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Assessment of grape volatile composition using fluorescence indices of leaves in vineyards affected by iron chlorosis

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ABSTRACT: Multiplex 330 (MX) is a portable, non-invasive fluorescence sensor that provides different multiparametric indices that are sensitive to the concentration of chlorophylls, flavonols, and anthocyanins on the leaf surface. This study investigated the use of these indices to assess the variability on free volatile composition of grapes (*Vitis vinifera* L.) in the field as well as other components of their quality potential in vineyards affected by iron deficiency chlorosis (IDC). Twenty non-irrigated Tempranillo/110 Richter vineyard subzones from non-affected to moderately affected by IDC were monitored in Ribera del Duero area (Spain) during two seasons. The results indicated that MX can characterize the spatial variation of leaf pigment concentrations, agronomic performance, and grape quality in vineyards affected by IDC. The MX indices measured at the leaf level close to harvest had better predictive values for the concentrations of free alcohols, volatile acids, C13-norisoprenoids, esters and acetates in the must than vine vigor, size or degree of technological maturity of the grapes. Our study demonstrates that the MX indices to estimate leaf pigment concentrations can be helpful to assess the technological maturity and free volatile composition of wine grapes in vineyards affected by IDC; nevertheless, the efficacy of the indexes may vary according to the year season.
Keywords: Multiplex, anthocyanin, aroma, chlorophyll, flavonol

Introduction

Iron deficiency chlorosis (IDC) is a common environmental stress in calcareous soils of the Mediterranean region, frequently inflicting high economic losses in grapevine (*Vitis vinifera* L.) production. IDC decreases the synthesis of chlorophylls (Val et al., 1987) and lowers the photosystem II efficiency (Bavaresco et al., 2006; Hailemichael et al., 2016). Therefore, high Fe stress levels compromise yield and vigor (Tagliavini and Rombolà, 2001; Echeverría et al., 2017), reducing the synthesis and accumulation of substances in the fruits throughout the maturation stage (Shi et al., 2018). However, weak to moderate Fe stress levels can positively affect wine grape quality, enhancing concentrations of sugars and phenolic compounds (Balint and Reynolds, 2017; González et al., 2019) while improving the aroma profile of the must (Sánchez et al., 2021).

In a vineyard, the spatial variation in soil conditions, such as the pH, texture, or the active limestone content, can cause a wide vine-to-vine variation in Fe status, affecting yield and grape composition (Martín et al., 2007; Li et al., 2017; González et al., 2019). The management of this variability within precision viticulture requires the use of methods that enable an accurate, fast, and non-invasive monitoring of the Fe status of vines. In recent years, interest in hand-held optical sensors has increased, such as Multiplex 330 (MX), developed by Force-A, to assess leaf pigment concentrations on a surface basis (Diago et al., 2016; Blank et al., 2018). The MX provides fluorescence-based indices related to chlorophyll, flavonol, and anthocyanin, which could be applied to monitor plant growth and stress responses (Schmidt et al., 2018). Nevertheless, these indices need testing in vineyards affected by IDC to estimate the quality potential of the grapes, including their volatile profile.

This study aimed to (i) investigate different multiparametric fluorescence indices related to the foliar level of chlorophylls, flavonols, and anthocyanins at the plant growth stage with the MX, reflecting the spatial variability between sampling sites in vineyards affected by IDC, and (ii) test in these vineyards if the leaf pigment indices could be helpful to assess the free volatile composition of grapes and other components of their quality potential in the field.

Materials and Methods

Study site

The study was carried out in Spain, during the 2017 and 2019 seasons, on 20 rainfed vineyard subzones (10 m × 10 m each) located in the western area of Ribera del Duero Appellation of Origin (41°38′34″ N, 4°09′27″ W, altitude 800 m). In the study site, the soils are calcareous, highly basic, loamy to clay loam in texture, and poor in organic matter, showing high variability in assimilable phosphorus (P), potassium (K), and magnesium (Mg) contents (Zarco-Tejada et al., 2013). The wide concentration ranges of assimilable Fe (2.3-6.4 mg kg^{-1}) and active limestone (3.3-16.0 %) in the soils, along with the presence of a sensitive rootstock (110-Richter), generate a variable incidence of IDC on the site with unaffected to moderately affected vineyards.

The study site has a continental Mediterranean climate, with very cold winters and hot and dry summers. Table 1 shows the monthly rainfall and temperature values registered in the studied years. The year 2017 was drier than 2019 (annual rainfall registered 273 mm *versus* 426 mm) and warmer (mean annual temperature was 12.7 °C *versus* 12.2 °C). In 2017, late frosts (–2.3 °C on 28 Apr and –0.7 °C on 1 May) strongly reduced the yield of vineyards in the site.

The study subzones were selected within 9.2 ha of the vineyard to keep the maximum variability among their nutrient status, as described Sánchez et al. (2021). All vineyard subzones comprised the Tempranillo grape variety, grafted on 110-Richter, in a plantation spacing of 3.0 m × 1.5 m (2222 plants ha^{-1}). Vines were trained on a vertical shoot positioned trellis and pruned on a bilateral cordon with an average load of 16 buds per plant.

Fluorescence sensor measurements

A Multiplex 330 sensor (Force A, Orsay Cedex, France) was used to obtain different indices sensitive to pigment concentrations per unit leaf area in the field and in a non-invasive way. The MX is a portable optical sensor that measures chlorophyll fluorescence emitted in red (RF, 670-690 nm) and far-red (FRF, 720-780 nm) spectral regions under excitation with light-emitting diodes at 515 nm (_G) or 630 nm (_R). Based on these signals, the sensor provides different fluorescence indexes. Specifically, we obtained SFR_R = FRF_R/RF_R, FLAV = log(FRF_R/FRF_UV) and ANTH_RG = log(FRF_R/FRF_G), related to the chlorophyll, flavonol and anthocyanin contents in the epidermis of plant tissues, respectively (Diago et al., 2016).

The MX measurements were taken each year in four sampling times: in the phenological stages of fruit set, pea size, and veraison, also one week before harvest. In every sampling, the values of each subzone were taken as the mean of the records obtained on 18 fully expanded leaves, randomly chosen at the mid-height of the canopy. Each leaf was measured once over a circular area of 8 cm in diameter.

Vigor, yield, and grape composition

Yield and winter pruning weight were determined each season in the study sites.

Harvesting was performed in all subzones on the same day each year. At harvest, a sample of 100 berries from each subzone was randomly collected, weighed, and used to determine the total soluble solid content (TSS), total acidity (TA), yeast assimilable nitrogen concentration, the pH, total polyphenol index, and color density of the must, according to the International Organization of Vine and Wine (2014).

Analysis of must volatiles by GC-MS

We obtained 300 mL of must of crusted grapes from each vineyard subzone that were frozen immediately to –20 °C until analysis. The analysis of volatile compounds was performed following the methodology proposed by Oliveira et al. (2008) with some modifications. We centrifuged (RCF = 9660, 20 min, 4 °C) 75 mL of grape juice per sample and filtered through a glass wool. Then, 3 µg of 4-nonanol (Merck, ref. 818773) as internal standard were added and passed through a LiChrolut EN cartridge (Merck, 500 mg, 40-120 µm) previously pre-conditioned (10 mL of dichloromethane, 5 mL of methanol and 10 mL of aqueous alcoholic solution 10 % v/v). The free volatile fraction was eluted with 5 mL of pentane-dichloromethane (2:1), dried in anhydrous sodium sulphate, and concentrated to 200 µL by solvent evaporation with N_2 before analysis.

Gas chromatograph (GC) Agilent GC 6890N coupled to mass spectrometer (MS) Agilent 5975C analyzed the volatile organic compounds. A volume of 1 µL was injected into a capillary column CP-Wax 52 CB (50 m × 0.25 mm, *i.e.*, 0.2 µm film thickness, Chrompack). The injector was programmed from 20 °C to 250 °C, at 180 °C min^{-1}. The oven temperature was held at 40 °C for 5 min then programmed to rise from 40 °C to 250 °C, at 3 °C min^{-1}. Then, held at 250 °C for 20 min and finally programmed to raise from 250 °C to 255 °C at 1 °C min^{-1}. The carrier gas was helium N60 (Air Liquide) at 103 kPa, corresponding to a linear speed of 180 cm s^{-1} at 150 °C. The detector was set to

Table 1 – Average monthly values of maximum (T_{max}), minimum (T_{min}), medium (T_{ave}) temperature (°C), and total precipitation (P, in mm) registered during 2017 and 2019 in the study area (41°38'32" N, 4°17'33" W, altitude 733 m – Valbuena de Duero, Valladolid, Spain).

Year	Variables	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
2017	T_{max}	9.2	12.7	16.5	21.1	24.2	30.7	30.7	30.3	25.5	24.1	14.1	9.2
	T_{min}	–3.8	1.0	1.6	3.0	8.6	13.0	12.7	12.3	8.1	5.6	–1.2	–0.6
	T_{ave}	2.0	6.5	8.6	12.3	16.6	22.1	22.1	21.5	16.7	14.3	5.6	3.9
	P	14.8	35.5	19.7	4.2	50.2	16.9	52.7	18.9	0	11.5	17.9	30.3
2019	T_{max}	9.3	15.0	17.0	15.9	22.7	28.0	31.5	30.5	24.8	20.4	11.4	10.8
	T_{min}	–3.1	–2.2	0.0	3.4	5.2	9.4	13.7	12.1	9.3	6.6	4.0	1.6
	T_{ave}	2.2	5.5	8.5	9.5	14.3	19.2	22.6	21.4	16.7	13.0	7.5	5.7
	P	31.6	5.0	12.8	53.0	5.2	14.9	38.1	32.8	25.7	54.5	64.7	88.0

electronic impact mode (70 eV), with an acquisition range from 29 to 360 m/z, and an acquisition rate of 610 ms.

The Search Free Software was used to identify volatile compounds by comparing mass spectra (Wiley and Nist libraries) and retention indices with those of pure standard compounds. All of the compounds were quantified as 4-nonanol equivalents.

Statistical analysis

The factorial analysis of variance (ANOVA) and the Tukey test, with a significance level of $p < 0.05$, were performed to evaluate the additive and interaction effects of season, measurement time, and vineyard subzones on the MX indices.

The relationships between the chlorophyll, flavonol, and anthocyanin indices with vine vigor, yield, and grape composition parameters were studied by calculating the Pearson's correlation coefficients. Additionally, a partial least squares (PLS) regression (Wold, 1966) was applied to show the relationships between the concentrations of different groups of volatile organic compounds in the must (dependent variables) and values of the MX indices, TSS, berry size, and vine vigor registered in studied subzones (independent variables), considering simultaneously 2017 and 2019 seasons. The data were standardized to have all of them in approximately the same scale.

All statistical analyses were performed using the Statistical Analysis System (SAS) version 9.2.

Results and Discussion

Variability in leaf pigment indices

The factorial analysis of variance (Table 2) showed that the year factor had substantial effects on the MX indices studied, with significant interactions between year × subzone and year × time of measurement. The average SFR_R in 2019 was lower than in 2017, while FLAV and ANTH_RG were higher. The meteorological conditions of the year can modify leaf pigment concentrations. The lower rainfall in May, June, and July 2019, compared to 2017 (Table 1), generated greater summer water stress; thus, vine leaves lost greenness and accumulated more flavonols and anthocyanins in the second season.

The measurement time modified the indices with the highest values of SFR_R and the lowest of FLAV and ANTH_RG at pea size (Table 2). The chlorophyll and flavonol concentrations in the leaves, on a surface basis, increased with leaf expansion and light exposure until veraison and then leaf chlorophyll usually decreased (Louis et al., 2009). The decrease of SFR_R after the maximum could be related to summer stress and the natural degradation of leaf chlorophylls in deciduous plants during the final part of the vegetative cycle (Casanova-Gascón et al., 2018). The increase of

Table 2 – Mean values of Multiplex leaf indices of chlorophylls (SFR_R), flavonols (FLAV) and anthocyanins (ANTH_RG), and significance of the three-way ANOVA on year, time of measurement and vineyard subzones.

	SFR_R	FLAV	ANTH_RG
Year (Y)			
2017	2.56 ± 0.20	1.90 ± 0.05	0.006 ± 0.015
2019	2.35 ± 0.22	1.94 ± 0.07	0.012 ± 0.019
Significance[1]	125.44***	38.00***	13.370***
Time of measurement (T)			
Fruit set	2.40 ± 0.22 c	1.93 ± 0.06 ab	0.020 ± 0.01 a
Pea size	2.51 ± 0.20 a	1.88 ± 0.06 c	–0.004 ± 0.01 b
Veraison	2.49 ± 0.25 ab	1.91 ± 0.07 b	0.010 ± 0.02 a
Harvesting	2.43 ± 0.25 bc	1.95 ± 0.05 a	0.013 ± 0.02 a
Significance[1]	7.19***	26.15***	36.90***
Subzones (S)			
F-value[1]	10.24***	3.34***	12.83***
Fruit set[2]	0.71	0.69	1.28
Pea size[2]	1.20	0.82	2.64*
Veraison[2]	3.06**	0.68	2.54*
Harvesting[2]	13.75***	0.85	5.14***
Interactions			
Y × T[1]	3.38*	36.97***	15.68***
Y × S[1]	4.23***	5.27***	3.14***
T × S[1]	1.91**	0.94	1.33

[1]F value in three-ways ANOVA on year, time of measurement and subzone; [2]F value for subzone variable in two-ways ANOVA (time or measurement is fixed); F values are significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; Means with the same letter within one column are not different (Tukey's test).

ANTH_RG from pea size to harvesting could be due to the cumulative effect of summer stress and IDC, which stimulated the biosynthesis of anthocyanins in the leaves (Caramanico et al., 2017).

The F-values for the MX indices studied in three-way ANOVA were significant for the subzone factor (Table 2). However, when the measurement time was fixed, the two-way ANOVA of FLAV was not significant for subzone variation in any phenological stage. In contrast, SFR_R and ANTH_RG were increasingly affected by the variability in the subzones from fruit set to harvesting. While the foliar chlorophyll content measured at veraison was a good indicator for IDC incidence in the study site (Sánchez et al., 2021), the homogeneous values of SFR_R registered at the fruit set did not distinguish differences in Fe status of the vines. The results in Table 2 show that in the last season, one week before harvest, the chlorophyll and anthocyanin indices best reflected the variations in the subzones.

Vigor, yield, and grape composition

In 2017, the average yield (0.44 kg m^{-2}) and winter pruning weight (0.15 kg m^{-2}) were lower than in 2019 (0.58 and 0.17 kg m^{-2}, respectively). Pruning weight was directly correlated with SFR_R measured close to harvest in both years and inversely correlated with FLAV and ANTH_RG in 2017. Yield only obtained significant *r* coefficients with SFR_R in 2019 (Table 3). The results on the SFR_R index agree with González et al. (2019) in the same study site. The authors compared subzones with high and low foliar chlorophyll content at the veraison stage. Our results suggest that SFR_R, and to a lesser extent FLAV and ANTH_RG, could be used to assess plant growth in vineyard zones under varying Fe stress.

Neither TSS nor yeast assimilable N concentration were correlated with the MX indices (Table 3). Nevertheless, SFR_R showed positive correlations with TA and negative with TSS/TA and with the pH in both seasons studied, while FLAV and ANTH_RG showed negative correlations with TA and positive with TSS/TA in 2017. The variability in vine vigor within the study site, correlated with SFR_R (Table 3), may have modified the microclimate conditions, generating greater sunlight exposure of leaves and clusters in most Fe-stressed subzones, thus advancing their ripening process (Sánchez et al., 2021).

Color intensity of the must was negatively correlated with SFR_R in both years studied and positively correlated with ANTH_RG in 2019. The increase of coloring matter of the grapes associated with decreasing values of SFR_R could be due to both a more favorable fruit microclimate in more Fe-stressed subzones (lower canopy density as SFR_R decreases) and a direct effect of IDC on promoting the biosynthesis of polyphenols (Bavaresco et al., 2005; González et al., 2019).

The results show that the MX chlorophyll index, measured one week before harvest, could be considered a good estimator of the technological maturity of the grapes in vineyards affected by IDC. Altogether, SFR_R seems a more interesting index to estimate TA, the pH, and color intensity of the must than FLAV and ANTH_RG. Diago et al. (2016) demonstrated the viability of assessing chlorophyll, epidermal flavonol, and N concentrations in grapevine leaves with MX indices for application in precision viticulture. Similarly, our results show that MX could have an the advantage of monitoring plant growth and response to IDC incidence in the vineyard. Moreover, from a management viewpoint, this fluorescence sensor could characterize the spatial variability in must quality to allow grapes from different quality zones to be batched for separate winemaking, increasing the economic value of the products.

Relationships between volatile profile and MX indices

Berry quality, mainly in wine grape varieties, is mainly dependent on secondary metabolites, namely polyphenols and volatiles. Accumulation of secondary metabolites is a plant defense strategy against biotic and abiotic stresses, modifying the phenolic and volatile profile (Ferrandino and Lovisolo, 2014).

In this work, 57 free volatile organic compounds were identified and quantified in the must samples (Table 4), with a total concentration of 1160.2 μg L^{-1} in 2017 and 1266.2 μg L^{-1} in 2019. Only 19 of these compounds were simultaneously detected in both seasons. Similarly, several studies have revealed a wide year-to-year variation on the volatile composition of must and wine from different grape varieties (Bureau et al., 2000; Vilanova et al., 2015).

Free alcohols, terpenes, and C_{13}-norisoprenoids are groups with a larger number of compounds determined (Table 4). Free volatiles directly contribute to the varietal wine aroma (Parker et al., 2018) and, along with volatile conjugates (aromatic precursors), represent the potential aroma of grapes (Liu et al., 2017; Ferreira and López, 2019). Higher alcohols contribute to desirable complexity of the wine if their concentration in wines does not exceed 300 mg L^{-1} (Rapp and Mandery, 1986). Terpenes play a fundamental role in the sweet and floral notes of aromatic grape varieties (Francis and Newton, 2005). C_{13}-norisoprenoids are also essential contributors to floral wine aroma because of their low odor threshold (Sefton et al., 2011).

Table 3 – Pearson's correlation coefficients between vigor, yield and must composition parameters with Multiplex indices of chlorophylls (SFR_R), flavonols (FLAV) and anthocyanins (ANTH_RG) recorded in grapevine leaves one week before harvest in 2017 and 2019 seasons.

	SFR_R		FLAV		ANTH_RG	
	2017	2019	2017	2019	2017	2019
Yield	0.18	0.44*	–0.36	–0.05	–0.20	–0.09
Pruning weight	0.63**	0.46*	–0.72***	0.21	–0.52*	–0.36
100-berry weight	0.41	0.41	–0.62**	0.27	–0.26	–0.28
Must composition						
Total soluble solids (TSS)	–0.29	–0.33	0.20	0.23	0.41	0.25
Total acidity (TA)	0.72***	0.56**	–0.86***	0.10	–0.61**	–0.26
TSS/TA	–0.73***	–0.66**	0.75***	–0.07	0.65**	0.33
pH	–0.52*	–0.67***	0.47*	–0.20	0.33	0.42
Yeast assimilable nitrogen	0.39	0.41	–0.19	0.07	–0.15	–0.31
Total polyphenol index	–0.11	–0.52*	0.29	–0.03	0.21	0.31
Colour density	–0.54*	–0.83***	0.40	–0.30	0.17	0.51*

Values are significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 4 – Pearson's correlation coefficients between concentrations of free volatile compounds in the must with Multiplex indices of chlorophylls (SFR_R), flavonols (FLAV) and anthocyanins (ANTH_RG) recorded in grapevine leaves one week before harvest in 2017 and 2019 seasons.

	SFR_R		FLAV		ANTH_RG	
Group / compound	2017	2019	2017	2019	2017	2019
Alcohols						
1-Butanol	–0.14	–0.62**	–0.22	–0.30	0.36	0.53*
(3 + 3)-Methyl-1-butanol		–0.41		–0.38		0.38
(2 + 3)-Methyl-1-butanol	0.34		–0.34		–0.20	
Benzyl alcohol	0.25	–0.14	–0.28	–0.22	–0.16	0.08
2-Phenyl-ethanol	0.05	–0.30	0.02	–0.19	0.06	0.29
3-Methyl-3-buten-1-ol+1-pentanol	0.05		0.02		–0.20	
1-Octanol	–0.50*		0.50*		0.59**	
1-Dodecanol		–0.58**		–0.38		0.27
3-Methyl-2-buten-1-ol	0.45		–0.29		–0.49*	
Total	0.30	–0.60**	–0.32	–0.40	–0.20	0.45*
C_6-Alcohols						
1-Hexanol	0.34	0.08	–0.21	–0.18	–0.28	–0.30
(E)-3-Hexen-1-ol	0.33	0.10	–0.32	–0.10	–0.22	0.06
(Z)-3-hexanol	0.38	0.29	–0.37	–0.18	–0.23	–0.28
(E)-2-Hexen-1-ol	0.41	0.07	–0.28	–0.24	–0.31	–0.22
(Z)-3-Hexen-1-ol	0.80***		–0.52*		–0.74***	
(Z)-2-Hexen-1-ol	0.16		–0.30		–0.02	
Total	0.58*	0.17	–0.43	–0.23	–0.44	–0.31
Aldehydes						
Hexanal	–0.25	–0.30	0.06	–0.19	0.22	0.37
(E)-2-Hexenal		–0.35		–0.31		0.32
Benzaldehyde	–0.23		–0.05		0.14	
Total	–0.25	–0.33	0.06	–0.25	0.22	0.36
Esters and acetates						
Isoamyl acetate	0.13	–0.51*	–0.38	–0.30	0.02	0.46*
Hexyl acetate		–0.47*		–0.22		0.39
Diethyl malate		–0.62**		–0.41		0.37
Ethyl hexanoate	–0.10		–0.35		0.20	
Ethyl octanoate	–0.39		–0.20		0.37	
2-Methyl-1-butyl acetate	0.35		–0.11		–0.34	
Total	0.13	–0.54*	–0.39	–0.32	0.02	0.45*
Terpenes						
Nerol	–0.47*	–0.58**	0.05	–0.46*	0.54*	0.38
Geraniol	0.21	–0.47*	–0.25	–0.25	–0.11	0.54*
Diendiol I	0.28		–0.22		–0.24	
Diendiol-II	0.02	–0.02	–0.22	0.27	0.11	0.11
(E)-8-hydroxylinalool		–0.61**		–0.31		0.49*
Terpinen-4-ol	–0.33		–0.15		0.37	
α-terpineol	–0.41		–0.06		0.50*	
Trans-Pyran-linalool oxide (LOP)	–0.04		–0.01		–0.05	
β-Citronellol	0.35		–0.28		–0.28	
Total	0.16	–0.64**	–0.25	–0.31	–0.08	0.56**
Volatile acids						
Hexanoic acid		–0.30		–0.18		0.40
(E)-2-hexenoic acid	0.30	–0.56**	–0.29	–0.20	–0.23	0.41
Nonanoic acid		–0.48*		–0.18		0.37
Decanoic acid		–0.59**		–0.17		0.36
Hexadecanoic acid	–0.18	–0.22	–0.05	–0.20	0.28	0.35
Geranic acid	0.11		–0.14		0.02	
Total	–0.09	–0.52*	–0.08	–0.25	0.19	0.47*
C_{13}-Norisoprenoids						
3-oxo-3,4-dihydro-actinidol i		–0.51*		–0.15		0.34

Continue...

Table 4 - Continuation.

3-oxo-3,4-dihydro-actinidol ii		–0.41		–0.16		0.32
3-oxo-3,4-dihydro-actinidol iii		–0.40		–0.19		0.50*
3-hydroxy-β-damascone		–0.51*		–0.23		0.53*
3-hidroxy-7,8-dihydro-β-ionol		–0.39		–0.17		0.27
4-Oxo-7,8-dihydro-β-ionol	–0.12	–0.10	0.20	0.10	0.14	0.16
3-Oxo-7,8-dihydro-α-ionol	0.09	–0.55*	–0.18	–0.19	0.00	0.41
3-hidroxy-7,8-dehydro-β-ionol		–0.58**		–0.31		0.65**
3-Oxo-α-ionol	0.20		–0.28		–0.06	
Total	0.08	–0.48*	–0.14	–0.18	0.02	0.43
Volatile phenols						
Guaiacol	0.15	–0.47*	–0.31	–0.16	–0.03	0.47*
4-Vinylphenol	0.17	–0.49*	–0.15	–0.16	–0.15	0.45*
Vanillin	–0.01		–0.26		–0.03	
Acetovanillone	–0.18		–0.04		0.24	
Total	0.06	–0.50*	–0.25	–0.17	0.02	0.50*
γ-Butyrolactone (lactone)	0.14	–0.10	–0.23	–0.10	–0.02	0.11
Acetoin (carbonyl compound)	0.05		0.13		–0.10	
Total free volatiles	0.46	–0.26	–0.49*	–0.35	–0.29	0.15

Values are significant at *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

The number of significant correlation coefficients between the concentrations of individual volatile compounds in the must and the MX indices were much higher in 2019 than in 2017 (Table 4). The total concentrations of free alcohols, esters and acetates, terpenes, volatile acids and volatile phenols in the must were directly correlated with ANTH_RG and inversely correlated with SFR_R in the 2019 season. Moreover, we detected negative correlations between the concentrations of some free alcohols (octanol, dodecanol and 1-dodecanol) and terpenes (such as nerol) with SFR_R and positive correlations with ANTH_RG. The concentration of some C_{13} norisoprenoids, such as 3-oxo-3,4-dihydro-actinidol i and 3-hidroxy-7,8-dehydro-β-ionol, and the total concentration of this family were negatively correlated with SFR_R in 2019, but these relationships were not observed for the compounds quantified in 2017.

The SFR_R index most correlated to the concentrations of free volatiles, registering significant *r* negative values in all groups, except for C_6-alcohols (Table 4). In general, ANTH_RG obtained correlation coefficients with the opposite sign to those observed for SFR_R. FLAV had very few correlations with individual volatile compound concentrations, although it was the only index presenting a significant *r* value with the total free volatiles (year 2017).

The total concentration of free C_6-alcohols in the must was correlated with SFR_R measured close to harvest in 2017. Increasing concentrations of (Z)-3-hexen-1-ol were strongly related with high values of SFR_R and lower values of FLAV and ANTH_RG. These volatile compounds are responsible, to some extent, for the undesirable green herbaceous and vegetable aromas perceived in wines when their concentration is high (González-Barreiro et al., 2015). The concentrations of C_6-alcohols in grapes decrease naturally throughout the ripening (Salinas et al., 2004); therefore, the lower presence of these compounds in musts from low SFR_R subzones might be due to the advance in maturation caused by less vigor in Fe-deficient plants (SFR_R were directly correlated with pruning weight and inversely with TSS/TA – Table 3). On the other hand, this decrease could also be explained by a decline in the lipoxygenase activity (Vannozzi et al., 2017), which is involved in the synthesis of C_6-alcohols. Iron is a cofactor that increases the catalytic activity of lipoxygenases (Podolyan et al., 2010).

The increase in the concentrations of terpenes and C_{13}-norisoprenoids associated to decreasing values of SFR_R (Table 4) agrees with Coelho et al. (2009), who studied the aroma of sparkling wines from vines cultivated in calcareous and non-calcareous soils. IDC could have not only a direct effect on promoting the biosynthesis of C_{13}-norisoprenoids in grapes (Vannozzi et al., 2017), but also an indirect effect through the microclimate. Fe-stressed plants have their vigor restricted, as explained above, and this increases the cluster exposure to sunlight. Greater fruit exposure, without excessive temperature, favors the C_{13}-norisoprenoids accumulation during ripening (Asproudi et al., 2016; Moreno et al., 2017). Moreover, in green berries, sunlight appears to increase the concentration of carotenoids, which are precursors of C_{13}-norisoprenoids (Bureau et al., 2000).

Similar to C_{13}-norisoprenoids, the concentration of total free volatile phenols, guaiacol and 4-vinylphenol increased with decreasing SFR_R values in 2019 (Table 4), in agreement with previous studies carried out by Sánchez et al. (2021) in the same site. According to the authors, moderate levels of Fe deficiency in vines increased concentrations of phenolic compounds in grapes. Volatile phenols, derived from hydroxycinnamic acid precursors in grape berry, exist mainly in glycosidic form, and its hydrolysis leads to several volatile compounds (Ristic et al., 2013).

In summary, our results indicate that vineyard subzones affected by Fe stress (lower foliar content of chlorophyll and higher content of flavonols and anthocyanins) tended to have a greater grape aromatic potential than the others. In this context, the MX indices showed a predictive value of the volatile composition of the must strongly dependent on the year.

Partial least squares regression

The PLS regression was applied to establish correlations between the free volatile concentrations in the must, leaf pigment indices, vine vigor, size and maturity grade of the grapes. This multivariate analysis technique extracts successive components to explain as many responses and predictor variations in the sample. The results obtained from the joint data of 2017 and 2019 include the representation of the variables regarding the two main components extracted and the amount of predictor and response variation for each component (Figure 1).

According to the loading weights of aldehydes, volatile phenols and terpenes (Figure 1) showed very poor results for free volatile organic compounds estimated by the first two PLS components. On the contrary, the concentration of free C_6–alcohols showed the highest correlations with PLS factors, keeping an inverse relationship with grape maturity (TSS) and a direct relationship with vine vigor (PW) and berry size (100BW).

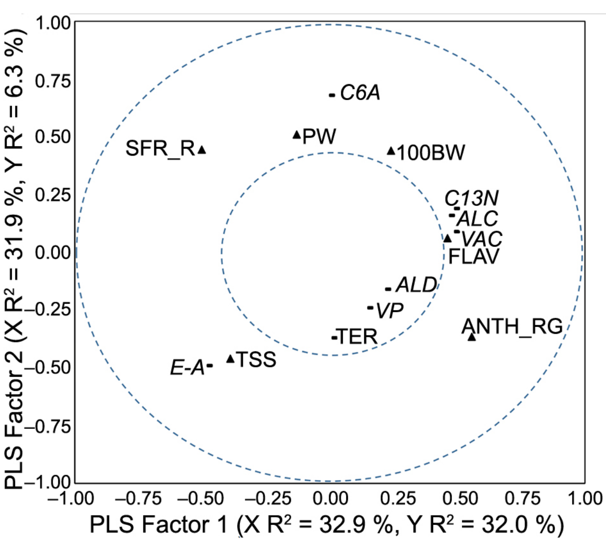

Figure 1 – The partial least square regression of the concentrations of free volatile compounds in the must (Y matrix) on the Multiplex indices and agronomic parameters measured in vineyard subzones (X matrix), considering joint data from 2017 and 2019 seasons. Y variables, in italics: alcohols (ALC), aldehydes (ALD) C_6-alcohols (C6A), esters and acetates (E-A), terpenes (TER), volatile acids (VAC), C_{13}-norisoprenoids (C13N), volatile phenols (VP). X variables, in bold: fluorescence indices related to foliar chlorophyll (SFR_R), flavonol (FLAV) and anthocyanin contents (ANTH_RG), pruning weight (PW), 100-berry weight (100BW) and total soluble solid content of the must (TSS).

SFR_R and ANTH_RG had opposite positions in the plot in Figure 1, indicating that these are variables with solid inverse correlation. Both indices would serve as predictors of the concentration of free alcohols, volatile acids, and C_{13}–norisoprenoids, with a slight advantage of ANTH_RG over SFR_R. The concentrations of these groups showed a higher correlation with TSS and MX indices than with 100BW. The results agree with those of Maoz et al. (2018), who reported changes in the volatile composition of Crimson Seedless table grapes related to the maturity level and not to the size.

The relative position of the variables in Figure 1 shows that FLAV had a lower predictive value than the other two MX indices, although it was the best to estimate the concentration of free esters and aldehydes. Overall, the set of MX indices improved the predictive value of the volatile profile versus TSS, PW, or 100BW. In fact, PW did not obtain correlations with the concentration of any group of free volatiles, except for C_6-alcohols.

Vegetation indices obtained from remote sensing platforms are currently the most used tool to monitor vineyards and to estimate berry composition in precision viticulture (Ferrer et al., 2020; Giovos et al., 2021). Nevertheless, our results suggest that foliar pigment indices could provide more helpful information to assess the grape quality potential than vegetation indices in vineyards affected by IDC.

Conclusions

This study provides evidence that the Multiplex 330 sensor showed changes in pigment composition of leaves over time induced in grapevines by Fe deficiency. The results obtained were strongly dependent on the year; however, they show that the MX indices could characterize the spatial variation of agronomic performance and grape quality potential in vineyards affected by IDC. The measurement time that could discriminate better among subzones was close to harvest.

To our knowledge, this is the first study evaluating the use of the Multiplex sensor to assess the must volatile composition in the vineyard. SFR_R, ANTH_RG and FLAV indices, measured in leaves close to harvest, could be a valuable tool to assess the aromatic potential of the grapes within vineyards affected by Fe deficiency. These indices showed an excellent predictive value for the concentrations of free alcohols, volatile acids, C_{13}–norisoprenoids, esters and acetates, although the efficiency of the indices may vary depending on the season. The results of the PLS regression analysis revealed that the MX indices were more effective to assess the free volatile composition of the must than vine vigor, size or technological maturity of the grapes.

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Authors' Contributions

Conceptualization: González, M.R.; Vilanova, M.; Martín, P. **Data acquisition:** Sánchez, R.; González, M.R.; Vilanova, M. **Data analysis:** González, M.R.; Martín, P. **Design of methodology:** González, M.R.; Vilanova, M.; Martín, P. **Writing and editing:** Sánchez, R.; González, M.R.; Vilanova, M.; Martín, P.

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