration of the crop (ETc.) throughout the growth season and during specific phenological stages (Chaves *et al*., 2010). Its application in grapevines induces the production of abscisic acid (ABA) which triggers several physiological responses, such as stomatal closure. This, in turn, reduces the photosynthetic rate and thus reduces plant growth (Ferrandino & Lovisolo, 2014; Osakabe *et al*., 2014).

Application of water deficit is a worldwide used strategy in red wine production. It reduces the size of the fruit, increases the skin/pulp ratio of the grape and affects the concentration of compounds from the solid part of the grape (Singleton, 1972; Roby *et al*., 2004; Chaves *et al*., 2010). Of particular importance is the accumulation of phenolic compounds that play a key role in defining the quality of a red wine, as they participate directly in its colour due to anthocyanins, and the astringency, bitterness, ageing capacity and colour stabilisation, due to flavonols (also known as condensed tannins or procyanidins) (Ribéreau-Gayon *et al*., 2006; Chaves *et al*., 2010; Casassa *et al*., 2015). It should be noted that the effect of water deficit on the composition of phenolic compounds depends on factors such as the intensity of the deficit (Kennedy *et al*., 2002), the moment of application (Matthews *et al*., 1990; Chaves *et al*., 2010), and the cultivar in question (Niculcea *et al*., 2014). Recent studies show that controlled water deficit influences the synthesis of these compounds at the level of gene expression along the phenylpropanoid pathway in the skin of the fruit, leading to an accumulation of flavonols, flavonols and anthocyanins (Villalobos-González *et al*., 2016; Cáceres-Mella *et al*., 2017).

To determine the optimum moment to harvest red wine grapes, as well as the sugar/acidity ratio (technological ripeness), it is also important that the grape has a high concentration of easily extractable phenolic compounds in the skin, such as anthocyanins and tannins, and that the seeds have a hard outer coating. This ensures lower extraction of unwanted tannins (which have a high degree of bitterness) during the

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winemaking process (Pourcel *et al.*, 2007). Not only is the concentration important, but also the composition of the phenolic compounds, especially that of tannins, as those from the seeds are bitter as they have a lower level of polymerisation and a higher percentage of galloylation, instead those from skins have a larger molecular size giving a better sensation of fullness in mouth (Brossaud *et al.*, 2001; Vidal *et al.*, 2003; Cheynier *et al.*, 2006; Monagas *et al.*, 2006). The optimum moment to harvest red wine grapes is therefore defined by technological and phenolic ripeness (Ribéreau-Gayon *et al.*, 2006). As the concentration of anthocyanins increases during ripening up to a maximum, after which it begins to decrease slightly (Pirie & Mullins, 1980; Castellarin & Di Gaspero, 2007), and as the maximum accumulation of tannins occurs at *veraison*, after which it decreases until harvest (Kennedy *et al.*, 2001; Harbertson *et al.*, 2002; Hanlin & Downey, 2009), the exact point at which there is a good ratio between these two types of ripeness is very important.

In warm climates, the two types of ripeness occur at different times, as technological ripeness precedes phenolic ripeness, leading to grapes with high sugar content and therefore, wines with a high level of alcohol and lower acidity. The earlier phenolic ripeness gives grapes without a high level of sugars, less dehydration and a lower risk of being rotten as there is less chance of their being affected by possible climate events, such as rainfall. Due principally that water deficit could speed up the accumulation of phenolic compounds allow an earlier harvest of grapes, the aim of this assay was to assess the effect of water deficit from three irrigation treatments on the phenolic and sensory composition of Cabernet Sauvignon wines harvested on two different dates.

Materials and methods

Chemical reagents and instrumentation

Methylcellulose (1500 cP, viscosity at 20 g L^{-1}) and gallic acid, caffeic acid, *p*-coumaric acid, caftaric acid, (+)-catechin, (−)-epicatechin, quercetin, myricetin, kaempferol and malvidin-3-glucoside standards were bought from Sigma-Aldrich (St. Louis, MO, USA). Sodium sulphate, potassium metabisulphite, vanillin (990 g L^{-1}), ethyl acetate, diethyl ether, sodium hydroxide, hydrochloric acid, sulphuric acid, HPLC grade acetonitrile, acetic acid, formic acid, methanol and 0.22 and 0.45 μm PVDF membrane were acquired from Merck (Darmstadt, Germany). All reagents were of analytic grade or above. Sep-Pak Environmental tC_{18} (900 mg) and Sep-Pak Plus Short tC_{18} (400 mg) cartridges were obtained from Waters (Milford, MA, USA). Anhydrous dibasic sodium phosphate and monobasic potassium phosphate were acquired from J.T. Baker (Phillipsburg, NJ, USA). Nitrogen gas was supplied by Indura S.A. (Santiago, Chile). Ultrapure water was obtained from the purification system Purelab Ultra MK2 (Elga Labwater, Lane End, UK).

pH was measured in an 8417N pH-meter (Hanna Instruments, Woonsocket, RI, USA). Low-molecular-weight phenol analysis was carried out in an Agilent 1100 series chromatograph (Agilent Technologies, Santa Clara, CA, USA), consisting of a G1315B diode array detector (DAD), a G1311A quaternary pump, a G1379A degasser and a G1329A autosampler. A Nova-Pak C_{18} reverse phase column (4 μm, 3.9 mm I.D. × 300 mm; Waters) was used for the analysis of individual phenolic compounds. The anthocyanins were analysed using an Agilent 1200 series system (Agilent Technologies) consisting of a G1315B DAD, a G1311A quaternary pump, a G1313A autosampler, a G1322A degasser and a G1316A thermostatted column compartment with a LiChro Cart 100 RP-18 reverse phase column (5 μm, 4.0 mm I.D. × 250 mm; Agilent Technologies). Absorbency was measured using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan).

Plant material and irrigation treatments

The field trials were carried out in 2015 on 12-year-old vines with ungrafted rootstock of the cultivar Cabernet Sauvignon at a commercial vineyard in the Maipo Valley, central Chile (latitude 33°42′30″S, longitude 70°36′12″W). The area has a semiarid Mediterranean climate characterised by cold winters with moderate precipitation, and warm dry summers. The 2015 season showed typical climate conditions for the area between January and April. The maximum and minimum temperatures for the period were 29.2 and 7.7 °C, respectively. The average ETp for the period was 3.2 mm day^{-1}.

The soils are colluvial in origin with a clay loam texture. The vineyard is run organically, with drip irrigation, vertical trellises oriented north–south, a plant density of 2666 plants ha^{-1} and with double Guyot pruning. The mean yield at the vineyard is 8 t ha^{-1}. The experiment used a fully randomised blocks design, with five replications. Each replication comprised a total of seven plants.

Prior to implementing the irrigation treatments with water deficit and to reduce the xylematic water potential of the plants, irrigation was suspended for 30 days before *veraison* to equal out the water potential of the plants up to 10 days before *veraison*, which was when the irrigation treatments began. The irrigation treatments are described in detail in Cáceres-Mella *et al.* (2017). They were implemented through a combination of drip emitters at different flow rates to generate the following xylematic water potential around midday:

T1, $\Psi_{stem} = -0.8$ MPa; T2, $\Psi_{stem} = -0.9$ MPa; T3, $\Psi_{stem} = -1.0$ MPa. The water status of the plants was checked weekly by measuring xylematic water potential at midday using a Scholander pressure chamber. Healthy adult leaves from the middle third of the trellis were selected for the measurements. They were placed in aluminised plastic bags for 90 min at midday. The wines were produced from two harvest dates, 55 DAV (days after *veraison*) and 64 DAV. The second harvest date was the commercial harvest of the entire vineyard (April 15, 2015).

Microvinification process

For winemaking and due to grape volume limitation, adjacent vineyard replicates were combined so that three wine replicates ($n = 3$) were produced from each irrigation treatment. On each harvest date, 20 kg of grapes was harvested manually per replication. The time of *veraison* was determined by visual observation and historical data on the vineyard. The stems were removed from the grapes, which were then crushed in an automatic destemmer (Eno 3; Enoitalia, Florence, Italy). The mass of the grape was placed in 25 L food grade plastic containers with the addition of sulphur anhydride (SO_2) at a dose of 1 g hL^{-1}, to eliminate any native yeasts. The pulp was fermented at 23–24 °C with Lamothe-Abiet yeasts (*Saccharomyces cerevisiae*) (Canejan, France) at a dose of 20 g hL^{-1} and pump down twice a day for 1 min each time. Once a residual sugar content <2 g L^{-1} was reached, the wines were pressed in a vertical wine press (Model 50; Enoitalia), separating the free-run juice from the wine press. Only the free-run juice was used to make the wine. Sulphite was then added to the wines at a dose of 30 mg L^{-1} of free SO_2, and the wines were then stabilised and decanted at 5 °C for 5 days. The wine was finally filtered through plates (FIL-H Inox 6; Magusa, Vilafranca del Penedès, Spain) adjusting the content of free SO_2 to 30 mg L^{-1}, if necessary. It was then bottled into 750 mL bottles. The same winemaking process was used for all treatments. Basic analysis of pH, total acidity and alcohol degree were carried out in accordance with official methods published by the OIV (2012).

Spectrophotometric analysis

Total phenols were measured using spectrophotometry at an optical density of 280 nm (Zamora, 2003). Total anthocyanins were coloured with bisulphite and measured using spectrophotometry at 520 nm (Zamora, 2003). Total tannins were measured using precipitation with methylcellulose, in accordance with Sarneckis *et al.* (2006). Colouring intensity was expressed as the sum of absorbencies at 420, 520 and 620 nm (Zamora, 2003).

Total flavan-3-ol content in monomeric, oligomeric and polymeric fractions

Tannin fractionation, a technique that separates tannins into monomeric, oligomeric and polymeric subunits according to their degree of polymerisation, was carried out according to the procedure described by Sun *et al.* (1998a), using tC_{18} Sep-Pak® cartridges. All steps in the method were verified in a previous study (Cáceres *et al.*, 2012). For each fraction obtained previously, flavonols were quantified using the modified vanillin assay described by Sun *et al.* (1998b). Briefly, a 2.5 mL aliquot of 1:3 (v/v) of sulphuric acid/methanol solution and 2.5 mL of 10 g L^{-1} vanillin in methanol were mixed with 1 mL of wine sample in test tubes. The tubes were incubated at 30 °C for either 15 min (for monomeric fraction) or a time sufficient to allow a maximal reaction (for oligomeric and polymeric fractions). The absorbance at 500 nm was measured. A blank was prepared by replacing the vanillin solution in the reaction mix with methanol.

HPLC-diode array detector analysis of anthocyanins

A 2 mL sample of wine was filtered through a PVDF membrane with a pore size of 0.22 μm, from which an aliquot of 150 μL was taken and used for reverse phase chromatographic separation. The DAD was adjusted to between 210 and 600 nm. The mobile phases used were as follows: (A) water/formic acid (90:10 v/v) and (B) acetonitrile. The gradient was applied at a flow of 1.1 mL min^{-1} from 0 to 22 min and 1.5 mL min^{-1} from 22 to 35 min in the following way: 96–85% A from 0 to 22 min, 85–15% A from 12 to 22 min and 85–70% A from 22 to 35 min. Quantification was carried out by measuring the area of the peak at 520 nm. The anthocyanins were quantified and expressed as mg L^{-1} of malvidin-3-glucoside. Calibration curves at 520 nm were obtained by injection of different volumes of standard solutions under the same conditions used for the samples (Fanzone *et al.*, 2012).

HPLC-diode array detector analysis of low-molecular-weight phenols

Phenolic compounds were extracted from 50 mL of wine by consecutive extraction with diethyl ether (3 × 20 mL) and ethyl acetate (3 × 20 mL). The resulting extract was evaporated until dry at 30 °C, dissolved in 2 mL of methanol/water solution (50:50 v/v) and filtered through a PVDF membrane with a pore size of 0.22 μm. A 25 μL aliquot of the final solution was then separated by reverse phase chromatography at 20 °C. The DAD detector was adjusted to between 210 and 360 nm. Two motile phases were used as follows: (A) water/acetic acid (98:2 v/v) and (B) water/acetonitrile/acetic acid (78:20:2) v/v). The

gradient was 1 mL min^{-1} from 0 to 55 min and 1.2 mL min^{-1} from 55 to 90 min as follows: 100–20% A from 0 to 55 min, 20–10% A from 55 to 57 min and 10–0% A from 57 to 90 min. Identification of specific compounds was carried out by comparing their absorption spectra and retention time with the respective standard. Quantification was carried out by the external standard method using commercial standard samples. Calibration curves were obtained by injecting the standards in the range of concentrations observed above. The methodology was applied under the same conditions as described in Peña-Neira *et al.* (2007).

Sensory evaluation of the wines

A descriptive sensory analysis was carried out 1 month after bottling in the Laboratory of Sensory Analysis from the Department of Agro-industry and Oenology of the University of Chile. The panel consisted of twelve trained judges (21–40 years of age). The wines were assessed in individual tasting booths equipped with a computer. Transparent glasses (Viticole; Arcoroc®, Arques, France) with 20 mL of wine were served at a temperature of 18–19 °C in a random order. The wines were given three-digit code numbers. Between each tasting the member of the panel rested for 30 s and rinsed out their mouth with water. A nonstructured scale of 0–15 was used, where 0 corresponded to very low intensity and 15 to high intensity for the respective attribute (Lawless & Heymann, 2010). These data were collected digitally using Fizz® software (Biosystemes, Couternon, France). The parameters were evaluated visually (colour intensity), via smell (red fruits aroma) and taste (bitterness, astringency, fullness and persistence).

Statistical analysis

The data analysis and interpretation included two parts: (i) Evaluation of the differences between irrigation treatments with water deficit for each harvest date separately, using ANOVA followed by the Tukey' HSD test if any differences were found and (ii) Evaluation of the differences between the harvests dates for each irrigation treatments separately, using a mean comparison test (*t*-Student). Pearson's correlation was used to relate the chemical composition and sensory analysis. All the analysis was performed in triplicate. The analysis used a value of $P < 0.05$ and MINITAB® version 17.1.0 (Minitab Inc., State College, PA, USA).

Results and discussion

Water status

The irrigation treatments began when all the plants had reached a xylematic water potential close to −1.4 MPa. Initially, the frequency of irrigation was weekly, as no rainfall was recorded during the fruits' growth and ripening stage. The irrigation treatments led to different xylematic water potential levels. The average potential during the season for T1 was $\Psi_{stem} = -0.85 \pm 0.02$ MPa, for T2 it was $\Psi_{stem} = -0.96 \pm 0.02$ MPa and finally, for T3 it was $\Psi_{stem} = -1.04 \pm 0.01$ MPa. T3 maintained the lowest water potential for the three treatments during the period. The values for water potential obtained in this study are common for winemaking in a Mediterranean climate (Choné *et al.*, 2001) and are considered as weak water deficit (T1) to moderate water deficit (T3) (Van Leeuwen *et al.*, 2009).

Wine chemical composition

Table 1 shows the results for the general chemical parameter analysis of the wine. In general, the wine from grapes harvested 64 DAV had a higher grade of alcohol. This may be due to the soluble solid content of the grapes, which varied from 21.1 to 24.0°Brix, with the berries harvested 64 DAV having a higher

Table 1 General analytical parameters and global phenolic composition of Cabernet Sauvignon wines

	55 DAV			64 DAV		
	T1	T2	T3	T1	T2	T3
Ethanol (% v/v)	12.3 ± 0.18 A	11.40 ± 0.15 B	12.50 ± 0.07 A	13.50 ± 0.07*	13.60 ± 0.11*	14.50 ± 0.17*
Titratable acidity (g L^{-1})	3.60 ± 0.04	3.40 ± 0.07	3.70 ± 0.07	3.80 ± 0.11	3.60 ± 0.07	3.70 ± 0.07
pH	3.70 ± 0.02	3.76 ± 0.06	3.79 ± 0.02	3.77 ± 0.02	3.70 ± 0.05	3.77 ± 0.03
Phenols (mg GAE L^{-1})	1509.61 ± 10.3	1439.66 ± 30.4	1523.70 ± 13.9	1557.20 ± 13.7*	1643.08 ± 25.9*	1632.80 ± 8.4*
Tannins (mg CE L^{-1})	1039.85 ± 59.3	933.18 ± 110.2	1076.17 ± 12.9	1056.98 ± 53.0	1150.31 ± 53.2	1149.28 ± 24.7
Anthocyanins (mg ME L^{-1})	645.38 ± 6.6 AB	620.29 ± 12.9 B	696.35 ± 8.8 A	655.79 ± 11.5	733.78 ± 36.2	738.08 ± 11.5
Colour intensity (a.u.)	14.88 ± 0.29	13.64 ± 0.42	15.59 ± 0.41	14.73 ± 0.13 C	16.50 ± 0.18 A*	15.58 ± 0.06 B

a.u., absorbance units; CE, (+)-catechin equivalent; CI, colour intensity; DAV, days after *veraison*; GAE, gallic acid equivalent; ME, malvidin equivalent; T1, $\Psi_{stem} = -0.8$ MPa; T2, $\Psi_{stem} = -0.9$ MPa; T3, $\Psi_{stem} = -1.0$ MPa.

Values are presented as mean ± standard error ($n = 3$). Different capital letters indicate difference between irrigation treatments for the same date.

*Difference between the same irrigation treatments for different dates ($P < 0.05$).

soluble solid value, especially in T3 (Talaverano *et al.*, 2018). This higher value of °Brix denotes a more advanced stage of ripeness for the later harvest (Kennedy *et al.*, 2000; Chaves *et al.*, 2010; Intrigliolo *et al.*, 2016). With regard to titratable acidity and pH, no differences were observed between irrigation treatments and harvest dates. Both attributes were found to be within normal ranges for vineyards in central Chile (Cáceres *et al.*, 2012).

With regard to the overall phenolic composition, differences were seen in the concentration of total phenols and total anthocyanins. The total phenol concentration varied between 1439.66 and 1643.08 mg L^{-1}, with generally higher values for the later harvest at 64 DAV. No differences were seen between the irrigation treatments. Bautista-Ortín *et al.* (2006) observed that as ripeness advances, the total phenolic composition increases. Total anthocyanins varied from 620.29 to 738.08 mg L^{-1}. Differences were only seen between irrigation treatments for the grapes harvested 55 DAV, with T3 producing a higher concentration of total anthocyanins than T2.

It should be noted that comparing harvest dates, no difference was found between the values for total tannins and total anthocyanins. This suggest that in principle, it is possible to obtain the same concentration levels of these two phenolic compounds even when harvesting earlier. The colour intensity of the wines varied from 13.64 to 16.50 a.u., and differences were observed between the irrigation treatments for the harvest at 64 DAV (Table 1).

Figure 1 shows the content of low-molecular-weight phenols grouped by family in wines. Table S1 shows details of the compounds that were quantified. In general, most differences were seen between the harvest dates (Fig. 1). The most abundant group of phenolic compounds was flavonoids (49.3–52.3%), followed by nonflavonoids (47.7–50.7%). The most common compounds in the samples were flavonols followed by tyrosol. Gallic acid was the main component of the hydroxybenzoic acids. The total content of hydroxybenzoic acids was similar for all irrigation treatments, showing no notable effect from the water deficit. Of the hydroxycinnamic acids, hexose ester from *trans p*-coumaric acid had the highest level and its highest concentration was found for T3 at 64 DAV. Regarding irrigation treatments, differences were only seen between total and individual concentrations of hydroxycinnamic acids at 64 DAV, although it should be noted that as with the hydroxybenzoic acids, the concentrations obtained were similar among treatments. These results are in agreement with Downey *et al.* (2006), who state that the accumulation of cinnamic acids appears to be indifferent to changes in the water status of the plant. Differences in higher alcohol concentrations were only seen between the harvest dates

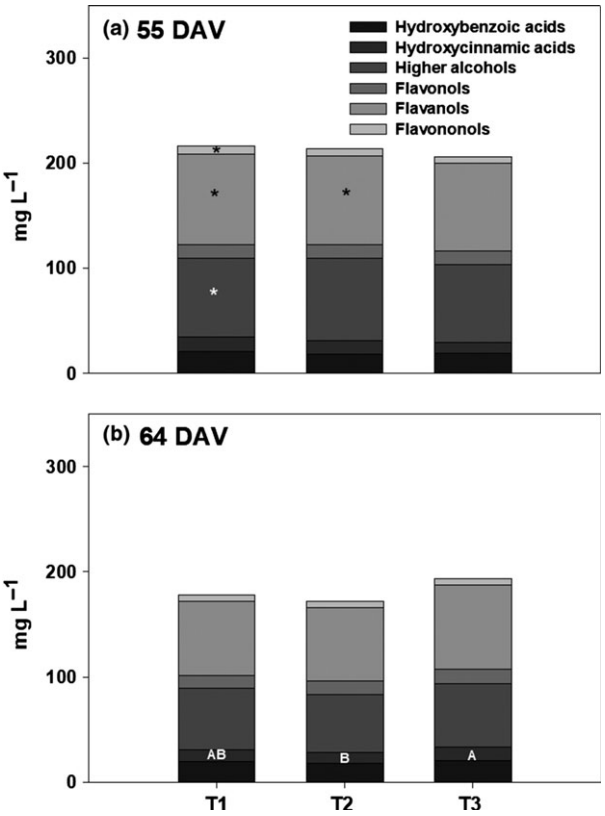

Figure 1 Low-molecular-weight phenolic compounds quantified in wine samples. Values are presented as mean ± standard error ($n = 3$). Different capital letters indicate significant difference between irrigation treatments for the same date. *indicates significant difference between the same irrigation treatment for different dates ($P < 0.05$). T1, $\Psi_{stem} = -0.8$ MPa; T2, $\Psi_{stem} = -0.9$ MPa; T3, $\Psi_{stem} = -1.0$ MPa. DAV, days after *veraison*.

for T1. In the case of stilbenes, these were detected at low concentrations. Stilbenes are produced by the fruits and the plant tissue in response to fungal attack and high exposure to u.v. rays, meaning the amount at harvest can vary (Jeandet *et al.*, 1991). In wine, the presence of stilbenes is important due to the beneficial properties to health, due principally that resveratrol could act as a powerful antioxidant, anticancer, anti-inflammatory and antidiabetes compound (Fernández-Mar *et al.*, 2012; Cavallini *et al.*, 2016). Deluc *et al.* (2011) found an increase in the biosynthesis of stilbenes, mainly t*rans*-resveratrol glycoside, in berries from vines subjected to water deficit levels similar to those of this study. The prediction amount of stilbene in the wine is not easy as there are many factors that affect its biosynthesis. It has been seen that the concentration in red wine can vary between undetectable to 14.3 mg L^{-1} *trans*-resveratrol (Fernández-Mar *et al.*, 2012). However, results show that treatments with

most restrictive condition of irrigation and an earlier harvest showed a higher amount of stilbenes, which may interesting from the point of view of increasing health benefits using less irrigation water in field.

Differences were not seen in the total flavonol content between the irrigation treatments and the harvest dates that is in agreement with previous results (Downey *et al*., 2006; Castellarin *et al*., 2007). With regard to total flavonol content, differences were only seen between the harvest dates. Procyanidin A1 was the main compound found, followed by procyanidin B3. The wines harvested 55 DAV produced higher flavonol content for T1 and T2, while for T3 the flavonol content on the two harvest dates showed no differences. This differs from the results of Kennedy *et al*. (2002) who found an increase in proanthocyanidin concentration in Cabernet Sauvignon wine as a result of the application of water deficit.

With regard of anthocyanins composition (Fig. 2), in general, differences were only seen between harvest dates, with none seen between the irrigation treatments. All the anthocyanins identified were present for all treatments. For the three irrigation treatments, the highest fraction quantified was that of glucosylated anthocyanins, with values in the range from 76.4% to 80.9%. Acetylates and coumaroylated fractions were found in quantities of 15.4–19.1% and 3.6–4.7%, respectively. Malvidin was the anthocyanin with highest presence in the samples, for all three forms, glucosylated, acetylated and coumaroylated (Table S2). Differences in concentration were only found between the harvest dates for the different anthocyanin groups (Fig. 2). At 55 DAV, T1 showed higher content levels for all anthocyanin fractions, compared to the samples harvested 64 DAV. T3 samples at 55 DAV had a higher concentration of the acetylated and coumaroylated fractions, while the T2 samples at 55 DAV had a lower concentration of the glucosylated fraction than that of the sample from 64 DAV. This means that in general, a higher concentration of anthocyanins was found for the earlier harvest, apart from the glucosylated fraction for T2, and that the most restricted treatment produced the same concentration of anthocyanins as the treatment with least water deficit (Fig. 2). This decrease in the content of total anthocyanins in last sample date is in agreement with previous studies (Cáceres *et al*., 2012). A later harvest could produce wines with more alcohol content and less anthocyanins, due to a degradation of anthocyanins in grape skins (Haselgrove *et al*., 2000).

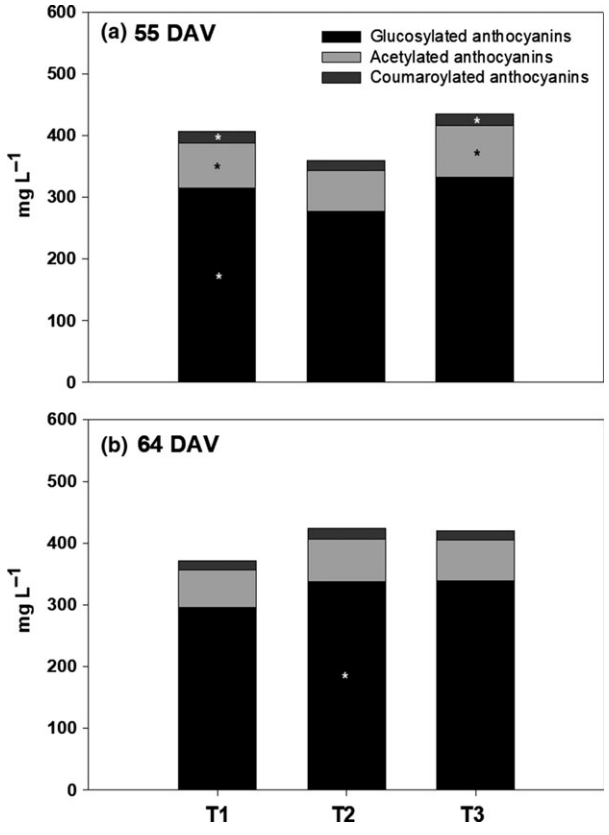

Figure 2 Anthocyanins quantified in wine samples. Values are presented as mean ± standard error ($n = 3$). *Indicates significant difference of parameter between the same irrigation treatments for different dates ($P < 0.05$). T1, $\Psi_{stem} = -0.8$ MPa; T2, $\Psi_{stem} = -0.9$ MPa; T3, $\Psi_{stem} = -1.0$ MPa. DAV: days after *veraison*.

The monomeric, oligomeric and polymeric proportions of flavan-3-ols in the wine samples were observed in Fig. 3. The percentage of proanthocyanidins in the oligomeric and polymeric fractions, as described in Sun *et al*. (1998a), varied from 4.7% to 10.2% and 88.4% to 94%, respectively. The monomeric fraction varied from 1.2% to 1.8%. Monomeric flavonols, represented by (+)-catechin, (−)-epicatechin, (−)-epigallocatechin and (−)-gallocatechin, showed no differences between irrigation treatments and harvest dates. In the oligomeric fraction, formed from 3 to 10 units of flavonols (Ribéreau-Gayon *et al*., 2006), differences were seen between the irrigation treatments at 55 DAV. For T3, a higher fraction of oligomers was found than for T2. Comparing harvest dates, there was an increase for T2 when going from 55 DAV to 64 DAV. In the polymeric fraction, differences were only seen between harvest dates for T3, with a decrease in polymer content at the later harvest date. Although Cáceres-Mella *et al*. (2017) found an increase in polymeric flavonol concentration and mDP (mean degree of polymerisation) during ripening in the skin of Cabernet Sauvignon berries subject to water deficit, the same behaviour was not found in the present wine samples. This discordance between prior results for grapes and the result for the wines may be due to the fact that at

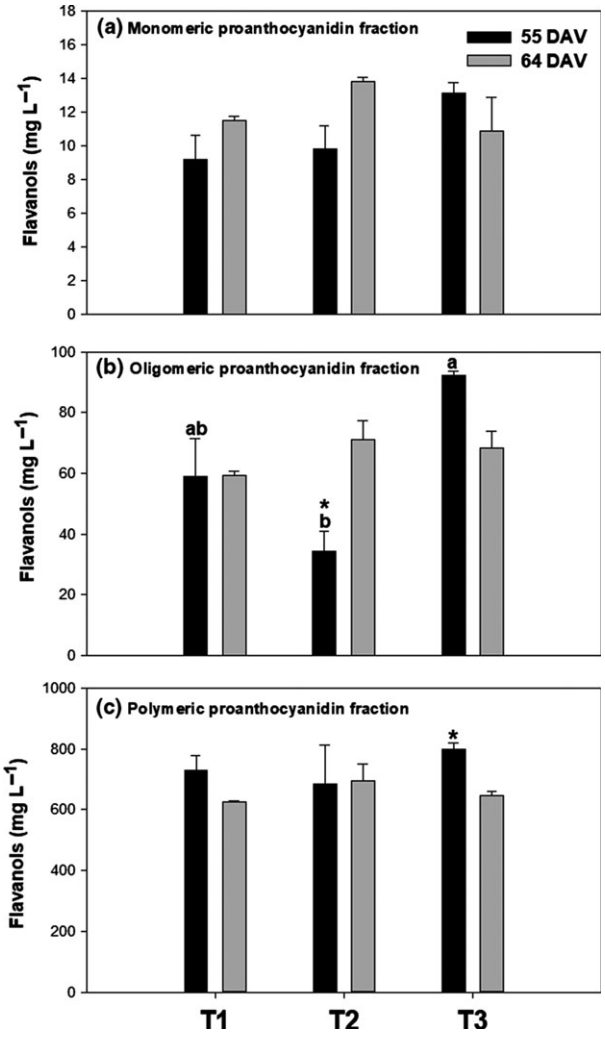

Figure 3 (a) Monomeric, (b) oligomeric and (c) polymeric proanthocyanidin fractions in wine samples. Values are presented as mean ± standard error ($n = 3$). Different capital letters above each bar indicate significant difference between irrigation treatments for the same date. *Indicates significant difference between the same irrigation treatment for different dates ($P < 0.05$). T1, $\Psi_{stem} = -0.8$ MPa; T2, $\Psi_{stem} = -0.9$ MPa; T3, $\Psi_{stem} = -1.0$ MPa. DAV, days after *veraison*.

harvest the amount of tannins in the berries is not a clear indication of the level of tannins that will be present in the wine (Harbertson *et al*., 2002; Bindon *et al*., 2013), and because the different tasks performed during winemaking can lead to variations in the extraction of phenolic compounds from the grape (Busse-Valverde *et al*., 2010). The interaction between tannins and cell walls from grape skins could limit the extraction from the solid parts of the berry and may have led to lower polymer concentrations in the wine from least irrigation treatments at 64 DAV, due principally that larger molecular sizes in the tannins increase their affinity for the cell wall, thus decreasing their extraction during the winemaking process (Bindon *et al*., 2010; Hanlin *et al*., 2010). Globally, water deficit and different harvest date only produced difference in concentration between proanthocyanidin fractions but not in the proportion of them.

Sensory characteristics of the wines

A sensory assessment was carried out to complement the chemical analysis of the wine. Table 2 shows the parameters assessed by the trained judges and their respective scores. In general, differences were seen between the irrigation treatments and the harvest dates. Only colour intensity, red fruits aromas and persistence showed differences between irrigation treatments. For the earlier harvest date (55 DAV), the T2 and T3 wines showed higher colour intensity, while for persistence T1 and T3 wines obtained a higher score than T2. For the remaining attributes, aroma of red fruits, bitterness, astringency and fullness, no differences were found between the irrigation treatments.

At the later harvest date, differences were seen in the aroma of red fruits for the different treatments. There were also differences in astringency and persistence between the two harvest dates for T2 wines, where the later date saw a higher score in both cases. The higher score can be associated with the increase in the oligomeric fraction, as shown in Fig. 3. In terms of sensory attributes, astringency increases with the increase in the degree of polymerisation of the molecules, producing an improvement in quality (Gawel, 1998; Cheynier *et al*., 2006).

Regarding the bitterness, no differences were seen between the irrigation treatments or the harvest dates. This is in line with the results in Fig. 3, where no significant differences were found between treatments for the monomeric fraction. Similarly, no differences were seen in the fullness of the wines between the irrigation treatments or the harvest dates. Finally, and based on the differences in the sensory assessment, the T3 wines showed the best attributes at the earlier harvest date (55 DAV), whereas the T2 wines had the best sensory attributes at the later date (64 DAV). To study possible connections between the analytical data and the sensory assessment of the wines, a correlation matrix was created, as shown in Table 3. A significant correlation was found between the persistence and total phenol and total tannin content. A positive correlation was also seen between the fullness and astringency, and between the fullness and persistence. There was also a negative correlation between flavonol content and the fullness of the wine, which can be associated with the size of the molecules quantified in the flavonol analysis by HPLC, which corresponds

Table 2 Sensory attributes in Cabernet Sauvignon wines

	55 DAV			64 DAV		
	T1	T2	T3	T1	T2	T3
Colour intensity	7.9 ± 0.6 B	10.8 ± 0.6 A*	11.0 ± 0.6 A*	9.3 ± 0.6 A	8.0 ± 0.5 AB	8.0 ± 0.5 B
Red fruits	8.4 ± 0.6	7.2 ± 0.6	8.4 ± 0.8	8.5 ± 0.7 AB	9.7 ± 0.4 A*	6.7 ± 0.5 B
Bitterness	5.9 ± 0.9	5.8 ± 0.9	6.2 ± 1.0	6.3 ± 0.9	6.4 ± 1.0	6.4 ± 0.6
Astringency	8.8 ± 0.6	7.2 ± 0.5	8.0 ± 0.5	8.7 ± 0.6	9.5 ± 0.8*	9.0 ± 0.6
Fullness	8.6 ± 0.7	7.7 ± 0.8	8.4 ± 0.7	9.4 ± 0.6	9.7 ± 0.6	8.1 ± 0.9
Persistency	9.5 ± 0.9 A	8.0 ± 0.6 B	9.5 ± 0.7 A	9.4 ± 0.7	10.5 ± 0.5*	9.9 ± 0.8

T1, Ψ_{stem} = −0.8 MPa; T2, Ψ_{stem} = −0.9 MPa; T3, Ψ_{stem} = −1.0 MPa; DAV, days after *veraison*.
Values are presented as mean score of twelve judges ± standard error. Different capital letters indicate significant difference of parameter between irrigation treatments for the same date.
*Difference between the same irrigation treatment for different dates ($P < 0.05$).

Table 3 Correlation matrix between phenolic composition and sensory attributes in Cabernet Sauvignon wines

	TP	TT	TA	Mon	Oli	Pol	Fla	GA	AA	CA	CI	RF	Bitt	Ast	Ful
TT	**0.552***	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TA	**0.869***	**0.609***	–	–	–	–	–	–	–	–	–	–	–	–	–
Mon	0.289	0.341	0.369	–	–	–	–	–	–	–	–	–	–	–	–
Oli	0.378	0.434	**0.541***	**0.715***	–	–	–	–	–	–	–	–	–	–	–
Pol	−0.135	**0.47***	0.067	0.264	0.335	–	–	–	–	–	–	–	–	–	–
Fla	**−0.512***	−0.17	−0.25	−0.196	−0.026	0.454	–	–	–	–	–	–	–	–	–
GA	**0.759***	**0.587***	**0.892***	0.352	**0.718***	0.213	−0.073	–	–	–	–	–	–	–	–
AA	−0.077	0.115	0.218	0.154	**0.537***	**0.577***	0.404	**0.482***	–	–	–	–	–	–	–
CA	−0.151	0.098	0.144	0.121	**0.477***	**0.583***	**0.595***	0.441	**0.883***	–	–	–	–	–	–
CI	−0.458	−0.4	−0.34	−0.117	−0.1	0.122	0.109	−0.315	0.326	0.104	–	–	–	–	–
RF	0.352	0.168	0.186	0.367	0.354	0.137	**−0.495***	0.327	0.288	0.21	−0.093	–	–	–	–
Bitt	0.367	0.001	0.207	−0.173	−0.017	0.011	−0.245	0.163	0.047	−0.092	0.128	0.197	–	–	–
Ast	0.357	0.404	0.193	0.239	0.291	−0.054	−0.334	0.321	−0.03	0.04	−0.218	0.352	0.123	–	–
Ful	0.371	0.341	0.184	0.092	−0.006	0.083	**−0.548***	0.093	−0.114	−0.167	−0.011	0.413	0.408	**0.57***	–
Per	**0.496***	**0.553***	0.406	0.307	0.297	0.198	−0.317	0.318	0.019	−0.032	−0.464	0.289	0.452	0.429	**0.535***

AA, acetylated anthocyanins; Ast, astringency; Bitt, bitterness; CA, coumaroylated anthocyanins; CI, colour intensity; Fla, flavanols; Ful, fullness; GA, glucosilated anthocyanins; Mon, monomeric proanthocyanidin; Oli, oligomeric proanthocyanidin; Pol, polymeric proanthocyanidin; RF, red fruits; TP, total phenols; TT, total tannins; TA, total anthocyanins.
*Bold values mean significance at $P \leq 0.05$.

to only units of smaller size, such as monomers of up to three subunits, while the fullness of the wine is represented by units of larger size (i.e. oligomers and polymers). In general, there are no great relationships between chemical and sensory parameters, which could infer that irrigation treatments and harvest date could enable the idea that can be obtain a wine with similar characteristic with an earlier harvest. Further studies in this area that take into account other compounds that may influence wine composition (i.e. polysaccharides), other climatic zones and the same assay in consecutive season are necessary to determine how the water deficit could modify the phenolic composition to provide a useful information for viticulturist and winemaker to achieve an optimal harvest date.

Conclusions

Under the present conditions, total phenols were much higher in 64 DAV in all irrigation treatments but in general slight, differences in phenolic composition were founded between irrigation treatments and harvest date, especially for total tannins and total anthocyanins. Moreover, the wines from grapes harvested 64 DAV had a higher grade of alcohol. An interesting result shows that treatments with most restrictive condition of irrigation and an earlier harvest showed a higher amount of stilbenes, enhance the wine health benefits using less irrigation water in field. The separation of proanthocyanidins by C_{18} Sep-Pak cartridges showed slight differences in concentration between proanthocyanidin fractions but not in the proportion

of them. In terms of sensory composition, the wines from T3 at 55 DAV gave a better colour and the same attributes for aroma of red fruits, bitterness, astringency, fullness and persistence, compared to wine from grapes harvested later (64 DAV). This is important in current viticulture, as in warm locations, the excessive accumulation of sugars in the grape berries, with the subsequent accumulation of alcohol in the wine, can lead to weaker acceptance from consumers. It was also possible to corroborate that the use of less water could increase the chemical and sensory quality of the wine, especially in a climate change context, where the water supply is thought to decline worldwide.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Low-molecular-weight phenolic compounds in wine samples.

Table S2. Anthocyanins quantified in wine samples.