

Irrigation and Leaf Removal Effects on Polyphenolic Content of Grapes and Wines Produced from cv. 'Agiorgitiko' (*Vitis vinifera* L.)

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Abstract

Vitis vinifera L. cv. 'Agiorgitiko' is one of the most important red grape varieties of Greece, cultivated almost exclusively in the region of Nemea in north-eastern Peloponnese. This work aimed to study the influence of some commonly applied viticultural practices on the polyphenolic composition of 'Agiorgitiko'. Leaf removal at veraison, irrigation, and a combination of both, were applied and the phenolic content of the grapes and of the produced wines was compared. The results showed that leaf removal decreased berry size, enhanced total anthocyanin, total phenol and malvidin 3-*O*-monoglucoside accumulation in skins and increased the amount of extractable anthocyanins in the juice. The combination of irrigation and leaf removal caused a significant increase in total phenols in the skin and in the amount of extractable anthocyanins in juice. As far as the produced wines were concerned, color intensity, tannin content and total polyphenols were increased due to leaf removal. Both irrigation and leaf removal resulted in wines with the highest concentration of malvidin 3-*O*-monoglucoside, although neither practice resulted in any significant difference in anthocyanin concentration of the wines. Vines where only irrigation was applied produced berries with reduced extractable anthocyanins, increased seed total phenols and lower wine total tannins. The study showed that increasing cluster sun exposure of 'Agiorgitiko' vines may be, overall, beneficial to the quality of the produced wine.

Keywords: anthocyanins, defoliation, irrigation, phenolic compounds, tannins

Introduction

Several strategies have been applied in order to increase the content of phenolic compounds in wine during the process of grape growing and wine making. Most of the viticultural practices applied in the vineyard are focusing on increasing light penetration and air circulation into the canopy because of the benefits that they present: reduced humidity (Haselgrove *et al.*, 2000), reduced risk of fungal and bacterial infection (Emmett *et al.*, 1992), increased berry temperature and possibly increased vine development, metabolite accumulation and wine quality (Bordelon *et al.*, 2008).

However, fruit zone defoliation effects on grape composition are not always consistent, depending on timing, severity of application, and grapevine genotype. Defoliation, either pre-bloom or post-bloom, has been adopted as an effective means for both yield control and wine quality improvement. The positive effect of pre-bloom leaf removal on grape composition has been often attributed to lower

cluster and berry size at harvest. Moreover, cell division in the berry skin seems to be sensitive to temperature, hence, exposed grapes have thicker berry skin and increased skin-to-pulp ratio (Palliotti *et al.*, 2011). At the whole vine level, pre-bloom defoliation was reported to increase leaf area-to-fruit ratio due to reduced fruit set and/or berry size and to leaf area recovery after veraison (Intrieri *et al.*, 2008). In contrast, fruit zone defoliation at berry set was found to reduce whole vine photosynthesis at an early stage (Petrie *et al.*, 2003). Moreover, post-bloom defoliation was reported to be ineffective in lowering cluster weight and berry number per cluster in 'Graciano' and 'Carignan', whereas final total leaf area per shoot was reduced, with no evident compensation for lateral leaf area (Tardaguila *et al.*, 2010).

In cool climates, increased sunlight exposure is related to enhanced anthocyanin and phenolic production (Ristic *et al.*, 2007). However, it is under consideration whether this practice can be beneficial to wine quality in warm to hot and sunny climates, since extended cluster exposure could sharply increase berry temperature

and thus reduce the anthocyanin content of the grapes (Lorrain *et al.*, 2011). Moreover, excessive sunlight exposure has been known to occasionally cause sunburn cluster damage without increasing total soluble solids or anthocyanin accumulation (Chorti *et al.*, 2010). The results reported by Petropoulos *et al.* (2011) regarding 'Agiorgitiko' wines suggested that the extreme hot and dry conditions may have a greater effect on grape phenolic content than any other viticultural practice, and therefore, viticultural practices increasing light exposure of grape bunches may not be beneficial for the wine quality.

In general, the results regarding leaf removal are variable since they may be influenced by many parameters such as training system, fruit load, vine age, fertility, cultivar, rootstock, irrigation practice and macroclimate (Main and Morris, 2004).

Irrigation management seems to be another important factor, and the most controllable one as it is, determining grape and wine quality (Feres and Evans, 2006), especially in arid and semi-arid areas, with the primary focus on grape phenolic compounds (Kennedy *et al.*, 2002; Ojeda *et al.*, 2002). Koundouras *et al.* (2009) found that water limitation, especially pre-veraison, notably increased anthocyanin concentration in skin, affecting biosynthesis directly, with the greater effect on malvidin 3-*O*-monoglucoside (Mlv).

However, studies have often shown contradictory effects of water on berry components, mainly because of different irrigation volumes, period of water application and environmental conditions, leading to variations in water availability (Koundouras *et al.*, 2009). Studies have shown that severe water deficit that usually occurs under semiarid conditions could be detrimental to fruit quality because of inadequate leaf area to ripen fruit or excessive grape exposure to sunlight leading to lower pigmentation in grapes as a result of increased berry temperature (Price *et al.*, 1995).

'Agiorgitiko' is one of the most important indigenous red grape varieties cultivated in Greece. In its region of origin, Nemea, 'Agiorgitiko' gives Denomination of Origin, deeply colored red wines (Koundouras *et al.*, 2006). The wine region of Nemea, where 'Agiorgitiko' is almost exclusively grown, has a Mediterranean type climate, characterized by high temperatures and water deficiency during the summer season. There is lack of information concerning the relationship between irrigation and leaf removal and wine quality due to the uniqueness of this grape variety and its limited cultivation area. In this study, the above practices were applied in the vineyard and the quality of the produced wines was determined based on two quality parameters of red wines, anthocyanin content and tannin composition.

Materials and Methods

Experimental conditions

The experiment was conducted in 2010 in a 12-year old commercial vineyard in Leontio sub-region of Nemea (north-eastern Peloponnese, Greece), at an altitude of 300 m above sea level. The soil in the location of the vineyard is clay and deep with good nutrient availability and water-retention capacity.

The vineyard was planted with *Vitis vinifera* L. cv. 'Agiorgitiko' grafted onto 1103P rootstock. Vine spacing was 1 m and row spacing was 2.5 m and the vines were trained to a bilateral cordon Royat consisting of four spurs per cordon, pruned to two buds per spur.

Four different treatments were applied: control (C), irrigation (I), leaf removal (LR), combination of irrigation and leaf removal (I/LR). Leaf removal consisted in removing all leaves around the

clusters at veraison, on both sides of the row, in order to obtain maximum sunlight exposure. Irrigation was applied by a drip irrigation system (one emitter every 33 cm) based on crop evapotranspiration with one application of 200 mm ha⁻¹ on 4/8/2010. Apart from leaf removal, canopy management was similar for all treatments and included shoot tucking and positioning and shoot topping to about 1.2 m above the bottom wire.

All four treatments were replicated four times. Harvest was conducted simultaneously for the four treatments on 15/9/10. On the same day, 750 berries were sampled from each replicate of each treatment. The sampled berries from two replicates of each treatment at a time were united, thus providing two replicates for each of the four treatments, which were subsequently vinified separately.

Phenolic content of grapes

Homogenized berries

A number of 50 berries from each treatment were homogenized using Ultra Turrax T25 at 24,000 rpm for 1 min. Total phenols and anthocyanin content were measured according to Iland *et al.* (2004); 1 g of the homogenate (in triplicate) was transferred into a centrifuge tube. An amount of 10 mL 50% v/v aqueous ethanol, pH 2, was then added and mixed for 1 h. After centrifugation at 3,500 rpm for 10 min, 0.5 mL of the supernatant was added to 10 mL 1M HCl and mixed thoroughly. After 3 h, absorbance at 520 nm and 280 nm was recorded.

Anthocyanin content, extractable anthocyanins and % contribution of skin and seed to total berry tannins (skin% and seed% respectively) were determined as described by Ribereau-Gayon *et al.* (1999), slightly modified. An amount of 20 g of the homogenate was macerated for 4 h with two different buffers. The pH of the first buffer was set at 1 while the pH of the second at 3.6. After 4 h, the macerated samples were centrifuged (4,000 rpm, 10 min) and then the anthocyanin and total phenolic content was measured on the supernatant.

Seed and skin extracts

Seeds and skins of 150 berries were removed by hand from grapes. Then they were freeze-dried and finally were ground to obtain fine powder.

The extraction of skin and seed tannins was carried out according to previously reported methods (Lorrain *et al.*, 2011). A 3 g portion of the obtained powder was firstly extracted with 25 mL mixture of acetone/water (80:20, v/v) for 3 h and then with 25 mL mixture of methanol/water (60:40, v/v) for 2.5 h. The centrifugal supernatants were combined and evaporated under reduced pressure at 30 °C to remove organic solvents; the residue was dissolved in water and lyophilised to obtain a crude tannin extract.

A part of the crude extracts was re-dissolved in a model solution (12% ethanol; 5 g L⁻¹ tartaric acid; pH 3.5 adjusted with 1 N NaOH) for the determination of Total Phenol Content by Folin Ciocalteu method (Waterman and Mole, 1994), antioxidant activity (Brand-Williams *et al.*, 1994) and total tannin estimation through the protein precipitation assay using bovine serum albumin- BSA (Harbertson *et al.*, 2003). Absorbance measurements were recorded on a Jasco V-530 UV/VIS spectrophotometer.

Anthocyanins were extracted with acidified methanol (0.1% HCl 12 N) from 1 g of dried skin powder at three successive

times (for 4, 18 and 24 h). After centrifugation, the supernatants were combined and analysed for total anthocyanins (Ribereau-Gayon *et al.*, 1999). HPLC analysis was carried out for the determination of monomeric anthocyanins on a Ristek pinnacle II C18, 250 × 4.6, 5 μm at a flow rate of 1 mL min⁻¹, using a 10 μL injection volume, detection at 520 nm, and the following elution program: 90% eluent A for 1 min, then from 90% to 50% in 22 min, from 50% to 5% in 10 min, which was kept isocratic for further 2 min. Eluent A was 10% aqueous formic acid and eluent B methanol. Identification and quantification was performed by establishing calibration curve for malvidin 3-O-monoglucoside (Mlv). Results were expressed as μg Mlv per g dry skin weight. All analyses were performed in triplicate.

Wines

The grapes used for the production of the experimental wines were harvested at their optimum technological maturity as judged by indices of sugar content (remain constant for two consecutive days). Duplicate fermentations were performed for each treatment. After crushing and destemming, 60 mg L⁻¹ SO₂ (as potassium metabisulfite) was added to the grapes. Lyophilized yeasts of the commercial strain F10 Laffort at 20 g hL⁻¹, previously hydrated in water (15 min, 38 °C) were also added. Beginning on the second day of fermentation, and for the following days, two punching downs per day were conducted.

After seven days of maceration at controlled temperature (23-25 °C), the wines were pressed and transferred to other tanks and spontaneous malolactic fermentation was completed after approximately three weeks. The wines were raked, cold-stabilized, supplemented with 50 mg L⁻¹ SO₂ (as potassium metabisulfite), filtered, bottled and stored at 15±2 °C in the dark until analysed.

Phenolic content of the wines

In wines several classical analytical parameters (% vol, hue, color intensity, total polyphenols - OD280) were determined after bottling according to the OIV methods (1990). In addition, their total phenolic content by Folin-Ciocalteu (Waterman and Mole, 1994), total anthocyanin content, ionization index, total tannins (Ribereau-Gayon *et al.*, 1999; Harbertson *et al.*, 2003), antioxidant activity (Brand-Williams *et al.*, 1994) and monomeric anthocyanins by HPLC (Kallithraka *et al.*, 2006) were also determined. All analyses were performed in triplicate.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using Statistica Version 7 (StatSoft Inc., Tulsa, OK, USA) and comparison of mean values was performed by Tuckey's honest significant difference (HSD) test when samples were significantly different after ANOVA ($P \leq 0.05$).

Results and Discussion

Growth components

Berries from LR were smaller (Fig. 1), although there was no difference among treatments in seed and skin weight which leads to conclude that they had lower pulp mass compared to the other treatments. According to Kotseridis *et al.* (2012) when leaf removal was applied before bloom, it had no effect on berry size in the case of 'Merlot' and 'Sangiovese' grapes, but reduced it in the

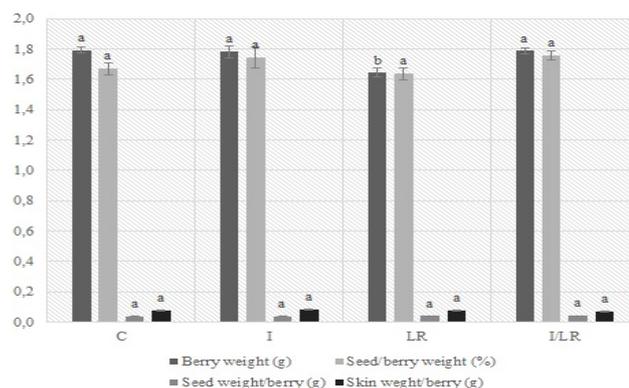


Fig. 1. Growth components measured at harvest. Different letters within a parameter denote significant differences (Tukey's test, $p \leq 0.05$).

case of 'Cabernet Sauvignon' grapes, leading to conclude that the effect may be cultivar-related. Irrigation had no effect on berry size, in contrast with studies showing that irrigation increases berry weight at harvest (Koundouras *et al.*, 2009). Moreover, Kyraleou *et al.* (2016a) observed a significant trend towards smaller berries in non-irrigated vines when compared with the full-irrigated ones. Similar results on the influence of water deficit on berry growth were reported previously for 'Shiraz' (Ojeda *et al.*, 2001), 'Cabernet Sauvignon' (Robby and Matthews, 2004), 'Cabernet franc' (Matthews and Anderson, 1989) and 'Tempranillo' (Santesteban *et al.*, 2011). This reduction was attributed to the decreased cell volume of the pericarp cells in water stressed berries leading to reduced skin cell wall extensibility and, therefore, to a reduced enlargement potential of berries (Kyraleou *et al.*, 2016a). However, water effects on berry components are often contrasting, mainly because of different irrigation volumes and environmental conditions, leading to variations in water availability.

Berry composition

Previous studies have shown contradictory results on anthocyanin accumulation of berries exposed to high light incidence. Although it is generally believed that elevated light exposure enhances anthocyanin accumulation (Dokoozlian and Kliewer, 1996; Jeong *et al.*, 2004), some papers reported a negative (Bergqvist *et al.*, 2001; Haselgrove *et al.*, 2000; Spayd *et al.*, 2002), or no effect whatsoever (Chorti *et al.*, 2010) in anthocyanin content. These differences observed could be attributed to different temperature conditions as well as cultivar specificity. It has been suggested that berry skin temperature has more influence on anthocyanin accumulation than light (Spayd *et al.* 2002; Mori *et al.* 2005; Tarara *et al.* 2008) and that the effect of temperature can vary greatly along developmental stages (Yamane *et al.* 2006). Incident solar radiation increases berry temperature and during the day it can result 11 °C greater or even more compared to berries naturally shaded by leaves, according to the time of day and weather conditions (Kliewer and Lider 1968; Reynolds *et al.* 1986).

In the present study, both treatments where leaf removal was applied (LR and I/LR) showed an increase in anthocyanin and total phenol content both per berry and per g of berry (Table 1). However, high-colored grapes *per se* are not enough in order to obtain high-colored wines, which may be dependent on the ease with which anthocyanins are extracted from grape skins into must

(Romero-Cascales *et al.*, 2005). Anthocyanin extractability is a very important parameter affecting the color of the produced wine. The lower the anthocyanin extractability index (%AE) is the higher the potential amount of anthocyanins extracted. As shown in Table 1, leaf removal (LR and I/LR) did not lead to different %AE compared to control, although it seems that the application of irrigation only results in lower anthocyanin extractability (higher %AE).

The LR treatment showed the highest anthocyanin content potential (total anthocyanins in mg L⁻¹ of juice and extractable anthocyanins in mg L⁻¹ of juice) as shown in Table 1 as well as the highest Mlv content. Kotsieridis *et al.* (2012) also found that leaf removal induced Mlv accumulation in 'Merlot' and 'Cabernet Sauvignon', although it had limited effect in 'Sangiovese'. The increased anthocyanin and Mlv content in vines that underwent defoliation (LR and I/LR) should probably be attributed to an enhanced anthocyanin biosynthesis caused by leaf removal rather than a result of reduced berry size, since the amount of anthocyanins per g of skin was higher in LR (followed by I/LR) and there were no differences observed in skin weight among the treatments, as already mentioned. According to Matus *et al.* (2009), increased exposure to solar radiation enhanced the specific anthocyanin biosynthetic gene encoding UDP-glucose:flavonoid 3-O-glucosyltransferase in 'Cabernet Sauvignon'. Similar results were obtained by Pertopoulos, *et al.* (2011) who observed that the concentration of malvidin-3-O-glucoside and its esters of 'Agiorgitiko' wines were positively influenced by leaf removal.

Irrigation and leaf removal also enhanced the contribution of both seed and skin tannins (Table 1).

Many studies using artificial shading, defoliation and other techniques have shown alterations in the production of tannins and anthocyanins (Bavaresco *et al.*, 2008; Tarara *et al.*, 2008). In cool climates, increased light penetration is related to enhanced anthocyanin and phenolic production (Ristic *et al.*, 2007). However it is under consideration whether this practice can be beneficial to wine quality in warm to hot and sunny climates (Dry *et al.*, 2009), since extended bunch exposure could sharply increase bunch temperature and thus reduce the anthocyanin content of grapes (Lorrain *et al.*, 2011).

Seed and skin extracts

The combination of leaf removal and irrigation caused a decrease in total tannin content of skins, but it had no similar effect on the seed tannin content (Table 2). No significant differences were observed among the other treatments. This result is in agreement with the results of a study conducted with 'Shiraz' grapes, where it was shown that at harvest, there were no significant

differences between the tannin content of shaded and non-shaded grapes (Downey *et al.*, 2003).

Regarding total phenolic content (TP), seeds on average contained higher amounts than skins (Table 2) in agreement with the findings of Kyrleou *et al.* (2016b). Moreover, irrigation (I) resulted in higher TP in seeds, while there were no differences among treatments in total phenolic content of the skins. According to Kyrleou *et al.* (2016b), irrigation had a significant effect on TP of both skin and seed samples. All irrigated samples contained significantly higher TP amounts compared to non-irrigated ones, indicating that irrigation may have a positive effect on total phenolic content of grapes.

Regarding total tannin content, seeds were richer than skins (Table 2), in agreement with the results presented previously (Kyrleou *et al.*, 2016b). Moreover, Kyrleou *et al.* (2016b) reported that the fully-irrigated seed samples were significantly richer in total tannins than the non-irrigated ones (in the first year of their study) while during the second year, no significant differences were observed among the two different irrigation regimes. In skin samples, irrigation did not result in any significant differences in their total tannin content during the first year of the study, while during the second year of the experiment irrigated skin samples were richer than non-irrigated ones.

In previous studies, water deficit did not alter the concentration of seed tannins in 'Shiraz' (Roby *et al.*, 2004) and 'Cabernet Sauvignon' (Koundouras *et al.*, 2009), while in two recent studies, irrigation resulted either in decreased content of tannins in 'Cabernet Sauvignon' seeds (Casassa *et al.*, 2015) or increased content of both seed and skin tannins in 'Shiraz' grapes (Bonada *et al.*, 2015).

Finally, the different treatments seem to have no effect on the antioxidant activity of neither seeds nor skins (Table 2), consistent with the results reported by Kyrleou *et al.*, (2016a) where no significant differences were detected concerning the antioxidant activity among the irrigated and non-irrigated samples, indicating that irrigation did not significantly affect this parameter.

Wine composition

As shown in Table 3, the vines of the LR treatment produced the wine with the highest color intensity, the highest ionization index and the lowest color hue, similar to the results reported by Gubler *et al.* (1992), Zoecklein *et al.* (1992) and Percival *et al.* (1994).

Although some differences were observed in total anthocyanin content of grapes, as shown above, this was not mirrored in the wine produced by those berries, since there were no differences in the anthocyanin content among treatments (Fig. 2). This could be

Table 1. Phenolic potential of berries

Parameter	C	I	LR	I/LR
Anthocyanins (mg/berry)	1.553±0.0222a	1.472±0.0379a	1.714±0.051b	1.558±0.037a
Anthocyanins (mg/berry g)	0.799±0.0133a	0.801±0.0184a	0.973±0.0266b	0.834±0.019a
Total Phenols (au/berry)	2.27±0.053a	2.32±0.062a	2.60±0.078b	2.43±0.048ab
Total phenols (au/berry g)	1.168±0.0326a	1.265±0.027ab	1.478±0.0338c	1.303±0.024b
Anthocyanin extractability (%AE)	27.012±0.892ab	35.966±1.367c	30.969±1.029b	24.84±0.760a
Total anthocyanins (mg L ⁻¹ juice)	350.36±2.21ab	342.94±14.24a	448.36±3.97c	374.37±4.07b
Extractable anthocyanins (mg L ⁻¹ juice)	255.66±2.81b	219.00±8.01a	309.44±4.77d	281.24±2.74c
mg malvidin g ⁻¹ dw skins	9.65±0.21c	8.43±0.16d	12.91±0.37a	11.02±0.36b
% contribution of seed tannins (seed%)	63.07±0.538b	73.61±0.779a	75.89±0.668a	75.58±0.581a
% contribution of skin tannins (skin%)	18.17±0.154b	26.38±0.779a	24.10±0.668a	24.41±0.581a

Note: Different letters within a row denote significant differences (Tukey's test, p ≤ 0.05). au, absorbance units; dw, dry weight

Table 2. Seed and skin extracts components

Parameter	C	I	LR	I/LR
Total tannins (mg catechin g ⁻¹ dw seeds)	34.78 ± 1.35a	34.85 ± 1.15a	37.05 ± 1.03a	37.01 ± 0.85a
Total tannins (mg catechin g ⁻¹ dw skins)	11.72 ± 0.342a	11.91 ± 0.409a	12.21 ± 0.417a	8.02 ± 1.157b
Total Phenols (mg gallic acid g ⁻¹ dw seeds)	96.51 ± 1.75a	105.84 ± 1.33b	94.21 ± 1.63a	97.19 ± 1.13a
Total phenols (mg gallic acid g ⁻¹ dw skins)	54.87 ± 1.116a	56.34 ± 1.912a	55.34 ± 1.616a	51.71 ± 0.908a
Antioxidant activity (mMtrolox g ⁻¹ dw seeds)	0.175 ± 0.002a	0.179 ± 0.002a	0.181 ± 0.003a	0.183 ± 0.001a
Antioxidant activity (mMtrolox g ⁻¹ dw skins)	0.20 ± 0.006a	0.21 ± 0.004a	0.20 ± 0.004a	0.1758 ± 0.026a

Note: Different letters within a row denote significant differences (Tukey's test, $p \leq 0.05$). au, absorbance units; dw, dry weight.

Table 3. Wine color components

Parameter	C	I	LR	I/LR
Ionization index %	12.54 ± 0.56c	12.32 ± 0.41c	22.97 ± 1.47a	16.38 ± 0.39b
Color hue	0.690 ± 0.015a	0.698 ± 0.011a	0.634b	0.67 ± 0.01ab
Color Intensity	4.71 ± 0.08c	4.74 ± 0.02c	7.73 ± 0.34a	5.83 ± 0.12b

Note: Different letters within a row denote significant differences (Tukey's test, $p \leq 0.05$).

a result of low extractability counterbalancing the higher anthocyanin content. The concentration of malvidin-3-glucoside is higher in I/LR treatment only in comparison to control.

Berries from LR treatment had the highest total polyphenols, followed by I/LR (Fig. 2). Those same two treatments were the ones with the highest total tannin content. Irrigation alone, on the other hand, led to the lowest total tannin content, in contrast with other study (Bonada *et al.*, 2015) that suggests that water availability has no effect on the content and concentration of tannins, phenolic substances and skin- and seed- derived tannins. Leaf removal seems to have caused a decrease in total phenols (expressed as gallic acid equivalents).

Conclusion

Leaf removal and irrigation are two viticultural practices widely applied in order to achieve better quality in grapes and wine. The results of this study demonstrate that the leaf removal of Agiorgitiko vines resulted in smaller grapes with increased total anthocyanin, total phenolic and malvidin 3-O-monoglucoside contents and in wines with higher color intensity and total tannin concentration. Moreover, when irrigation was combined with leaf removal anthocyanin extractability was increased significantly. Vines where only irrigation was applied produced berries with reduced anthocyanin extractability, increased seed total phenols and decreased wine total tannins. Overall, increasing cluster sun exposure of 'Agiorgitiko' vines may be, beneficial to the quality parameters of the produced wine.

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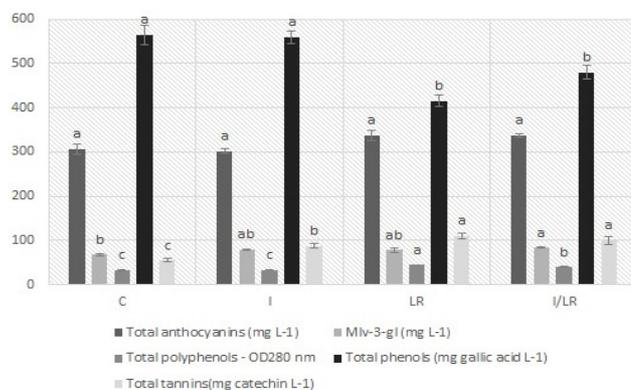


Fig. 2. Wine components measured at harvest. Different letters within a parameter denote significant differences (Tukey's test, $p \leq 0.05$).

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