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Volatile compounds and phenolic composition of skins and seeds of 'Cabernet Sauvignon' grapes under different deficit irrigation regimes

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Summary

Aroma compounds and skin and seed polyphenols are determinants of wine composition. The aim of this study was to determine the effect of different post-veraison deficit irrigation strategies on volatile profile and the chemical composition of grape skin and seeds in a 'Cabernet Sauvignon' vineyard in Valencia (Spain). Besides a non-irrigated regime (rainfed), irrigation treatments consisted of replacing 25, 50 and 75 % of the estimated crop evapotranspiration (ET_C). When compared to rainfed vines, watering during post-veraison at 75 % of the ET_C, decreased concentrations of alcohols but increased those of aldehydes such as hexanal, related to herbaceous (non-desirable) aromas in wines. Irrigating at 25 % or 50 % of ET_C resulted in similar concentrations of grape volatile compounds than rainfed vines. There was also a general trend in a reduction in skin to flesh ratio as irrigation regime increased. The concentration of skin anthocyanins and tannins increased with water applications, but seed tannins decreased in the most irrigated regimes. This suggests different effects of water stress on skin and seed polyphenol synthesis and accumulation. For the tannin content, water stress provoked higher tannin mean degree polymerization values, which positively affect must astringency. Under the experimental conditions of the present study, watering at 50 % ET_C during post-veraison is the recommended irrigation strategy for optimizing grape composition and improving yield in comparison with rainfed vines.

K e y w o r d s : aroma compounds; anthocyanins; tannins; *Vitis vinifera*; water stress.

Introduction

Grape ripening is a complex process affected by many external and endogenous vine factors among which vine water status plays a crucial role (Jackson and Lombard 1993). As a consequence, mainly in semi-arid terroirs, supplemental irrigation application modifies the chemical composition of the berry, mainly the concentrations of glucose, fructose, organic acid and mineral elements (Esteban *et al.* 1999), but also anthocyanins and total polyphenols (Sipiora and Granda 1998). Indeed, some studies pointed out that water deficit improved wine sensory attributes due to an increment of fruity aromas (Chapman *et al.* 2005, Koundouras *et al.* 2006).

In relation to phenolic compounds, in general, applying a water deficit will result in an increase of anthocyanins concentration and Total Polyphenolic Index (TPI) (Bravdo *et al.* 1985). Some studies tested the effect of a post-veraison water stress in 'Cabernet Sauvignon' finding inferior levels of phenolic compounds, anthocyanins and tannins on less-stressed vines (Matthews *et al.* 1990, Nadal and Arola 1995). Similarly, in other cultivars such as 'Bobal', supplemental irrigation decreased grape and wine phenolics (Salón *et al.* 2005), while in 'Tempranillo' the effects of irrigation on grape color and anthocyanins are dependent on the timing and severity of water stress (Intrigliolo and Castel 2009). In this sense, Castellarin *et al.* (2007) found that water stress might positively affect the anthocyanins synthesis pathway. On the other hand, Ojeda *et al.* (2002), working with 'Syrah', observed that a severe water deficit before veraison provoked a decrease of anthocyanin synthesis and Romero *et al.* (2010) in 'Monastrell' found that an extremely severe water stress was detrimental to the total grape phenolic concentration. Indeed, the effect of water stress on grape phenolics is far from being consistent among experimental studies. Usually, it is not clear if the reported effects were caused by berry dehydration, a higher skin to pulp ratio, or a change in the compound metabolism.

The final wine aroma, which plays a decisive role determining wine character and quality, is the result of a large sequence of biological, biochemical and technological reactions (Canuti *et al.* 2009). Nearly 800 volatile compounds are present in wines at concentrations varying from nanograms to hundreds of miligrams per litre (Ribéreau-Gayon *et al.* 2006). Grape aromas are constituted by volatile compounds including alcohols, aldehydes, esters, acids, terpenes, norisoprenoids, thiols and carbonyl compounds. Grape skin

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contains more than half of these aromatic substances and their precursors (Canuti *et al.* 2009), whereas the flesh is an important source of nitrogen compounds, not as rich as the skin in terms of aromatic substances, but also accumulates alcohols, aldehydes and esters (Hernández-Orte *et al.* 2011).

The cultivar used in the present study, 'Cabernet Sauvignon', is a high-quality variety grown worldwide and characterized by small clusters with hard, small and spherical berries and a good skin/flesh ratio from an enological point of view. The study of the aromatic potential of 'Cabernet Sauvignon' presents a big challenge due to the low concentration of the aromatic compounds. The majority of the volatile compounds of this variety contain esters, alcohols and aldehydes (Canuti *et al.* 2009, Forde *et al.* 2011). In a previous study by Intrigliolo *et al.* (2016), the effects of deficit irrigation regimes on 'Cabernet Sauvignon' vine performance and general must composition were investigated in an irrigation trial conducted within the DO Valencia (Spain). It was found that in general supplemental irrigation decreased the must concentration of total polyphenols and anthocyanins in particular. However, it was not elucidated whether this was due to a concentration effect because berries from water-stressed vines suffered some dehydration, or if there was a direct effect of water stress on polyphenol synthesis. Therefore, the aim of the current study was to determine the effect of different post-veraison deficit irrigation regimes on the chemical composition of skin and seeds and also in the volatile profile of 'Cabernet Sauvignon' grapes.

Material and Methods

Site description: The experiment was carried out in 2010 and 2011, in a 2.7 ha *Vitis vinifera* L. (cv. 'Cabernet Sauvignon'), grafted on 161-49 rootstock, commercial vineyard located near Moixent (38°52' N; 0°44' W; elevation 550 m), Valencia, Spain. Vines were planted in 2,000 and spaced 3 by 1.3 m between rows and vines, respectively (2,564 vines·ha^{-1}). The vineyard was deficit irrigated at 35 % of the estimated crop evapotranspiration (ETc) since plantation time using a drip-irrigation system with a single drip line located next to the vine trunks, tied to the trellis at 70 cm height from the vineyard floor. The drip lines had emitters of 2.3 L·h^{-1} placed at 1.25 m distance. Vines were trained to a vertical trellis on a bilateral cordon system, east-west oriented. Canopy management practices, all manually performed, included shoot thinning and shoot-tip cutting. The soil has a sandy loam texture, it is highly calcareous, and with low fertility. Weather conditions were assessed with an automated meteorological station located 11 km away from the plot. Climate is continental with an average annual rainfall of 697 mm, of which about 65 % falls during the dormant period. Evolution of the meteorological conditions during the two years of experiment are shown in Fig. 1 (suppl. data) and the description of the weather station can be found in Intrigliolo *et al.* (2016)

Irrigation treatments: The experiment was laid out in a randomized block design with four treatments in three replications. There were, therefore, a total of 12 experimental plots; each one had 15 rows with 38 vines per row. Approximately 308 central vines per each experimental unit were used for berry sampling and composition analysis. Irrigation was scheduled using the approach suggested by Allen *et al.* (1998). On a weekly basis crop water needs (ET_C) were estimated using the reference evapotranspiration (ET_0) and an adjusted crop coefficient (K_C); $ET_C=ET_0*K_C$. At full canopy growth, based on the report by Williams and Ayars (2005), the estimated K_C value to refill the potential water needs was considered to be 0.6. Rainfall occurring during the growing season was also considered when scheduling irrigation, and the effective precipitation was estimated to be 70 % of the total rainfall. Irrigation frequency varied from 2 to 4 times per week, depending on the weekly total irrigation amount to be applied. In-line water meters were used for measuring the irrigation water application for each irrigated experimental plot (*i.e.* three water meters per treatment).

The four irrigation treatments studied were as follows: 1) R = Rainfed, receiving only rainfall water, 2) 0.25 ET_C, where water was applied to replace only 25 % of the ET_C, 3) 0.50 ET_C, where irrigation replaced half of the estimated ET_C and 4) 0.75 ET_C, where irrigation replaced 75 % of the estimated crop evapotranspiration. In all treatments, irrigation did not start until veraison, which was assessed visually when at least 50 % of the clusters were at veraison stage. It was considered to occur on July 31st and August 4th in 2010 and 2011, respectively. All treatments were fertilized at a rate of 15-10-30 kg·ha^{-1} of N, P and K, respectively. Field practices were those commonly used in the area, including shoot trimming applied after fruit set. Midday stem water potential determinations (Ψ_{stem}) were carried out every two weeks on two representative vines per experimental plot and one leaf per vine, using a pressure chamber (PMS, model 600, Albany, Oregon, USA). The post-veraison water stress integral (ΣYs) (Myers 1988), was calculated as:

$$\Sigma\Psi s = \sum_{i=0}^{i=1} \left| (\Psi s_{i,\,i+1} - c) \right| n \text{ , (MPa*day)}$$

where $\Psi_{Si,i+1}$ is the Ψ_{stem} value at the end of each interval i,i + 1; c = -0.6 MPa, a value considered indicative of absence of water stress during the post-veraison period (Intrigliolo *et al.* 2009).

Two sets of 400 berries, one for flesh and seed evaluation, and another one for volatile compound analysis, randomly selected from each experimental plot at harvest, were collected and frozen at -18 °C before analytical determinations.

Volatile compounds extraction and identification: Volatile compounds were extracted by purge and trap thermal desorption (Overton and Manura 1994, Escriche *et al.* 2009). The grapes were refridgerated and defrosted before analysis. After the manually extraction of seeds, flesh and skin were blended at room temperature using a ULTRA-TURRAX® (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Two samples of this blending (20 g) were placed in a purging vessel flask and spiked with 10 µL 4-methyl, 2-pentanol (1 mg·L^{-1}) as an internal standard. From the bottom of the vessel, purified nitrogen was forced through a porous frit during 15 min at 60 °C at 100 mL·min^{-1}. The stream of bubbles passed through the sample collecting

the volatile compounds, which were trapped in a 100 mg porous polymer (TenaxTA, 20-35 mesh) and packed into a glass tube placed at the end of the system. The volatile compounds were thermally desorbed using a direct thermal desorber (TurboMatrix TD, Perkin Elmer, Massachusetts, USA). Desorption was performed under a 10 mL min^{-1} helium flow at 220 °C for 10 min. Volatile compounds were then cryofocused in a cold trap at -30 °C and transferred directly onto the head of the capillary column by heating the cold trap up to 250 °C (at 99 °C s^{-1}).

The separation of the volatile compounds was performed by gas chromatography-mass spectrometry (GC-MS) using a Trace GC 2000 (ThermoQuest Italia S.p.A., Italy) equipped with a DB-WAX capillary column (SGE, Australia) of 60 m length, 0.32 mm internal diameter and 1.0 µm film thickness. Helium, at a constant flow rate of 50 mL·min^{-1} was used as a carrier gas. The temperature was programmed to increase from 40 °C to 50 °C at 2 °C min^{-1}, from 50 °C to 130 °C at 4 °C·min^{-1}, from 130 °C to 180 °C at 8 °C·min^{-1} and finally to 220 °C at 8 °C·min^{-1}. Once the volatile compounds were separated, they were analyzed with a mass spectrometer (Finnigan TRACETM MS, TermoQuest, Austin, USA) at an electron impact of 70 eV with a mass range of m/z 41-457. Two extracts were obtained for each sample.

The identification of isolated volatile compounds was carried out by comparing their mass spectra, retention times and linear retention indices with those obtained from authentic standards: 2-butanol, 1-propanol, 2-methyl-1-propanol, 1-pentanol, 1-hexanol, (Z) 3-hexen-1-ol, 2-octanol, 1-heptanol, 3-methyl-butanal, hexanal, heptanal, (E) 2-hexenal, ethyl acetate, d-limonene and 3,7-dimethyl- 1,6-octadien-3-ol (Sigma-Aldrich (St. Louis, Missouri, USA), Acros Organics (Geel, Belgium) and Fluka (Buchs, Switzerland)). The compounds for which it was not possible to find authentic standards were tentatively identified by comparing their mass spectra with spectral data from the National Institute of Standards and Technology 2002 library (80 % percent probability value was always considered as a minimum), their linear retention indices and their spectral data published in the literature.

The results were expressed in µg of compound per g of grape fresh weight, considering as 1 the response factor for each compound. The values were calculated as the ratios between the peak areas of each compound and the peak area of the internal standard. These ratios were the variables used in the statistical analysis.

Skin compounds extraction: Skin parameters determined in the current study were total anthocyanins, TPI using the extraction methodology described in Ribéreau-Gayon *et al.* (2006) and total condensate tannins by analyzing the proanthocyanidin mean degree of polymerization (mDP), using the methodology described by Kennedy and Jones (2001). The skins were manually separated from the berry flesh of the frozen berries, rinsed with distilled-deionized water and extracted with a 90 % ethanol, 10 % water and 5 g·L^{-1} tartaric acid hydroalcoholic solution (1:10 skin/solvent) at 50 °C with 75 rpm stirring for 2 h.

To minimize proanthocyanidin oxidation, solutions were sparged with nitrogen and the extraction was carried out in the dark. The extracts were crystal-wood filtered and then lyophilized to a dry powder. Analytical determinations for each extract were performed in duplicate, which were then averaged to obtain a value to work with later.

Following Kennedy and Jones (2001) methodology, the crude proanthocyanidins were purified using Toyopearl TSK HW 40-F size exclusion media (Tosoh, Japan), packed in an Omnifit column (250 x 25 mm) that was equilibrated with 1:1 MeOH/water containing 0.1% v/v trifluoroacetic acid. The proanthocyanidin powder was dissolved in a minimum amount of this mobile phase and then applied to the column. The column was then rinsed with five column volumes of the mobile phase to remove carbohydrate and low-molecular-weight flavan-3-ol monomer material. The proanthocyanidins were then eluted with three column volumes of 2:1 acetone/water containing 0.1 % v/v trifluoroacetic acid. The eluent was concentrated under reduced pressure at 35 °C to remove acetone, and then lyophilized to a dry powder.

Seed extraction methodology: Seeds were manually separated from the berry flesh, rinsed with distilled-deionized water, dried and weighted. A 3 g sample was horizontally placed in a Falcon tube with 50 mL 2:1 acetone/water for maceration during 24 hours at room temperature and 75 rpm stirring. The eluent was concentrated under reduced pressure at 35 °C to remove acetone, and then lyophilized to a dry powder.

Tannin main degree polymeration estimation in skin and seeds: A 5 mg sample of the dry powder with the proanthocyanidin of interest, was reacted in a solution of 0.1 N HCl in MeOH, containing 50 g L^{-1} phloroglucinol and 10 g L^{-1} ascorbic acid at 50 °C for 20 min, and then combined with 2 volumes of 80 mM aqueous sodium acetate to stop the reaction.

Phloroglucinol adducts were analyzed by a reversed-phase HPLC JASCO MD-2010 Plus diode array detector (JASCO, Tokyo, Japan), equipped with a degasser, a quaternary gradient pump, an automatic injector and a thermal stable compartment for the column and a diode array detector (195 to 600 nm). A LC-Net II/ADC hardware interface between the system components and PC was also used (JASCO, Tokyo, Japan). The chromatographic column was a Gemini NX (particle size 5 µm, 250 x 4.6 mm) purchased from Phenomenex (Torrance, California, USA). The method used a binary gradient with mobile phases containing 1 % v/v aqueous acetic acid (mobile phase A) and 100 % MeOH (mobile phase B). Eluting peaks were monitored at 280 nm. The elution conditions were 1.0 mL·min^{-1}; 5 % B for 10 min, a linear gradient from 5 to 20 % B in 20 min, a linear gradient from 20 to 40 % B in 25 min. The column was then washed with 90 % B for 10 min and re-equilibrated with 5 % B for 5 min before the next injection (Kennedy and Jones 2001).

To calculate the apparent mDP, the sum of all subunits (flavan-3-ol monomer and phloroglucinol adduct, in moles) was divided by the sum of all flavan-3-ol monomers (in moles). In the case of galloylation percent (% G), it was calculated by dividing the total galloylated proantocyanadin by all identified proantocyanadins and multiplied by 100. To conclude, the average molecular weight (aMW) was estimated by the response factor relative to (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin-3-o-gallate (Extrasynthese, Lyon Nord, France).

Statistical Analysis: Data were analyzed by two-way analysis of variance (ANOVA) with irrigation treatment and year as factors, using the Statgraphics Centurion XVI V16.1.15 software (Statpoint Technologies, Inc., The Plains, Virginia, USA). When the differences were statistically significant at $P < 0.05$, Duncan multiple range tests at $P < 0.05$ were performed. In this analysis, the homogenous groups indicate statistical differences between types of treatment and year. Principal components analysis (PCA) was applied to evaluate the overall effect of the irrigation strategy on the volatile fraction during the two years of study, using Unscrambler® 10 (CAMO Software AS., Oslo, Norway).

Results and Discussion

Weather conditions and vine water stress: The two experimental seasons had different rainfall patterns with more precipitation occurring during the ripening period of the 2010 campaign than in 2011 when basically during the summer months no rainfall occurred (Fig. 1, suppl. data). Peak monthly ET_0 values were slightly lower in 2010 than in 2011. A detailed description of the seasonal water stress experienced by the different irrigation treatments is described in detail in our companion paper by Intrigliolo *et al.* (2016), where the effects of the imposed irrigation treatments on yield and its components are also reported. In the present manuscript, Tab. 1 (suppl. data) summarizes the integral of water stress determined by the midday stem water potential measurements. Rainfed vines suffered the highest cumulative water stress both years being followed by the watered treatments, in a decreasing trend, when increasing water applications. In average, $0.25ET_C$ were 20 % less stressed than R and $0.50ET_C$ and $0.75ET_C$ accounted a 40 % lower stress level from veraison to harvest than rainfed vines.

Volatile compound fraction identification: Twenty-seven major volatile compounds were identified in grape samples, including alcohols (12), aldehydes (6), esters (7) and terpenes (2) (Tab. 2, suppl. data). Their corresponding retention time ranges (RT), linear retention indices calculated ranges (RI) and the methodology used for compound identification are also included.

C_6 compounds, as well as the alcohols 1-hexanol and 2-ethyl-1-hexanol, and the aldehydes hexanal and (e) 2-hexenal, were abundant in the samples analyzed, consistent with other 'Cabernet Sauvignon' studies (Canuti *et al.* 2009, Kalua and Boss 2009, Zhu *et al.* 2012). Xu *et al.* (2015) found a positive correlation between heptanal and short-term precipitation, which could explain absence of this compound during 2011, a dryer vintage. However, other volatile compounds and the specific esters and terpenes identified in the present study, were not reported by these authors. This is expected considering that, even within the same variety, the final berry composition depends on many site-specific factors and variables (Ribéreau-Gayon *et al.* 2006). In this sense, the odorants 3-alkyl-2-methoxypyrazines were not identified in the present research most likely because grapes were picked at an advanced ripening stage. It is known in fact that the concentration of 3-alkyl-2-methoxypyrazines sharply decreases during the berry ripening period (Ryona *et al.* 2008).

Irrigation strategy influence in the volatile fraction: The average values of the volatile compounds analysed in the grapes, as well as the ANOVA result (homogeneous groups, F-ratio and statistical significance) for the two factors (irrigation treatments and years) and their interaction are showed in Tab. 3. In general, results indicate that for all treatments, 2010 grape samples contained a greater number of volatile compounds (a total of 27), and in a higher concentration, than those found in 2011 samples (only 11). In fact, all the identified compounds in 2011 were also found in 2010. Of the 27 identified compounds, 25 of them showed significant differences between years, whereas only five compounds showed these differences between treatments. The interaction between factors was not significant in any case. In order to evaluate the global effect of the type of treatment on the volatile profile from a descriptive point of view, PCA was performed.

Fig. 2 shows the PCA (A: scores and B: loadings) for the two principal components in which the code for each point in the figure corresponds to: treatment-year. In the score plot, proximity between samples reflects similar behavior in terms of the volatile profile. There are two clearly separated groups on the score plot (Fig. 2A) differentiated

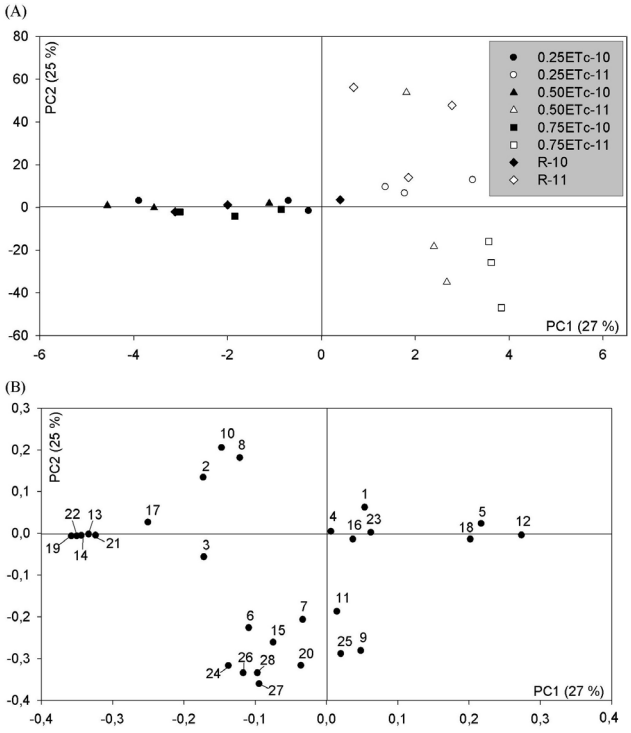

Fig. 2: Principal component analysis of volatile compounds found in 2010 and 2011. (**A**). Plot of the two principal component scores. (**B**) Plot of the two principal component loadings. Legend for the plots: 1 = 2-methyl-propanal; 2 = 2-propanone; 3 = methylacetate; 4 = 2-methylbutanal; 5 = 3-methylbutanal; 6 = ethylpropionate; 7 = ethyl-2-methylpropionate; 8 = 2-butanol; 9 = ethylbutanoate; 10 = 1-propanol; 11 = ethyl-3-methylbutanoate; 12 = hexanal; 13 = 2-methyl-1-propanol; 14 = 1-butanol; 15 = heptanal; 16 = limonene; 17 = 3-methyl-1-butanol; 18 = (E) 2-hexenal; 19 = 1-pentanol; 20 = hexylacetate; 21 = 1-hexanol; 22 = (E) 3-hexen-1-ol; 23 = (Z) 3-hexen-1-ol; 24 = 2 octanol; 25 = ethyloctanoate; 26 = 1-heptanol; 27 = 2-ethyl-1-hexanol; 28 = 3,7-dimethyl-1,6-octadien-3-ol.

by the first component (PC1): the 2010 samples located on the left and the 2011 samples on the right. PC1 explained 27 % and was positively correlated with hexanal, (E) 2-hexenal, 3-methyl-butanal, 1-hexanol, 1-butanol, 1-pentanol, among others. The second component (PC2) explained 25 % and was positively correlated with 2-ethyl-1-hexanol, hexylacetate, 3,7-dimethyl-1,6-octadien-3-ol and others. PC2 differentiates the treatment applied to a certain extent. In 2011 there is more differentiation among the irrigation treatments that in 2010 when all irrigation regimes were aligned along the horizontal axis showing no treatment differentiation. This different effect of irrigation regime according to the experimental season could be attributed to the different rainfall patterns between seasons (Fig. 1, suppl. data). In 2011, during the ripening period (August to October), there was basically no rainfall and therefore the irrigation regimes imposed determined more the vine water status than in 2010, as reported in Intrigliolo *et al.* (2016). The differences among years in volatile compounds are difficult to be explained as many factors can occur. For instance in 2010 grapes were picked with higher probable alcohol degree (%) (i.e. more advanced technological maturity) than in 2011. In this sense, Salinas *et al.* (2004) in Monastrell and Canuti *et al.* (2009) and Kalua and Boss (2009) in 'Cabernet Sauvignon', obtained a higher number of volatile compounds by the end of the ripening period than in the first stages of maturity. In the present experiment, alcohols and aldehydes, both situated on the extremes of the PC1 (Fig. 2B), are the compounds that influenced most on the differentiation between years.

In general, the content of alcoholic components is higher during 2010, especially for 2-methyl 1-propanol, 1-butanol, 1-pentanol, 1-hexanol and (Z) 3-hexen-1-ol. On the other hand, in 2011 some aldehydes, as hexanal, 3-methyl-butanal and (E) 2-hexenal, were found in higher concentration than in 2010. Similar results were found by Canuti *et al.* (2009) and Kalua and Boss (2009) when identifying the volatile compounds at different stages of grape maturity in 'Cabernet Sauvignon', obtained greater levels of aldehydes in early stages of ripening. Meanwhile alcohol compounds as 1-hexanol, increased along the ripening process. It is important to point out that some compounds, as C_6 alcohols and aldehydes are responsible for the grape herbaceous aromas (Ribéreau-Gayon *et al.* 2006, Yang *et al.* 2009, Gómez García-Carpintero et al. 2012). Therefore, it is possible that the volatile compounds of 2011 grapes could generate wines with a non-desirable green odor.

Table 3

Volatile compound relative content (mg·g^{-1} grape; assuming a response factor equal to one) and ANOVA results by irrigation treatment (T), year (Y) and theirs interaction (homogeneous groups, F ratio and statistic signification)

Volatile Compound	T				Year		ANOVA		
	R	0.25ET$_c$	0.50ET$_c$	0.75ET$_c$	2010	2011	T	Y	T*Y
Alcohols									
2-Butanol	0.100	0.083	0.089	0.060	0.170	nd	ns	128***	ns
1-Propanol	0.057	0.065	0.064	0.039	0.113	nd	ns	86**	ns
2-Methyl-1-propanol	0.32	0.32	0.35	0.23	0.51 b	0.094 a	ns	146***	ns
1-Butanol	0.29	0.22	0.28	0.27	0.49 b	0.072 a	ns	774***	ns
3-Methyl-1-butanol	0.78 b	0.74 b	0.73 b	0.61 a	0.782 b	0.645 a	7***	21***	ns
1-Pentanol	0.43	0.41	0.55	0.50	0.94 b	0.036 a	ns	349***	ns
1-Hexanol	0.59 b	0.56 ab	0.61 b	0.49 a	0.90 b	0.21 a	3.93*	750***	ns
(Z) 3-Hexen-1-ol	0.071	0.058	0.074	0.074	0.13 b	0.011 a	ns	412***	ns
(E) 2-Hexen-1-ol	0.029	0.028	0.029	0.022	0.025	0.028	ns	ns	ns
2-Octanol	1.20	1.05	1.19	1.35	1.20	nd	ns	834***	ns
1-Heptanol	0.19	0.16	0.19	0.24	0.39	nd	ns	307***	ns
2-Ethyl-1- hexanol	0.018	0.015	0.017	0.023	0.040	nd	ns	243***	ns
Aldehydes									
2-Methyl-propanal	0.013	0.011	0.012	0.011	0.024	nd	ns	576	ns
2-Methyl-butanal	0.045 a	0.062 b	0.037 a	0.037 a	0.056 b	0.035 a	5**	14***	ns
3-Methyl-butanal	0.108	0.087	0.089	0.091	0.077 a	0.111 b	ns	12**	ns
Hexanal	0.43 ab	0.44 ab	0.38 a	0.50 b	0.52 b	0.43 a	3*	17***	ns
Heptanal	0.031	0.027	0.035	0.042	0.068	nd	ns	218**	ns
(E) 2-Hexenal	0.52 b	0.37 a	0.37 a	0.50 b	0.44	0.50	3*	ns	ns
Esters									
Methyl acetate	0.18	0.17	0.20	0.18	0.36	nd	ns	482**	ns
Ethyl propionate	0.031	0.033	0.033	0.034	0.066	nd	ns	1009**	ns
Ethyl 2-methylpropionate	0.050	0.052	0.055	0.056	0.106	nd	ns	997**	ns
Ethyl butanoate	0.057	0.065	0.060	0.067	0.12	nd	ns	722***	ns
Ethyl 3-methylbutanoate	0.14	0.15	0.15	0.16	0.30	nd	ns	798**	ns
Hexyl acetate	0.067	0.066	0.069	0.077	0.14	nd	ns	353**	ns
Ethyl octanoate	0.22	0.19	0.16	0.25	0.41	nd	ns	217***	ns
Terpenes									
D-limonene	0.19	0.23	0.20	0.20	0.41	nd	ns	153***	ns
3,7-Dimethyl- 1,6-octadien-3-ol	0.38	0.26	0.33	0.42	0.35	nd	ns	167***	ns

T: treatment; Y: year; nd: not detected; ns: non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

For the study of the irrigation strategy influence on the volatile compound content, a PCA was performed taking into consideration only the 2011 data, when treatments were less influenced by rainfall (Fig. 3; A: scores and B: loadings). The scores plot shows a clear differentiation between the

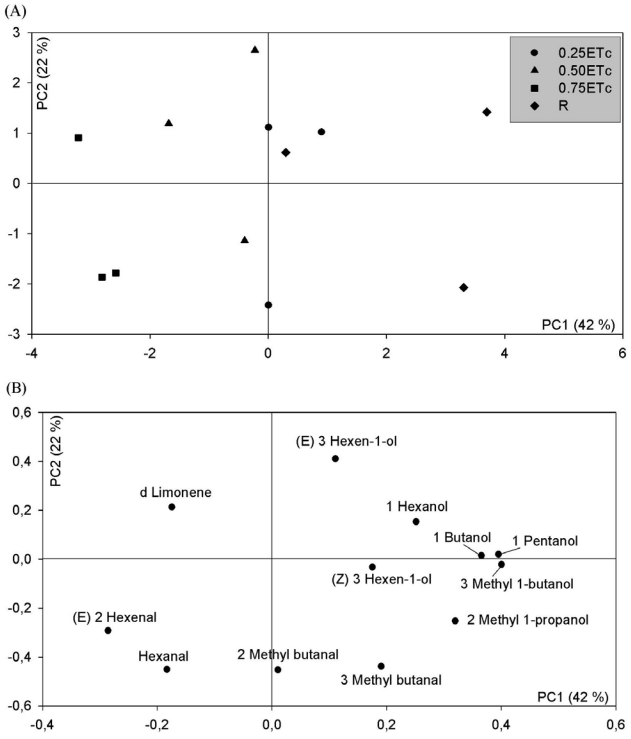

Fig. 3: Principal component analysis of volatile compounds found in 2011. (**A**). Plot of the two principal component scores. (**B**) Plot of the two principal component loadings.

most extreme treatments, rainfed (R) and 0.75ET$_C$. The most irrigated treatment was located on the left side of PC1 meanwhile those less irrigated (R and 0.25ET$_C$) were distributed on the right side of PC1. The least irrigated treatments resulted in greater alcoholic compounds content: 2-methyl-1-propanol, 1-butanol, 3-methyl-1-butanol and 1-pentanol. There are few studies relating irrigation with grape aromatic potential, but all of them expose an inverse relation between applied water and the aromatic potential (Oliveira *et al.* 2004, Koundouras *et al.* 2009, Song *et al.* 2012). In the present study, aldehydes content: hexanal and (E) 2-hexenal were obtained in higher concentration in the less stressed vines represented by the 0.75 ET$_C$ treatment. The herbaceous aromas in wines are not desirable and the presence of elevated concentrations of aldehydes could be considered as a defect. Therefore, the most irrigated treatment could be the least proper strategy for a good quality winemaking in terms of pleasant aroma. In contrast, less watered strategies imply a higher number of alcoholic compounds which, at low concentrations, gives sweet and soft sensations, allowing the development of pleasant aromas. In the present study, 0.50 ETc treatment, with the lowest C_6 aldehyde content and a higher number of alcoholic compounds, could be the most adequate strategy in terms of grape aroma potential.

Skin and seed evaluation: Results of the determinations of flesh and skin weight, skin/flesh ratio, anthocyanin and tannin concentrations expressed as milligrams per skin weight (mg·g^{-1}) and the correspondent ANOVA results by treatment, year and their interaction, can be found in Tab. 4. According to Junquera *et al.* (2012), flesh weight is the yield component most influenced by water restrictions, but our results did not show a proportional relation between flesh weight and irrigation applications, especially for 2010 when no significant differences were found due to the spring rainfall accounted that year, as previously mentioned. Even during 2011 when the most irrigated treatment was the one with heavier berries, no clear proportional effect was found between water applied and flesh weight. These results could be due to the irrigation regimes only applied from veraison to harvest, a non-water affected stage for flesh weight development (McCarthy 2000). Several studies have found that berry weight is affected when water deficit is applied from set to veraison but there is no effect when applied from veraison to harvest (Kennedy *et al.* 2002, Ojeda *et al.* 2002, Acevedo-Opazo *et al.* 2010, Santesteban *et al.* 2011).

Regarding the skin weight, the Year factor was highly significant because the 2010 skin weights are double than those from 2011, while there is no influence of the irrigation treatment on the skin weight (Tab. 4). In terms of skin/flesh weight ratio, in general, the effect of irrigation treatment had a decreasing trend with irrigation applications (Tab. 4). Similarly, Kennedy *et al.* (2002) and Zarrouk *et al.* (2012), reported that water deficits produce greater skin/flesh ratios on berries. These greater ratios in water-stressed berries often result in wines with higher colour intensity and TPI (Acevedo-Opazo *et al*. 2010). Regarding the two experimental seasons, much higher percentage of skin weight with respect to the flesh was obtained in 2010 than in 2011, which might explain why musts from the 2010 campaign were much richer in terms of phenolic content than in 2011, as reported in our previous study (Intrigliolo *et al.* 2016).

Table 4

Grape components for a 'Cabernet Sauvignon' vineyard in the rainfed application and in the treatments watered at 25, 50 and 75 % of the estimated crop evapotranspiration (ET$_C$) during the post-veraison period during each season and averaged over the 2010 and 2011 period. For the analysis of the data across years, the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T*year factor was statistically significant at $P < 0.05$ differences between treatment means were not explored

Parameter	T	2010	2011	Average	F-Ratio and signification		
					T	Year	T*-Year
Flesh weight (g)	Rainfed	1.02	1.07 ab	1.04	ns	7*	ns
	0.25ET$_C$	1.07	1.10 ab	1.08			
	0.50ET$_C$	1.06	1.05 a	1.08			
	0.75ET$_C$	1.03	1.14 b	1.08			
Skin weight (g)	Rainfed	0.37	0.17	0.27	ns	527***	3*
	0.25ET$_C$	0.33	0.18	0.25			
	0.50ET$_C$	0.36	0.15	0.24			
	0.75ET$_C$	0.32	0.16	0.24			
% weight skin/flesh	Rainfed	36.85 b	16.21	26.53 b	3*	474***	ns
	0.25ET$_C$	31.32 a	16.30	23.81 a			
	0.50ET$_C$	33.97 ab	14.65	24.31 ab			
	0.75ET$_C$	31.58 a	14.68	23.13 a			
Anthocyanins (mg·g^{-1} of skin)	Rainfed	3.61 a	3.43 a	3.52 a	4.39**	4**	5**
	0.25ET$_C$	4.79 b	3.31 a	4.05 b			
	0.50ET$_C$	3.97 a	4.32 b	4.16 b			
	0.75ET$_C$	4.74 b	3.94 ab	4.34 b			
Tannins (mg·g^{-1} of skin)	Rainfed	5.31 a	8.50 b	7.18 a	4.77**	ns	19***
	0.25ET$_C$	9.93 c	6.31 a	8.12 b			
	0.50ET$_C$	7.64 b	8.63 b	8.13 b			
	0.75ET$_C$	10.24 c	7.72 b	8.98 b			

Within each column means followed by a different letter are significantly different at $P < 0.05$ based on Dunnett's t test. $^{*}p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

On average, skin anthocyanin concentration (mg·g^{-1}) was lower in the R treatment, both years, than in the rest of the treatments (Tab. 4), but no differences were found with 0.50ETc in 2010 and 0.25ETc and 0.75ETc in 2011 for this trait. But when Intrigliolo *et al.* (2016) described total grape phenolic composition at harvest for the same vintages as in the present study, R treatment had the highest total anthocyanin concentration being progressively reduced when irrigation was increasing. Therefore with the sight of both experiments, this means that the milligrams per gram of flesh remain the same meanwhile the proportion skin to flesh of the rainfed treatment made R the one with the highest anthocyanin concentration. There are similar studies where water deficits increase phenolic compounds (Esteban *et al.* 2001, Acevedo-Opazo *et al.* 2010) due to smaller berries and a greater proportion of skin to flesh (Kennedy *et al.* 2002).

The tannin content of the skins, expressed as milligrams per gram of skin (Tab. 4), were different in both years and so was also the effect of irrigation among experimental seasons. In 2010, there was an intensification of skin tannins per gram of skin when water supply increased, being significant in the lowest concentration for R treatment. When Intrigliolo *et al.* (2016) described the total tannin content (as mg·L^{-1}), an inverse relation to the irrigation treatments was found, which means that the final tannin concentration of the must was higher for the non-irrigated treatment even when milligrams per gram of skin were lower due to a greater skin to flesh ratio, previously mentioned. The effects of irrigation on tannins skin concentration was however less consistent

in 2011, when only the 0.25 ETc treatment significantly differed from the others. As it was said before, during grape ripening, changes take place in concentration and structure of the grape tannins. In that way, the skin tannins increase along the ripening period, meanwhile seed tannins decrease. During this process, also an increase of tannin polymerization will occur causing higher mDP values and lower %G (Kennedy and Jones 2001). The astringency of the wine is related, among others, with the tannin mDP of the grapes, so high grape mDP values might result in less astringent wines. Galloylation percentage, which indicates the epicatechin-3-O-gallate proportion on the tannin molecule, also affects the astringency and is mostly found in seeds. Several authors indicate that the mDP is less than 10 in seeds with polymers constituted by (+)- catechin, (-)- epicatechin and epicatechin-3-O-gallate with a lower proportion of (-)- epicatechin gallate (Adams 2006); and more than 20 in skins, depending on the determination technique used and the cultivar studied, mDP values can vary from 2.3 to 15.1 (Kennedy *et al.* 2000, Downey *et al.* 2003, Monagas *et al.* 2003, Moreno *et al.* 2008, Casassa *et al.* 2013).

Tannins are mainly found in seeds and, their structures will influence wine composition. For that reason, the seed tannin mDP, galloylation percent and aMW results, calculated according to Kennedy and Jones (2001), are listed in Tab. 5. In the present experiment, 2010 tannin mDP and aMW in seeds from all treatments showed no differences among treatments but these values were smaller than the ones from 2011. These results agree with Kennedy *et al.* (2002) studies, which showed that the less available water for grapes, the greater mDP of the tannins (and so its molecular weight). As the rainfall registered in 2011 was lower than in 2010 (Fig. 1, suppl. data), also the 2011 mDPs and aMWs were higher, where the most irrigated one (0.75ETc) was the one with the lowest degree of polymerization and molecular weight, which means more astringency. Regarding the seed galloylation percentage, no differences were found among treatments in any of the two years of study but the values were higher in 2011 than in 2010 (Tab. 5). In 2011, the tannin mDP, galloylation percentage and the average molecular weight (aMW) of the grapes skin were calculated and can be found in Tab. 6. Higher skin mDP values were obtained when reducing water applications with the non-irrigated treatment significantly being different from the rest of treatments. For the galloylation percentage, the least irrigated treatments (R and 0.25ETc) showed a lower value than the most irrigated ones (0.50ETc and 0.75ETc). These results agree with similar reports by Kennedy *et al.* (2002), grape skins with high polymerized tannins might allow elaborating less astringent wines but with also high tannin concentration.

Table 5

Concentration of seed polymeric proanthocyanidins for a 'Cabernet Sauvignon' vineyard in the rainfed application and in the treatments watered at 25, 50 and 75 % of the estimated crop evapotranspiration (ET_C) during the post-veraison period during each season and averaged over the 2010 to 2011 period determined by HPLC-phloroglucinolysis. For the analysis of the data across years, the statistical significance of the effects of year and treatment by year interaction are also indicated. When the T*year factor was statistically significant at $P < 0.05$ differences between treatment means were not explored

Parameter	T	2010	2011	Average	F-Ratio and signification		
					T	Year	T*-Year
Tannin mDP	Rainfed	4.35	5.82 b	5.23 b	5.90**	77.13***	ns
	0.25ET_C	4.31	5.78 b	5.19 b			
	0.50ET_C	4.35	5.43 b	5.00 b			
	0.75ET_C	4.05	4.73 a	4.44 a			
Galloylation % of tannins	Rainfed	13.06	15.57	14.57	ns	79.11***	ns
	0.25ET_C	13.76	16.18	15.22			
	0.50ET_C	13.36	15.86	14.86			
	0.75ET_C	13.33	15.91	14.88			
Tannin aMW	Rainfed	1322	1837 b	1631 b	5.77*	95.07***	ns
	0.25ET_C	1336	1821 b	1627 b			
	0.50ET_C	1345	1709 b	1563 ab			
	0.75ET_C	1251	1490 a	1394 a			

Within each column means followed by a different letter are significantly different at $P < 0.05$ based on Dunnett's *t* test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 6

Concentration of skin polymeric proanthocyanidins for a 'Cabernet Sauvignon' vineyard in the rainfed application and in the treatments watered at 25, 50 and 75 % of the estimated crop evapotranspiration (ET_C) during the post-veraison period during 2011 period determined by HPLC-phloroglucinolysis

Parameter	T	2011	F-Ratio	*P*-Value
Tannin mDP	Rainfed	11.89 b	15.21***	0.000
	0.25ET_C	9.28 a		
	0.50ET_C	9.14 a		
	0.75ET_C	7.23 a		
Galloylation % of tannins	Rainfed	3.59 a	5.12**	0.008
	0.25ET_C	3.87 ab		
	0.50ET_C	4.47 c		
	0.75ET_C	4.37 bc		
Tannin aMW	Rainfed	3256 c	14.96***	0.000
	0.25ET_C	2755 b		
	0.50ET_C	2721 b		
	0.75ET_C	2150 a		

Within each column means followed by a different letter are significantly different at $P < 0.05$ based on Dunnett's *t* test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Conclusions

In the present study, the low watered treatments would result in a more adequate strategy due to increasing of the volatile compound content, unlike the most irrigated treatment which seems to produce an increase of herbaceous related aromas (hexanal and (E) 2-hexenal) not desirable for wines. Berries subjected to the most stressed treatment (rainfed) had a lower anthocyanin and tannin concentration per gram of skin compared with those berries watered using a reduced stress strategy (0.75 ET_C), but this does not affect the final must due to a skin to flesh proportion effect. Regarding the astringency, seed and skin tannins, were lower in those less irrigated treatments. Berry seeds and skins from those plots subjected to the highest level of irrigation (0.75 ET_C), had a lower tannin polymeration, which means the most astringents berries of the experiment but with no effect on the gallolyation percentage. From a practical point of view, it can be concluded that watering at 50 % of the ET_C during the post-veraison period, is a recommended irrigation strategy for optimizing grape composition and improving yield in comparisons with rainfed vines.

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