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Influence of vertical training systems on warm climate red winemaking: wine parameters, polyphenols, volatile composition, and sensory analysis

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ABSTRACT

Aim: A study was performed on the influence three vertical training systems had on wine composition in warm climates, analysing the wine's polyphenol and volatile compound contents and sensory properties.
Methods and results: The polyphenols and volatile compound content of wines was analysed together with their sensory properties. The effect of different training systems (double Guyot (CT), Smart-Dyson variation (SM), and Triple cordon (TC)) was studied in Tempranillo (TEMP), Syrah (SYR) and Tintilla de Rota (TR) cultivars to determine their influence on red winemaking over a two-year period. Statistical analysis was conducted with Cultivar and Year as factors. TC delayed grape ripening and reduced the alcohol in the wine when grapes from the three training systems were picked on the same date. Overall, no differences were found in polyphenol content. Higher alcohol acetates, ethyl esters of branched acids and various esters were found to be influenced, and some of these compounds are related to ripening. A limited impact was found on the sensory properties of the wines.
Conclusions: Under the test conditions, irrespective of the cultivar, the alcohol content of the wines was modified by the training systems through delayed ripening.
The training system affected certain polyphenol compounds and the ester profile of the wines. Some of these compounds are related to ripening. Therefore, differences were due to delayed ripening caused by training management.
Significance and impact of the study: Training systems have an impact on the oenological parameters of wines and the quantity of the polyphenols and volatile compounds they contain. In this regard, cultural practices such as training system may be used to optimise berry and wine quality (Teixeira *et al.*, 2013). However, the relationship between management practices and secondary metabolites such as the phenolics and volatile compounds produced by plants in warm climates is not well known.

KEYWORDS

vertical training systems, wine composition, polyphenol, warm climate, volatile compound

Additional tables can be downloaded from https://oeno-one.eu/article/view/2123

INTRODUCTION

Vine training systems involve the physical manipulation of plants to directly manage their canopies. All aspects of vine growth, development, yield, and fruit composition may be affected by modifications in training (Reynolds and Vanden Heuvel, 2009).

Polyphenolic compounds, such as anthocyanins, flavonols or catechins (flavan-3-ols), are secondary metabolites that play an important role in determining the quality of wine, contributing strongly to sensory characteristics such as colour and astringency (Arnold *et al.*, 1980). In addition to the technological tools such as the winemaking processes (Ricardo-da-Silva *et al.*, 1992; Spranger *et al.*, 2004), or the ageing and storage conditions of wine (Pérez-Magariño and González-San José, 2004; Sun *et al.*, 2011), the content of polyphenolic compounds in wine is mainly influenced by the grape cultivar and the degree of berry ripening (Pérez-Magariño and González-San José, 2004; Ricardo-da-Silva *et al.*, 1992), together with the vine-growing method and related training systems (Jackson and Lombard, 1993; Pérez-Lamela *et al.*, 2007; Peterlunger *et al.*, 2002). Another important group of secondary metabolites affecting wine quality are volatile compounds. Several studies demonstrated how training systems influenced the accumulation of aromatic precursors and varietal compounds in grapes and wines (Hernandez-Orte *et al.*, 2015; Lee *et al.*, 2007). Other authors reported that light conditions, which can be adapted by the training system, promoted the accumulation or suppression of C6 esters in wines (Xu *et al.*, 2015). Moreover, alterations to the canopy, a vine characteristic intrinsically linked to the training system, have been shown to induce changes in aromatic profiles, impacting on the sensory attributes of wine (Šuklje *et al.*, 2014). Among all aroma compounds in wine, esters have grown in popularity in recent years due to insights into their sensory contribution to the typical fruity aroma of red wines (Hernandez-Orte, *et al.*, 2015; Escudero *et al.*, 2007).

Polyphenolic and aromatic profiles are strongly dependent on the genetic basis of grapes (Guerrero *et al.*, 2009; Iriti and Faoro, 2006; Polaskova *et al.*, 2008; Ribéreau-Gayon *et al.*, 2006) and can be modulated by viticultural and/or winemaking practices based on the genetic plasticity of cultivars (Hernandez-Orte, *et al.*, 2015; Pozo-Bayón *et al.*, 2009).

The aim of this multidisciplinary study is to evaluate the influence that three vertical training systems used in a warm climate have on the sensory analysis of wine and its composition, including polyphenols and volatile compounds. A three-factor experimental design was used on wines from three vertical training systems with three red varieties, over a period of two years and under the same winemaking conditions. This study contributes to the increasing body of knowledge regarding the relationship between wine composition and the training systems, so it may be useful in the near future to mitigate the effects of climate change on vineyards while maintaining wine quality.

MATERIALS AND METHODS

1. Chemicals and reagents

Malvidin-3-*O*-glucoside was purchased from Polyphenols A.S. (Sandnes, Norway); quercetin-3-rutinoside was purchased from Merck (Darmstadt, Germany); *E*-Resveratrol was purchased from Sigma-Aldrich (Steinheim, Germany); catechin, gallic acid and caffeic acid were purchased from Sigma (Madrid, Spain). Analytical grades of formic acid and methanol (MeOH) were supplied by Panreac (Barcelona, Spain). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this study.

HPLC-grade ethanol was obtained from J.T. Baker Chemicals B. V. (Denventer, Holland). Sodium chloride, ACS reagent grade (purity ≥ 99.8%) and the standard volatile compounds listed in Supplementary Table 2 were purchased from Sigma-Aldrich (Madrid, Spain).

2. Plant material

The experiments were conducted in 2014 and 2015 on three red wine grape cultivars: Tempranillo (TEMP, Spanish national cultivar), Syrah (SYR, international cultivar), and Tintilla de Rota (TR, local cultivar) (*Vitis vinifera L.*). The vineyard is located on a trial site of the IFAPA Centre "Rancho de La Merced", Jerez de la Frontera (south-west Spain, longitude 06:00:58W, latitude 36:45:29N) at 35 m above sea level, in an area with a limestone soil composed of 19% sand, 38.5% clay and 42.5% silt. The vines were planted in 2009 on 140 Ru, with a planting density of 3200 vines/ha, and a vine spacing of 2.50 m between rows and 1.25 m between vines. They are north–south oriented,

not irrigated and the ratio of canopy height to alley is maintained below 1:1.

The systems are abbreviated as follows: single cordon double Guyot (CT); Smart-Dyson variation (SM), with some similarities with the Ruakura Twin Two Tier (Smart *et al.*, 1990); and Triple cordon double Guyot (TC), known as the Te Kauwhata Three Tier trellis (Smart *et al.*, 1990).

3. Winemaking experimental procedure

Approximately 200 kg of grapes were harvested manually when optimum maturity was achieved in the CT sample. The harvest date for the three training systems – CT, SM and TC – was the same, while the date varied for the TEMP, SYR and TR cultivars. The decision was made to compare the composition of the grapes from the CT training system at full ripeness with those from the SM and TC training systems, assuming these would reach full ripeness on the same date. The grapes were transported to the winery, divided into 100 kg duplicates, and stored at low temperature (15ºC) for 24 hours. After this period, they were destemmed and crushed. The must and solid were pumped into a 100 L stainless steel tank, potassium metabisulphite was added (50 mg/kg SO_2, Solfosol, Sepsa-Enartis, Spain), and the pH was adjusted to 3.6 with tartaric acid (Sepsa-Enartis) as in standard winemaking. Yeast was inoculated (20 g/hL, ES488, Sepsa-Enartis) and alcoholic fermentation was controlled at 25ºC. The fermentation cap was punched down once daily during the alcoholic fermentation. The solid was then pressed in a pneumatic press (Willmes, Germany). The free-run and press wine were combined in a 100 L tank. The reducing sugars were analysed to ensure levels of under 2 g/L before malolactic fermentation. To conduct malolactic fermentation, *Oenococcus oeni* (1 g/hL, Challenge Easy ML, Sepsa-Enartis) and nutrients (20 g/hL Nutriferm ML, Sepsa-Enartis) were added. Finally, at the end of the malolactic fermentation process the wine was racked, the SO_2 was corrected to reach 50 mg/kg of SO_2 (Solfosol, Sepsa-Enartis) and the wine was bottled with no filtration.

Grapes were harvested from three vineyard lines for each cultivar (TEMP, SYR and TR), each training system (CT, SM and TC) and in two seasons (2014 and 2015). As above, the winemaking was conducted in duplicates, so a total of 18 fermentations were performed each year.

4. Wine oenological parameters

The wines were analysed for alcohol by volume (Alc/vol (%)), volatile acidity, glycerol, iron, copper, and zinc. Malic acid (MH_2) was analysed in the must before malolactic fermentation. Colour intensity (sum of absorbance at 420, 520 and 620 nm), hue, CIELab coordinates (L*, a*and b*) and dry extract were recorded based on the recommendations in the *OIV Compendium of International Methods of Analysis of Wines and Must* (OIV, 2014). The analyses were performed in duplicate.

5. Wine polyphenols

Analysis by direct injection was performed according to a previous protocol with slight modifications (Guerrero, *et al.*, 2009). Before analysis, the wine samples were filtered through a 0.22 µm filter (PTFE Teknokroma, Barcelona, Spain). The samples (20 µL) were analysed using a Jasco PU-2089 HPLC quaternary gradient pump and an MD-2010 Plus diode array detector. Separations were achieved on a Synergi 4µ Hydro-RP 80A (Phenomenex, USA) (RP-18, 150×2 mm; 4 µm particle size) and a guard column of the same material, at 30ºC. The mobile phase consisted of water with 5% formic acid (solvent A) and HPLC-grade methanol (solvent B) at a flow rate of 1 mL/min. The elution programme involved gradient elution from 20% B to reach 32% at 30 min, 40% B at 45 min, and 95% B at 75 min. Chromatograms were recorded at 520, 360, 320, 306 and 280 nm. Anthocyanins (ΣANT) were identified by their UV spectra and retention time, and quantified as malvidin 3-*O*-glucoside at 520 nm. Flavan-3-ols (ΣCAT) were identified by their UV spectra and retention time, and quantified as catechin at 280 nm. Stilbenes (ΣSTB) were identified by their UV spectra and retention time, and quantified as *E*-Resveratrol at 306 nm. Flavonols (ΣFLAV) were identified by their UV spectra and retention time, and quantified as quercetin-3-rutinoside at 360 nm. Hidroxibenzoic acids (ΣHBEN) were identified at 280 nm and quantified as gallic acid. Hydroxycinnamic acids (ΣHCIN) were identified by their UV spectra and retention time, and quantified as caffeic acid at 320 nm.

6. Wine volatile compounds

The wine samples (25 mL) were spiked with 20 µL of internal standard mix solution at 200 mg/L of isotopically labelled esters supplied by CDN isotopes (Pointe-Claire, Canada): [$^2H_3$]-ethyl butyrate, [$^2H_{11}$]-ethyl hexanoate, [$^2H_{15}$]-ethyl octanoate and [$^2H_5$]-ethyl cinnamate. Then, 10 mL of the spiked samples diluted 1:14 with Mili-Q water were placed into a 20 mL SPME vial containing 3.5 g of NaCl. The capped vials were homogenised for 30 seconds in a vortex shaker, placed into a Combipal autosampler tray (CTC Analytics) and analysed by HS-SPME-GC-MS. A previously conditioned 100 µm PDMS fibre (Supelco, Bellefont, PA, USA) was used. The vials were stirred at 500 rpm for 2 min at 40ºC. Extraction was set at 40ºC for 30 min and desorption was performed at 250ºC for 15 min. The fibre was desorbed into a Trace GC ultra-gas chromatograph (Thermo Fisher Scientific S.p.A., Rodano, Milan, Italy) coupled to an ISQ Single Quadrupole MS spectrometer (Thermo Fisher Scientific, Austin, Texas, USA). The injection mode was splitless for 0.75 min. The column was a BP21 of 50 m × 0.32 mm, 0.25 µm film thickness (SGE Analytical Science, UK). The carrier gas was helium at a column-head pressure of 8 psi. The oven temperature was programmed at 40ºC for 5 min, raised to 220ºC at 3ºC/min, and then kept constant for 30 min. The MS transfer line and source temperature were 230ºC and 200ºC, respectively. The mass spectrometer operated in electron ionisation mode at 70 eV using selected-ion-monitoring (SIM) mode. Identification was carried out by comparing retention times and mass spectra with those of pure standards. Esters were grouped according to their origin and chemical structure into seven groups: ethyl esters of fatty acids (ΣEEFA), higher alcohol acetates (ΣHAA), ethyl esters of branched acids (ΣEEBA), cinnamates (ΣCINN), methyl esters of fatty acids (ΣMEFA), isoamyl esters of fatty acids (ΣIEFA), and miscellaneous esters (ΣME).

7. Wine sensory analysis

The triangle test was conducted to confirm that there were no differences between the wine duplicates. One wine duplicate was used for the descriptive sensory analysis.

A descriptive sensory analysis was performed by 10 experts. Forty-two descriptors (six on colour, 26 on aroma and 10 on taste) were rated 0, 1, 2, or 3 on an ordinal scale. Only descriptors that showed differences were studied: the purple colour descriptor; the aroma intensity, red fruit, black fruit, tree fruit, overripe fruit, and spicy aroma descriptors; and the bitter, astringent, balanced, and persistence taste descriptors.

8. Statistics

Statistix 9.1 version was used for the data treatment. The Shaphiro Wilks test of normality ($W>0.05$ and $P<0.05$) was conducted on each parameter to ensure linearity. A three-way factorial ANOVA [training system (CT, SM and TC), cultivar (TEMP, SYR and TR) and year (2014 and 2015)] was performed to detect two-ways interactions. Interactions were represented as follows. Training × Cultivar interaction for two years: CT-TEMP, SM-TEMP, TC-TEMP, CT-SYR, SM-SYR, TC-SYR, CT-TR, SM-TR and TC-TR. Training × Year interaction for three cultivars: CT-2014, SM-2014, TC-2014, CT-2015, SM-2015 and TC-2015. Cultivar × Year interaction for three training systems: TEMP-2014, SYR-2014, TR-2014, TEMP-2015, SYR-2015 and TC-2015. An LSD all-pairwise comparison test at $P<0.05$ was used for post-hoc test grouping.

Pearson correlations were determined between parameters.

To study the results from the wine sensory analysis, the Kruskal-Wallis one-way ANOVA was run on Statistix 9.1 software. An all-pairwise comparison test at $P<0.05$ was used for post-hoc test grouping.

RESULTS AND DISCUSSION

The results were divided into two tables. Table 1 shows the results of the ANOVA for the Training system, Cultivar and Year factors together with the double interaction *significance*. Table 2 shows the results of the double interactions.

1. Training method

The oenological parameters of the wines and their composition of polyphenols and volatile compounds were studied to establish the effects of the different training systems. Total acidity and pH showed no differences, as tartaric addition was conducted to adjust pH in the winemaking process (see Material and Methods section).

TABLE 1. Training system, cultivar and year three-way ANOVA on oenological parameters, polyphenols and volatile composition.

	Training				Cultivar				Year			Interactions		
	CT	SM	TC	P-Value	TEMP	SYR	TR	P-Value	2014	2015	P-Value	T-C	T-Y	C-Y
Enological parameters														
Alc/vol (%)	13.8	13.5	12.9	***	11.8	13.3	15.0	***	15.0	11.7	***	NS	**	*
MH2(g/L)	2.0	1.9	1.9	NS	2.5	2.0	1.3	***	2.2	1.7	***	***	NS	**
Volatile acidity (g/L AcH)	0.54	0.49	0.47	***	0.43	0.48	0.60	***	0.56	0.44	***	***	NS	***
Glycerol (g/L)	9.57	9.22	9.04	*	8.08	9.37	10.38	***	10.45	8.1	***	NS	NS	***
Fe (ppm)	0.83	0.75	0.8	NS	0.84	0.64	0.9	***	0.85	0.73	***	**	NS	***
Cu (ppm)	0.11	0.11	0.1	NS	0.13	0.09	0.1	***	0.14	0.07	***	*	NS	**
Zn (ppm)	0.28	0.24	0.22	NS	0.18	0.25	0.31	***	0.36	0.13	***	NS	NS	**
Color intensity	1.037	1.048	1.039	NS	0.770	1.068	1.286	***	1.345	0.739	***	NS	NS	NS
Hue	0.59	0.57	0.57	NS	0.56	0.56	0.61	***	0.57	0.58	NS	**	NS	***
L*	50.99	50.55	51.98	NS	61.24	48.11	44.18	***	42.71	59.63	***	NS	*	***
a*	50.68	51.43	49.72	NS	45.69	52.43	53.71	***	57.19	44.03	***	**	**	***
b*	1.758	1.887	2.105	NS	-1.789	-1.609	9.148	***	6.082	-2.249	***	NS	NS	**
Dry extract (g/L)	23.67	22.76	21.20	***	18.63	22.83	26.17	***	24.93	20.16	***	NS	NS	**
Polyphenols (mg/L)														
ΣANT	426.5	418.6	410.9	NS	373.4	475.5	407.1	***	429.8	407.5	**	NS	*	***
ΣCAT	82.6	77.8	70.9	*	45.6	153.8	32.0	***	95.0	59.3	***	**	NS	***
ΣSTB	1.3	1.1	1.4	NS	1.5	0.0	2.4	***	1.7	0.9	***	**	**	***
ΣFLAV	141.6	142.2	129.7	NS	93.6	189.1	130.8	***	181.4	94.3	***	NS	NS	***
ΣHBEN	34.2	37.1	37.0	NS	45.5	15.3	47.5	***	33.1	39.1	*	NS	NS	**
ΣHCIN	28.4	25.7	27.5	*	40.9	26.0	14.7	***	31.2	23.3	***	*	**	**
Volatile compounds (µg/L)														
ΣEEFA	1408	1354	1263	NS	1355	1475	1194	***	1398	1285	*	NS	NS	***
ΣHAA	2277	2283	1976	*	3310	2660	565	***	2014	2343	**	NS	*	***
ΣEBBA	126	142	148	***	111	148	156	***	110	167	***	NS	NS	***
ΣCINN	0.5	0.5	0.5	NS	0.5	0.5	0.4	*	0.8	0.2	***	NS	NS	**
ΣMEFA	1.8	1.7	1.6	NS	1.2	2.0	1.9	***	2.2	1.3	***	NS	NS	***
ΣIEFA	1.7	1.7	1.6	NS	1.6	1.8	1.5	**	2.2	1.1	***	NS	NS	***
ΣME	178	188	194	*	146	231	183	***	170	203	***	**	*	***

Training systems: CT, Control; SM, Smart; TC, Triple cordon. Red grape cultivars: TEMP, Tempranillo; SYR, Syrah; TR, Tintilla de Rota. Double factor interaction level of significance: T-C, training system and cultivar interactions; T-Y, training system and year interactions; C-Y, cultivar and year interaction. Polyphenols were grouped according to their polyphenol subgroup: anthocyanins (ΣANT), flavan-3-ols (ΣCAT), stilbenes (ΣSTB), flavonols (ΣFLAV), hidroxibenzoic acids (ΣHBEN), and hydroxycinnamic acids (ΣHCIN). Esters were grouped according to their origin and chemical structure into seven groups: ethyl esters of fatty acids (ΣEEFA), higher alcohol acetates (ΣHAA), ethyl esters of branched acids (ΣEEBA), cinnamates (ΣCINN), methyl esters of fatty acids (ΣMEFA), isoamyl esters of fatty acids (ΣIEFA) and miscellaneous esters (ΣME). a, b or c for the same parameter denote significant differences, $p < 0.05$. Analyses of variance, level of significance (p-value, T-C, T-Y, and C-Y): NS (not significant), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

1.1. Alc/vol (%)

Alcohol by volume is an essential parameter in wines. The TC wine clearly showed a lower degree of alcohol compared with CT and SM (Table 1). This was undoubtedly related to the ripeness of the grapes at harvest. The ºBé of the CT, SM and TC grapes at harvest was 13.8, 13.5 and 12.9, respectively (data not shown).

As no double interaction "Training × Cultivar" was found (Table 2), this shows that under the test conditions alcohol by volume of wine can be modified by the training system irrespective of the cultivar. Nevertheless, the training system interacted with the Year factor and no differences were found in 2014 between the training systems. The alcohol by volume of the three training systems in 2014 was more than 2° higher than in 2015 (Table 2). In 2015, the cultivar trend observed in the simple interaction "Training" was maintained. TC-2015 showed the lowest value (10.8% alc./vol). Differences between years may be related with the harvest dates as these differed in each year and depended on the full ripeness of the CT grapes. In this parameter, TC was more variable depending on the year.

1.2. Malic acid

Malic acid (MH_2) was analysed after alcoholic fermentation and before malolactic fermentation. No significant differences were found between training systems. This parameter did not show any differences in the degree of ripeness between training systems, in contrast with the results for alcohol by volume. Malic acid levels decrease during ripening (Ribéreau-Gayon, *et al.*, 2006). Thus, a lower malic acid concentration was expected in those wines with a higher alcohol by volume. Malic acid after malolactic fermentation showed no differences as this acid was totally consumed by the bacteria (data not shown).

1.3. Volatile acidity

The average level of acetic acid (main volatile acid) in a young dry table wine is less than 400 mg/L, though levels may range from undetectable to 3g/L (Ribéreau-Gayon, *et al.*, 2006). The aroma threshold for acetic acid in red wine varies from 600 to 900 mg/L, depending on the cultivar and style. The amount of volatile acidity found in healthy grapes is negligible because it is a by-product of microbial metabolism. Although this parameter is mainly affecting by winemaking (Ribéreau-Gayon, *et al.*, 2006), it showed variation in the three ANOVA factors and their interactions. Volatile acidity decreased in the order CT, SM and TC. However, this parameter was below the limits of quality wines in the samples tested.

1.4. Glycerol

Glycerol is typically found at concentrations of 4–10 g/L in dry wine (Ribéreau-Gayon, *et al.*, 2006). It is the most abundant compound in wine after water and ethanol. Several parameters have been shown to influence the glycerol levels in wine. These include grape ripeness, the microbial flora on grape berries and cellar equipment, as well as pH, fermentation temperature, nitrogen source and yeast strain (Scanes *et al.*, 1998). The wines made under the CT training system showed a higher glycerol concentration than the SM and TC wines. This may be explained by the higher degree of ripeness of the CT grapes. Glycerol is an interesting parameter in sensory evaluation. It is related to body and a smooth taste in wines. In general, higher glycerol levels are considered to improve wine quality. No interactions were observed between the training system and either cultivar or year, so differences were related directly with the training system.

1.5. Metals

Fe and Cu were analysed due to their importance in wine oxidation. Zinc is a trace element that plays a major role in auxin metabolism and therefore in plant growth. Zinc deficiency causes a decrease in plant size, as well as a change in the arrangement and colour of leaves (Ribéreau-Gayon *et al.*, 2006). No significant differences were found in Fe, Cu and Zn between the training systems. This result may be related to the greater influence of factors such as edaphology and phytosanitary treatments rather than the training system, cultivar or year, and to the fact that the detected values were low.

1.6. Colour intensity, Hue, L*, a* and b*

Vine-growing practices have been shown to strongly influence wine colour (Pérez-Lamela *et al.*, 2007). The colour of red wines is one of their most important sensory attributes and is related to the extraction of anthocyanins from grape skins during winemaking. These are accumulated in the berry skins during grape ripening, and

several viticultural factors such as cultivar, climate, soil conditions, canopy management, crop level and irrigation have been related to anthocyanin accumulation (Jackson and Lombard, 1993). Regarding the effect of the training system on the colour parameter in wine, no significant influence was found for colour intensity, hue, L*, a* and b*. The influence of the training system on colour was difficult to study due to the high influence of the cultivar factor on anthocyanin and subsequently, on colour.

In the literature, pruning and training systems were found to affect the colour characteristics of wine. Cordon systems, represented by CT in the current work, have been demonstrated to increase the proportion of colour compounds by 10%, 20% and 70% in the Souson, Mencia and Brancellao cultivar, respectively, compared with the Guyot system (Pérez-Lamela *et al.*, 2007). The results in the current research showed that the colours of TEMP, SYR and TR cultivars were not as influenced by the training system as those in previous studies.

1.7. Dry extract

Red wines generally contain approximately 20–30 g/L of dry extract. Significant differences were observed in this parameter according to the training system. TC showed the lowest value and CT and SM were included in the same group with a higher value. Double interactions with training were not found. This parameter may be a key factor in the differences found in other parameters. Pearson's correlation was conducted on this parameter (data not shown) with alcohol by volume, volatile acidity, glycerol, zinc, colour intensity and b*, showing a correlation factor of 0.9420, 0.8134, 0.9347, 0.7697, 0.8465, and 0.8496, respectively. However, only the correlation between dry extract and either alcohol by volume and glycerol is meaningful in oenology. The post-hoc grouping analysis of the dry extract of the training system only concurred with alcohol and glycerol, which also showed significant differences, grouping together SM and TC instead of CT and SM, as was the case in the dry extract parameter.

1.8. Polyphenols

Polyphenols families are shown in Table 1. Small differences were found in the total concentration of anthocyanins, stilbenes, flavonols and hydroxybenzoic acid. Differences were observed in individual compounds such as delphinidin-3-*O*-glucoside, petunidin-3-*O*-acetylglucoside, peonidin-3-*O*-acetylglucoside, cyanidin-3-*O*-coumaroylglucoside, peonidin-3-*O*-coumaroylglucoside, malvidin-3-*O*-coumaroylglucoside, catechin, *E*-piceid, myricetin-3-*O*-glucoronide, myricetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoronide, kaempherol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside and syringetin-3-*O*-glucoside (Supplementary Table 1). However, their contribution to the total concentration of their family was either minor or compensated for. In addition, the influence of cultivar and year regarding polyphenols was remarkable.

Significant differences were found in catechins and hydroxycinnamic acid compounds. The compounds studied were catechin, epigallocatechin gallate and epicatechin (Supplementary Table 1). A higher concentration was found in CT than TC, and a concentration three times was found in SYR where CT, SM and TC were separated statistically in decreasing order. No clear trend was found for the TEMP and TR cultivars.

In the ΣHCYN group, caffeic acid and the hydroxycinnamic acid derivatives caffeoyl-tartaric acid and coumaroyl-tartaric acid were analysed. The main contributor was caffeoyl-tartaric acid. Differences in hydroxycinnamic acids were found between CT with the highest levels and SM with the lowest (Table 1).

Dependence of the polyphenolic compounds on the training system was discussed by Coletta *et al.* (2014), but the extent of this was influenced by the cultivar. The training system induced significant differences in all the polyphenols families, changing the quantitative ratios between them, which were in turn related to the sensory characteristics of the wine (Coletta *et al.*, 2014). The importance of the training system to Pinot Noir grapes and their related wine composition has been assessed, as has the effect of the degree of grape ripening on wine colour and the level of flavanols and anthocyanins (Pérez-Magariño and González-San José, 2004). Polyphenols increase with grape maturation, although the accumulation of these phenolic compounds and their patterns of evolution varied considerably with the cultivar (Navarro *et al.*, 2008). Calò *et al.* (1994) found anthocyanin levels to be affected by the training system.

Conversely, other authors have found that the training system (Guyot and spur pruned) does not influence the tannin and anthocyanin contents in Nero d`Avola grapes (Corona *et al.*, 2004). Another study comparing grapes from two different harvests revealed the absence of a direct relationship between changes in the polyphenol content of red wine and the training system, although slight differences in wine colour were found (González-Neves *et al.*, 2004). The quality of wines produced from Cabernet-Sauvignon grapes cultivated with different training systems was found to be similar (Jackson and Lombard, 1993). More specifically, the content of polyphenolic compounds was observed to vary according to the training system, and an increased content of these molecules was found in sun-exposed Pinot Noir grapes. Different results were also obtained for Pinot Noir in the Friuli Hills in the North-East of Italy (Peterlunger *et al.*, 2002). This study found the influence of the training system on the composition of grapes and on wine quality to be minimal, while the relevance of light exposure was confirmed.

Under the experimental conditions presented, it was clear that the training system influenced the level of polyphenol compounds in the wines. CT training provided a higher polyphenol concentration of catechins and hydroxycinnamic acids. Nevertheless, no differences were found in anthocyanins and flavonols, the main polyphenols in wine. The important influence of cultivar on polyphenols indicates that the influence of training system on wine polyphenols must be studied for each cultivar individually, and according to the literature, in a specific region and terroir.

1.9. Volatile compounds

The volatile compounds in wine depend on viticultural, climatic and winemaking factors. It has already been described that aroma compounds depend on vine-growing practices (Jackson and Lombard, 1993; Reynolds and Vanden Heuvel, 2009). However, although many aroma compounds have been identified, our understanding of the role viticulture plays in their evolution remains limited (Robinson *et al.*, 2014).

The sum of volatile ester groups is shown in Table 1. Supplementary Table 2 shows the differences in single volatile compounds. TC training produced the wines with the highest contents of ethyl esters of fatty acids (ΣEEFA) and the lowest higher alcohol acetates (ΣHAA), while the wines produced under CT treatment showed the lowest values for ethyl esters of fatty acids and miscellaneous esters (ΣME). The SM wines presented high contents of ethyl esters of fatty acids (Table 1). The compounds most affected by the training systems (Supplementary Table 2) were ethyl butyrate, propyl acetate, isoamyl acetate, ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate, and ethyl propanoate. However, the underlying molecular mechanism behind such influences needs to be determined (Xu, *et al.*, 2015).

Regarding ethyl esters of fatty acids, ethyl butyrate was affected by training management, the highest levels found in wines from CT training (13%). Indeed, this compound was not subject to the interactions Training × Year and Training × Cultivar. Thus, the response of the cultivars to training during the two years was consistent. In contrast, the concentrations of propyl acetate and isoamyl acetate compounds belonging to the higher alcohol acetates group were affected by training. It is thought that vineyard treatment may have an indirect impact on higher alcohol acetates by influencing and modulating the composition of grape amino acids, ammonium or lipids (Šuklje, *et al.*, 2014). Wines produced under TC training conditions showed a 15% lower content of higher alcohol acetates as a result of lower amounts of propyl and isoamyl acetates (Supplementary Table 2). ΣHAA showed significant Training × Cultivar interaction differences. The training system affected ΣHAA depending on cultivars – the TR cultivar being the least influenced by the training methodology (Table 2). Furthermore, no *significant* interactions of Training × Year and Training × Cultivar were found for propyl acetate. Therefore, training had a similar effect on propyl acetate levels in the cultivars during both years (Supplementary Table 2).

Ethyl esters of branched acids (ΣEEBA) were also affected by training. The TC and SM wines showed higher ΣEEBA contents. The main contributors to the changes observed were ethyl isobutyrate, ethyl 2-methylbutyrate and ethyl isovalerate (Supplementary Table 2). No interactions of Training × Cultivar were observed for ethyl isobutyrate, indicating that the cultivars responded similarly to training. Nevertheless, the significant Training × Year

TABLE 2. Training system, cultivar and year three-way ANOVA double interactions on oenological parameters, polyphenols and volatile composition.

	Training-cultivar									Training year						Cultivar-year					
	CT-TEMP	CT-SYR	CT-TR	SM-TEMP	SM-SYR	SM-TR	TC-TEMP	TC-SYR	TC-TR	CT-2014	SM-2014	TC-2014	CT-2015	SM-2015	TC-2015	TEMP-2014	SYR-2014	TR-2014	TEMP-2015	SYR-2015	TR-2015
Enological parameters																					
Alc/vol (%)	12.3	14.0	15.2	11.8	13.4	15.2	11.3	12.6	14.7	15.2	14.9	14.9	12.4	12.0	10.8	13.1	15.2	16.8	10.5	11.5	13.2
MH2(g/L)	3.0	2.0	1.5	2.2	2.0	1.7	2.4	1.9	1.4	2.2	2.2	2.2	1.8	1.7	1.6	2.7	2.3	1.5	2.3	1.6	1.2
Volatile acidity (g/L AcH)	0.47	0.45	0.69	0.42	0.49	0.57	0.39	0.49	0.54	0.61	0.56	0.53	0.47	0.43	0.42	0.41	0.6	0.68	0.45	0.36	0.52
Glycerol (g/L)	8.35	9.7	10.67	8.1	9.4	10.17	7.79	9.03	10.3	10.68	10.33	10.34	8.47	8.11	7.74	8.93	11.08	11.35	7.23	7.67	9.41
Fe (ppm)	0.91	0.66	0.93	0.86	0.65	0.74	0.75	0.61	1.03	0.9	0.77	0.9	0.77	0.73	0.69	0.8	0.76	1.01	0.89	0.51	0.8
Cu (ppm)	0.13	0.1	0.1	0.12	0.09	0.11	0.14	0.07	0.1	0.14	0.15	0.14	0.08	0.07	0.07	0.16	0.12	0.15	0.1	0.06	0.06
Zn (ppm)	0.22	0.26	0.36	0.16	0.27	0.3	0.16	0.23	0.28	0.38	0.38	0.33	0.17	0.11	0.12	0.27	0.34	0.48	0.09	0.17	0.14
Color intensity	0.829	1.045	1.238	0.741	1.115	1.290	0.741	1.046	1.331	1.294	1.329	1.411	0.780	0.768	0.668	1.116	1.297	1.621	0.425	0.840	0.951
Hue	0.55	0.59	0.62	0.55	0.55	0.62	0.57	0.55	0.59	0.58	0.57	0.56	0.59	0.57	0.58	0.49	0.58	0.64	0.63	0.54	0.58
L*	58.56	49.43	44.98	61.20	46.18	44.27	63.95	48.72	43.28	44.13	42.56	41.46	57.85	58.54	62.51	47.86	41.81	38.48	74.61	54.41	49.88
a*	48.55	51.07	52.43	46.56	53.91	53.81	41.96	52.32	54.88	56.16	57.27	58.13	45.20	45.58	41.31	59.71	56.00	55.85	31.67	48.86	51.56
b*	-1.713	-1.730	8.715	-2.908	-0.900	9.468	-0.748	-2.198	9.260	5.452	6.482	6.313	-1.937	-2.708	-2.103	0.058	3.538	14.650	-3.637	-6.757	3.645
Dry extract (g/L)	19.29	24.80	26.93	18.85	22.47	26.97	17.76	21.23	24.61	26.07	24.89	23.81	21.28	20.63	18.59	19.94	25.93	28.91	17.33	19.73	23.43
Polyphenols (mg/L)																					
ΣANT	378.5	482.4	418.6	376.8	485.4	393.5	364.8	458.7	409.2	433.2	421.4	434.7	419.8	415.7	387.1	438.0	480.1	371.2	308.7	471.0	442.9
ΣCAT	45.1	174.3	28.4	44.0	153.7	35.7	47.6	133.4	31.8	103.8	94.7	86.3	61.4	60.9	55.5	56.5	183.4	45.0	34.6	124.2	19.0
ΣSTB	1.5	0.0	2.4	1.5	0.0	1.9	1.4	0.0	2.8	1.8	1.4	2.0	0.8	0.9	0.8	1.2	0.0	4.0	1.8	0.0	0.8
ΣFLAV	94.0	200.3	130.4	104.4	192.2	129.9	82.4	174.8	132.0	183.2	187.7	173.3	100.0	96.7	86.1	123.4	246.9	173.9	63.8	131.3	87.7
ΣHBEN	47.9	13.0	41.7	43.9	18.3	49.0	44.7	14.6	51.7	30.1	36.5	32.7	38.3	37.7	41.3	35.8	15.1	48.5	55.3	15.6	46.4
ΣHCIN	40.6	29.0	15.5	40.4	24.8	12.1	41.7	24.3	16.5	34.1	28.6	30.8	22.6	22.9	24.2	44.9	28.1	20.4	36.8	24.0	9.0
Volatile compounds (µg/L)																					
ΣEEFA	1411	1597	1216	1398	1492	1171	1258	1336	1196	1481	1402	1311	1335	1305	1215	1450	1249	1494	1260	1701	894
ΣHAA	3305	2965	561	3629	2673	546	2996	2343	589	2249	2100	1694	2305	2466	2258	3370	2021	652	3249	3300	479
ΣEBBA	108	134	136	113	152	161	114	160	170	98	115	116	153	169	179	96	121	113	126	176	199
ΣCINN	0.6	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.4	0.8	0.7	0.7	0.2	0.2	0.2	0.9	0.7	0.7	0.2	0.2	0.2
ΣMEFA	1.3	2.1	2.1	1.3	1.9	1.8	1.1	2.0	1.9	2.4	2.1	2.0	1.3	1.2	1.3	1.6	2.2	2.8	0.9	1.8	1.1
ΣIEFA	1.6	1.9	1.5	1.8	1.8	1.4	1.6	1.9	1.5	2.2	2.2	2.1	1.1	1.1	1.2	2.4	2.0	2.1	0.9	1.7	0.8
ΣME	152	209	172	146	230	188	140	254	189	175	169	166	181	207	222	140	181	189	153	281	176

interaction indicated changes in ethyl isobutyrate concentrations between both years. Other compounds (ethyl 2-methylbutyrate and ethyl isovalerate) showed interaction effects (Training × Cultivar). The wines obtained from the TEMP cultivar were less affected by the training regarding these two compounds, while SYR and TR wines showed different contents according to the CT and TC training methods.

Regarding the miscellaneous esters (ΣME) group of volatiles, ethyl propanoate was affected by training. The wines produced under the CT training system showed the lowest values for this compound. Interactions of Training × Year and Training × Cultivar were found for ethyl propanoate, indicating different behaviour in each cultivar.

In the literature, comparisons of vertical training systems with the Smart-Dyson, and Geneva Double-Curtain training systems report clear differences in the concentrations of 1-hexanol, linalool and b-damascenone in Viognier grapes (Zoecklein *et al.*, 2008). Another study into the effect of training systems on volatiles in Traminette grapes in Ohio, USA showed that Smart-Dyson grapes had the lowest levels of C6 aldehydes and monoterpenes, and that grapes grown using a vertical training system produced the highest amount of geraniol and C6 aldehydes (Ji and Dami, 2008). In the study of Primitivo grapes from Four Rays and Bilateral Guyot training in southern Italy, differences in C6 composition were also observed and were later reflected in their corresponding wines (Fragasso *et al.*, 2012). Fatty acid-derived volatile compounds have been described as affected by training systems that provide different growing conditions, but the influence varies in different wine regions (Fragasso, *et al.*, 2012; Ji and Dami, 2008; Zoecklein, *et al.*, 2008).

Factorial ANOVA with three factors (training system: CT, SM and TC), cultivar: TEMP, SYR and TR) and year: 2014 and 2015) was conducted up to two-way interactions. Interaction were represented as follows. Training × Cultivar interaction in two years: CT-TEMP, SM-TEMP, TC-TEMP, CT-SYR, SM-SYR, TC-SYR, CT-TR, SM-TR and TC-TR. Training × Year interaction in three cultivars: CT-2014, SM-2014, TC-2014, CT-2015, SM-2015 and TC-2015. Cultivar × Year interaction in three training systems: TEMP-2014, SYR-2014, TR-2014, TEMP-2015, SYR-2015 and TC-2015. Polyphenols were grouped according to their polyphenol subgroup: anthocyanins (ΣANT), flavan-3-ols (ΣCAT), stilbenes (ΣSTB), flavonols (ΣFLAV), hidroxibenzoic acids (ΣHBEN), and hydroxycinnamic acids (ΣHCIN). Esters were grouped according to their origin and chemical structure into seven groups: ethyl esters of fatty acids (ΣEEFA), higher alcohol acetates (ΣHAA), ethyl esters of branched acids (ΣEEBA), cinnamates (ΣCINN), methyl esters of fatty acids (ΣMEFA), isoamyl esters of fatty acids (ΣIEFA) and miscellaneous esters (ΣME). a, b or c for the same parameter denote significant differences ($p<0.05$).

The study by Zoecklein *et al.* 2008, found that Smart-Dyson wines had the most abundant ethyl hexanoate and 1-hexanol, and indicated that better light conditions seem to be beneficial for the accumulation of C6 esters in wines. It was later concluded that wines made from grapes from better-lit environments perform better with regard to C6 esters (Xu, *et al.*, 2015). In contrast with data found in the literature, under our conditions higher values of ethyl hexanoate were found in the CT and SM wines than in the TC wines, although these differences were not significant.

2. Cultivar

The results clearly indicate that different cultivars have a great effect on the composition of wines. Although the red wines were produced from grapes grown in the same plot, in the same climate conditions and following the same winemaking processes, all the parameters studied were affected with the highest level of *significance* by the Cultivar factor (see Table 1 and Supplementary Table 1). This effect was considered before conducting the experiment and was the main reason to consider cultivar as a factor. Although it might mask small differences between training systems, it was more important to observe if the training system affected the different cultivars. The aim was to determinate the parameters that were singularly altered by the training system. Nonetheless, the main effects of the cultivar on wine composition are discussed below.

2.1. Alc/vol (%)

Differences in alcohol by volume of around 1% were found between cultivars. TR showed the highest alcohol by volume followed by SYR and TEMP. Due to the differences found in the

interaction Cultivar × Year, TEMP was less affected by the Year factor than SYR and TR (Table 2). TEMP decreased 2.5% from 2014 to 2015 whereas SYR and TR decreased 3.7% and 3.6%, respectively. Thus, under experimental conditions it was observed that TEMP was more resilient to year-on-year changes with regard to this parameter.

2.2. Glycerol

The TR wines were more glyceric than the SYR and TEMP wines, the latter presenting the least glycerol. Statistical classification of glycerol content correlated with ºAlc. In the Cultivar × Year interaction, TR-2014 and SYR-2014 were the most glyceric wines, followed by TR-2015 and TEMP-2014, and a fourth group composed of TEMP-2015 and SYR-2015. A difference of 4.12 g/L was found between TR-2014 and TEMP-2015. However, the cultivars behaved differently in the two years. SYR showed the highest decrease depending on the Year factor.

2.3. Colour intensity, Hue, L*, a* and b*

TR showed the highest colour intensity and TEMP the lowest. Again, TR showed the highest value for hue whereas TEMP and SYR showed similarly lower values. Hue correlates with the yellow-red colour of wine. The lower the hue value, the higher the proportion of red colour to yellow.

Regarding CIELab parameters, the lightness value, L*, represents the darkest black at L* = 0, and the brightest white at L* = 100. The colour channels, a* and b*, represent true neutral grey values at a* = 0 and b* = 0. The a* axis represents the green–red component, with green in the negative direction and red in the positive direction. The b* axis represents the blue–yellow component, with blue in the negative direction and yellow in the positive direction. TEMP showed lighter wines, SYR showed redder wines and TR redder and more yellow wines in agreement with the absorbance data.

2.4. Dry extract

A clear discrimination between cultivars was observed regarding dry extract: TR > SYR > TEMP. This trend was not affected by the Training interaction factor. However, cultivars had an influence on the Year factor. Under the experimental conditions, the dry extract value of the TEMP wines seemed to be less influenced by year-on-year climate conditions (Table 2).

2.5. Polyphenols

Significant differences were found regarding the cultivar. TEMP showed lower anthocyanin and flavonol values. TR showed the highest amount of stilbenes. SYR showed the highest values for anthocyanins, catechins and flavonols. However, these differences were only found in both years for catechins and flavonols. This is in agreement with the extensively studied influence of cultivar and year on the polyphenol profile of wines.

Regarding anthocyanin, the cultivar trend was SYR > TR > TEMP, although this varied year-on-year (Table 2). In 2014, the trend was SYR > TEMP > TR. The difference between years was mainly due to the TEMP-2015 value, which was surprisingly low.

SYR showed the highest values for catechins in both years (Table 2). However, a decrease was produced between years, the percentage dependent on the cultivar.

The fact that stilbenes were not detected in the SYR wines, and that TR-2014 showed the highest stilbene value, had a strong influence on the trends and interactions involving stilbenes. A special case was *E*-piceid, which was only identified in the TR cultivar : where TC was separated from SM, these training systems showed the highest and lowest concentrations, higher alcohol acetates (ΣHAA) and cinnamates (ΣCINN), respectively, and the lowest levels of ethyl esters of branched acids (ΣEEBA), methyl esters of fatty acids (ΣMEFA) and miscellaneous esters (ΣME). The SYR wines showed the highest concentrations of ethyl esters of fatty acids (ΣEEFA), ethyl esters of branched acids (ΣEBBA), methyl esters of fatty acids (ΣMEFA), isoamyl esters of fatty acids (ΣIEFA), and miscellaneous esters volatiles groups (ΣME). Meanwhile, the TR wines presented the highest levels of ethyl esters of branched acids (ΣEBBA) and methyl esters of fatty acids (ΣMEFA) and the lowest values for volatiles belonging to higher alcohol acetates (ΣHAA).

Variations in ethyl esters of fatty acids related to cultivar have also been reported in the literature (Antalick, *et al.*, 2015).

Higher alcohol acetates concentrations grouped each variety independently. The TEMP wines

showed the highest concentrations of acetates (isoamyl) followed by the SYR wines (Table 1, Supplementary Table 2). TR showed almost six-fold lower hexyl acetate concentrations than TEMP. The different composition of higher alcohol acetates in wines could be linked to variations in the composition of grapes from different cultivars. Amino acids, phenolics and unsaturated fatty acids (regulators of lipoxygenase activities and alcohol acetyltransferase activities in yeasts) modify the synthesis of higher alcohol acetates (Antalick, *et al.*, 2015; Sumby *et al.*, 2010). Quantitative differences in isoamyl and hexyl acetates have been reported to modify the "aromatic/sensory" profile of red wines (Lytra *et al.*, 2012).

Differences in the concentration of ethyl esters of branched acids were observed according to the cultivar. The SYR and TR wines presented the highest values for this group of volatile compounds. Ethyl isobutyrate, with the main contribution, followed by 2-methylbutyrate and isovalerate contents were responsible for these differences (TR>SYR>TEMP). Ethyl leucate showed a different pattern, the highest values being observed for the wines obtained from the SYR grapes. The SYR and TR wines showed higher values of ethyl isobutyrate and ethyl leucate, irrespective of the training used, as no Training × Cultivar interactions were found. Variations in the yeast redox metabolism and amino acid composition of grapes have been suggested to contribute to varietal differences in groups of ethyl esters of branched acids (Antalick *et al.*, 2015). Furthermore, different phenolic compositions might also contribute to the differences observed in the ethyl esters of branched acid group between the wines from the three cultivars. However, this hypothesis should be studied in greater depth.

3. Year

The data in Table 1 (Year) represent the averages from the wines from three red grape cultivars from three different training systems. Interestingly, all the studied parameters were higher in 2014 than in 2015, except for L*, ΣHBEN, ΣHAA, ΣEBBA and ΣME. As with the cultivar factor, the year had a clear influence on the described data; this is known as the "vintage effect". The year factor is a source of error whereby pre-harvest and oenological factors interact in wineries. Year factor was considered to orthogonalise the importance of year variability. It is important to note that robust conclusions are found in warm climates when considering the high variability in wine composition between years.

Alcohol by volume was much higher in 2014. Increased ripeness might be expected in 2014 due to the climate conditions (data not shown). Late ripening being responsible for increased alcohol by volume can be discarded due to results for malic acid, which was surprisingly low in 2015 (Table 1).

Despite differences in volatile acidity, the concentrations of Fe, Cu and Zn were under legal quality limits in both years. Glycerol was highly affected by year-on-year conditions and can be related to alcohol by volume as was mentioned above for the cultivar results. The wines in 2014 had a higher colour intensity (observed by colour intensity and L* value) and were redder and more yellow than those in 2015. However, red and yellow were compensated for by hue, which did not present significant differences.

The higher alcohol by volume must have contributed to a stronger polyphenol extraction during solid-liquid contact during maceration and alcoholic fermentation. The exception was ΣHBEN in 2015, showing a higher concentration than in 2014.

Climate conditions have also been described to significantly affect aroma development (Lee *et al.*, 2007). All the ester groups were impacted by the year factor (climatic conditions) as were most of the compounds analysed (Hernandez-Orte *et al.*, 2015). This shows the importance of year factor in warm viticultural areas (Herrera *et al.*, 2015). ΣHAA, ΣEEBA and ΣME were significantly higher and ΣEEFA, ΣCINN, ΣMEFA, and ΣIEFA lower in 2015 than in 2014.

No differences were found in the compounds ethyl octanoate, hexyl acetate, ethyl isovalerate and ethyl leucate year-on-year (Supplementary Table 2). Cultivar × Year interaction was significant for all the aroma compounds except methyl decanoate and isobutyl hexanoate.

3. Sensory analysis

Firstly, a statistical analysis was performed for the oenological parameters, polyphenols and volatile compounds. The only significant differences found were for the "Black fruit" and

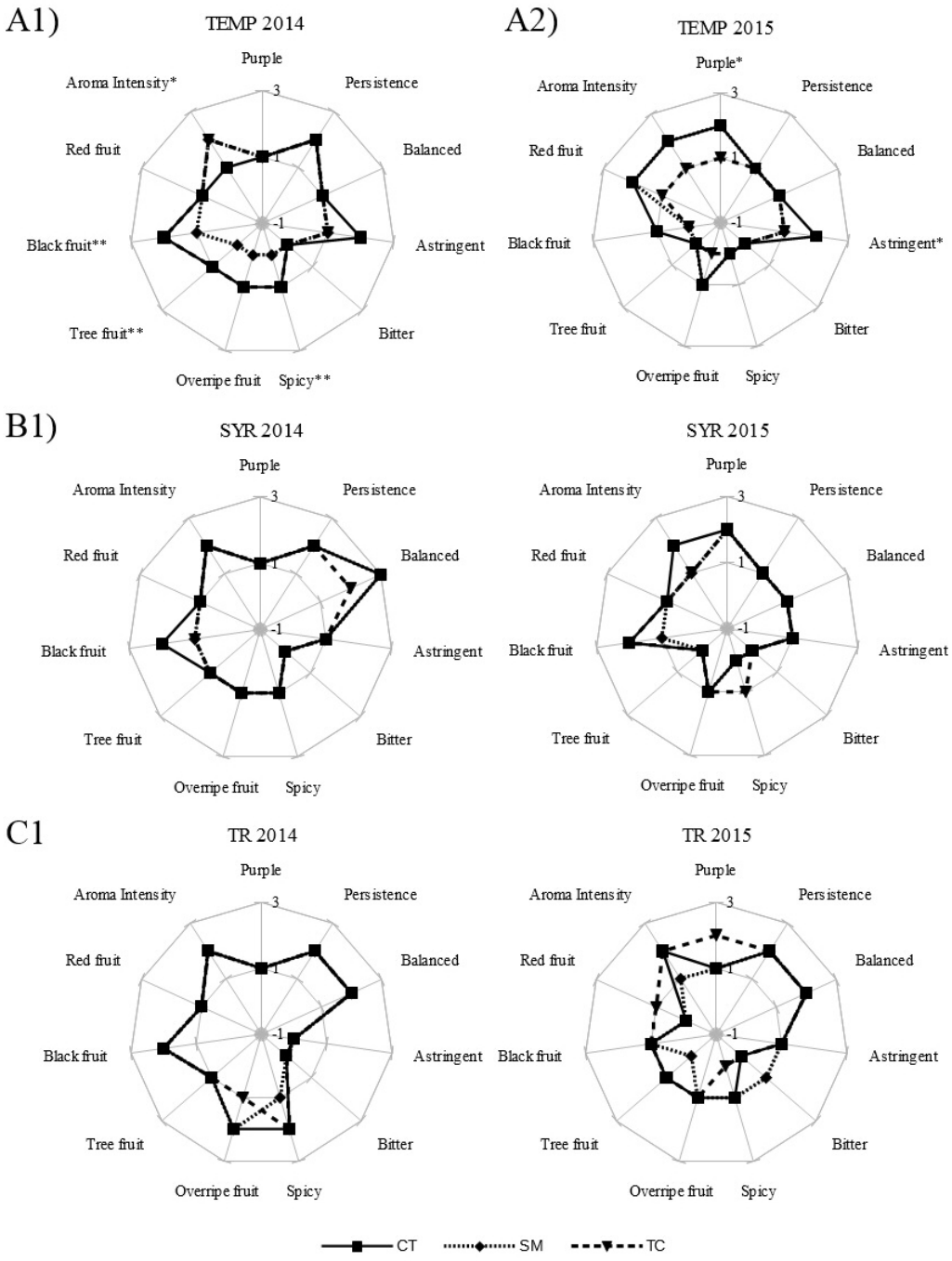

FIGURE 1. Mode representation of sensory analysis of wines from the three training systems divided by cultivar and year.

Training systems: CT, Control; SM, Smart; TC, Triple cordon. Cultivars: A) Tempranillo, TEMP; B) Syrah, SYR; C) Tintilla de Rota, TR. Year: 1) 2014; and 2) 2015. Kruskal-Wallis one-way analysis of variance, level of significance (p-value using Chi-squared approximation): *p<0.05 and **p<0.01.

"Tree fruit" descriptors (data not shown). "Tree fruit" aroma after post-hoc test application at p=0.05 could separate CT with the highest rate, from SM with the lowest rate, whereas it was not possible to differentiate TC. "Black fruit" could not differentiate training systems at the studied p-value.

However, due to the variability in sensory analysis, another statistical analysis of sensory properties was conducted in which cultivar and year factor were considered separately. Figure 1 shows mode results for sensory data divided vertically by cultivar and horizontally divided by year. Year differences are evident at a glance

whereas cultivar differences in the same year seem to be subtle.

Despite the graphical differences between the training systems that are shown in Figure 1, statistically significant differences were only observed in the TEMP cultivar for aroma intensity, black fruit, and tree fruit in 2014, and for purple colour and astringency in 2015. Differences in black and tree fruit attributes converged on the same post-hoc results. The TC training system, with the highest black and tree fruit attributes, was separated from SM, whereas CT could not be differentiated. These results are different from those of the first statistical analysis, where TC and SM could not be separated. In the case of the TEMP wines, the aroma intensity descriptor in both 2014 and 2015 could not distinguish between training systems at the *significance* level studied. The spicy descriptor was only significant in TEMP 2014. Despite presenting significant differences, the post-hoc test could not separate samples by purple colour and astringency.

The results of the sensory analysis indicated that there are some changes in wine sensory aroma when different training systems are used; these differences were not evident year-on-year, however. The differences in aroma were subtle and more likely in the wines from the TEMP cultivar than the SYR or TR. The differences were dependent on the Year factor.

CONCLUSIONS

The fact that it is possible for training systems to reduce the alcohol by volume of wines offers the wine industry a new tool for obtaining the lower alcohol red wines that are popular among consumers. This may be especially useful in warm climates where wines with a higher alcohol by volume are obtained, a phenomenon that is expected to increase due to climate change. Alcohol by volume seems to be related to ripening and SM and TC training systems are thought to delay the ripening period. This is of interest in warm climates, where ripening occurs in a short period.

Regarding oenological parameters, TC resulted in a less alcoholic wine with lower volatile acidity, glycerol and dry extract than CT. No significant differences were found in the other oenological parameters.

The training system used influenced the polyphenol composition of the wines. CT training showed a higher polyphenol concentration of catechins and hydroxycinnamic acids. However, significant differences were not found for anthocyanins and flavonols, which are the major polyphenols in wine. The influence of cultivar and year were stronger than that of the training system, making it difficult to reach definite conclusions.

Training influenced the ester profile, modifying the concentrations of higher alcohol acetates, ethyl esters of branched acids and miscellaneous esters volatile groups. Some of these compounds seem to be related to ripening. Therefore, training management can result in delayed ripening.

Winemakers and the wine industry will find information in this work to enable them to modulate the composition of red wine through the application of different training systems. The same winemaking procedure provided red wines with a similar structure and whose sensory properties were little influenced by the cultivar and year.

This study contributes to enhancing the knowledge about the relationship between wine composition and training systems. The results enable the raw material in winemaking to be modified by the training system employed. It is up to oenologists to take advantage of these differences to produce wines with varying characteristics. The information provided could be exploited in both viticultural and oenological fields to ameliorate the effects on vineyards of climate change with the aim of maintaining wine quality.

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