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Effect of viticulture practices on concentration of polyphenolic compounds and Total Antioxidant Capacity of Southern Italy red wines

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**ABSTRACT**

This study aims to assess the effect of three wine grape varieties, three training systems and two bud loads on the Total Antioxidant Capacity (TAC) and polyphenolic composition of Southern Italy red wines produced, during two vintages. Primitivo, Malvasia nera of Brindisi-Lecce and Montepulciano as grape varieties, single Guyot (SG), single spur pruned low cordon (SLC) and single spur pruned high wire cordon (HSLC) as training systems, 8 and 12 buds/plant as bud loads were compared. Significant differences in polyphenolic families are shown by the grape varieties and modifying the vine growing practices. Moreover, results demonstrated that varieties influence the TAC (indicating the Malvasia as the more effective one), that SLC leads to the lowest level of TAC and, that 8 buds/plant increases it. The relationship between antioxidant indexes and the concentration of single polyphenolic families was evaluated finding the highest correlation degrees with total polyphenols and proanthocyanidins family.

**Keywords:** Total Antioxidant Capacity, TEAC, BRAI, red grape, wine, grape variety, polyphenolic compounds, training system, bud load.
1. Introduction

Polyphenolic compounds, as anthocyanins, flavonols, or flavan-3-ols, play an important role in determining the quality of wine, particularly contributing to the sensory characteristics as color and astringency (Arnold, Noble & Singleton, 1980). Moreover, they are recognized to be the main responsible of the beneficial health effects of wine, since they can directly protect the cardiovascular system against the effect of the free radicals produced by human aerobic metabolism (Virgili & Contestabile, 2000; Halpern, Dahlgren, Laakso, Seppanen-Laakso, Dahlgren & McAnulty, 1998).

Apart from technological tools, such as winemaking process (Ricardo-da-Silva, Rosec, Bourzeix, Mourgues & Moutounet, 1992; Spranger et al., 2004) or aging and storage conditions of wine (Pérez-Magariño & González-San José, 2004; Sun et al., 2011; Tsanova-Savova, Dimov & Riharova, 2002), polyphenolic compounds content in wine can be mainly influenced by grape variety and berry ripening degree (Pérez-Magariño et al., 2004; Ricardo-da-Silva et al., 1992), together with vine growing methods and related training systems (Jackson & Lombard, 1993; Pérez-Lamela, García-Falcón, Simal-Gándara, Orriols-Fernández, 2007; Pérez-Magariño et al., 2004; Peterlunger, Celotti, Da Dalt, Stefanelli, Gollino & Zironi, 2002). The effect of the grape ripening degree on wine color and flavanols and anthocyanins level has been assessed (Pérez-Magariño et al., 2004) as well as the importance of training system on Pinot Noir grape and related wine composition. Particularly, anthocyanins content was studied (Peterlunger et al., 2002). However, few researches have been carried out concerning the relationships among the polyphenolic compounds concentration, the Total Antioxidant Capacity (henceforth: TAC) of wine and the viticulture practices. Therefore, this study is aimed to evaluate the relationship between the vine growing practices – namely, training system and bud load level – and both the polyphenolic
composition and the TAC of three Southern Italy red wines, Primitivo, Malvasia nera of Brindisi-Lecce (henceforth: Malvasia B/L) and Montepulciano, in two consecutive vintages (2005 and 2006). The effect of the viticultural practices was evaluated also on yield components and on grape quality.

The polyphenolic compounds were determined by specific spectrophotometric analysis, which are very widespread, due to their simplicity and low cost, to compare the wine polyphenolic changes related to environmental conditions or specific chemical or agrarian treatments or aging (Tabart, Kevers, Pincemail, Defraigne & Dommes, 2010; Baiano, Terracone, Gambacorta & La Notte, 2009; Ough & Amerine, 1988). As for the redox capacity, the TAC was evaluated by way of BRAI (Briggs-Rauscher Antioxidant Index) and TEAC (Trolox Equivalent Antioxidant Capacity) methods. The first one uses the reaction of polyphenolic molecules with the small hydroperoxyl radical HOO’ (Prentesi, Toso & Berto, 2005), whereas TEAC is based on the reduction of a bulky cationic radical, ABTS*+ (2,2’-azinobis-(3-ethylbenzthiazoline-6-sulfate)) (Prior, Wu & Schaich, 2005).

2. Materials and methods

2.1 Viticultural practices

2.1.1 Plant material

The research was carried out in 2005 and 2006 in a vineyard located in the same farm on a flat country (60 m above sea level), in the rural area of Brindisi, Apulia region, Italy (Latitude 40°37’43”32 N; Longitude 17°56’15”36).

The “1103 Paulsen” rootstock (Vitis berlandieri × V. Rupestris) was planted in 2002 and, one year later, V. vinifera L. Primitivo (Zinfandel), a medium - ripening red wine grape, (3rd decay of August -
1st decay of September) Malvasia nera of Brindisi-Lecce (Malvasia Br/Le), and Montepulciano, two medium-late ripening red wine grape (2nd-3rd decay of September) were grafted on it. The plant density was 6.250 vines ha\(^{-1}\) and the vine spacing was 2.0 m between rows and 0.8 m within each row. The rows were oriented NE-SW. The site was characterized by clay-sandy soil (sand 49.7%, clay 16.4% and silt 34%) deep and very homogeneous, with a 0 - 1% slope. The organic matter content was 1.3%.

Two bud-loads - 8 buds/plant (8b/p) and 12 buds/plant (12 b/p) - and three training systems - single Guyot (SG), single spur pruned low cordon (SLC) and single spur pruned high wire cordon (HSLC), with cordon wire height at 150 cm - were imposed. The vines were cane pruned in SG and spur pruned in both, SLC and HSLC. Varieties, bud loads and training systems were arranged as a split plot design with three replications. The variety represented the main plots (Primitivo, Malvasia B/L, Montepulciano; three randomized levels); training system (SG, SLC and HSLC; three randomized levels) represented the subplots; and bud load (8b/p and 12b/p; two randomized levels) represented the sub-subplots.

Each of the three replications consisted of 36 rows of 180 vines. The main plot (variety) included 12 rows of 240 vines each, whereas the subplot (training system) was made by 4 rows of 120 vines each and sub-subplot (bud-load) was 4 rows of 60 vines each. Only the central 50 vines of the middle two rows for each subplot were used to collect the experimental data. In the area a Mediterranean climate prevails, with an annual rainfall of about 650 mm, so water irrigation is necessary mainly from June to September. Irrigation is required because rain generally falls in the dormant phase of the growing season during which the water soil profile is not sufficient to supply the vineyard evapotranspiration.

Vineyards were irrigated according to a controlled water deficit (CWD), which counterbalanced about 24% of crop evapotranspiration. Starting 10 days after the end of veraison (at 100% of berry coloring) and until harvest, all vineyards were irrigated 4 times and the interval between irrigation cycle was approximately 15 days. A volume of 150, 200, 150, 100 m\(^3\) ha\(^{-1}\) of water in the scheduled irrigation was applied on 12th and 31st July, 15th August, and 10th September in 2005 whereas on 20th July, 5th
and 20\textsuperscript{th} August, and 15\textsuperscript{th} September on 2006. Primitivo on 19\textsuperscript{th} and 25\textsuperscript{th} September was harvested in 2005 and 2006, respectively. Both, Malvasia and Montepulciano, on 7\textsuperscript{th} and 12\textsuperscript{th} October were harvested in 2005 and 2006, respectively. Grapes were harvested at commercial maturity and during each harvest time, grapes coming from the training systems and the bud loads were harvested together.

2.1.2 Plant water status

From the middle of July to the harvest time the midday stem water potential ($\psi_s$) was measured in the two years of study (2005 and 2006) at the same stages (pre and post irrigations), and mean values are reported in Table 1. The measures were performed on two mature and non-transpiring leaves per vine, that had been bagged with plastic sheets inserted into aluminum foil at least 1 h before measurement. The bagging used to prevent the leaf transpiration, equaling the leaf water potential to the stem water potential (Begg & Turner, 1970). Leaves were then detached and their $\psi_s$ was measured immediately in the field by a model 600-pressure chamber instrument (PMS Instrument Company, Albany, Oregon, USA). The measurements of stem $\psi$ were collected during the steady period of the water potential diurnal curve (from 11.00 am to 14.00 pm).

2.1.3 Yield and fruit components.

Yield (kg vine\textsuperscript{-1}) was determined at harvest averaging five vines yield per sub-subplot at harvesting. The mean cluster weight was calculated on 30 clusters (10 clusters per replicate, sampled from 10 different vines). The average berry weight was determined on 150 berries (50 berries per replicate). then the berries were hand-crushed, and the juice was collected and centrifuged at 1556 g for 5 min at 20\textdegree C, to measure soluble solids by a portable refractometer (Atago PR32, Norfolk, Virginia, U.S.A.). Titratable acidity (expressed as g/L of tartaric acid) was analyzed by titration according to the EEC Regulation 2676/90. The juice pH was also measured. In winter, the pruning weight of five vines for
each replicate was determined and the Ravaz Index (Kliewer & Dokoozlian, 2005; Ravaz, 1903) and
the yield – pruning weight ratio was calculated.

2.1.4 Sampling and winemaking

Grapes were harvested and processed separately in the same wine cellar. For each treatment, about 50
kg of grapes were destemmed/crushed and collected in stainless steel 50 L tanks. Before starting the
fermentation, the crushed grapes were supplied with potassium metabisulphite, 6 g/100 kg (equivalent
to 30 mg/kg of total SO$_2$) and dry yeasts, Activeflore C F33 (Laffort, Floirac, France), 20 g/100 kg.
During the alcoholic fermentation, two daily gentle punching down of the cap of the pulp into the
fermenting juice were made. After running-off and light pressure of the marcs, the wine was
inoculated (25 g/100 L) by a commercial strain of Oenococcus oeni, Uvaferm alpha (Lallemand
oenologie, France), and placed at 20 °C. After the malolactic fermentation, the wine was decanted and
a second aliquot of potassium metabisulphite (equivalent to 30 mg/kg of total SO$_2$) was added. Finally,
after a second decantation performed in March-April the wine was bottled in 1 liter glass bottles with a
crown cap and stored in dark condition at room temperature. For each vintage, the analyzes were
conducted within a year, starting from the sixth month after bottling.

2.2 Analytical procedures

2.2.1 Chemicals

Folin-Ciocalteu reagent, hydrogen peroxide (30 % w/w), malonic acid (purity grade >99%),
gallic acid (purity grade >98%), sodium pyrosulfite (purity grade 98%) and ethanol
(analytical grade 99.8%) were purchased from Fluka (ordered by Sigma Aldrich, Milan,
Italy), and Na$_2$CO$_3$ (purity >99%) was from Merk (Darmstadt, Germany). Ethanol 96%,
methanol 99%, hydrochloric acid 37%, sulfuric acid 95%-97%, o-vanillin (purity 99%), (+)-catechin monohydrate (purity 98.5%), malvidin-3-glucoside chloride (purity >90%), cyanidin chloride (purity >95%), iron(II) sulfate heptahydrate (purity >99.0%), ABTS, 2,2’-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid), diammonium salt (purity grade >98%) and Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (purity grade >98%) were Sigma-Aldrich products (Milan, Italy). Potassium periodate (purity grade 99.7-100.4%) and starch were from Riedel-de Haën (ordered by Sigma Aldrich, Milan, Italy). Manganese sulfate monohydrate was from Carlo Erba Reagents (Milan, Italy).

2.2.2 Spectrophotometric measurements of polyphenolic compounds
Phenolic compounds in wine were determined by means of a Jasco V-550 Uv-vis double-beam spectrophotometer (quartz, optical path length 1.000 cm; JASCO International Co., Ltd., Tokyo, Japan).

The Folin–Ciocalteu method (Ough et al., 1988) was used for the analysis of the total polyphenolic compounds (TPP) here expressed as mg/L of gallic acid equivalent. The determination of the total flavonoids (TF), total anthocyanins (TA), flavonoids without anthocyanins (FNA) and proanthocyanidins (PrA) were realized following the methods of Di Stefano, Cravero and Gentilini (1989). The content of TF and FNA is expressed as (+)-catechin equivalents (mg/L), the TA are expressed as malvidin-3-glucoside equivalents (mg/L) and the PrA as cyanidin chloride (mg/L). The flavan-3-ols (F) were quantified as described by Baiano et al. (2009)and are expressed as (+)-catechin equivalents (mg/L). Other experimental details are reported in the Supplementary Material file.

2.2.3 BRAI measurement
The Briggs-Rauscher (BR) oscillating reaction was used to measure the total antioxidant ability of each wine; the BR test environment is prepared mixing the reagents as fully described (Prenesti et al., 2005 and ref. therein). The oscillating regime was potentiometrically monitored using a combined platinum electrode (Metrohm mod. 6.0402.100 (LE)) and a Metrohm automatic computer-assisted potentiometric apparatus (Basic Titrino 794). The reaction temperature was controlled (25°C) by means of a thermostat (model DI-G Haake). When antioxidant free-radical scavengers (i.e. wine samples) were added to an oscillating BR mixture, a break of the oscillating regime occurred, and the corresponding so-called “inhibition time” ($t_{\text{inhib}}$) varied linearly with their concentration.

The values of $t_{\text{inhib}}$ of reference standard solution (gallic acid) and wine samples were plotted versus the mass of gallic acid, for the calibration, or the volume of wine injected in the BR mixture, respectively, obtaining linear trends. The experimental points were fitted with straight-lines equations. BRAI (Briggs-Rauscher Antioxidant Index) was expressed as the ratio of the slopes of the straight-line equations obtained for each sample, $\text{slope}_{\text{sample}} = \frac{t_{\text{inhib}}}{\text{mL}_{\text{sample}}}$, and for the standard molecule, $\text{slope}_{\text{standard}} = \frac{t_{\text{inhib}}}{\text{mg}_{\text{gallic acid}}}$. The ratio of slopes thus obtained, $\frac{\text{mg}_{\text{gallic acid}}}{\text{mL}_{\text{sample}}}$, is finally referred to 100 ml of beverage (Prenesti et al., 2005).

2.2.4 TEAC measurement

As previously described (De Beer, Joubert, Gelderblom & Manley, 2003), the ABTS$^{+}$ radical solution was prepared, every six days, by ABTS oxidation with peroxodisulfate ion whilst the Trolox stock solution was prepared in ethanol. Two mL of ABTS$^{+}$ solution and 20 µL sample or Trolox standard solution were added into the spectrophotometric cell. As the reducing
compounds, contained in both the standard and wines, turn the ABTS$^{+*}$ solution from intense blue to colorless, the absorbance decrease at 734 nm after 4 minutes of reaction at room temperature was recorded. The values of $A_{734}$ were plotted versus the mass of Trolox, for the calibration, or the volume of wine, obtaining linear trends. The experimental points were fitted with straight-lines equations. TEAC was expressed as the ratio of the slopes of the straight-line equations obtained for each sample, $\text{slope}_{\text{sample}} = A_{734}/\mu L_{\text{sample}}$, and for the standard molecule, $\text{slope}_{\text{standard}} = A_{734}/\mu g_{\text{Trolox}}$ (Greco, 2013). The ratio of slopes thus obtained, $\mu g_{\text{Trolox}}/\mu L_{\text{sample}}$, is finally referred to 100 mL of beverage. This analytical procedure allows to compare TEAC values obtained from different wine dilutions and to check the linearity of the analytical response.

2.3 Statistical analysis

Analysis of variance (ANOVA) was made using a three-way model to separate the effect of variety, training system and bud-load and their significant interactions. F test was used to compare the averages within the bud-load factor. The Fisher LSD multiple range test was used to compare the means within variety and training system and to compare the significant interactive effects. The correlations and the regression analyses between the TAC estimated according to the two methods and the polyphenolic compounds, as well as ANOVA and Fisher LSD test, were performed using the software Statistica ver. 6.1 (Statsoft, Tulsa, UK).

3. Results and discussion

3.1 Yield components and fruit composition
Yield components and qualitative parameters of grapes, as affected by variety, training system and bud-load, and mediated along the two vintages (2005 and 2006), are reported in Table 2. A significant influence of the variety was found; Montepulciano and Primitivo grapes showed high level of total acidity and yield component together with the consequent lower soluble solids content. Conversely, Malvasia Br/Le was characterized by the highest concentration of sugars and lower total acidity.

Considering that Malvasia vine had water stress index ($\psi_s = -1.14$) higher than that of Primitivo and Montepulciano ($\psi_s = -1.24$ and -1.29, respectively), this finding seems in agreement with Reynolds, Lowrey, Tomek, Hakimi & De Savigny (2007), who observed an increment of sugar content in grapes of an Ontario Chardonnay vineyard characterized by the decrease of $\psi_s$ during its growth cycle. Furthermore, Malvasia variety showed the lowest Ravaz Index value to highlight less capability than Primitivo and Montepulciano in producing the maximum yield per canopy weight.

Several studies about the training system effect on yield and composition of grapes have been conducted with opposite results depending on the agronomic and pedoclimatic conditions. No differences on yield and soluble solids in grapes of Barbera trained on various trellis systems were found (Bernizzoni, Gatti, Civardi & Poni, 2009); conversely, the training system significantly modified the yield, vine size, and canopy density of Pinot Noir and Traminette, although it only slightly influenced the berry composition, such as soluble solids, pH, titratable acidity and monoterpenes (Peterlunger et al., 2002; Bordelon, Skinkis & Howard, 2008). It is worth noting that in this study a very significant ($P < 0.01$) impact of the training system factor on yield, bunch weight, pruning weight and Ravaz Index was revealed. Single spur pruned low cordon (SLC) allowed to produce the highest quantity of grape and the highest bunch weight without exceeding in canopy growth, respect to single armed Guyot (SG). The small canopy development registered in high trellis (HSLC) with the highest yield/pruning weight ratio is to be pointed out; this behavior should be probably related to the canopy architecture and to the height of the cluster zone (which could have favored, as sink, the bunches to the canopy) rather than to the high level of $\psi_s$ (-0.95 and -1.39) registered in the first and second period of the growth cycle, respectively.
Finally, no significant influence on any measured parameters was determined by the bud-load factor.

3.2 Relation between polyphenolic compounds and viticultural practices

The influence of variety, training system and bud-load, together with their interactions, on the polyphenolic compounds content are reported in Tables 3. Very significant differences in polyphenolic families are shown by the variety and training system factors: the highest amounts of compounds was generally quantified in Malvasia Br/Le and Montepulciano or HSLC and SG trellis system. In agreement with the literature data (Esteban, Villanueva & Lissarague., 2001; Crupi et al., 2012), a genetic dependence of total anthocyanins was evident in this research, because the highest levels of these compounds were found in Malvasia Br/Le (361 ± 48) and Montepulciano (382 ± 56), in the two vintages (2005, 2006), irrespective to \( \psi_s \) values (Table 1), training system or bud-load, as confirmed by the absence of significant interactions among the three factors (Table 3).

On the contrary, the water stress and viticultural practices seemed to play a more decisive role on the other polyphenolic compounds.

Indeed, the highest values of TPP (2987 ± 155), TF (2286 ± 135), FNA (1788 ± 135), F (1453 ± 137) and PrA (2947 ± 157) were registered in Malvasia trained on the HSLC system, which were characterized by mild water stress condition (\( \psi_s = -1.30 \)) in the late ripening period. According to the literature (Dokoozlian & Kliewer, 1996; Figueiredo-González, Cancho-Grande, Boso, Santiago, Martínez & Simal-Gándara, 2013), mild \( \psi_s \) values can cause a lower canopy density, with a consequent cluster exposition to sunlight, resulting in an improvement of total polyphenols concentration.

Excepting for total polyphenols, no significant effect by the bud-load factor was observed; on the contrary, a positive interaction between variety and bud-load was observed, with polyphenolic
compounds concentration higher in 8 b/p than in 12 b/p, both in Primitivo and Montepulciano (Table 3).

3.3 Relation between TAC and viticultural practices

Table 3 reports the effect of variety, training system and bud-load on the TAC of wines. Significant differences in the TAC among varieties, training systems and bud load were found, except in the case of the BRAI index in the training system factor. This is probably caused by the high dispersion of the BRAI values due to the difference between the results from the two bud-load levels of the same training system. Both the antioxidant capacity parameters result sensitive to the bud-load, whereas the bud-load variance component did not determine any significant effect on single polyphenolic families. This apparent discrepancy can be explained observing that for the single family the 8 b/p gives higher values for the most of samples but these increments are small and, very probably, with no statistical significance. If all polyphenolic compounds (TPP parameter) are taken into account, as in the case of antioxidant indexes, the small increments become significant, moreover it could exists a synergic effect between polyphenolic molecules, as described in previous studies (Kirakosyan et al., 2010). The more marked differences in varieties were highlighted. The varieties showed two different levels of TAC with BRAI test and three different levels with the TEAC one. Therefore, a dependence of the amount and the antioxidant capacity of polyphenolic compounds from the training system is stated, but its extent is influenced by the variety. This is in accordance with observation of Jackson and Lombard (1993): the quality of wines produced from grape of Cabernet Sauvignon cultivated with different training systems is similar, whereas the color and the aroma of Pinot Noir depend upon the vine growing practices. In particular, for the Pinot Noir differences in polyphenolic compounds content
were observed varying the training system and an increased content of these molecules with the light exposure of berries. Different results were also obtained for Pinot Noir in the Friuli (a region in the North-East of Italy) hills (Peterlunger et al., 2002). In this case, the influence of the training system on the composition of grapes and on wine quality resulted minimal, nevertheless the relevance of the light exposure was confirmed.

The interactions between the factors, reported in Figure 2 and in Table S1 of the Supplementary Material file, show that there is a sensitivity to bud load, in particular for Primitivo, but there is no interaction between variety and training system on both BRAI or TEAC values.

The wines derived from SG and HSLC training systems show higher values of TAC with respect to the wines obtained from SLC method. The relation between each antioxidant index and various concentration values were evaluated by the Pearson test (Table 4). Particularly, a strong correlation between TEAC and TPP, or PrA, or F was found, showing $r$ of 0.957, 0.938 and 0.896 respectively. Conversely, BRAI index showed a weaker significant correlation with the same polyphenol families, never exceeding 0.763 which was the highest Pearson’ coefficient value registered between BRAI and PrA. These results could be linked to the difference in the reaction mechanism involved in BRAI and TEAC methods, as reported in the Introduction. Figure 1 presents the results for the fit of the regression model where each index refers to the most related polyphenolic families and to the total polyphenol content. These results confirm the direct relation between the TAC and the total amount of polyphenolic compounds and, in particular, the TAC seems to be more strictly related to PrA family.

4. Conclusions

The results obtained evidenced that:
i) the grape varieties, the training system and the bud load can affect the polyphenolic compounds concentration of the wine and, therefore, the TAC,

ii) the grape varieties show different sensitivity to the type of viticultural practice.

The training systems and pruning level that provide higher polyphenolic compounds contents and, consequently, higher TAC values were identified. Moreover, training system induces significant differences also in all the polyphenolic families changing the quantitative ratios between them, which are strictly related to the sensory characteristics of wine. These information could be taken into account in both viticultural and oenological fields in order to affect the concentration, the type of polyphenolic substances and the related redox behavior.

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References


Figure 1 Variations of TAC in relation to TPP (a), PrA (b), F (c), TA (d), TF (e) concentration for TEAC index and in relation to TPP (f), PrA (g), F (h), TA (i) concentration or BRAI index.
Figure 2 Significant interactions between bud load and trellis system on BRAI (a), between bud load and variety on BRAI (b) and TEAC (c), between bud load, variety and trellis system on BRAI (d) and TEAC (e). Vertical bars denote 0.95 confidence intervals.
### TABLES

**Table 1** Midday stem ($\psi_s$) water potential values of vines during the 2005 and 2006 vintages, as affected by variety, training system and bud-load. For each stage the two years average value is shown.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Irrigation stage</th>
<th>Pre – irrigation</th>
<th>6 - days after irrigation</th>
<th>Pre – irrigation</th>
<th>6 - days after irrigation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12th July - 5th August (1st - 2nd irrigation)</td>
<td></td>
<td></td>
<td>15th August - 15th September (3rd, 4th irrigation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12th July - 5th August (1st - 2nd irrigation)</td>
<td></td>
<td></td>
<td>15th August - 15th September (3rd, 4th irrigation)</td>
<td></td>
</tr>
<tr>
<td>Primitivo</td>
<td>-1.11 ±0.16(^a)</td>
<td>-0.84 ±0.14</td>
<td>-1.61 ±0.24</td>
<td>-1.24 ±0.21</td>
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<td>Malvasia Br/Le</td>
<td>-0.98 ±0.18</td>
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<td>-1.31 ±0.25</td>
<td>-1.14 ±0.21</td>
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<tr>
<td>Montepulciano</td>
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<td>-0.85 ±0.10</td>
<td>-1.52 ±0.22</td>
<td>-1.29 ±0.10</td>
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</tr>
</tbody>
</table>

**Training system\(^c\)**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Pre – irrigation</th>
<th>6 - days after irrigation</th>
<th>Pre – irrigation</th>
<th>6 - days after irrigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSLC</td>
<td>-0.95 ±0.20</td>
<td>-0.89 ±0.21</td>
<td>-1.36 ±0.27</td>
<td>-1.25 ±0.24</td>
</tr>
<tr>
<td>SLC</td>
<td>-1.13 ±0.08</td>
<td>-0.96 ±0.15</td>
<td>-1.68 ±0.26</td>
<td>-1.32 ±0.20</td>
</tr>
<tr>
<td>SG</td>
<td>-1.07 ±0.13</td>
<td>-0.81 ±0.12</td>
<td>-1.43 ±0.12</td>
<td>-1.27 ±0.27</td>
</tr>
</tbody>
</table>

**Bud load**

<table>
<thead>
<tr>
<th>Variety</th>
<th>Pre – irrigation</th>
<th>6 - days after irrigation</th>
<th>Pre – irrigation</th>
<th>6 - days after irrigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 buds/plant</td>
<td>-1.18 ±0.24</td>
<td>-0.90 ±0.28</td>
<td>-1.53 ±0.24</td>
<td>-1.40 ±0.18</td>
</tr>
<tr>
<td>8 buds/plant</td>
<td>-1.08 ±0.18</td>
<td>-0.75 ±0.27</td>
<td>-1.51 ±0.22</td>
<td>-1.38 ±0.23</td>
</tr>
</tbody>
</table>

\(^a\)Means of three replicates;  
\(^b\)Standard deviation at $p \leq 0.05$  
\(^c\)HSLC = single spur pruned high wire cordon; SLC = single spur pruned low cordon; SG = single Guyot.
Table 2 Influence of variety, training system and bud-load on the yield components and the main qualitative characteristics of grapes.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Yield (kg/vine)</th>
<th>s.d.</th>
<th>Bunch weight (g)</th>
<th>s.d.</th>
<th>Berry weight (g)</th>
<th>s.d.</th>
<th>Soluble solids (Brix)</th>
<th>s.d.</th>
<th>pH</th>
<th>s.d.</th>
<th>Titratable acidity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>s.d.</th>
<th>Pruning weight (kg)</th>
<th>s.d.</th>
<th>Ravaz Index</th>
<th>s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variety</strong></td>
<td></td>
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</tr>
<tr>
<td>Primitivo</td>
<td>1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>106.96</td>
<td>27.07</td>
<td>0.86</td>
<td>0.15</td>
<td>21.63</td>
<td>1.78</td>
<td>3.35</td>
<td>0.12</td>
<td>6.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86</td>
<td>0.49&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.18</td>
<td>3.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.02</td>
</tr>
<tr>
<td>Malvasia Br/Le</td>
<td>1.13</td>
<td>0.53</td>
<td>89.91</td>
<td>59.09</td>
<td>0.82</td>
<td>0.15</td>
<td>24.63</td>
<td>1.21</td>
<td>3.56</td>
<td>0.13</td>
<td>4.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.36</td>
<td>0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21</td>
<td>2.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.46</td>
</tr>
<tr>
<td>Montepulciano</td>
<td>1.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.46</td>
<td>111.51</td>
<td>30.52</td>
<td>0.90</td>
<td>0.11</td>
<td>21.00</td>
<td>0.84</td>
<td>3.24</td>
<td>0.12</td>
<td>5.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34</td>
<td>0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.018</td>
<td>5.56&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>n.s.</td>
<td></td>
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</tr>
<tr>
<td>HSLC</td>
<td>1.39</td>
<td>0.40</td>
<td>75.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.81</td>
<td>0.85</td>
<td>0.15</td>
<td>21.73</td>
<td>2.01</td>
<td>3.38</td>
<td>0.19</td>
<td>5.97</td>
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<td>0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13</td>
<td>5.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.15</td>
</tr>
<tr>
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<td>1.78 a</td>
<td>0.57</td>
<td>130.26 a</td>
<td>37.89</td>
<td>0.91</td>
<td>0.15</td>
<td>22.65</td>
<td>2.28</td>
<td>3.41</td>
<td>0.18</td>
<td>5.87</td>
<td>1.28</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21</td>
<td>3.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.35</td>
</tr>
<tr>
<td>SG</td>
<td>1.18 b</td>
<td>0.55</td>
<td>102.34 ab</td>
<td>24.33</td>
<td>0.81</td>
<td>0.11</td>
<td>22.88</td>
<td>1.82</td>
<td>3.37</td>
<td>0.19</td>
<td>5.52</td>
<td>0.86</td>
<td>0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18</td>
<td>2.40&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>n.s.</td>
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<tr>
<td><strong>Bud-load</strong></td>
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</tr>
<tr>
<td>12 buds/plant</td>
<td>1.58</td>
<td>0.62</td>
<td>99.24</td>
<td>24.40</td>
<td>0.84</td>
<td>0.15</td>
<td>21.90</td>
<td>2.24</td>
<td>3.38</td>
<td>0.19</td>
<td>5.57</td>
<td>0.87</td>
<td>0.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.22</td>
<td>3.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.23</td>
</tr>
<tr>
<td>8 buds/plant</td>
<td>1.32</td>
<td>0.48</td>
<td>106.36</td>
<td>54.12</td>
<td>0.87</td>
<td>0.12</td>
<td>22.94</td>
<td>1.77</td>
<td>3.39</td>
<td>0.17</td>
<td>6.01</td>
<td>1.23</td>
<td>0.42&lt;sup&gt;ab&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> expressed as g/L of tartaric acid

<sup>b</sup> Means of three replicates;

<sup>c</sup> Different letters in the same line are significantly different at 5% level (Tukey HSD test);

<sup>d</sup> Standard deviation at p ≤ 0.05;

<sup>e</sup> HSLC = single spur pruned high wire cordon; SLC = single spur pruned low cordon; SG = single Guyot.

* = P<0.05, ** = P<0.01, *** = P<0.001, n.s. = not significant.
Table 3. Total polyphenols (TPP), total flavonoids (TF), flavonoids without anthocyanins (FNA), anthocyanins (TA), flavans (F), proanthocyanidins (PrA), BRAI and TEAC antioxidant indexes in wines as affected by variety, training system and bud-load.

<table>
<thead>
<tr>
<th>Factors</th>
<th>TPP s.d.</th>
<th>TF s.d.</th>
<th>FNA s.d.</th>
<th>TA s.d.</th>
<th>F s.d.</th>
<th>PrA s.d.</th>
<th>BRAI s.d.</th>
<th>TEAC s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Primitivo</td>
<td>2273 b</td>
<td>±325</td>
<td>1931 b</td>
<td>±381</td>
<td>1526 a</td>
<td>±311</td>
<td>278 b</td>
<td>±69</td>
</tr>
<tr>
<td>Malvasia Br/Le</td>
<td>2958 a</td>
<td>±168</td>
<td>2204 a</td>
<td>±157</td>
<td>1678 a</td>
<td>±162</td>
<td>361 a</td>
<td>±48</td>
</tr>
<tr>
<td>Montepulciano</td>
<td>2765 a</td>
<td>±168</td>
<td>1896 b</td>
<td>±153</td>
<td>1302 b</td>
<td>±155</td>
<td>382 a</td>
<td>±56</td>
</tr>
<tr>
<td>Significance</td>
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<td>**</td>
<td>***</td>
<td>**</td>
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<td></td>
</tr>
<tr>
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<td>±430</td>
<td>2019 a</td>
<td>±254</td>
<td>1542 a</td>
<td>±242</td>
<td>302 b</td>
<td>±70</td>
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<td>SLC</td>
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<td>±433</td>
<td>1879 b</td>
<td>±271</td>
<td>1371 b</td>
<td>±213</td>
<td>348 ab</td>
<td>±77</td>
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<tr>
<td>SG</td>
<td>2731 ab</td>
<td>±256</td>
<td>2134 a</td>
<td>±284</td>
<td>1594 a</td>
<td>±299</td>
<td>371 a</td>
<td>±56</td>
</tr>
<tr>
<td>Significance</td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>**</td>
</tr>
<tr>
<td>Bud load</td>
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<tr>
<td>12 buds/plant</td>
<td>2566 ±424</td>
<td>1985 ±293</td>
<td>1471 ±254</td>
<td>335 ±129</td>
<td>1110 ±232</td>
<td>2601 ±547</td>
<td>2795 ±740</td>
<td>425 ±75</td>
</tr>
<tr>
<td>8 buds/plant</td>
<td>2764 ±335</td>
<td>2036 ±281</td>
<td>1533 ±280</td>
<td>345 ±126</td>
<td>1214 ±196</td>
<td>2618 ±419</td>
<td>3003 ±458</td>
<td>450 ±56</td>
</tr>
<tr>
<td>Significance</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
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<tr>
<td>Interactions</td>
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</tr>
<tr>
<td>Variety × T. system</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Variety × Bud load</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
<td>*</td>
<td>n.s.</td>
</tr>
<tr>
<td>T. system × Bud load</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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<td>n.s.</td>
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<td>n.s.</td>
</tr>
<tr>
<td>Variety × T. system × Bud load</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* Values followed by the same letters within columns did not differ significantly at P < 0.05, using the Fisher LSD multiple range test. Not significant interactions are not listed.

b HSLC: single spur pruned high wire cordon; SLC: single spur pruned low cordon SG: single armed Guyot;

For the interactions of BRAI and TEAC see Figure 1 and Supplementary Material file.

* = P<0.05, ** = P<0.01, *** = P<0.001, n.s. = not significant.
Table 4 Correlation analysis of TEAC and BRAI indexes with TPP (Total polyphenols); TF (Total flavonoids); FNA (Flavonoids without anthocyanins); F (Flavan-3-ols); PrA (Proanthocyanidins); TA (Total anthocyanins).

<table>
<thead>
<tr>
<th></th>
<th>TPP</th>
<th>TF</th>
<th>FNA</th>
<th>TA</th>
<th>F</th>
<th>PrA</th>
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</thead>
<tbody>
<tr>
<td>TEAC</td>
<td>0.957195</td>
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<td>0.625571</td>
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<tr>
<td>BRAI</td>
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<td>0.197713</td>
<td>0.719223</td>
<td>0.716563</td>
<td>0.765939</td>
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</tbody>
</table>

Bold values indicate significance at P<0.05