

Texture properties and phenol extractability indices of the grape berry under pre- and post-veraison water deficit (*Vitis vinifera* L. 'Kékfrankos')

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Summary

Optimal water supply is one of the most important factors in quality wine making. However, water availability is limited in several wine regions and water shortage is getting even worse due to climate change, especially under arid climate conditions. Therefore, proper water management of the vineyards, the amount and the timing of irrigation will play a crucial role in sustainable viticulture in the near future. In this study, the effect of timing of moderate water deficit on berry texture characteristics and phenolic maturity were investigated. 'Kékfrankos' grapevines were submitted to different water regimes: moderate water deficit from berry set until veraison (WD1), moderate water deficit from veraison until harvest (WD2), no water deficit (C). Concentration of the phenolic components of the grape berry skins and berry mixture for the Glories indices were measured by spectrophotometer (UVmini-1240 CE UV-VIS, Shimadzu, Japan). Cell and seed maturity indices (CMI%, SMI%) were also calculated. Berry texture characteristics were monitored by a TA.XT Plus Texture Analyser (Stable Micro System, UK). Berry skin and seed texture properties were affected by water regimes. Skin and seed hardness of WD1 were significantly lower than those of WD2. Significant differences were found in berry hardness between the treatments (C>WD1>WD2). Berry gumminess, resilience and chewiness of WD2 were lower compared to C and WD1. Anthocyanin extractability was higher when plants were not subjected to post-veraison water limitations (WD1). Furthermore, the seed maturity index was lower when water deficit occurred between berry set and veraison. Pre-veraison water deficit resulted in delayed ripening, softer seeds and thus higher phenol extractability compared to WD2 and C. Late seasonal water deficit resulted in thicker skins, which was accompanied by lower anthocyanin extractability than in WD1 and C. It seems that anthocyanin extractability is influenced by the actual water status of the grapevine rather than skin textural properties. However, there are some connections between skin texture parameters and maturity indices.

Key words: pre- and post-veraison; water deficit; phenolic maturity; extractability; berry texture characteristics.

Introduction

Grape yield and berry maturity is strongly influenced by water deficit. One of the most common phenomena of water deficit is the decreased berry size. In parallel, water restriction after veraison increases skin thickness, and thus results in higher skin/flesh ratio irrespective of berry size (ROBY and MATTHEWS 2004). Also, berry sugar concentration decreases as a result of drought stress (MATTHEWS and ANDERSON 1989), however moderate water deficit may lead to increased berry sugar accumulation due to the modified sink-source relations and changes in assimilate partitioning (ZSÓFI *et al.* 2011, NICULCEA *et al.* 2014). Beside the changes of basic yield and quality parameters, water deficit induces large metabolic changes in the grape berry. Several studies have reported that the concentration of hormones in the grape berry (*i.e.* ABA, IAA) is strongly affected by water supply and the timing of water deficit. A similar phenomenon was observed in the case of total soluble solids, acidity and the concentration of some amino acids and total anthocyanin (NICULCEA *et al.* 2014, ZARROUK *et al.* 2016). However, the sampling time (growing stages) and the cultivar play an important role in responses to water deficit. Indeed, it has been shown that different water regimes have a great effect on the concentration of berry skin volatile compounds. In particular, water deficit after veraison decreased the concentration of the unpleasant volatile components responsible for the herbaceous odour of 'Cabernet Sauvignon' wines (GARCÍA-ESPARZA *et al.* 2018). A research conducted on 'Gewürztraminer' indicated that moderate water deficit after veraison has a positive effect on the concentration of important free terpenes (geraniol, citronellol) in the grape berries; however, this phenomenon was not transcriptionally regulated (KOVALENKO *et al.* 2021). In contrast, ZUFFEREY *et al.* (2020) found that there were no differences between the irrigated and non-irrigated treatments in aromatic precursors of 'Arvine' grape must.

During the red wine making process, grape phenolic maturity, including skin anthocyanin concentration and extractability, is essential. Water deficit has a significant effect on the concentration of several secondary metabolites of the berry skin (OJEDA *et al.* 2002, CASTELLARIN *et al.* 2007a and 2007b, HOCHBERG *et al.* 2015). Indeed, phenolic maturity is affected by mild to moderate water deficit during berry development and ripening. It has a positive effect on the concentration of skin phenolic components,

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such as anthocyanins and flavan-3-ols. These phenomena occur due to the changes in the metabolic pathway of flavonoid compounds (CASTELLARIN *et al.* 2007a and 2007b) and the increase of skin/flesh ratio of the grape berry (ROBY and MATTHEWS 2004). Recently, several studies have reported that a certain level of post-veraison water deficit increases the concentration of proanthocyanidins and the mean degree of polymerization (mDP) in the berry skin (CÁCERES-MELLA *et al.* 2017, CALDERAN *et al.* 2021). Very similar results were obtained by KYRALEOU *et al.* (2017) when 'Cabernet Sauvignon' was submitted to regulated deficit irrigation from berry set. In contrast, in the case of 'Merlot', the anthocyanin concentration of the skin extraction solution was higher in the control compared to the stressed treatment. On the other hand, in the same study, opposite results were obtained in the case of 'Sangiovese' (COCCO *et al.* 2020). Furthermore, it seems that the extent, the timing and the duration of water restriction can modify such effects (OJEDA *et al.* 2002, ROBY *et al.* 2004, OLLÉ *et al.* 2011). Studies have shown that pre- and post-veraison water deficit both result in a significant increase in anthocyanin and flavan-3-ols concentration in the berry skin (OJEDA *et al.* 2002, OLLÉ *et al.* 2011). However, these effects may be influenced by co-occurring effects such as heat (ZARROUK *et al.* 2016), UV radiation (MARTÍNEZ-LÜSCHER *et al.* 2014, ALONSO *et al.* 2016) and CO₂ concentration (KIZILDENIZ *et al.* 2015). In addition, different vintage climatic conditions can also influence skin anthocyanin concentration and its extractability, in spite of identical irrigation regimes (KOUNDOURAS *et al.* 2013, INTRIGLIOLO *et al.* 2016, VILANOVA *et al.* 2019).

Cell Maturity Index - CMI% (also known as Anthocyanin Extractability - EA%) of the grape berry has been studied intensively recently and the results have presented evidence that this aspect of phenolic maturity is influenced by many factors. Several authors have shown that this parameter strongly depends on grape ripening grade and berry physiological stage (FOURNAND *et al.* 2006, ROLLE *et al.* 2009, HERNANDEZ-HIERRO *et al.* 2012). Similarly, anthocyanin extractability from the grape berry may strongly vary with genotype (ROMERO-CASCALES *et al.* 2005, ORTEGA-REGULES *et al.* 2008, RÍO SEGADE *et al.* 2008b). Furthermore, differences have been found among terroirs in this parameter in the case of 'Barbera' as well (TORCHIO *et al.* 2010, RÍO SEGADE *et al.* 2011b); however no detailed description of the environmental conditions of the growing areas were presented.

In several studies, it was shown that anthocyanin extractability correlated to berry skin structure (ORTEGA-REGULES *et al.* 2006, HERNANDEZ-HIERRO *et al.* 2012) and skin texture characteristics (ROLLE *et al.* 2008, RÍO SEGADE *et al.* 2011a). Indeed, berry skin thickness and hardness showed a close correlation with anthocyanin extractability in these works (RÍO SEGADE *et al.* 2008b, RÍO SEGADE *et al.* 2011a, ROLLE *et al.* 2011), and therefore it seems the textural behaviour of the grape berry skin has a significant impact on wine phenolic concentration. Some research also focused on grape seed maturity index and texture properties. It was shown that there are certain differences between cultivars in seed texture properties. However, seed mechanical pa-

rameters are also significantly influenced by the vintage characteristics and ripening grade. In addition, it seems that the changes in seed maturity indices during ripening are also affected by the genotype (LETAIEF *et al.* 2008b, RÍO SEGADE *et al.* 2008a, RÍO SEGADE *et al.* 2008b). Scientific evidence of the connection between seed maturity and texture parameters is rare; however, it was also reported that there is some relationship between seed maturity index and some seed mechanical properties (ROLLE *et al.* 2012).

Recently, it has been shown that moderate and severe water deficit after veraison results in significant changes in berry texture properties (ZSÓFI *et al.* 2014, ZSÓFI *et al.* 2015). For example, berry hardness is strongly influenced by water supply. Furthermore, in drought-stressed berries skin thickness and skin hardness are generally greater compared to the well-watered treatments; however, these differences are highly influenced by the harvest time. Anyway, it seems that cultivars, environmental conditions, berry skin structure and texture are all relevant factors in creating grape phenolic maturity, particularly anthocyanin extractability. However, no scientific results have been presented yet about the possible relationship between timing of water deficit, berry texture characteristics and the extractability of phenolics from the grape berry skin and the seeds.

The aim of this present study is to describe the effect of pre- and post veraison moderate water deficit on berry and seed texture properties, and seed (SMI) and cell maturity indices (CMI, anthocyanin extractability).

Material and Methods

Experimental design: Six-year-old gobelet trained 'Kékfrankos' (*Vitis vinifera* L.) grapevines grafted on 'Teleki-Kober 5BB' rootstocks were submitted to pre-, and post-veraison water deficit in 2013. The experiment was carried out on potted grapevines in Eger, Hungary in a greenhouse of the Research Institute for Viticulture and Enology, as was also described in our previous studies (VILLANGÓ *et al.* 2013). Briefly: the greenhouse was opened at the front during the experiment; furthermore, the air temperature of the greenhouse was half-controlled by an automatic system, which regulated the opening of the upper windows. Based on the measurements of the infrared gas analyser, air temperature of the greenhouse ranged between 27-32 °C at midday, depending on the outside temperature. At the same time, leaf temperature of the plants was slightly lower (between 24-30 °C). Also, photosynthetically active radiation (PAR) was always above saturating light intensities (1300-1500 µE) and relative humidity ranged between 70-85 % during the measurements. Plants were planted into 50 L white plastic containers in a mixture of perlite (20 %), loamy soil (30 %) and peat (50 %) (v/v) with a completely randomised arrangement of the pots.

Three treatments were applied during the experiments: control (full irrigation during the whole vegetation period, nil stress, C), moderate water deficit from berry set to veraison (WD1) and moderate water deficit from veraison until harvest (WD2). The WD1 phase was 36 d (02 June –

08 July), the WD2 phase was 35 d (04 July – 08 August) long. The water regimes were defined by the leaf daily stomatal conductance (g_s) as described by several authors (FLEXAS and MEDRANO 2002, CIFRE *et al.* 2005, GALMÉS *et al.* 2007, POU *et al.* 2008): nil stress (g_s above 150 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) and moderate stress (g_s between 50-150 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$). Irrigation was carried out twice a day, early in the morning and in the afternoon. Eight plants were kept as control with irrigation twice a day (in the evening and in the morning). In the case of another eight plants, irrigation was stopped after berry set, and they were kept under moderate water deficit ($g_s =$ between 50-150 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) until veraison. After veraison these plants were rewatered and they were fully irrigated similarly to the control plants until harvest ($g_s =$ above 150 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$). In parallel, irrigation was stopped from veraison for another eight plants to adjust moderate water stress treatments until harvest. The daily water loss was measured by a scale (Kern, DS 100K1, Balingen, Germany).

During the experiment, the same canopy management was applied. Three shoots, and two clusters per shoot were left in each pot respectively. A thin bamboo stick was used to fix each shoot. Lateral shoots of the plants were removed during plant development from each treatment. Shoot tips were cut after the 14th leaves unfolded. The leaves had been fully developed already by the time when water restriction for the WD1 treatment started.

Physiological measurements: Changes in leaf gas-exchange of the treatments were monitored daily (except on cloudy days) in the morning, 11:00 (local time) by a CIRAS-1 infrared gas-analyser (PP System, UK) until the moderate water deficit was achieved. After the desired water deficit treatment was achieved the weights of the pots were recorded. All pots of the water deficit treatments were weighted twice a day during the rest of the experiment and the water loss was calculated. The level of water stress was maintained by watering the plants with the amount of daily water loss each day until the end of the experiment. Also, gas-exchange was monitored in this period, in order to check the plant response of the treatments.

Measurements were taken on different plants (one leaf per plant), on mature (between the 7th and 10th level from the basal leaves), undamaged leaves that had grown fully-exposed to the sun. All measurements were taken on ambient, saturating light intensities within 1 h and on the same side (south) of the canopy in order to obtain comparable data (VILLANGÓ *et al.* 2013, ZSÓFI *et al.* 2014). The reference CO_2 concentrations of the gas analyser were between 360-370 ppm during the measurements.

Berry sampling: For each treatment, the harvest was made at the same time on 8th of August (Fig. 1). Grape clusters were harvested from the plants of the treatments, berries were removed with pedicels from the clusters and visually tested before analysis. 24 clusters of four plants (six clusters per plant) per treatment were harvested, respectively. Berries for measurements were taken from each cluster (textural analyses: 2-3 berries/cluster; analytical measurements, skin and seed weight: 1-2 berries/cluster; average berry weight: 5-6 berries/cluster (ZSÓFI *et al.* 2014). 100 berries were selected to measure average berry

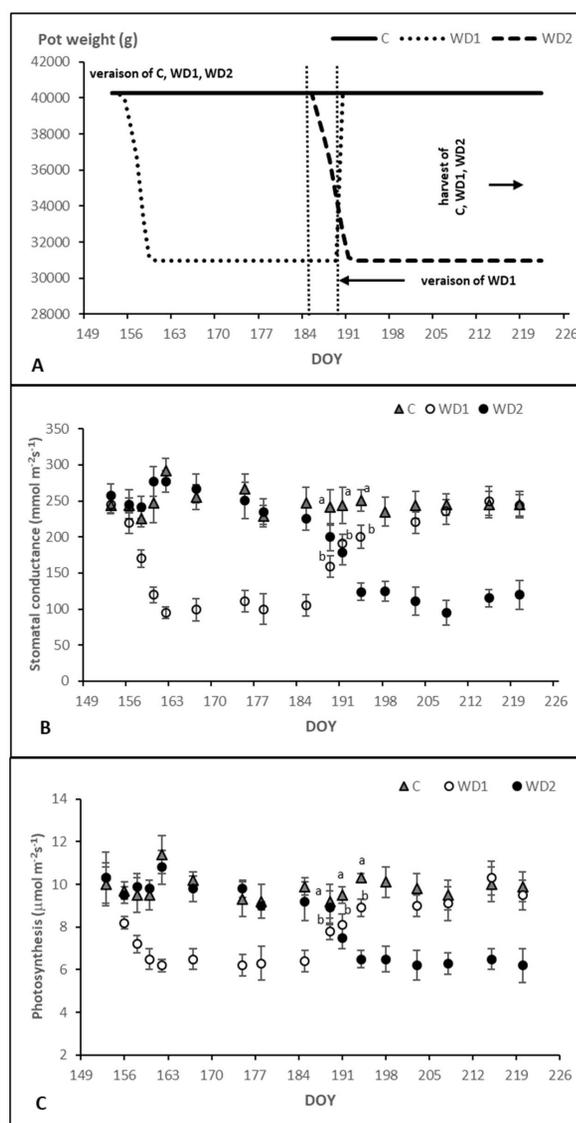


Fig. 1: Experimental design (A), changes in stomatal conductance (B) and net photosynthesis (C) during the experiment. Changes in pot weights during the experiment between berry set and harvest according to the treatments. WD1 – moderate water deficit between berry set and veraison; WD2 – moderate water deficit between veraison and harvest; C – control, irrigation during the complete growing season (A). Please note, that berry colorization of WD1 treatment delayed 5 days compared to C and WD2. Each g_s and P_n symbols represent the average of 4-8 replicates. The starting dates of the water supply treatments and the dates of harvest are indicated by arrows. There were significant differences between the treatments after achievement of the desired water deficit according to Duncan's test. Different roman letters indicate significant differences between the WD1 and the control treatments after rewatering ($P < 0,05$).

weight. Skin and seed weight of 40 berries was also measured by an analytical scale (Kern EG 300-3 M, Albstadt, Germany).

Basic analytical measurements: Approximately, one kg of berries per treatment was divided into three parts, then crunched and pressed to obtain juice for basic analytical measurements. The analytical methods recommended by the OIV (2019) were used to determine sugar concentration, titratable acidity and the pH of the grapes.

Assessment of grape cell (CMI) and seed maturity indices (SMI): A total of 225 berries were selected separately for the measurements of the CMI and SMI indices. These berries were subdivided into two equal groups for the pH 1 and pH 3.4 solutions. The measurement was done in triplicate, and 25 berries were used for each repetition (VILLANGÓ *et al.* 2015). The phenolic potential of the grapes was calculated according to the method described by SAINT-CRICQ *et al.* (1998). This involved grinding the grapes with a blender and macerating for four hours with buffer solutions at two pH values (1.0 and 3.4). The original method proposed a buffer of pH 3.2, but this was adjusted to 3.4, as this is more relevant to the grapes from this region. The indices of phenolic maturity were calculated according to GLORIES and AUGUSTIN (1993): potential anthocyanins (A1), extractable anthocyanins (A3.4), cell maturity index (CMI%) and seed maturity index (SMI%). All the measurements were done in triplicate. The following equations were used:

$$\text{CMI (\%)} = [(A1 - A3.4) / A1] \times 100$$

$$\text{SMI (\%)} = [(A280 - ((A3.4 / 1000) \times 40)) / A280] \times 100.$$

Determination of phenolic components in grape skin extracts: Skins of 40 berries/treatment were peeled in order to measure their phenolic composition. The extraction of phenolics from grape skins was carried out according to SUN *et al.* (1996). The following solvent was used during the maceration: methanol:water (60:40) with 1% HCl-methanol. From this solvent 20 mL was used for each sample. The maceration of skins took place for 48 h in a dark room. The total amount of skins of ten berries was used for one replicate and four replicates were done for each treatment. After that the samples were filtrated and stored in a cool and dark place before the analysis. Phenolic components were measured by spectrophotometer (UVmini-1240 CE UV-VIS, Shimadzu, Japan) at 280 nm, and 520 nm wavelengths depending on

the applied assay presented below. The bisulphite bleaching method was used to determine the anthocyanin content of grape skin extracts (RIBÉREAU-GAYON *et al.* 2006). Total phenolics of the grape skin extracts were analysed by the Folin-Ciocalteu method (SINGLETON and ROSSI 1965). Results are expressed in gallic acid equivalents (GAE mg·L⁻¹). Catechin was measured with the vanillin assay according to AMERINE and OUGH (1980).

Measurements of berry mechanical properties: A TA.XTplus Texture Analyser (Stable Micro System, Surrey, UK) with an HDP/90 platform and 30 kg load cell was used to follow grape mechanical properties. Fifty berries were used for each type of mechanical measurements, respectively. The Exponent 5.1 software was used for data evaluation. All operative conditions were applied according to (LETAIEF *et al.* 2008a, LETAIEF *et al.* 2008b) (Tab. 1). Briefly: P/35 probe was used to determine berry hardness (BH, N). Berries of approximately the same size, along with their pedicel, were gently removed from the cluster; they were laid on the plate of the analyser. After this, they were compressed to 25 % of their diameter. A P/2N needle was applied to conduct a puncture test. Also, berries with their pedicel were removed from the cluster, laid on the plate of the analyser and then punctured on the lateral face (LETAIEF *et al.* 2008a). Skin break force (F_{sk} , N), skin break energy (W_{sk} , mJ) and Young modulus of berry skin (E_{sk} , N·mm⁻¹) were calculated from the puncture test by macros. Berry skin thickness was measured using P/2 probe with 2 mm diameter. For this measurement, approximately 0.25 cm² skin was removed from the lateral face of the berry. The skin was carefully and gently cleaned from pulp, it was placed on the platform and the test was conducted as described by other authors previously (RÍO SEGADÉ *et al.* 2008a). For seed hardness tests one seed was removed from the berry, placed on the platform on its lateral side and the test was performed. Seed break force (F_{sk} , N), seed break energy (W_{sk} , mJ) and Young's modulus of the seed (E_{sk} , N/mm) was calculated by macros.

Table 1

Operative conditions of the berry texture analyses; measured parameters and their acronyms (after LETAIEF *et al.* (2008a))

	Probe	Test speed	Compression	Mechanical property
Berry skin thickness	P/2 2 mm ø	0.2 mm·s ⁻¹	-	S_{psk} : berry skin thickness (mm)
Berry skin hardness	P/2N needle	1 mm·s ⁻¹	3 mm	F_{sk} : berry skin break force (N) W_{sk} : berry skin break energy (mJ) E_{sk} : Young's modulus of the skin (N·mm ⁻¹)
Seed hardness	P/35 35 mm ø	1 mm·s ⁻¹	50 % of the seed ø	F_s : seed break force (N) W_s : seed break energy (mJ) E_s : Young's modulus of the seed (N·mm ⁻¹)
Berry hardness	P/35 35 mm ø	1 mm·s ⁻¹	25 % of the berry ø	BH: measure of force necessary to attain a given deformation (N) BCo: berry cohesiveness: (A2 + A2W)/(A1 + A1W) (strength of internal bonds making up berry body) BG: berry gumminess (N): BH* BCo (force necessary to disintegrate a semisolid food until ready for swallowing) BS: berry springiness (mm): D2 (distance recovered by sample during time comprised between the end of the first bite and the start of the second bite) BCh: berry chewiness (mJ): BH* BCo* BS (energy necessary to chew a solid food until ready for swallowing) BR: berry resilience: (A1W/A1) (how well berry fights to regain original position)

Statistical analyses: Statistical analysis was conducted by the Sigma Stat (Systat Software Inc., San Jose, CA, USA) 8.0 software. Values were compared by one-way ANOVA test and Duncan's multiple range test was used for mean separation.

Results

Gas-exchange measurements: Stomatal conductance (g_s), net-photosynthesis (P_n) and transpiration rate of the moderately stressed plant were gradually decreased due to deficit irrigation. During the gas-exchange measurements there were no significant differences between the samplings with regard to light intensity (PAR), relative humidity (RH) and air temperature (T) (please see the description of the greenhouse conditions).

Water deficit was achieved by the 8th day in the case of WD1 and the 10th day in the case of the WD2 treatment after the irrigation stopped. Average g_s values of moderate water deficit treatments ranged between 95-129 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Average g_s values of the non-stressed plants ranged between 205-292 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. As a consequence, stomatal responses induced decreased CO_2 -fixation and transpiration rate per unit leaf area in both water stressed treatments. During the experiment the average net assimilation rate of the non-stressed treatment was ranging between 8.5-11.4 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, values of the moderately stressed plants were between 6.2-7.0 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The average transpiration rate of the non-stressed treatment was between 3.7-4.3 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, values of the moderately stressed plants were between 2.1-3.0 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. There were significant differences between the water stressed and control plants in stomatal conductance and net photosynthesis after the desired water deficit was achieved. Interestingly, after rewatering of WD1 treatment the recovery of g_s and P_n was relatively slow. In fact, there were significantly lower values of this treatment compared to the control after veraison (Fig. 1).

Average berry, skin and seed weight, juice sugar concentration, pH and titratable acidity: Average berry weight of the WD1 treatment was significantly lower compared to the WD2 and the control plants. Also, a significant difference was found in this parameter between WD2 and the non-stressed berries. Control berries had the greatest average skin weight and no differences were found between the water stressed treatments. However, the WD1 treatment presented the greatest skin/flesh ratio (0.30) compared to WD2 (0.26) and the non-stressed (0.25) plants. Taking the average cluster weight, control grapevines produced the heaviest clusters. They were followed by the WD2 and finally the WD1 treatment (Tab. 2).

Sugar concentration of the grape juice was the highest in the non-stressed treatments and the lowest in the WD2 berries. Interestingly, there were significant differences between WD1 and C berries in this parameter. Similarly, the lowest pH and the highest titratable acidity were presented by the WD2 plants. In contrast, the lowest acidity levels were measured in juice pressed from WD1 berries, but

Table 2

Changes in basic yield and quality parameters according to the treatments (average cluster weight $n = 10$, average berry weight $n = 40$, average skin weight $n = 40$, skin to flesh ratio $n = 40$, sugar concentration, titratable acidity and pH $n = 3$). Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit

Parameter	Treatments		
	C	WD1	WD2
Sugar ($\text{g}\cdot\text{L}^{-1}$)	233.67a	208.67b	182.67c
Titratable acidity ($\text{g}\cdot\text{L}^{-1}$)	8.07b	6.57c	10.03a
pH	3.43a	3.51a	3.26b
Average cluster weight (g)	229.05a	144.18c	183.25b
Average berry weight (g)	1.77a	1.13c	1.33b
Average berry skin weight (g)	0.34a	0.26b	0.28b
Berry skin/flesh ratio	0.25b	0.30a	0.26b

in the case of pH there was not any significant difference compared to C berries (Tab. 2).

Berry, skin and seed textural properties: Berry hardness (BH) of the water stressed grape was consistently lower, while there were significantly higher values in the case of WD1 treatments than in WD2 (Fig. 2). No visible shrinkage symptoms were detected on any berries irrespective of the treatments. A similar pattern was shown in the case of some further berry texture parameters derived from the berry hardness test. Berry gumminess (BG) and berry chewiness (BCh) were the highest in the WD1 treatment, followed by control and WD2. No significant differences were found in berry cohesiveness (BCo) and berry springiness (Bs) between the treatments. WD2 represented the lowest berry resilience (Br) value (Tab. 3).

Skin thickness (S_{psk}) was the lowest in the WD1 treatment and the highest values were measured in berries sub-

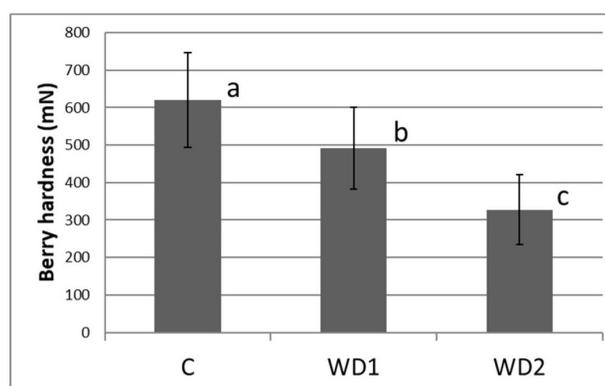


Fig. 2: Berry hardness of the treatments measured by the double compression test. Each column represents the average of 50 replicates. Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit.

mitted to post-veraison water deficit (Fig. 3). Skin break force (F_{sk}) decreased significantly because of pre-veraison water deficit. Interestingly, F_{sk} values of the WD1 treatment were significantly lower compared to the control and WD2. The skin Young's modulus (E_{sk}) was highest in the case of the WD1 berries, followed by the non-stressed treatment, and the lowest values were measured in the WD2 berries.

Table 3

Changes in berry texture parameters of the treatments derived from the results of double compression test (berry cohesiveness, gumminess, springiness, chewiness and resilience). Each value represents the average of 50 replicates. Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit

Parameter	Treatments		
	C	WD1	WD2
Berry cohesiveness	0.557	0.579	0.579
Berry gumminess (N)	3.305a	3.349a	2.076b
Berry springiness (mm)	0.025	0.026	0.024
Berry chewiness (mJ)	0.087a	0.088a	0.052b
Berry resilience	0.248a	0.256a	0.220b

Berry skin break energy (W_{sk}) showed its highest values in the case of WD2, and significantly lower values were recorded in the C and WD1 berries (Tab. 4).

Seed mechanical properties showed significant differences among the treatments. Significant differences were found between the control and water deficit treatments in seed hardness (F_s). Interestingly, F_s values of the pre-veraison water deficit treatment were significantly lower compared to control and WD2 seeds. Trends observed in the case of seed elasticity (E_s) and seed break energy (W_s) were different. WD2 had an increasing impact on E_s values, while WD1 berries remained on the same level as C. W_s showed statistically significant differences amongst treatments, where WD2 values were the highest (Tab. 4).

CMI and SMI indices: The Glories indices, which provide a prediction of phenolic compounds in the

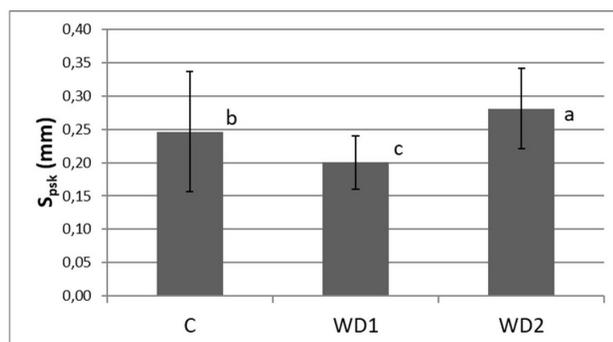


Fig. 3: Berry skin thickness of the treatments. Each column represents the average of 50 replicates. Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit.

resulting wines, are given in Tab. 5. CMI indices showed lower values in the C and WD1 berries than in WD2. No differences were found between C and WD1 treatments in this parameter. In contrast, the highest CMI indices were measured in WD2 treatments. Interestingly, in the case of seed maturity indices (SMI), no differences were found between C and WD2, and they were both significantly higher compared to the WD1 berries.

Grape skin phenolics: Moderate water deficit resulted in significant changes in the concentration of the phenolic components (total anthocyanin, catechin, total polyphenol) of the berry skin. The concentration of total anthocyanins, catechin and total polyphenols increased in WD1 and WD2 treatments compared to the control. Both water deficit treatments showed significantly higher values than the well-watered grapevines with slightly higher values of the WD1 (Tab. 6). Berry colorization of the WD1 treatment was delayed by 5 d, compared to C and WD2.

Discussion

Mild to moderate water deficit has a great effect on grape yield and quality. Water deficit decreases cluster and berry weight depending on the stress level. Beside the grape berry weight reduction, the skin to flesh/berry ratio increases (CÁCERES-MELLA *et al.* 2017). However, the sen-

Table 4

Results of the skin and seed texture parameters. Each value represents the average of 50 replicates. Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). F_{sk} : berry skin break force, E_{sk} : Young's modulus of the skin, W_{sk} : berry skin break energy, F_s : seed break force, E_s : Young's modulus of the seed, W_s : seed break energy. C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit

Treatments	Skin texture parameters			Seed texture parameters		
	F_{sk} (N)	E_{sk} (N·mm ⁻¹)	W_{sk} (mJ)	F_s (N)	E_s (N·mm ⁻¹)	W_s (mJ)
C	0.714a	0.554b	0.518b	23.85b	47.54b	4.131c
WD1	0.622b	0.656a	0.341c	20.38c	45.34b	5.153b
WD2	0.754a	0.444c	0.585a	28.49a	52.17a	6.504a

Table 5

Anthocyanin extractability and seed maturity index of the treatments. Each value represents the average of three replicates. Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). A1: potential (total) anthocyanins, A3.4: extractable anthocyanins at wine pH, CMI%: cell maturity index, SMI%: seed maturity index, C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit

Parameter	Treatments		
	C	WD1	WD2
A1 (mg·L ⁻¹)	570.0b	739.5a	756.5a
A3.4 (mg·L ⁻¹)	306.2b	419.6a	301.1b
CMI%	45.65b	42.95b	60.19a
SMI%	64.51a	52.93b	65.19a

Table 6

Total anthocyanin, catechin and total polyphenol levels of the treatments. Each value represents the average of four replicates. Different letters indicate significant differences between the treatments according to Duncan's test ($P < 0.05$). C: control, WD1: pre-veraison water deficit, WD2: post-veraison water deficit

Parameter	Treatments		
	C	WD1	WD2
Total anthocyanin (mg·kg ⁻¹)	6072.6b	8778.2a	8650.3a
Catechin (mg·kg ⁻¹)	7219.4b	9991.1a	9502.7a
Total polyphenol (mg·kg ⁻¹)	7915.8b	9815.1a	9700.2a

sitivity of berry growth to water deficit seems to be lower than that of the shoot organs (MATTHEWS and NUZZO 2007). Water restrictions influences the basic quality parameters as well as the berry phenolic maturity (ROBY *et al.* 2004). Indeed, in our experiments, we have found typical water stress phenomena with regard to yield (average berry and cluster weight) and quality; however, the timing of water restriction has a big impact on these characteristics. MATTHEWS and ANDERSON (1989) and OJEDA *et al.* (2001) obtained very similar results. They found that early (between anthesis and veraison) and late (between veraison and harvest) water deficit decrease yield, berry size and diameter. Furthermore, early water deficit resulted in smaller berries, compared to water stress after veraison. Both water deficit treatments induced decreased pericarp cell volume. However, early water restriction may have had a great impact on pericarp cell wall structure as well and thus it resulted in an irreversible decrease in cell volume. Opposite results were obtained by OLLÉ *et al.* (2011) with regard to the effect of early water deficit on berry weight. They found no significant differences between the control and the water stressed treatment before veraison, but in that study the duration and the extent of the stress level before veraison was not the same as the water deficit after veraison. Anyway, our findings indicated a significant decrease in flesh volume in the case WD1 berries, and thus it resulted in the highest skin to flesh ratio among the treatments in

accordance with the findings of NICULCEA *et al.* (2014) in the case of 'Tempranillo'. Indeed, the ratio of the WD1 flesh weight reduction compared to the control was significantly higher (36 %) than the decrease of the berry skin weight (23 %) (Tab. 2).

The relationship between berry size and berry sugar concentration has been widely studied. Several studies have shown that there is a close correlation between berry sugar concentration (or °Brix), berry sugar content and berry size (ROBY *et al.* 2004, MATTHEWS and NUZZO 2007, FERRER *et al.* 2014, BIGARD *et al.* 2019, MIRÁS-AVALOS *et al.* 2019). The sugar content (g·berry⁻¹) is higher in heavier berries; however, an inverse relationship can be obtained if sugar concentration (g·L⁻¹) is plotted against berry size. Indeed, the smaller the berries the higher the sugar concentration (SCIENZA *et al.* 1978, ROBY *et al.* 2004) due to the dilution of sugars. This negative correlation is still valid under water deficit irrespective of the stress level, but the slope of the linear regression can be different (ROBY *et al.* 2004, MIRÁS-AVALOS *et al.* 2019). There are also studies reporting the lack of this relationship between berry size and sugar concentration (FERRER *et al.* 2014, BIGARD *et al.* 2019). One possible reason of that could be the different distributions of berry sizes in the population between the experimental plots (BIGARD *et al.* 2019).

In our study, the lower sugar concentration of the WD2 berries was due to the decreased photosynthetic activity. This result is in line with previous studies (MATTHEWS and ANDERSON 1988, ZSÓFI *et al.* 2014). They found that berries of the water-stressed treatments presented lower sugar concentration, compared to the non-stressed treatment. Other experiments, conducted under field grown conditions, have shown that mild to moderate water deficit during the ripening induce higher berry sugar concentration compared to the well-watered vines. This is probably due to the reduced berry size, the combined effects of change in assimilate partitioning and the modified sink-source ratio of the grapevine (ZSÓFI *et al.* 2011). Interestingly, the sugar concentration of the WD1 berries was significantly lower compared to the control, despite full irrigation during the ripening period. In parallel, berry hardness (BH) of WD1 presented lower values compared to the control. This parameter is highly influenced by water supply, as shown in a previous study (ZSÓFI *et al.* 2014). These results suggest that after rewatering, pre-veraison water deficit has an impact on berry water household during the ripening period and sugar accumulation. One possible reason for the lower berry sugar concentration of WD1 treatment could be the post effect of water deficit on stomatal conductance. It was shown by POU *et al.* (2008) that after rewatering, stomatal conductance of the water stressed plants was lower for several days as a result of the still increased ABA concentration in the xylem sap. The same phenomenon was shown in the case of kidney bean (MIYASHITA *et al.* 2005) and bell pepper (CAMPOS *et al.* 2014). MIYASHITA *et al.* (2005) has shown, that there is a close relationship between the timing of rewatering and the effectiveness of stomatal conductance and photosynthesis recovery. Notably, the longer the water deficit period the slower the recovery of these parameters. Indeed, in our gas-exchange measurements

after rewatering indicate, that there were lower stomatal conductance and net photosynthesis of the WD1 treatment compared to the control. However, this difference disappeared by the end of the experiment. A further explanation for this observation could be the relationship between final berry size and reduced phloem transport. MCCARTHY and COOMBE (1999) suggest that phloem flow impeded after the maximum berry weight has been achieved. Indeed, the smallest berries have been found in the case of WD1 treatments, probably due to the decreased cell volume. This phenomenon was described as an irreversible process (OJEDA *et al.* 2001, OLLAT *et al.* 2002) resulting in stronger limitation of WD1 berry expansion compared to the control. In addition, water deficit induces increased phenolic concentration in the berry skin. These phenolics bound to basic cell wall compartments such as polysaccharides and proteins. They further stiffen the cell wall, and thus limit cell expansion (KELLER 2010). The lower berry hardness of WD1 berries can be explained in a very similar way. We have no direct evidence to prove the background of this phenomenon; however, the impeded phloem flow (because of the achieved final berry weight (MCCARTHY and COOMBE 1999) may also cause decreased turgor pressure, and thus lower berry hardness. This hypothesis is supported by our previous findings, which show the relationship between berry size and berry hardness (ZSÓFI *et al.* 2015). In this study it is shown that the bigger the berry, the higher the BH, irrespective of the level of water supply.

Concentration of skin phenolics showed a pattern similar to that in previous studies (OJEDA *et al.* 2002, OLLÉ *et al.* 2011). In our experiment, both water deficit treatments induced stronger polyphenol synthesis, which is a common phenomenon in drought exposed grape berries (CASTELLARIN *et al.* 2007a and 2007b). However, the concentration of the different anthocyanin derivatives can be very different according to the timing and the strength of water deficit. Indeed, the concentration of some anthocyanin derivatives (e.g. malvidin-glucosides) is significantly influenced by early water deficit (OLLÉ *et al.* 2011). Furthermore, GARCÍA-ESPARZA *et al.* (2018) concluded that the optimal level of deficit irrigation after veraison (based on ETc) improves skin anthocyanin concentration. In our study the concentration of all phenolic parameters, measured by spectrophotometer, was higher in the water stressed treatments than in the control in accordance with other findings (OJEDA *et al.* 2002, OLLÉ *et al.* 2011). However, it seems that this phenomenon can be influenced by the genotype, as was reported by NICULCEA *et al.* (2014) in the case of 'Tempranillo' and 'Graciano'. Similar results were obtained when we measured the pH1 solution for the CMI indices (Tab. 5). Furthermore, it seems that water supply has a significant effect on anthocyanin extractability and the cell maturity index. Indeed, according to the findings of NICULCEA *et al.* (2014) pre- and post-veraison water deficit increased the CMI indices of both examined varieties, and the concentration of extractable anthocyanins ($\text{mg}\cdot\text{kg}^{-1}$) decreased as a result of early water restriction. Other authors obtained similar results about the relationship between water deficit and anthocyanin extractability, and they reported that this phenomenon was affected by

the irrigation scheduling (INTRIGLIOLO *et al.* 2016). They found some differences between the irrigation treatments in anthocyanin extractability (% and $\text{mg}\cdot\text{L}^{-1}$); however, the vintage effect also played a role in creating the phenolic ripeness of the grape berries. Anyway, in our experiment CMI was not affected by early water deficit, but the extractability ($\text{mg}\cdot\text{L}^{-1}$) was the highest in this treatment. The WD2 treatment presented the highest CMI index, and no differences were found between C and WD1. Similarly, the SMI index was the highest in WD2 and, interestingly, it did not differ from the control treatment. Very similar results were obtained by INTRIGLIOLO *et al.* (2016); notably, no differences were found in the SMI index between the irrigation treatments applied after veraison. However, in the third experimental year, a slight increasing trend could be observed in parallel with the higher water supply. The lower SMI and CMI indices of the WD1 treatment and the lower anthocyanin extractability of WD2 may be explained partly by berry texture parameters, discussed later in this paper.

Skin thickness was the highest in WD2 and the lowest in WD1 berries. Cell expansion and cell wall metabolism are sensitive to drought during the first phase of berry growth (OJEDA *et al.* 2001, GRIMPLET *et al.* 2007), and this may have resulted in thinner berry skin. Water deficit during the ripening period resulted in a higher Sp_{sk} compared to the control vines. ZSÓFI *et al.* (2014) obtained similar results in a previous study. In this work, a significant increase was observed in skin thickness of water stressed berries between two harvest dates. Also, the higher relative skin mass of the water stressed berries explained by the increased cell wall/apoplast volume (ROBY and MATTHEWS 2004) as a common response of plant tissues on water deficit (CUTLER *et al.* 1977, PATAKAS and NOITSAKIS 1999).

Berry skin hardness was higher in the case of the WD2 treatment than in C, and both of them were significantly higher compared to WD1 berries. The lower F_{sk} values in WD1 treatment suggest that there is no relationship between the phenolic concentration of the berry skin and its hardness. Indeed, significantly higher concentration of the phenolic compounds was found in pre-veraison water deficit treatment (Tab. 6). In contrast, in the control berry skins much less phenolic components were accompanied by higher F_{sk} values, compared to the WD1 treatment. The fact that skin break force was significantly higher in WD2 than in WD1 (and there was a slightly higher phenolic concentration in WD1 than in WD2) suggests that phenolic components do not play a significant role in skin textural behaviour. Similar conclusions can be made in the case of the derived parameters of the puncture test (E_{sk} and W_{sk}).

In previous studies, skin break force and skin thickness was shown as a reliable parameter to predict anthocyanin extractability. RÍO SEGADE *et al.* (2011a and 2011b) showed that thinner berry skin seems to release greater amount of red pigments. ROLLE *et al.* (2008) also showed that in a model solution the extracted anthocyanin content was higher in grapes with higher skin hardness. In our study, in agreement with RÍO SEGADE *et al.* 2011a and 2011b, relatively high anthocyanin extractability was accompanied by the WD1 treatment, where the skin thickness was the

lowest. However, no differences were found in the cell maturity index between WD1 and C in spite of the obvious differences in Sp_{sk} between the two treatments. In WD2 berries, the anthocyanin extractability was the lowest compared to C and WD1. Therefore, it seems, the thicker berry skin (as a result of post-veraison water deficit) leads to the reduction of anthocyanin extractability, assumingly due to the increased apoplast/cell wall volume. Furthermore, as ORTEGA-REGULES *et al.* (2006) indicated in the case of 'Monastrell', cell-wall composition has a significant effect on anthocyanin extractability and it strongly depends on the ripening stage and the geographical origin of the grape. Taking the results of skin break force, no clear relationship was found between F_{sk} and cell maturity indices in the context of water deficit treatments. It seems that under this experimental condition, anthocyanin extractability is influenced by the actual water status of the grapevine rather than skin textural properties. Indeed, there were no significant differences in CMI indices between the control and WD1 berries; however there were differences in several texture parameters (Sp_{sk} , F_{sk} , E_{sk} , W_{sk}) between the two treatments.

Measurements of seed mechanical properties presented a significantly lower F_s in WD1 seeds compared to the control and the WD2. In addition, water deficit after veraison resulted in harder seeds compared to the control. These results are in agreement with our previous study and it has been already discussed (ZSÓFI *et al.* 2014). In parallel, the seed maturity index was significantly lower in the WD1, compared to C and WD2. This means that the release of the phenolic components from the seeds was relatively higher in WD1 than WD2 and C. Thus, this suggests that water deficit before veraison increases the extractability of the phenolic components from the seed. This phenomenon is probably due to the changes in berry development under early water deficit and, thus, to the delayed ripening of the berries as well as the seeds. Anyway, under our experimental conditions a lower SMI index accompanied softer seeds, which may suggest some connection between extractability of phenolics from the seeds and its texture parameters. The relationship between seed texture and phenolic extractability is supported by other authors as well. ROLLE *et al.* (2012) examined these parameters during ripening on the 'Cabernet Sauvignon' grape variety. In this study, the authors did not find differences in seed hardness (F_s) and seed break energy (W_s) between the ripening stages. However, Young's modulus of elasticity (E_s) of the seeds showed significant changes during the ripening processes, in parallel with phenol extractability. These results suggest that seed springiness may influence the release of phenolics from the seeds during the winemaking process (ROLLE *et al.* 2012).

Conclusion

Timing of water shortage resulted in significant differences in several physical and quality berry parameters. Berry hardness strongly depends on the timing of water supply and this suggests that pre-veraison water deficit still has an effect on berry water household after rewatering.

In addition, timing of the water shortage has a significant effect on the skin and seed texture parameters. It seems that post-veraison water deficit has a negative effect on anthocyanin extractability. In contrast, the anthocyanin extractability was higher under pre-veraison water deficit when the skin thickness was lower. Seed hardness was the lowest when the plants were submitted to early water deficit and this was accompanied by higher phenolic release. In order to better understand the relationships between water deficit, berry texture properties and phenol extractability further investigations are needed.

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