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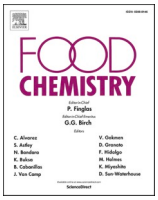

Volatile composition and sensory properties of wines from vineyards affected by iron chlorosis

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ABSTRACT

Recent studies have shown that mild to moderate iron chlorosis can have positive effects on grape quality potential, including volatile profile. The main objective of this work was to investigate, for the first time, how moderate iron stress in grapevines affects the presence of volatile organic compounds (VOCs) in wines. The study was carried out during 2018–2019 seasons, in 20 Tempranillo vineyard subzones with different degree of iron deficiency, located in Ribera del Duero (North-Central Spain). The results showed that moderate iron stress increased in wines the concentrations of VOCs associated with floral notes, such as 2-phenylacetaldehyde, 2-phenylethanol and 2-phenylethyl acetate, while reducing the presence of C6-alcohols, responsible for green-herbaceous aroma.

A favourable reduction of pH and a betterment of parameters related to colour were detected in wines from iron deficient subzones. Chlorosis incidence was associated to improvements in wine sensory attributes as layer intensity, black fruit and aroma intensity.

1. Introduction

The sensory quality of a wine depends mainly on its aroma, and it is the result of a multitude interactions between all its chemical components (Chira et al., 2011). Many volatile compounds are responsible for the aroma of wine (alcohols, esters, aldehydes, ketones, fatty acids, terpenes, lactones, furanic compounds, etc.). Some of these compounds, even at very low concentrations, play a very important role in the aroma and sensory characteristics of wine; however other volatile compounds present in higher concentration have a low sensory impact (Sánchez-Palomo et al., 2019).

The many chemical compounds that contribute to flavour and aroma in wine are determined partly in the vineyard through complex and poorly understood interplay among the natural environment, vineyard management practices and vine genotypes (Jackson & Lombard, 1993; Torres et al., 2021; Van Leeuwen et al., 2004). Viticultural practices aim primarily at producing quality grapes that would reflect varietal flavours and aromas and/or characters typical for a specific region or terroir (Styger et al., 2011; Torres et al., 2021).

Iron deficiency (iron chlorosis) in calcareous soils, are a common environmental stress in Mediterranean area. Iron chlorosis leads to a decrease in the synthesis of photosynthetic pigments and a lowering of the efficiency of photosystem II (Bavaresco et al., 2006; Hailemichael et al., 2016). This reduces the carbon assimilation in vines, which can modify the composition of grapes, then affecting the aroma and sensory characteristics of the wine. Although iron chlorosis generally leads to poor must quality (Shi et al., 2018; Veliksar et al., 2005), the plants affected restrict vegetative growth, thus less yield and smaller berries, thereby concentrating constituents as sugars and phenolics (González et al., 2019). Recently, it has been shown that mild to moderate iron deficiency in the vineyard can have positive effects on aromatic profile of Tempranillo grapes, by increasing the concentration of some terpenes, C13-norisoprenoids, volatile acids and volatile phenols (Sánchez et al., 2021), and also contribute to enhance the quality of wines, decreasing the pH and improving sensory attributes (Sánchez et al., 2020).

However, no research papers have been found studying the effects of iron deficiency on the volatile composition of wines. Based on previous results on aroma grape, it would be interesting to explore the impact of iron chlorosis on the aroma of the wine, together with its sensory properties, to provide useful information to assess quality potential of the vineyard in the framework of precision viticulture. The objective of this work was to investigate the effects of the incidence of iron

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deficiency chlorosis in the vineyard on volatile composition and sensory characteristics of Tempranillo wines.

2. Materials and methods

2.1. Chemicals

Standards for identification and quantification of VOCs were purchased from Sigma-Aldrich (Madrid, Spain) and Fluka (Buchs, Switzerland) with purity > 98%. Helium (99.9997%) for gas chromatography was provided by Carburos Metálicos S.A. (Valladolid, Spain). The remaining chemicals were analytical quality grade and purchased from Panreac (Madrid, Spain).

2.2. Study site and iron chlorosis incidence

The study was conducted in 2018 and 2019, on 20 non-irrigated vineyard subzones located in Pesquera de Duero (latitude 41°38′34″ N, longitude 4°09′27″ W, Ribera del Duero Appellation of Origin area, North Central Spain). The region has Mediterranean climate, with low temperatures in winter, hot and dry summers. The year 2019 was more humid than 2018 in the study site (annual precipitation registered 664 mm versus 426 mm), and the mean annual temperature was of 12.1 °C and 12.2 °C, respectively.

The soils in the study are simultaneously calcareous, very basic and poor in organic matter. Concentrations of active carbonate (3–16%) and diethylenetriaminepentaacetic acid (DTPA) extractable iron (2.3–6.4 mg/kg) were highly heterogeneous within the area. Such soil properties, along with the presence of a lime sensitive rootstock as 110-Richter, led to different levels of iron deficiency chlorosis in the vineyards, from unaffected to moderately affected.

The studied subzones (10 m × 10 m each) were selected after a previous visual screening of the spatial variability on canopy size and colour. Different soil physicochemical properties were chosen to ensure maximum variability in nutrient status across the sites, according to the purposes of the investigation. The vineyards correspond to Tempranillo cultivar, 15 to 20 years old, grafted on 110-Richter rootstock. Vines were spaced 3.0 m × 1.5 m, trained in a vertical shoot positioning and pruned with a mean load of 16 buds per plant. The average yield of subzones was 4.0 t/ha in 2018 and 5.8 t/ha in 2019.

Each season, foliar chlorophyll content per leaf area unit (Chl) was recorded in the study subzones, one week before harvest, following Hailemichael et al. (2016). Data were obtained from readings of a CL-01 portable colorimeter (Hansatech Instruments Ltd., Norfolk, UK) in 30 leaves taken at random in each subzone. Finally, the subzones were classified into groups, with high and low Chl, considering as limit value the median of the variable throughout the two years studied (98.2 μg/cm^2).

2.3. Winemaking process

The grape harvest was carried out when the grapes presented proper maturity levels and sanitary conditions. It was performed in all subzones on the same day in each year.

The wines were elaborated following a classical pattern of development of red wine through microvinification process. A total of 40 wines were elaborated per vintage, 10 from each of the subzones (high and low Chl) by duplicated (10x2x2). All grapes from each subzone were harvested, destemmed, crushed and properly blended. A representative sample of 15 kg of grape/must were fermented in 25 L steel tanks with the addition of sulfur anhydride (SO_2) at a dose of 50 mg/kg, to eliminate any native yeasts. Skin and must were fermented at about 24 °C with *Saccharomyces cerevisiae* (Zymaflore RX60; Laffort, Bordeaux, France) at a dose of 30 g/hL and pump down once a day. Once a residual sugar content was below 2 g/L, the wines were pressed in a pneumatic press (maximum pressure 0.2 MPa) and 0.01 g/L of *Oenococus oeni* lactic acid bacteria (SB3 Instant; Laffort) were inoculated to induce malolactic fermentation (MLF). After completion of MLF, the wines were racked off gross lees, and corrected to about 25 mg/L of free sulfur dioxide. The wines were bottled in 750 mL bottles and stored for approximately 2 months at 13 °C prior to chemical, volatile and sensory analysis.

2.4. Must and wine chemical composition

pH, total and volatile acidity, alcoholic degree and CIELab colour coordinates were assayed in duplicated applying methods recommended by the International Organization of Vine and Wine (OIV, 2020). Colour intensity was determined from absorbance values measured at 420, 520 and 620 nm and total polyphenol index was calculated at 280 nm (Lan Optics 2000 UV, Labolan, Spain) (Zamora, 2003), and these analyses were also performed by duplicate. Total tannin and anthocyanin contents were also quantified by triplicated following the methodologies described by Hidalgo (2011).

Before the vinification, the total soluble solids and yeast assimilable nitrogen (YAN) concentrations in the musts were determined following OIV methods (OIV, 2020).

2.5. Quantification of volatile organic compounds (VOCs) of wine by headspace-solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS)

The analysis of wine VOCs was performed using a CombiPal RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland) connected with a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a 5977 mass selective detector (Agilent Technologies). HS-SPME was carried out for the extraction of wine volatile compounds following the method proposed by Massera et al. (2012) with minor modifications. 5 mL of wine saturated with 3 g of sodium chloride (NaCl) and 50 μL of methyl nonanoate (0.059 mg/L) as internal standard were added into a vial of 20 mL sealed with a magnetic screw cap provided with a PTFE/silicone septa. Before volatile extraction, each sample was equilibrated at 40 °C for 15 min with agitation (250 rpm). Prior to analysis, a 50/30 μm DVB/CAR/PDMS fibre (Supelco, Inc., Bellefonte, PA, USA) was preconditioned at 270 °C for 15 min before daily use. Then, the fibre was exposed to head space of vial at 40 °C for 30 min with agitation (250 rpm). Previously, the fibre was preconditioned at 270 °C for 15 min before daily use. After extraction, the fibre was inserted into the injector of chromatograph, and desorption of volatiles was carried out at 250 °C for 15 min. The injector temperature was 250 °C, working in splitless mode (1 min). A HP-Innowax column (60 m, 0.250 mm, 0.5 μm) (J &W Scientific, Folsom, CA, USA) was employed for the volatile separation. Helium gas was used at a pressure of 22.413 psi and a flow of 1.2 mL/min. The oven temperature was held at 40 °C for 5 min and increased at 2.5 °C/min to 230 °C, then was held for 20 min. MS detector was operated in full scan mode over a range of m/z of 30–500. The identification of volatile compounds was based on the comparison of their GC mass spectra with pure standards and/or with spectral data from the NIST08 y Wiley7 libraries. Quantification was carried out using the internal standard quantification method using standards. The calibration graphs were carried out using five concentrations of each standard in a synthetic wine (12% v/v ethanol, 5 g/L tartaric acid, pH 3.8). For some compounds, whose standards were not available, quantification was performed as equivalents (Ayestarán et al., 2019; Sieiro et al., 2014) of 2-octanol. Samples were analysed in triplicated.

2.6. Wine sensory analysis

A trained panel composed of eleven assessors (five men and six women; average age: 21 years) participated in this study. The panel of tasters was selected and trained using ISO 8586 (2012) as a reference and the results have already been published (Sánchez et al., 2020). All panelists had previously participated in wine sensory descriptive

Table 1
Mean values and standard deviations of physicochemical properties of wines obtained from vineyard subzones with high and low foliar chlorophyll content (Chl) in 2018 and 2019 and F-values of analysis of variance.

Code	Variable	2018		2019		F-values		
		Low-Chl	High-Chl	Low-Chl	High-Chl	Chl	Y	Chl × Y
pH	pH	**3.97 ± 0.09^{b}**	**4.12 ± 0.09^{a}**	3.91 ± 0.06	3.94 ± 0.09	25.8***	36.4***	9.78**
TA	Total acidity (g/L)	4.31 ± 0.23	4.15 ± 0.27	4.02 ± 0.22	4.10 ± 0.22	0.577 ^{ns}	9.12**	4.58*
VA	Volatile acidity (g/L)	0.47 ± 0.04	0.46 ± 0.03	0.44 ± 0.03	0.44 ± 0.04	0.446 ^{ns}	7.00*	0.26 ^{ns}
AD	Alcoholic degree (%, v/v)	13.8 ± 0.9	13.5 ± 1.0	12.4 ± 0.7	12.1 ± 0.6	2.46 ^{ns}	54.9***	0.000 ^{ns}
L	Clarity	**54.2 ± 4.6^{b}**	**60.3 ± 3.3^{a}**	**59.5 ± 4.4^{b}**	**65.9 ± 3.9^{a}**	41.2***	30.9***	0.030 ^{ns}
a	a*	**40.5 ± 4.4^{a}**	**34.7 ± 2.6^{b}**	**41.0 ± 4.7^{a}**	**34.8 ± 3.3^{b}**	42.1***	0.087 ^{ns}	0.066 ^{ns}
b	b*	**6.52 ± 1.89^{a}**	**3.29 ± 1.06^{b}**	−0.68 ± 1.81	−1.02 ± 1.51	2.44***	224***	14.0***
CI	Colour intensity	**1.93 ± 0.26^{a}**	**1.57 ± 0.17^{b}**	**1.56 ± 0.23^{a}**	**1.25 ± 0.18^{b}**	43.5***	45.4***	0.210 ^{ns}
TPI	Total polyphenol index	**68.0 ± 9.1^{a}**	**61.0 ± 6.9^{b}**	**47.3 ± 5.4^{b}**	**54.3 ± 9.7^{a}**	0.000 ^{ns}	54.0***	14.1***
TT	Total tannins (g/L)	1.90 ± 0.37	1.83 ± 0.29	2.26 ± 0.81	2.10 ± 1.09	0.227 ^{ns}	1.90 ^{ns}	0.040 ^{ns}
TA	Total anthocyanins (mg/L)	694 ± 146	677 ± 75	738 ± 109	701 ± 116	0.726 ^{ns}	1.10 ^{ns}	0.090 ^{ns}

Bold and different letters indicate significant differences between subzones (Low and High Foliar Chlorophyll Level) for each year ($P < 0.05$, Tukey test). *Significant ($P < 0.05$); **significant ($P < 0.01$); ***significant ($P < 0.001$); ns: No significant ($p \geq 0,05$). Chl: Chlorophyll Level. Y: Year.

analysis studies (Sánchez et al., 2020). The descriptive sensory analysis was carried out in the Sensory Science Laboratory of the School of Agricultural Engineering (University of Valladolid, Palencia, Spain) in individual booths. The samples were served as 25 mL aliquots in standardized wineglasses (ISO, 1977), 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 questionnaire was comprised 15 sensory descriptors grouped in three visual descriptors (limpidity, tonality, and layer intensity), seven olfactory descriptors (aroma intensity, red fruit, black fruit, herbaceous, lactic, acetic, 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 where 0 corresponded to very low intensity and 10 to high intensity for the respective attribute. Evaluations were carried out in two sessions every year of the study in which the judges tasted all the wines in duplicate, according to a randomized complete block design.2

2.7. Statistical analysis

IBM SPSS Statistical version 24.0 (IBM Corp. in Armonk, NY) and Statgraphics Centurion XVIII (StatPoint Technologies, Inc.) were used for all statistical analyses. A variance analysis was carried out to study the effects of foliar chlorophyll level and season on chemical composition, volatile profile and sensory attributes of the wines. The Tukey test calculated at a confidence level of 95% was employed to determine significant differences among means. Principal component analysis (PCA) was carried out with the significant data of chemical, volatile and sensory composition as variables and the wines from 2018 and 2019 vintages as samples.

3. Results and discussion

3.1. Wine chemical composition

Table 1 shows the basic chemical composition of Tempranillo wines affected by iron chlorosis and by year of study (2018 and 2019 vintages). The results of factorial ANOVA show that the effect of year on chemical composition of the wine was significant in all studied variables except for red/green colour component (a*), total tannins and total anthocyanins. This was probably because the different meteorological conditions of the two study years elicited significant variations in the grape composition. In fact, the average total soluble solids content of the must in 2019 was significantly lower than in 2018 (22.6 versus 24.6 ^{o}Brix, respectively). The incidence of iron chlorosis in the study area had impact on pH, clarity (L), a*, blue/yellow colour component (b*) and colour intensity.

In 2018 vintage a*, b*, colour intensity and total polyphenol index showed significantly higher values in wines from vines with low-Chl than those from high-Chl. Also, in 2018 vintage wines from low-Chl subzones had lower values for pH and L. In 2019 vintage a* and colour intensity also showed higher values in wines from vines with low-Chl than those from high-Chl, while total polyphenol index presented significantly lower values in wines from low-Chl subzones than those from high-Chl.

According to the results mentioned above, low-moderate levels of iron chlorosis might have a positive effect on wine quality, since a lower pH would generate an improved sensory perception in 2018 vintage wines. Due to climate change, no-balanced wines with low levels of acidity and too mature odours is obtained in some regions and, therefore the wine quality parameters are also below the optimum (Vila-Crespo et al., 2010). Consequently, the higher perception of acidity in the wines could be associated with a higher wine quality. The lower pH and enhanced red colour detected in low-Chl subzones confirm the results found in the same study area for 2016 and 2017 seasons, both in musts (Sánchez et al., 2021) and wines (Sánchez et al., 2020). Although iron deficiency can enhance the concentrations of polyphenols in the must (Bavaresco et al., 2010; González et al., 2019), its effect in the study area is dependent on water status of the vineyard (Sánchez et al., 2020).

3.2. VOCs composition

Sixty-seven VOCs were identified in wine samples, being the volatile profiles of wines manly composed of ethyl esters, alcohols and acetates. Significant differences between both vintages were observed for the 97% (65 out 67) of VOCs, except for ethyl 4-hydroxybutanoate and ethyl nonanoate. In comparison with 2019 vintage, wines from 2018 vintage showed the highest concentrations of acetates, acids, alcohols, aldehydes, C6-alcohols and ethyl esters, and the lowest concentrations of methyl and branched esters. Other studies also revealed inter-annual variation on wine VOCs (Bouzas-Cid et al., 2018; Gutiérrez-Gamboa et al., 2021). This could be directly related to the meteorological conditions from year to year, which modify the grape composition (González et al., 2019).

The concentrations of wine VOCs were also influenced by the iron status of vines. The effect of the incidence of iron chlorosis on concentration of total volatiles was not significant. However, 26 specific volatile compounds from the different chemical families analysed, except for acids, showed significant differences between low-Chl and high-Chl subzones. Overall, higher concentrations of these volatiles were observed in wines from vines with high-Chl than those from low-Chl.

Wines from vineyards affected by moderate iron deficiency chlorosis showed the highest concentrations of some higher alcohols, such as octan-1-ol in 2018 and 2-methylpropan-1-ol, 3-methylbutan-1-ol and

Table 2
Mean values and standard deviations of wine volatile organic compounds (VOCs) (mg/L) obtained from vineyard subzones with high and low foliar chlorophyll content (Chl) in 2018 and 2019 and F-values of analysis of variance.

Variable		2018		2019		F-values		
Code	IUPAC name	Low-Chl	High-Chl	Low-Chl	High-Chl	Chl	Y	Chl × Y
1	3-methylbutanoic acid[i]	0.073 ± 0.041	0.041 ± 0.028	0.008 ± 0.009	0.009 ± 0.007	3.87 ^{ns}	38.5***	4.22*
2	Hexanoic acid	0.456 ± 0.187	0.535 ± 0.222	0.302 ± 0.064	0.303 ± 0.069	0.667 ^{ns}	15.5***	0.641 ^{ns}
3	Octanoic acid	1.60 ± 0.42	1.39 ± 0.40	0.796 ± 0.155	0.792 ± 0.202	1.17 ^{ns}	48.2***	1.09 ^{ns}
4	Nonanoic acid	nd	nd	0.006 ± 0.005	0.009 ± 0.008	1.02 ^{ns}	22.8***	1.02 ^{ns}
5	Decanoic acid	**0.239 ± 0.068^{a}**	**0.168 ± 0.038^{b}**	0.360 ± 0.063	0.364 ± 0.096	2.28 ^{ns}	48.8***	2.76 ^{ns}
6	*Total acids*	2.37 ± 0.56	2.13 ± 0.62	1.47 ± 0.27	1.48 ± 0.37	0.586 ^{ns}	25.6***	0.624 ^{ns}
7	Propan-1-ol	0.218 ± 0.142	0.259 ± 0.120	**0.023 ± 0.010^{b}**	**0.032 ± 0.008^{a}**	0.746 ^{ns}	52.7***	0.303 ^{ns}
8	2-methylpropan-1-ol	88.2 ± 22.6	93.3 ± 29.3	**19.4 ± 5.5^{a}**	**15.0 ± 2.5^{b}**	0.003 ^{ns}	148***	0.623 ^{ns}
9	3-methylsulfanylpropan-1-ol[i]	nd	nd	0.018 ± 0.004	0.017 ± 0.008	0.169 ^{ns}	123***	0.169 ^{ns}
10	Butan-1-ol	0.245 ± 0.184	0.418 ± 0.252	nd	nd	2.97 ^{ns}	43.6***	2.97 ^{ns}
11	3-methylbutan-1-ol	54.0 ± 15.0	60.2 ± 18.9	**12.6 ± 3.5^{a}**	**9.69 ± 1.58^{b}**	0.003 ^{ns}	149***	0.623 ^{ns}
12	Butan-2-ol	0.245 ± 0.184	0.418 ± 0.252	nd	nd	2.97 ^{ns}	43.6***	2.97 ^{ns}
13	(2R)-3-methylbutan-2-ol[i]	0.018 ± 0.008	0.011 ± 0.008	nd	nd	3.11 ^{ns}	63.5***	3.11 ^{ns}
14	Pentan-1-ol	0.004 ± 0.003	0.005 ± 0.003	nd	nd	1.22 ^{ns}	44.5***	1.22 ^{ns}
15	(E)-pent-3-en-1-ol	0.004 ± 0.004	0.003 ± 0.002	nd	nd	1.59 ^{ns}	25.5***	1.59 ^{ns}
16	(3S)-3-methylpentan-1-ol[i]	0.025 ± 0.008	0.021 ± 0.006	**0.005 ± 0.005^{a}**	**0.001 ± 0.002^{b}**	4.82*	126***	0.017 ^{ns}
17	Heptan-1-ol	0.462 ± 0.133	0.433 ± 0.166	0.028 ± 0.008	0.028 ± 0.006	0.187 ^{ns}	151***	0.180 ^{ns}
18	Octan-1-ol	**0.083 ± 0.020^{b}**	**0.118 ± 0.026^{a}**	0.050 ± 0.023	0.059 ± 0.017	9.03**	42.0***	3.32 ^{ns}
19	Nonan-1-ol[i]	nd	nd	0.023 ± 0.017	0.026 ± 0.022	0.126 ^{ns}	26.6***	0.126 ^{ns}
20	Decan-1-ol	nd	nd	0.017 ± 0.011	0.019 ± 0.005	0.223 ^{ns}	74.2***	0.223 ^{ns}
21	2-phenylethanol	**66.6 ± 29.6^{a}**	**41.6 ± 11.1^{b}**	**11.3 ± 1.9^{a}**	**8.85 ± 2.71^{b}**	8.20*	84.4***	5.56 ^{ns}
22	*Total higher alcohols*	213 ± 55	197 ± 55	**43.4 ± 10.6^{a}**	**33.7 ± 5.9^{b}**	1.08 ^{ns}	178***	0.069 ^{ns}
23	Hexan-1-ol	**2.29 ± 0.40^{b}**	**2.89 ± 0.62^{a}**	0.367 ± 0.087	0.403 ± 0.082	6.72*	334***	5.25*
24	Hexan-2-ol	0.029 ± 0.005	0.029 ± 0.008	nd	nd	0.002 ^{ns}	393***	0.002 ^{ns}
25	(E)-hex-3-en-1-ol[i]	0.016 ± 0.007	0.016 ± 0.009	nd	nd	0.004 ^{ns}	82.2***	0.004 ^{ns}
26	(Z)-hex-3-en-1-ol[i]	**0.202 ± 0.022^{b}**	**0.288 ± 0.046^{a}**	0.057 ± 0.013	0.068 ± 0.018	28.2***	392***	16.4***
27	2-ethylhexan-1-ol[i]	0.075 ± 0.050	0.050 ± 0.027	nd	nd	2.16 ^{ns}	51.2***	2.16 ^{ns}
28	*Total C6-alcohols*	**2.62 ± 0.40^{b}**	**3.27 ± 0.66^{a}**	0.423 ± 0.099	0.471 ± 0.099	7.54**	386***	5.61*
29	Benzaldehyde	0.085 ± 0.024	0.084 ± 0.018	0.020 ± 0.009	0.015 ± 0.013	0.221 ^{ns}	151***	0.110 ^{ns}
30	2-phenylacetaldehyde	nd	nd	**0.010 ± 0.008^{a}**	**0.003 ± 0.007^{b}**	4.61*	14.8**	4.61*
31	Pentanal[i]	0.011 ± 0.004	0.011 ± 0.004	nd	nd	0.123 ^{ns}	142***	0.123 ^{ns}
32	*Total aldehydes*	0.096 ± 0.025	0.094 ± 0.019	0.030 ± 0.009	0.018 ± 0.017	1.25 ^{ns}	147***	0.771 ^{ns}
33	2-phenylethyl acetate	**0.756 ± 0.383^{a}**	**0.399 ± 0.092^{b}**	0.190 ± 0.052	0.153 ± 0.038	10.8*	46.2***	7.17*
34	Ethenyl acetate[i]	4.36 ± 1.75	5.15 ± 2.10	0.605 ± 0.244	0.644 ± 0.330	0.881 ^{ns}	87.0***	0.722 ^{ns}
35	2-methylpropyl acetate[i]	**0.465 ± 0.474^{b}**	**0.992 ± 0.488^{a}**	0.013 ± 0.042	0.058 ± 0.062	6.95*	40.7***	4.93*
36	3-methylbutyl acetate	**0.892 ± 0.671^{b}**	**1.77 ± 0.63^{a}**	**0.172 ± 0.021^{b}**	**0.214 ± 0.042^{a}**	10.0**	61.3***	8.27**
37	Hexyl acetate	**0.026 ± 0.016^{b}**	**0.047 ± 0.019^{a}**	nd	0.000 ± 0.001	7.38*	82.8***	6.77*
38	*Total acetates*	6.49 ± 1.97	8.35 ± 2.44	0.980 ± 0.239	1.07 ± 0.37	3.68 ^{ns}	159***	3.03*
39	1-O-ethyl 4-O-(3-methylbutyl) butanedioate[i]	0.133 ± 0.082	0.122 ± 0.066	0.126 ± 0.031	0.120 ± 0.035	0.210 ^{ns}	0.062 ^{ns}	0.024 ^{ns}
40	4-O-butyl 1-O-ethyl butanedioate[i]	nd	nd	0.007 ± 0.008	0.003 ± 0.006	1.45 ^{ns}	10.2***	1.45 ^{ns}
41	2-methylpropyl octanoate[i]	nd	nd	0.002 ± 0.001	0.002 ± 0.001	2.03 ^{ns}	46.0***	2.03 ^{ns}
42	3-methylbutyl hexanoate[i]	0.007 ± 0.002	0.010 ± 0.002	0.010 ± 0.004	0.012 ± 0.003	4.42*	5.05*	0.081 ^{ns}
43	Methyl octanoate[i]	**0.013 ± 0.004^{b}**	**0.019 ± 0.007^{a}**	**0.007 ± 0.003^{b}**	**0.012 ± 0.004^{a}**	13.9**	20.9***	0.382 ^{ns}
44	2-methylbutyl octanoate	nd	nd	0.013 ± 0.014	0.024 ± 0.018	1.83 ^{ns}	23.4***	1.83 ^{ns}
45	3-methylbutyl octanoate	0.010 ± 0.008	0.015 ± 0.010	0.037 ± 0.010	0.042 ± 0.009	2.06 ^{ns}	73.2***	0.004 ^{ns}
46	Methyl decanoate[i]	nd	nd	**0.003 ± 0.004^{b}**	**0.008 ± 0.003^{a}**	6.69*	44.0***	6.69*
47	3-methylbutyl decanoate	nd	nd	0.040 ± 0.011	0.046 ± 0.012	1.10 ^{ns}	236***	1.10 ^{ns}
48	*Total methyl and branched esters*	0.164 ± 0.083	0.166 ± 0.078	0.238 ± 0.045	0.265 ± 0.045	0.490 ^{ns}	17.3***	0.339 ^{ns}
49	Ethyl acetate	66.2 ± 59.4	119 ± 55	18.1 ± 5.9	19.7 ± 3.3	4.51*	33.2***	4.01 ^{ns}
50	Ethyl 2-hydroxypropanoate[i]	**44.6 ± 11.4^{b}**	**65.7 ± 19.8^{a}**	17.3 ± 5.4	19.1 ± 2.8	8.86**	91.9***	6.24*
51	Ethyl 3-hydroxybutanoate[i]	0.131 ± 0.052	0.132 ± 0.055	nd	nd	0.002 ^{ns}	120***	0.002 ^{ns}
52	Ethyl (E)-but-2-enoate[i]	**0.010 ± 0.006^{b}**	**0.021 ± 0.007^{a}**	nd	nd	14.1**	95.1***	15.2***
53	Diethyl butanedioate	96.1 ± 16.9	96.1 ± 20.2	17.0 ± 4.7	18.0 ± 2.9	0.015 ^{ns}	331***	0.011 ^{ns}
54	Ethyl 4-hydroxybutanoate[i]	**0.053 ± 0.054^{a}**	**0.014 ± 0.019^{b}**	0.023 ± 0.010	0.016 ± 0.006	6.78*	2.66 ^{ns}	3.42 ^{ns}
55	Ethyl butanoate	0.279 ± 0.359	0.545 ± 0.243	0.080 ± 0.024	0.076 ± 0.013	3.81 ^{ns}	24.8***	4.01 ^{ns}
56	Ethyl hexanoate	**2.74 ± 0.80^{b}**	**3.69 ± 0.38^{a}**	0.723 ± 0.149	0.733 ± 0.120	12.0**	326***	11.6**
57	Ethyl heptanoate	0.013 ± 0.002	0.012 ± 0.004	**0.005 ± 0.003^{a}**	**0.002 ± 0.003^{b}**	5.71*	76.7***	1.65 ^{ns}
58	Ethyl octanoate	10.6 ± 3.3	14.2 ± 4.0	4.88 ± 1.26	5.66 ± 0.80	6.36*	66.4***	2.68 ^{ns}
59	Ethyl nonanoate	0.066 ± 0.033	0.112 ± 0.062	**0.084 ± 0.059^{b}**	**0.134 ± 0.023^{a}**	9.49**	1.60 ^{ns}	0.027 ^{ns}
60	Ethyl decanoate	**4.48 ± 1.67^{b}**	**6.43 ± 1.95^{a}**	3.60 ± 1.08	4.35 ± 0.79	8.38*	10.0**	1.64 ^{ns}
61	Ethyl dec-9-enoate[i]	0.261 ± 0.171	0.395 ± 0.328	0.195 ± 0.051	0.238 ± 0.054	2.06 ^{ns}	3.26*	0.529 ^{ns}
62	Ethyl undecanoate	nd	nd	0.086 ± 0.033	0.129 ± 0.029	8.67**	214***	8.67**
63	Ethyl dodecanoate	0.896 ± 0.460	0.953 ± 0.618	**5.77 ± 1.52^{b}**	**7.60 ± 1.91^{a}**	4.88*	182***	4.30 ^{ns}
64	Ethyl tetradecanoate[i]	nd	nd	0.751 ± 0.158	0.874 ± 0.130	3.22 ^{ns}	560***	3.22 ^{ns}
65	Ethyl hexadecanoate[i]	nd	nd	2.47 ± 0.43	2.71 ± 0.54	1.02 ^{ns}	507***	1.02 ^{ns}
66	Ethyl (E)-hexadec-9-enoate[i]	nd	nd	0.006 ± 0.004	0.007 ± 0.005	0.681 ^{ns}	34.7***	0.681 ^{ns}
67	Ethyl octadecanoate[i]	nd	nd	0.110 ± 0.027	0.116 ± 0.039	0.123 ^{ns}	201***	0.123 ^{ns}
68	*Total ethyl esters*	**226 ± 74^{b}**	**307 ± 88^{a}**	71.2 ± 18.2	79.4 ± 7.6	5.65*	104***	3.76 ^{ns}
69	3-hydroxybutan-2-one	**0.042 ± 0.013^{b}**	**0.059 ± 0.013^{a}**	0.007 ± 0.014	0.007 ± 0.005	4.76*	128***	5.10*
70	octan-2-one[i]	nd	nd	0.000 ± 0.004	0.000 ± 0.002	2.76 ^{ns}	5.71 ^{ns}	2.76 ^{ns}
71	5-ethoxyoxolan-2-one[i]	nd	nd	**0.014 ± 0.012^{a}**	**0.005 ± 0.006^{b}**	4.17*	19.3***	4.17*
72	Oxolan-2-one[i]	0.181 ± 0.041	0.156 ± 0.026	0.004 ± 0.012	0.003 ± 0.004	2.83 ^{ns}	457***	2.42 ^{ns}

(*continued on next page*)

Table 2 (*continued*)

Variable		2018		2019		F-values		
Code	IUPAC name	Low-Chl	High-Chl	Low-Chl	High-Chl	Chl	Y	Chl × Y
73	2,4-ditert-butylphenol	nd	nd	1.56 ± 0.60	2.15 ± 0.90	2.66 ^{ns}	106***	2.66 ^{ns}
74	3,7-dimethyloct-6-enyl acetate	nd	nd	0.118 ± 0.065	0.112 ± 0.039	0.055 ^{ns}	82.8***	0.055 ^{ns}
75	Total volatiles	451 ± 97	518 ± 142	120 ± 21	119 ± 12	1.40 ^{ns}	171***	1.46 ^{ns}

[i] Quantified as 2-octanol equivalents. Bold and different letters indicate significant differences between subzones (Low and High Foliar Chlorophyll Level) for each year ($P < 0.05$, Tukey test). *Significant ($P < 0.05$); **significant ($P < 0.01$); ***significant ($P < 0.001$); ns: No significant ($p \geq 0,05$). Chl: Chlorophyll Level. Y: Year; nd: not detected

Table 3
Mean values and standard deviations of wine sensory variables obtained from vineyard subzones with high and low foliar chlorophyll content (Chl) in 2018 and 2019 and F-values of analysis of variance.

Code	Variable	2018		2019		F-values		
		Low-Chl	High-Chl	Low-Chl	High-Chl	Chl	Y	Chl × Y
D1	Limpidity	7.79 ± 0.98	7.67 ± 1.00	8.23 ± 1.46	8.33 ± 1.46	0.003 ^{ns}	18.0***	0.681 ^{ns}
D2	Tonality	7.18 ± 1.13	7.08 ± 1.22	8.30 ± 1.12	8.20 ± 1.18	0.778 ^{ns}	91.0***	0.000 ^{ns}
D3	Layer intensity	**7.62 ± 1.05^{a}**	**6.91 ± 1.50^{b}**	**8.32 ± 1.44^{a}**	**7.72 ± 1.42^{b}**	21.6***	28.6***	0.141 ^{ns}
D4	Aroma intensity	6.44 ± 1.20	6.69 ± 6.01	**6.96 ± 1.22^{a}**	**6.60 ± 1.36^{b}**	0.025 ^{ns}	0.435 ^{ns}	0.863 ^{ns}
D5	Red fruit	5.68 ± 1.52	5.69 ± 1.38	5.88 ± 1.37	5.99 ± 1.42	0.166 ^{ns}	3.12 ^{ns}	0.115 ^{ns}
D6	Black fruit	5.99 ± 1.43	5.70 ± 1.26	6.14 ± 1.39	5.85 ± 1.57	4.13*	1.08 ^{ns}	0.001 ^{ns}
D7	Herbaceous	4.88 ± 1.45	4.68 ± 1.45	**5.28 ± 1.55^{a}**	**4.74 ± 1.78^{b}**	5.21*	2.09 ^{ns}	1.16 ^{ns}
D8	Lactic	5.02 ± 1.75	5.31 ± 1.57	4.96 ± 1.46	5.15 ± 1.44	2.32 ^{ns}	0.509 ^{ns}	0.108 ^{ns}
D9	Acetic	3.72 ± 1.58	3.68 ± 1.43	3.15 ± 1.53	2.79 ± 1.49	1.76 ^{ns}	23.1***	1.06 ^{ns}
D10	Alcoholic	5.27 ± 1.81	5.47 ± 1.39	4.98 ± 1.80	4.62 ± 1.65	0.221 ^{ns}	11.5**	2.67 ^{ns}
D11	Flavour intensity	6.58 ± 1.26	6.18 ± 1.64	6.44 ± 1.39	6.35 ± 1.41	2.78 ^{ns}	0.016 ^{ns}	1.11 ^{ns}
D12	Bitter	6.04 ± 1.68	5.53 ± 1.82	5.38 ± 1.63	5.42 ± 1.77	1.79 ^{ns}	4.95*	2.46 ^{ns}
D13	Acidity	6.11 ± 1.94	5.70 ± 1.77	5.24 ± 1.53	5.12 ± 1.80	2.25 ^{ns}	16.6***	0.664 ^{ns}
D14	Astringency	5.72 ± 1.89	5.25 ± 2.06	5.66 ± 1.79	5.29 ± 1.99	4.61*	0.002 ^{ns}	0.055 ^{ns}
D15	Persistence	6.19 ± 1.38	6.29 ± 1.61	5.95 ± 1.40	5.94 ± 1.56	0.072 ^{ns}	3.84 ^{ns}	0.129 ^{ns}

Bold and different letters indicate significant differences between subzones (Low and High Foliar Chlorophyll Level) for each year ($P < 0.05$, Tukey test). *Significant ($P < 0.05$); **significant ($P < 0.01$); ***significant ($P < 0.001$); ns: No significant ($p \geq 0,05$). Chl: Chlorophyll Level. Y: Year.

(3S)-3-methylpentan-1-ol in 2019. These compounds, also known as fusel alcohols, are synthetized by yeast during alcoholic fermentation, either from catabolism of amino acids through the Ehrlich pathway or by formation of α-keto-acids during amino acid synthesis from sugar (Swiegers et al., 2005). These volatiles impart alcoholic and ethereal notes and positively contribute to the complex aroma of wines at low concentration (<300 mg/L) (Bakker & Clarke, 2011). Moreover, in 2018 and 2019, moderate iron stress increases the concentration of 2-phenylethanol, a pleasant alcohol characterized by a rose-like aroma (Cordente et al., 2018).

Regarding C6-alcohols, in 2018, vineyard subzones with low-Chl produced wines with lower concentrations of hexan-1-ol and (Z)-hex-3-en-1-ol than those with high-Chl. Similarly, results found in the same area of study disclosed that low-Chl vines provided musts with lower concentrations of unpleasant C6-alcohols, especially hexan-1-ol (Sánchez et al., 2021). These C6-alcohols are responsible for green herbaceous and vegetable aromas, and they are derived from the action of lipoxygenases on fatty acids. Sánchez et al., (2021) attributed the lowest concentration of C6-alcohols in must from vines with low-Chl to a decrease in the activity of iron-containing lipoxygenases. Previously, Vannozzi et al. (2017) reported a reduction in the expression of lipoxygenase gen in iron stressed vines.

Among the aldehydes analysed, 2-phenylacetaldehyde was affected by chlorosis and, in 2019, its concentration was higher in wines from low-Chl subzones than those from high-Chl subzones.

Esters are usually secondary aromas arising from the wine fermentation and they are responsible for the fruity and floral character in wine. The synthesis of acetate esters come from an enzymatic condensation between compounds derived from acetic acid and ethanol, while ethyl esters are produced by reaction of medium chain fatty acid and ethanol (Bakker & Clarke, 2011; Saerens et al., 2008).

The incidence of iron chlorosis had impact on the concentration of some acetates such as 2-phenylethyl acetate, 2-methylpropyl acetate, 3-methylbutyl acetate and hexyl acetate. In 2018, the concentration of 2-methylpropyl acetate, 3-methylbutyl acetate and hexyl acetate were significantly higher in wines from high-Chl subzones than low-Chl subzones, while less concentration on 2-phenylethyl acetate, an acetate ester which imparts rose-like aroma (Cordente et al., 2018), was observed in high-Chl subzones. However, in 2019 only 3-methylbutyl acetate showed significant differences between both subzones.

These results and those discussed above suggested that a moderate iron deficiency chlorosis increases the concentrations of pleasure floral volatiles in wine, such as 2-phenylacetaldehyde and 2-phenylethanol and its acetate ester 2-phenylethyl acetate with a rose-like aroma (Cordente et al., 2018; Swiegers et al., 2005).

In general, the highest concentrations of esters were also observed in wines produced from high-Chl subzones. In 2018, methyl octanoate, ethyl 2-hydroxypropanoate, ethyl (E)-but-2-enoate, ethyl hexanoate, ethyl decanoate and total ethyl esters showed the highest concentrations in wines from high-Chl subzones. In 2019, similar behaviour was observed in methyl octanoate, methyl decanoate, ethyl nonanoate and ethyl dodecanoate. However, wines from high-Chl subzones had the lowest concentrations of two ethyl esters, ethyl 4-hydroxybutanoate and ethyl heptanoate in 2018 and 2019, respectively.

It has reported that YAN in must affects ester formation during alcoholic fermentation, increasing ester concentration with a rise in YAN (Carrau et al., 2008; Vilanova et al., 2012). The recorded values of YAN in the musts from low-Chl subzones was lower than high-Chl subzones, both in 2018 (132.8 versus 161.7 mg/L) and 2019 seasons (140.9 versus 190.2 mg/L). These results agree with those previous studies carried out in the same area, which reported that iron deficiency restricted nitrogen availability to vines (González et al., 2019) and reduced YAN in musts (Sánchez et al., 2021). Thus, the highest concentration of esters found in wines from high-Chl subzones could be related to the increased concentration of must YAN.

Iron chlorosis was also associated to some compounds produced

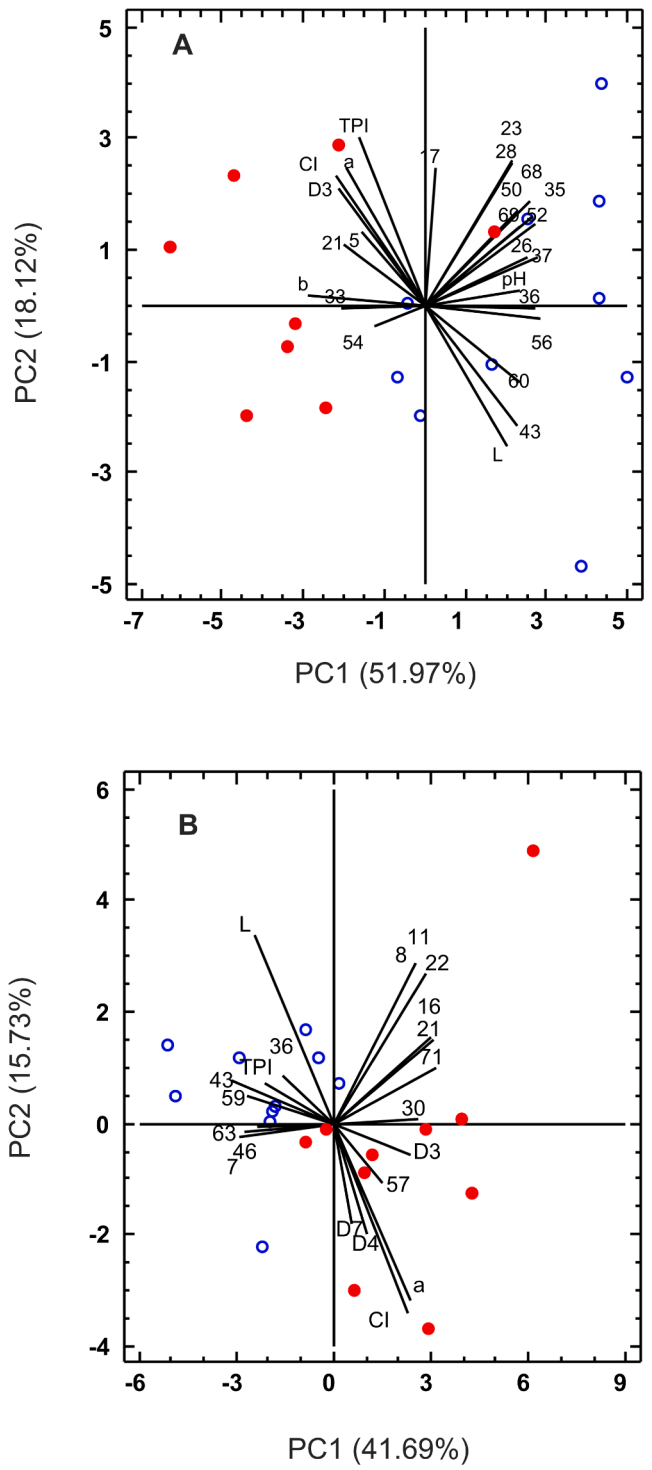

Fig. 1. Principal component analysis biplot of loadings (basic, VOCs and sensory composition) and wine samples from 2018 and 2019 vintages (subfigures A y B, respectively). Codification of samples: red filled circle (low-chlorophyll level) and blue circle (high-chlorophyll level). Codifications of variables: see Tables 1–3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during malolactic fermentation. In 2019, the concentration of the lactone 5-ethoxyoxolan-2-one significantly increased due to iron stress, adding fruity flavours to the wines (García et al., 2003). In 2018, wines from high-Chl subzones had a higher concentration of 3-hydroxybutan-2-one (acetoin). This volatile presents notes of butter and contributes to the wine aromatic complexity at concentrations above 5–7 mg/L (Martineau & Henick-Kling, 1995).

3.3. Sensory variables

The sensory variable results provided interannual differences, as was observed in wine chemical and VOCs composition (Tables 1 and 2). In 7 of 15 sensory attributes studied (Table 3) presented significant differences in the comparison of the two vintages: in the three visual descriptors (limpidity, tonality and layer intensity), in two olfactory descriptors (acetic and alcoholic), and in two descriptors in the mouth (bitter and acidity). In 2019 vintage the wines showed values higher in the three visual descriptors, while the other four sensory attributes presented lower intensity. Furthermore, the effect of iron chlorosis on sensory variables was significant in 4 of the 15 attributes studied: layer intensity, black fruit, herbaceous and astringency.

The wines of 2018 and 2019 vintages from low-Chl subzones showed significantly higher scores in layer intensity than those from high-Chl (Table 3), according to the higher colour intensity and total polyphenol index detected in those wines (Ribéreau-Gayon et al., 2006). Moreover, in 2019, aroma intensity showed higher scores in wines from low-Chl vines than those from high-Chl. These results agree with those obtained by Sánchez et al. (2020) and confirm that mild to moderate iron stress in vineyards can contribute to improve sensory attributes as layer intensity and aroma intensity.

Concerning herbaceous descriptor, we observed the wines from low Chl subzones had higher scores than those from high Chl (Table 3), although they had lower concentrations of the total C6-alcohols (Table 2). In addition to C6-alcohols, other volatiles derived from the oxidation of fatty acids could be responsible for the high score in herbaceous descriptor observed in wines from low-Chl subzones. C6-adehydes, such as hexanal and the two hexenals, trans-(E)-hex-2-enal and cis-(Z)-hex-3-enal) (Hatanaka, 1993) and some powerful aroma C9-aldehydes, such as E-2-nonenal and (E,Z)-2,6-nonadienal, also play an important role in the vegetable and herbaceous characteristics of grapes and wines (Ferreira & Lopez, 2019).

Other important sensory attribute in the quality of red wines is astringency. The perception of this attribute depends on factors such as pH (Kallithraka et al., 2007). In the present work we have observed in both vintages that in wines from low-Chl subzones, as the pH is lower than those from high-Chl subzones (Table 1), the perception of astringency from the sensory point of view is higher (Table 3). Fontoin et al. (2008) demonstrated that pH is a dominant variable affecting astringency.

3.4. Principal component analysis

PCA was applied to identify the main variables that determine the differentiation between wines from high-Chl and low-Chl subzones and to obtain a general overview of the effect of iron status of vines and vintage on wine quality variables (Fig. 1). This analysis was performed based on physicochemical, VOCs and sensory characteristics of the wines from 2018 and 2019 vintages using the statistically significant variables previously identified by factorial ANOVA (Tables 1–3).

PCA from the data of 2018 vintage was carried out (Fig. 1a). The first principal component (PC1) and second principal component (PC2) expressed 51.97% and 18.12% of physicochemical, VOCs and sensory composition of wines, respectively. According to PC1, wines from low-Chl subzones were placed at negative side while wines from high-Chl subzones were positioned at positive side. Parameters associated to wine colour, such as a*, b*, colour intensity, total polyphenol index and layer intensity, as well as high concentrations of 2-phenylethanol and 2-phenylethyl acetate were highly related to the wines from low-Chl subzones. In contrast, ethyl hexanoate, hexyl acetate, ethyl (E)-but-2-enoate, 3-methylbutyl acetate, 2-methylpropyl acetate, VOCs with fruity notes, were highly associated with the wines from high-Chl subzones.

PCA of the data of 2019 displays that PC1 and PC2 explain 41.69%

and 15.73% of total variance, respectively (Fig. 1b). Overall, wines from low-Chl subzones were positioned at the positive side of PC1 and negative of PC2. Parameters associated to wine colour, such as a*, colour intensity and layer intensity, ethyl heptanoate, as well as aroma intensity and the olfactory descriptor herbaceous were highly related to these wines. In the space defined by negative PC1 and positive PC2 were localized the wines from high-Chl subzones. These wines were highly associated with L, total polyphenol index, and methyl octanoate, ethyl nonanoate, ethyl dodecanoate and 3-methylbutyl acetate (VOCs with fruity notes).

4. Conclusions

Our results confirm that a moderate incidence of iron chlorosis in the vineyard can produce a favourable reduction of pH, increase of colour intensity, and enhance sensory attributes of Tempranillo wine, as layer intensity, black fruit and aroma intensity. Moreover, we have shown that the positive effects of iron stress on wine quality can also be detected in its volatile profile.

To our knowledge, this is the first research evaluating the impact of iron deficiency in the vineyard on VOCs of the wine. A moderate level of iron stress in grapevines increased the concentrations of VOCs associated with floral notes, as 2-phenylacetaldehyde, 2-phenylethanol and 2-phenylethyl acetate in the wines, and reduced the presence of C6-alcohols, responsible for green-herbaceous aroma. As negative effect, wines from chlorotic zones reduced the concentration of esters.

The information provided can be useful to evaluate the quality potential of vineyards affected by iron chlorosis. More studies would be interesting to contrast these results under different soil, climate conditions and grape varieties.

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CRediT authorship contribution statement

Ramón Sánchez: Investigation, Methodology. **José Manuel Rodríguez-Nogales:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **Encarnación Fernández-Fernández:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. **María Rosa González:** Conceptualization, Investigation, Methodology, Formal analysis, Funding acquisition. **Laura Medina-Trujillo:** Investigation, Methodology. **Pedro Martín:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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