RELATIONSHIPS BETWEEN CHLOROPHYLL CONTENT OF VINE LEAVES, PREDAWN LEAF WATER POTENTIAL AT VERAISON, AND CHEMICAL AND SENSORY ATTRIBUTES OF WINE

Running title: Iron and water status affect wine quality

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Abstract

BACKGROUND: Water deficit and iron deficiency (iron chlorosis) are common environmental stresses, which affect the grapevine production in the Mediterranean area. Studies on the impact of both stresses, when they act simultaneously, are rare. The main objective of the present investigation was to evaluate the combined effects of the incidence of iron chlorosis and the vine water status on quality of Tempranillo wine. For this, twenty non-irrigated vineyard subzones (10 m x 10 m each), from non-affected to moderately affected by iron chlorosis, were monitored in Ribera del Duero area (North-Central Spain) during two consecutive seasons.

RESULTS: Factorial ANOVAs were performed to study the effects of predawn leaf water potential and foliar chlorophyll content, both measured at veraison, on chemical and sensory characteristics of wine. With an impact much greater than water status, the incidence of iron stress decreased pH of the wine and enhanced sensory attributes as tonality, layer intensity, flavour intensity and persistence in the mouth. There were increases in red colour, astringency and persistence of the wine associated to chlorosis, although they might be restricted in water deficit conditions.

CONCLUSION: The results have demonstrated that mild to moderate iron stress can have positive effects on chemical and sensory attributes of Tempranillo wine. Measurements of foliar chlorophyll content at veraison could be very useful to map quality potential in rainfed vineyards affected by iron deficiency.

Keywords: grape, iron chlorosis, quality, Tempranillo, *Vitis vinifera* L., water deficit.

Introduction

Lime induced iron deficiency and water deficit are common environmental stresses in calcareous soils of Mediterranean area, which cause serious economic losses in grapevine production. In different ways, both stresses decline photosynthetic activity in plants. While iron deficiency leads to a decrease in the synthesis of photosynthetic pigments[1] and a lowering of the efficiency of photosystem II,[2,3] water deficit causes stomatal closure, reducing the availability of CO_2 in leaf mesophyll.[4] The loss of photosynthetic capacity generally depresses yield and vigour both in iron[5,6] and water stressed grapevines,[7,8] and reduces the synthesis and accumulation of substances in the fruit during ripening.[9] Nevertheless, moderate stress levels can have positive effects on grape quality, as the plants restrict vegetative growth, obtain less yield and smaller berries, thus concentrating constituents as sugars or anthocyanins in them.[10,11]

Recent studies in Ribera del Duero Designation of Origin (Spain) have demonstrated that mild to moderate iron deficiency can improve phenolic content and aromatic potential of Tempranillo grapes.[12,13] It would be interesting to study to what extent these improvements are transferred to the composition and sensory characteristics of the wine. To the best of our knowledge, there are currently no studies on how iron deficiency affects chemical and sensory quality of wine.

There is limited knowledge regarding the effect of vine water status on the sensory profile of wines and its influence is not clear.[11,14] Most of the studies correspond to irrigation experiments,[15,16] while the effects of spatial variability in vine water status within a non-irrigated vineyard have received limited attention.[17] On the other hand, the study of the relationships between specific attributes of the wine with grape maturity and agronomic performance could provide valuable information for precision viticulture, to improve the management of vineyards affected by iron and water stress.

Sensory properties are major factors affecting quality perception and consumer's acceptance of wines, so that sensory characterization of the wines allows the validation of the sensory impact of agronomic and oenological practices. Different approaches have been used to assess the sensory characteristics of wines. Conventional profiling is often employed since it is able to describe the products with a high level of precision.[18,19] To ensure this, two main phases are needed: the training phase that includes the checking of panel performance, and the measurement phase.

The objectives of this work were (i) to evaluate the additive and interaction effects of the incidence of iron deficiency and the vine water status at veraison on the chemical and sensory characteristics of Tempranillo wine and (ii) to study the relationships between wine quality with vine vigour, size and maturity of the grapes within rainfed vineyards affected by iron chlorosis.

Materials and methods

Study site description and field data collection

The study was conducted in 2016 and 2017 seasons, on 20 non-irrigated vineyard subzones located in Pesquera de Duero (latitude 41º 38’ 34’’N, longitude 4º 09’ 27’’W, Ribera del Duero area, North Central Spain). The subzones (10 m x 10 m each) correspond to Tempranillo variety grafted on 110-Richter rootstock and cultivated under non-irrigated conditions. The vines, 15 to 20 years old, are spaced 3.0 m x 1.5 m (2222 plants ha^{-1}) and trained in a vertical shoot positioning system. Eight spurs per vine, with two buds per spur, were retained during winter pruning.

The study site has Mediterranean climate, with low temperatures in winter and hot and dry summers. The mean annual temperature is 12.3 °C and the total annual rainfall is 427 mm, of which 71 mm correspond to June, July and August. The average temperature registered in 2017 (12.7 °C) was higher than in 2016 (11.9 °C), while the rainfall from 1 April to 30 September was lower (142 mm vs 176 mm). In 2017, late frosts affected irregularly the subzones, restricting the yield.

The soils in the study area have medium to medium-weighted texture, are calcareous, very basic and poor in organic matter.[20] Concentrations of active carbonate (33–160 g kg^{-1}) and diethylenetriaminepentaacetic acid extractable iron (2.3–6.4 mg kg^{-1}) were highly heterogeneous within the area. Such soil properties, along with the presence of a lime sensitive rootstock, led to different levels of iron deficiency chlorosis in the vineyards, from unaffected to moderately-affected.[3] On the other hand, the differences in topography, texture and root explorable depth of the soils ensured a broad variability of grapevine water status within the study area.

Each season, data on foliar chlorophyll content (Chl), predawn leaf water potential (LWP) and leaf area index (LAI) were obtained in the study subzones at veraison stage, with 75 % of coloured berries (23-24 August 2016 and 9-10 August 2017). Chlorophyll content per leaf area unit (µg cm^{-2}) was obtained following González *et al*.[12] from readings of a CL-01 portable colorimeter (Hansatech Instruments Ltd., Norfolk, UK). Data were recorded in 30 leaves in each subzone, always choosing the fourth or fifth leaf counting from the first sheet of the apex. Measurements of LWP were taken during the two hours before dawn with a Scholander pressure chamber (Solfranc Technologies SL, Spain) in six fully expanded leaves in each subzone. LAI was measured according to Sánchez-de-Miguel *et al*.[21] from 20 representative shoots in each subzone, using a CID-202 portable laser leaf area meter (CID Bio-science, Inc. USA).

Harvesting was performed manually in all subzones on the same day in each year, after the average value of total soluble solid content of the must reached 22 °Brix (29 September 2016 and 8 September 2017). At that moment, yield was determined, and a sample of 20 kg of grape bunches were randomly collected throughout each subzone for microvinification.

Winemaking protocol

The harvested clusters from each subzone were vinified, in duplicate, following traditional red winemaking method. For each replicate, 15 kg of grape/must were fermented at about 24 °C, in 25-L steel tanks, adding initially sulphur dioxide at 50 mg kg^{-1} and *Saccharomyces cerevisiae* (Zymaflore RX60, Laffort) at 30 g hL^{-1}. After alcoholic fermentation, wines were pressed using a pneumatic press (maximum pressure = 0.2 MPa) and inoculated with 0.01 g L^{-1} *Oenococus oeni* lactic acid bacteria (SB3 Instant, Laffort) to induce malolactic fermentation. Then, wines were racked and free SO_2 was adjusted to 25 mg L^{-1}, transferred to 0.75-L bottles and stored at 13 °C until analysis. Wines were analysed approximately two months after bottling.

Chemical analysis

Total acidity, pH, alcoholic degree, CIELAB coordinates and total polyphenol index of wines were determined according to the principles and methods stablished by the International Organization of Vine and Wine.[22] Total anthocyanin and total tannin contents were also determined.[23]

Wine sensory analysis

Quantitative descriptive sensory analysis was performed by a panel composed of 11 trained assessors (5 men and 6 women; average age: 21 years) following the method of Stone et al.[24] The assessors were chosen based on their availability and sensory experience, according to ISO 8586.[25] They underwent 12 hours of basic training and 16 hours of specific training, which involved identification of appropriate descriptive terms with reference samples, the use of intensity scales and recognition and scoring of sensory attributes using eight different commercial young red wines. The sensory analysis was carried out in the Sensory Science Laboratory of the Agricultural Engineering College at the University of Valladolid, Palencia (Spain), in individual booths designed in accordance with ISO 8589.[26] In all the sessions, the samples were served as 25 mL aliquots in standardized wineglasses,[27] which were coded with 3-digit numbers. The serving temperature of the samples was 15±1 °C. Water was provided to rinse mouth between evaluations.

The final questionnaire comprised 13 sensory descriptors[28,29] grouped in three visual descriptors (limpidity, tonality, layer intensity), five olfactory descriptors (aroma intensity, red fruit, herbaceous, lactic and alcoholic) and five descriptors in the mouth (flavour intensity, bitter, acidity, astringency and persistence). The different descriptors were quantified using 10-cm unstructured intensity scales.[30]

To evaluate the panel performance, we used seven different commercial young red wines in triplicate, served in balanced order using a randomized complete block design, for four one-hour sessions. The parameters of the assessors' choice were the discriminant capacity, the reproducibility and the homogeneity of the panel using the ISO 8586[25] as a reference.

Finally, for development of the sensory profile, trained assessors evaluated twenty experimental red wine samples in duplicate each year in a randomized order.

Statistical analysis

The panel performance was analysed by three-way ANOVA in which assessors, samples, repetitions and their interaction (assessors x repetition and assessors x sample) were considered as explaining factors. The results were used to compare the performance of the assessors in relation with the discriminatory capacity, the reproducibility of the answers and the agreement among assessors. These data were analyzed using the statistics package SPSS version 15.0 for Windows (SPSS Inc., Chicago, USA).

Factorial analysis of variance (ANOVA) and Tukey's test were performed to separate the effects of season, water status and iron chlorosis incidence on chemical and sensory properties of wines. For this, the subzones were previously classified into groups with high and low LWP, and with high and low Chl. The limit values for segmentation were the median of both explanatory variables in the subzones throughout the two years studied (-0.737 MPa and 99.9 μg cm^{-2}, respectively), so that the statistical design was as balanced as possible. Pearson correlation coefficients were used to study the relationships among variables. The data analysis was performed with version 9.2 of SAS statistical software (Statistical Analysis System).

Results and discussion

Panel performance

Following the ISO 8586,[25] the discriminant power of the panel was verified with the ANOVA F values for the sample factor, which were statistically significant for all attributes of the tasting sheet except for flavour intensity, acidity and persistence (data not shown). The trained assessors were able to give reproducible answers since the F values for repetition were not significant in 10 of the 13 descriptors considered, and F values for the interaction assessor x repetition were always not significant, except for astringency. The homogeneity of the evaluation among panelists, estimated with F values for assessors x samples,[25,31] was satisfactory for most descriptors, although the group was less concordant for tonality, aroma intensity, herbaceous, alcoholic and flavour intensity. In summary, the presented results indicated that panel had acceptable performance on discriminant capacity, reproducibility and homogeneity.

Effects of iron and water stress

In the present investigation LWP values, registered at veraison, were considered as representative of water status of subzones, once they have been well correlated with productive and qualitative potential of the vineyard in previous studies.[3,12] The mean values of LWP in different study subzones (Table 1) indicated moderate to severe water deficit, according to ranges from Van Leeuwen *et al.*[7] The variability of water status at the beginning of the ripening was clearly wider in 2017 than in 2016 (the coefficients of variation of LWP were 24.0 % and 13.4 %, respectively). The rainfall registered from veraison to harvest in 2017 (18.9 mm) was higher than in 2016 (9.0 mm), and also the average temperature (20.6 °C vs 19.3 °C). The cumulative potential evapotranspiration (Penman-Monteith equation) throughout the ripening was slightly lower in the second season (152 mm vs 156 mm).

Mean values of Chl increased from 2016 to 2017 (Table 1). Chlorosis of the leaves, the main symptom of iron deficiency, is highly correlated with total limestone levels of the soil within the study area.[32] Chl is also related to the nutritional level of nitrogen since active calcium carbonate reduces soil organic matter turnover, limiting soil nitrogen on offer to the vines.[33] According to previous studies carried out in the same area,[3,12] no consistent correlations were obtained between Chl and LWP in 2016 ($r = 0.04$, $p > 0.05$) and 2017 ($r = 0.12$, $p > 0.05$) so that both variables could be considered independent.

The results of factorial ANOVA (Table 2) show that the effects of season on composition and chromatic characteristics of the wine were always significant. This was probably due to the different meteorological conditions of both study years generated significant variations in the vineyard performance and grape composition parameters. In fact, the spring frost damages and reduced water availability during the vegetative cycle decreased pruning weight in 2017, in relation to 2016 values (0.15 kg m^{-2} vs 0.26 kg m^{-2}, $p < 0.05$). The frosts occurred in 2017 drastically restricted the number of clusters per plant, and consequently the yield, as compared to that registered in 2016 (0.44 kg m^{-2} vs 0.75 kg m^{-2}, $p < 0.05$). Values of 100 berry weight also were lower in 2017 (144 g vs 177 g, $p < 0.05$). Under these conditions, the grapes of the first season reached a lower degree of maturity than in the second. As a consequence, average values of alcoholic degree (11.5 % vol.) and pH (3.8) of wines in 2016 were lower than those registered in 2017 (13.9 % vol. and 4.2, respectively), while total acidity values were higher (5.6 g L^{-1} in 2016 vs 3.7 g L^{-1} in 2017, $p < 0.05$). Total anthocyanin content (500 mg L^{-1}) and total tannin content of wines (1.43 g L^{-1}) in 2016 were lower than those in 2017 (665 mg L^{-1} and 2.03 g L^{-1}, respectively). The average value of chroma in 2016 (35.3) was higher than in 2017 (22.1).

Although the interannual differences in wine composition were clear (Table 2), significant effects of the season were detected only on 4 of 13 sensory attributes studied. The wines from 2017 season had greater limpidity (7.1 vs 6.8) and lower tonality scores (5.5 vs 7.2) than in 2016, and were less alcoholic in the olfactory evaluation (5.5 vs 5.8). The higher tannin content of the wines in 2017, as mentioned above, made them more astringent than those of 2016 (5.0 vs 4.8).

The factorial ANOVA (Table 2) shows that the incidence of iron chlorosis in the study area had a much greater impact on wine composition and sensory attributes than vine water status at the beginning of fruit ripening. When the musts from the subzones with different LWP were analyzed in both seasons, no significant differences in the soluble solids content or total acidity were detected,[13] although an increase in water deficit during the ripening period should tend to enhance the maturity level of the grapes.[7,8,11] Nevertheless, in 2017 low *versus* high LWP subzones significantly increased extractable anthocyanin content in grapes, potassium content and colour intensity of the must. The rise in anthocyanin concentration might be a consequence of both an increase in fruit exposure to sunlight (lower canopy density), and a direct stimulation of anthocyanin biosynthesis enzymes in more water stressed plants.[34] The rise in potassium content in the must from low LWP subzones, probably related to a greater translocation of K^+ cations from leaves to grapes in intense water stress conditions,[35] did not cause significant variations in the pH of the wine (Table 2).

Similar to our results, several authors did not find clear differences in the basic composition of wines elaborated with grapes from vineyards with different water status zones in non-irrigation[14,17] and irrigation assays.[15] Although it is well established that vine water deficit has significant effects on chemical composition of grape,[16] very often the compositional variations in grapes do not consistently translate into the corresponding wines,[36,37] since winemaking involves a set of complex oenological processes, which might impair it.[38] In any case, water status had additive effects with iron chlorosis incidence on wine astringency and persistence, with significant interaction effect in these two descriptors (Table 2). The subzones with low LWP reached higher scores of limpidity in 2017 (7.3 vs 7.0) and bitter in 2016 (5.4 vs 5.0) than those with high LWP. Casassa *et al*.[38] also observed that a moderate water stress increased the perceived bitter of Cabernet Sauvignon wines.

Table 3 shows the significant mean separations of composition and sensory attributes of the wine considering Chl as explanatory variable. On interannual average, wines from low-Chl subzones obtained higher acidity scores. Moreover, these subzones produced wines with lower pH than the high-Chl ones in the two years studied. According to this, low-moderate levels of iron availability might have some positive effects on wine quality, since a lower pH would generate (i) an improved perception of acidity and better sugar/acid balance, (ii) an enhanced quality of red colour, (iii) a reduction of colour and aroma evolution by oxidation, and (iv) a greater stability against biological spoilage.[39] There were significant differences between alcoholic degrees in wines from subzones with low and high Chl (14.4% vs 13.5 %, $p < 0.05$) in 2017 and no differences in 2016. The results on pH and alcoholic degree of wine agree with those obtained in must,[13] the incidence of iron deficiency tended to advance fruit ripening, increasing total soluble solids content and reducing total acidity of the must.

Total polyphenol index in low-Chl was higher than in high-Chl subzones (62.7 vs 55.2) in 2017, in agreement with other authors[12,40] who reported enhanced concentrations of polyphenols in must from vines affected by iron chlorosis. In both seasons, total polyphenol index was positively correlated with tonality ($r = 0.50$), layer intensity ($r = 0.60$) and astringency ($r = 0.71$) attributes.

Regarding the colour parameters of wine, chroma and red colour component significantly increased as a consequence of iron deficiency, while luminosity decreased (Table 3). These results seem to be in contradiction with the showed mean separation of average anthocyanin concentration (higher values in low-Chl subzones). However, they agree with those reported by Balint and Reynolds,[11] who found a negative correlation between anthocyanin concentration and colour density in wines. In fact, wines with the highest concentration of anthocyanins do not necessarily present the highest intensity of colour. It is well known that anthocyanins are responsible for the highest intense of red colour of wines but they can present other colours according to pH and their relationship with other polyphenols.[36]

From the sensory analysis point of view, low-Chl subzones obtained high scores on tonality, layer intensity, flavour intensity, acidity, astringency and persistence than high-Chl subzones regardless of study year (Table 3), with significant mean separations on flavour intensity, astringency and persistence in 2017. High scores on tonality and layer intensity in wines from low-Chl subzones are consistent with their higher values in red colour and lower in pH. On the other hand, Sánchez *et al.*[13] reported that the incidence of iron chlorosis increased the concentrations of some specific C_{13}-norisoprenoids and volatile phenols in the musts. This could explain, at least in part, the higher scores on flavour intensity in wines from low-Chl subzones.

Colour intensity, red to green component of wine colour and astringency scores were subjected to a LWP x Chl interaction (Figure 1), so that differences between low and high Chl values were detected ($p < 0.05$) only in subzones with better water status at veraison. The same mean separation was observed in persistence scores in 2017. These results completely agree with the Chl x LWP interaction on total phenolic content in grapes, detected previously.[13] The increase in red colour, astringency and persistence of the wine from plants with low Chl might be due to a direct effect of iron deficiency on biosynthesis of polyphenols in grapes through shikimate pathway,[10] although this effect could be limited in water deficit conditions.

Relationships with vigour, berry size and grape maturity

With more water availability during the vegetative cycle, vineyard subzones registered in 2016 values of pruning weight and yield significantly higher than in 2017, as mentioned above. Under these conditions, chemical and sensory attributes of the wine were homogeneous within the subzones and not correlated with LAI in the first season (Table 4). However, in the second, higher LAI values were related to lower concentrations of alcohol, polyphenols and tannins in the wine, and lower layer intensity, flavour intensity, persistence and astringency scores. This agree with the results of Balint and Reynolds[11], who found that ethanol concentration, anthocyanins and red colour of wines were negatively correlated with grapevine vigour. The results of 2017 in the present study suggest that more favourable leaf and cluster microclimate (less LAI values) could have contributed significantly to a better phenolic maturation of the fruits. As water stress restrict vegetative growth in vines, the proportion of well-exposed leaf surface increases and the yield decreases,[11,41] thus helping to balance source-sink relationship during ripening period,[42] and concentrating constituents as sugars and phenolic compounds in the grapes. On the other hand, a less dense canopy in water stressed plants favours the exposure of clusters to sunlight, which contribute to enhance colour intensity and anthocyanin content of the berries.[34,43] Furthermore, much of the basal leaves can become senescent during ripening period in more water stressed vines, which also increases cluster exposure.

Berry weight had few significant correlation coefficients with chemical and sensory attributes of the wine in the study area (Table 4). It has been proven that the water and iron stress levels registered in the vineyard subzones in 2016 and 2017 did not produce smaller berries,[13] which would have increased skin/pulp ratio and therefore polyphenol and anthocyanin content in grapes. Thus, the higher polyphenol content and the improvements on sensory attributes (as colour and flavour intensity), in wines from more stressed zones, could be mainly associated to an increase in the biosynthesis of phenolic compounds in grapes, caused by moderate iron and water deficit.[10,34] With varying intensity depending on the growing cycle conditions, an increase in fruit exposure to sunlight (lower canopy density) in stressed plants could indirectly stimulate the activity of polyphenol biosynthesis enzymes.[11]

The grape maturity level, estimated with Brix degree of the must, was positively correlated with total polyphenol index and chroma in 2016, and with alcoholic degree, total polyphenol index, total tannins, and alcoholic, astringency and persistence attributes of wine in 2017 (Table 4). It is generally considered that ripeness strongly influences the alcoholic degree, phenolic composition and colour of red wines.[44] Cadot *et al.*[45] reported that astringency, alcoholic and persistence attributes significantly increased with grape maturity level in Cabernet Franc wines. Astringency of Tempranillo wines from riper grapes was also greater and it was positively correlated with the concentration of tannins.[46] Wines from riper Cabernet Sauvignon grapes had an increased alcoholic degree as well as higher total polyphenol index, total tannins, chroma and astringency index compared to wines from less mature grapes.[47]

Some sensory attributes such as astringency and persistence are directly associated with the phenolic composition of wine and thus affected by grape of ripeness.[48] In 2017, we found a positive correlations between the alcoholic degree with the astringency ($r = 0.74$) and persistence ($r = 0.64$) descriptors. It has been demonstrated that higher concentrations of ethanol increases the astringency of proanthocyanidins and the extraction of colour and phenolic compounds.[49]

Conclusions

The presented results confirm that mild to moderate iron stress in vineyard can contribute to enhance wine quality, decreasing the pH and improving sensory attributes as tonality, layer intensity, flavour intensity and persistence in the mouth. In this context, measurements of foliar chlorophyll content at veraison could be very useful to distinguish subzones with different quality potential in vineyards affected by iron deficiency.

Compared with the incidence of iron chlorosis, vine water status had little impact on the composition and chromatic characteristics of the wine in the study area, although it significantly affected sensory descriptors as limpidity, astringency and persistence. The interaction effects detected between both explanatory variables suggest that increase in red colour, astringency and persistence of the wine associated to iron chlorosis incidence might be restricted if the water deficit reaches a certain level.

The total polyphenol content, colour intensity, astringency and persistence of the wine were independent of berry size and directly correlated with the grape technological maturity in the study area.

Acknowledgments

This research was supported by RTA2014-00077-C02-02 (from INIA and the Spanish Ministry of Economy and Competitiveness) and VA013P17 (Junta de Castilla y León) projects, and co-financed with FEDER Funds. The authors are grateful to Bodegas Emilio Moro S.L. for their collaboration to conduct this investigation.

Conflict of interest

The authors declare no conflict of interest.

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Figure legend

Figure 1: Mean comparison of C* and a* coordinates (CIELAB colour space), astringency (AST) and persistence scores (PER) in sensory evaluation of wines from vineyard subzones with different foliar chlorophyll content (Chl) and predawn leaf water potential (LWP) at veraison.

Tables

Table 1. Mean values and standard deviations of foliar chlorophyll content (Chl) and predawn leaf water potential (LWP) measured at veraison in different vineyard subzones.

Year	Subzone type	Chl ($\mu g\ cm^{-2}$)	LWP (MPa)
2016	High Chl and high LWP	111.53 ± 10.44	-0.65 ± 0.05
	High Chl and low LWP	114.07 ± 13.96	-0.77 ± 0.02
	Low Chl and high LWP	72.67 ± 24.79	-0.63 ± 0.09
	Low Chl and low LWP	82.66 ± 15.40	-0.79 ± 0.05
	Average	93.54 ± 23.70 b	-0.71 ± 0.09 a
2017	High Chl and high LWP	123.92 ± 22.08	-0.57 ± 0.20
	High Chl and low LWP	117.31 ± 23.19	-0.79 ± 0.06
	Low Chl and high LWP	95.00 ± 2.15	-0.64 ± 0.08
	Low Chl and low LWP	89.18 ± 6.89	-0.82 ± 0.07
	Average	108.95 ± 22.91 a	-0.71 ± 0.17 a

In each column, means followed by the same letter are not significant different (p < 0.05, Tukey's test).

Table 2. F-values of factorial analysis of variance of composition and sensory parameters of wines obtained from vineyard subzones with high and low predawn leaf water potential (LWP) and high and low foliar chlorophyll content at veraison (Chl), in 2016 and 2017.

Variables	**Model**	**Year**	**Chl**	**LWP**	**LWP*Chl**
Wine composition					
Alcoholic degree	27.41 ***	108.77 ***	3.90 *	2.29	2.86
Total acidity	11.06 ***	40.94 ***	0.25	0.50	1.67
pH	25.23 ***	72.35 ***	12.10 **	0.05	1.21
Total polyphenol index	3.34 *	5.38 *	3.36	0.00	6.15
Total anthocyanins	5.39 **	9.76 **	6.16 *	2.16	0.59
Total tannins	9.18 ***	34.54 ***	3.44	2.63	2.34
CIELAB coordinates					
C*	13.82 ***	24.79 ***	11.84 **	2.09	8.67 **
H	11.47 ***	36.35 ***	1.63	0.41	2.34
L	6.45 ***	4.47 *	11.43 **	2.63	5.64 *
a*	14.00 ***	26.39 ***	12.19 **	2.54	6.80 *
b*	30.94 ***	118.32 ***	0.00	0.02	0.01
Sensory attributes					
Limpidity	6.63 ***	16.90 ***	0.84	5.44 *	0.01
Tonality	23.03 ***	75.00 ***	3.51 *	0.82	0.08
Layer intensity	1.96	0.44	4.25 *	1.41	1.64
Aroma intensity	0.43	0.05	0.03	0.78	0.71
Red fruit	2.13	1.90	2.35	3.58	0.23
Herbaceous	1.87	3.69	1.15	0.96	0.15
Lactic	0.79	2.06	0.03	0.01	0.81
Alcoholic	2.72 *	6.53 *	1.05	0.19	1.39
Flavour intensity	2.48 *	1.22	9.50 **	0.22	0.26
Bitter	1.41	1.12	1.70	3.67	0.94
Acidity	1.61	0.52	4.70 *	0.06	0.09
Astringency	5.93 **	5.22 *	14.36 ***	5.64 *	5.60 *
Persistence	6.30 ***	3.60	10.73 **	6.43 *	3.39 *

***** Significant $p < 0.05$; ** Significant $p < 0.01$; *** Significant $p < 0.001$.

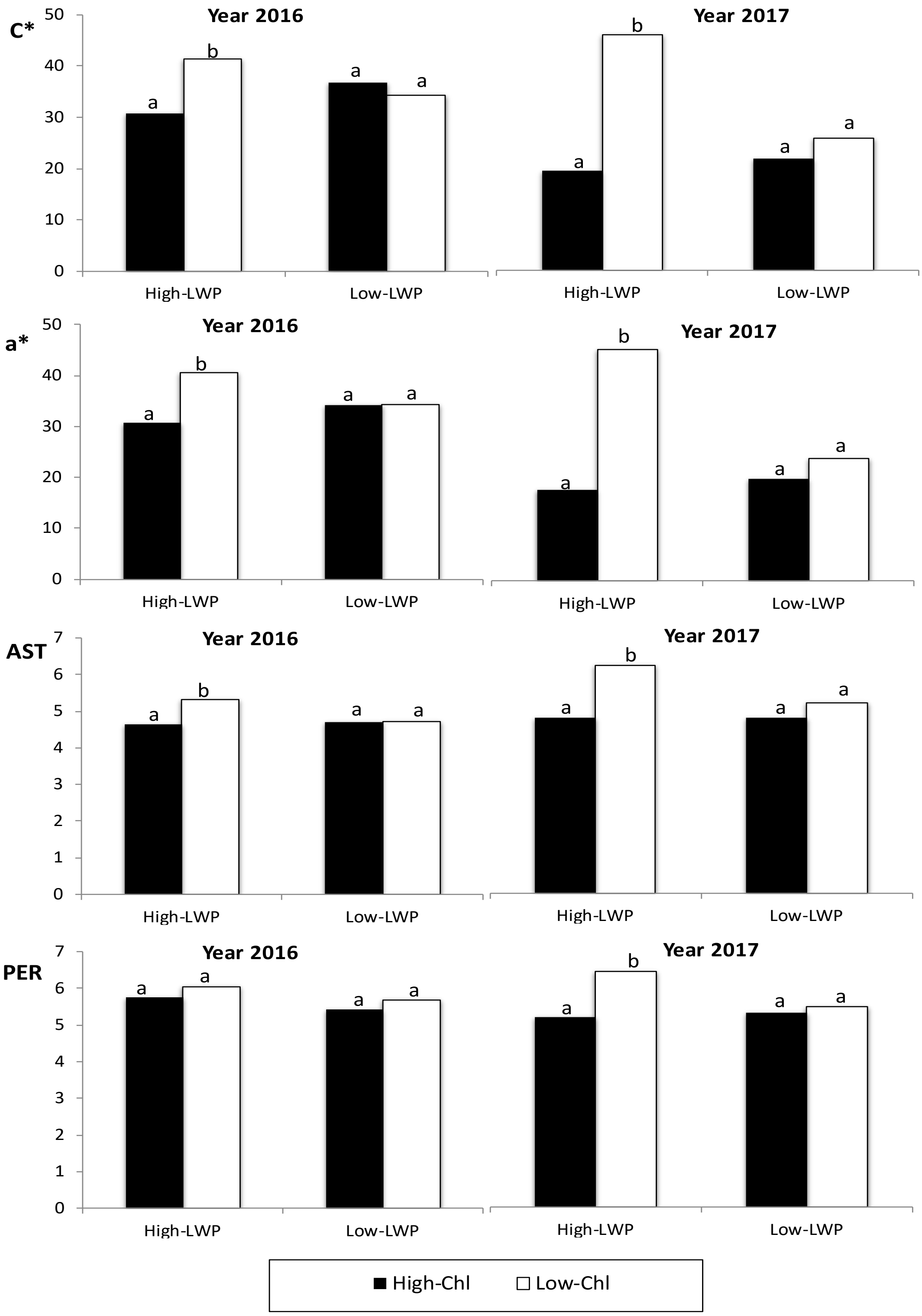
Table 3: Significant mean separations of wine quality variables ($p < 0.05$, test Tukey) obtained in High and low foliar chlorophyll content at veraison (Chl) in the years studied.

Variables	**2016**		**2017**		**Average**	
	Low Chl	**High Chl**	**Low Chl**	**High Chl**	**Low Chl**	**High Chl**
Wine composition						
Alcoholic degree (% v/v)			14.4	13.5		
pH	3.8	3.9	4.1	4.2	3.9	4.1
Total polyphenol index			62.7	55.2		
Total anthocyanins (mg/L)					521	639
CIELAB coordinates						
C*			27.7	18.4	33.3	24.5
L			68.5	79.9	67.1	75.7
a*	37.1	31.9	26.3	16.7	32.6	23.2
Sensory attribute scores						
Tonality					6.8	6.1
Layer intensity					6.7	6.2
Flavour intensity			6.5	6.0	6.4	6.1
Acidity					5.9	5.6
Astringency			5.5	4.8	5.2	4.7
Persistence			5.8	5.2	5.8	5.4

Table 4. Pearson correlation coefficients between composition and sensory attributes of wines with vine vegetative development, size and maturity of the grapes.

Year	Variable	Leaf area index	100 berry weight	Total soluble solids of the must
2016	**Alcoholic degree**	-0.07	0.14	0.50 *
	Total acidity	-0.18	0.13	0.15
	pH	0.51 *	0.03	0.18
	Total polyphenol index	-0.28	0.03	0.43 *
	Total anthocyanins	0.42	-0.31	0.26
	Total tannins	-0.02	-0.21	0.30
	Chroma (C*)	-0.29	-0.10	0.48 **
	Hue (H)	-0.38	-0.19	-0.13
	Limpidity	-0.20	-0.16	-0.51
	Tonality	0.08	0.02	0.51
	Layer intensity	0.01	0.00	0.70
	Aroma intensity	-0.16	0.52 *	0.22
	Red fruit	-0.20	-0.02	-0.05
	Herbaceous	0.02	0.29	0.24
	Lactic	-0.08	0.47 *	-0.12
	Alcoholic	0.07	0.36	0.47
	Flavour intensity	-0.05	0.34	0.35
	Bitter	-0.21	0.11	0.18
	Acidity	-0.03	0.15	0.37
	Astringency	-0.36	0.26	0.45
	Persistence	-0.15	0.31	0.47
2017	**Alcoholic degree**	-0.47 *	-0.17	0.86 ***
	Total acidity	0.54 *	0.57 **	-0.27
	pH	0.29	0.05	0.09
	Total polyphenol index	-0.60 **	-0.28	0.63 ***
	Total anthocyanins	-0.02	-0.22	-0.33
	Total tannins	-0.45 *	0.04	0.49 ***
	Chroma (C*)	-0.37	-0.05	0.45
	Hue (H)	0.39	-0.03	-0.17
	Limpidity	0.21	0.08	0.27
	Tonality	-0.40	-0.09	0.12
	Layer intensity	-0.48 *	-0.13	0.52
	Aroma intensity	-0.13	0.06	0.04
	Red fruit	0.02	0.32	0.21
	Herbaceous	0.03	0.02	0.16
	Lactic	0.02	-0.36	-0.24
	Alcoholic	-0.20	-0.14	0.18 **
	Flavour intensity	-0.69 **	-0.51 *	0.09
	Bitter	-0.10	-0.14	0.12
	Acidity	-0.07	-0.18	-0.02
	Astringency	-0.52 *	-0.22	0.49 **
	Persistence	-0.51 *	-0.10	0.47 *

* Significant $p < 0.05$; ** Significant $p < 0.01$; *** Significant $p < 0.001$.



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557 Figure 1.