



## Relationship between physical and chemical parameters for four commercial grape varieties from the Bierzo region (Spain)

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### ABSTRACT

Four *Vitis vinifera* L. varieties (Mencía, Cabernet Sauvignon, Tempranillo and Merlot) were evaluated for viticultural characteristics in the vineyards of a winery located in the Bierzo Protected Designation of Origin (PDO) region of León in Spain. Must parameters (total soluble solids, pH, titratable acidity, total phenolic content, colour intensity and colour tonality), production parameters (mean berry weight, yield, number of clusters and mean cluster weight) and vigour parameters (shoot pruning weight, Ravaz index, number of shoots, mean weight of shoots, mean length of shoots, mean number of buds per shoot, mean length of internodes, and mean diameter of shoots) were measured during four seasons (2007, 2008, 2009 and 2010). The grape samples were taken 3–5 days after harvest with similar degree brix levels. Must and production parameters differed significantly among varieties and seasons. Although total soluble solids and pH values differed significantly between varieties, the range for the two measurements was small. Mencía yielded the highest grape production while Tempranillo had the lowest, with differences due primarily to mean berry weight and mean cluster weight. Tempranillo and Cabernet had the highest vigour values. Ravaz index values were highest (>10) for Mencía and lowest (<4) for Tempranillo, Merlot and Cabernet. Mencía was the variety best suited to the growing area and the preferential variety for the wines made in the Bierzo PDO. Tempranillo adapted most poorly to the growing conditions. The varietal evolution trend was sharper than the temporal evolution trend.

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### 1. Introduction

Viticulture is a key economic sector in the Spanish autonomous community of Castilla y León, particularly so in the Bierzo region, which has 3683 ha registered under the Bierzo Protected Designation of Origin (PDO) label. To successfully compete in the market, vineyards and plots have to be properly characterized and winery production has to be optimized, which, in turn, requires knowing the specific characteristics of the wine produced by individual

plots (Zamora, 2003). Wine quality, among other things, depends on must characteristics such as pH, total acidity, total soluble solids (TSS) and also alcohol content, acidity, colour, etc. (de Andrés-de Prado et al., 2007), as these values reveal the characteristics of the resulting wines; e.g., a higher TSS will produce wines with a higher alcohol content.

The decision as to harvesting date is mostly dependent on TSS (reflecting sucrose dissolved in must), since grape maturity is indicated by must sugar (mainly sucrose) content as determined by the refractive index for the sample (Girard and Soto, 2004). TSS provides an estimate of the probable alcohol yield and so guides appropriate grape selection for good quality wines (Albuquerque et al., 2004, 2007). In the *Vitis vinifera* varieties, the main carbohydrates are glucose and fructose, although there are also small quantities of sucrose and other carbohydrates. Considerable scientific effort has been invested in understanding the complex physical and biochemical changes that occur in grape berries during the growth cycle (Coombe, 1992; Coombe and McCarthy, 2000). Tartaric and malic acids constitute almost the totality of organic acids in grapes (Coombe, 1992; Beriashvili and Beriashvili, 1996; Saxton et al., 2009), which typically contain around 80% tartaric acid (Lavee and Nir, 1986; Hunter et al., 1991), 10% malic acid and

**Abbreviations:** TSS, total soluble solids (°Brix); TA, titratable acidity (g/L); TPC, total phenolic content ( $A_{280}$ ); CI, colour intensity ( $A_{420} + A_{520} + A_{620}$ ); CT, colour tonality ( $A_{420}/A_{520}$ ); BW, mean berry weight ( $\times 10^{-3}$  kg); Y, yield ( $\times 10^{-3}$  kg); NC, number of clusters; CW, mean cluster weight ( $\times 10^{-3}$  kg); PW, shoot pruning weight ( $\times 10^{-3}$  kg); RI, Ravaz index (Y/PW); NS, number of shoots; WS, mean weight of shoots ( $\times 10^{-3}$  kg); LS, mean length of shoots ( $\times 10^{-2}$  m); NB, mean number of buds per shoot; LI, mean length of internodes ( $\times 10^{-2}$  m); DS, mean diameter of shoots ( $\times 10^{-3}$  m); PDO, Protected Designation of Origin.

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small amounts of p-coumaric and other acids. The typical titratable acidity (tartaric acid equivalent) (TA) of unripe grapes is around 40 g/L, but this falls to below 10 g/L in ripe grapes. Grape acidity is inversely related to carbohydrate content; in the course of ripening, grape glucose and fructose concentrations increase while acid concentrations fall (Saxton et al., 2009).

The organoleptic characteristics of the must are strongly related to content in phenols. Wine colour is due to a range of chemical compounds with different compositions and that develop simultaneously (Gómez-Plaza et al., 2000; Arnous et al., 2002; Gil-Muñoz et al., 2009). Knowing the chromatic characteristics of the must in terms of total phenolic content (TPC), colour intensity (CI) and colour tonality (CT) is important because these determine whether a particular must is suitable for wine to be aged in barrels. Anthocyanins and tannins are mainly responsible for colour in red wines (Gil-Muñoz et al., 2009). Anthocyanins are accumulated during grape ripening, predominately in the berry skins (Dimitrovska et al., 2011); content is variable depending on grape variety, climate and soil and agricultural practices. However, genetic factors determine the specific anthocyanin profile for each grape variety (Dimitrovska et al., 2011).

Vigour is a very important viticultural quality as it indicates the vegetative growth of the plant, specifically, the vegetative biomass produced annually by shoots. Some authors have demonstrated the existence of an inverse relationship between grapevine vigour and the resulting composition of the must in terms of soluble solids, tannins and polyphenols (Cortell et al., 2005, 2008).

Carey et al. (2008a), for a South African study, demonstrated the influence of climate conditions in identifying the natural terroirs – land characterized by a stable group of values relating to the topography, climate, substrate and soil (Carey et al., 2008a) – representing the agricultural aptitude of a site. Climate, soil and topography affect phenology, growth, yield, berry composition and wine-related parameters for Cabernet-Sauvignon (Carey et al., 2008b). Lower acidity in Cabernet-Sauvignon wines is predominantly related to higher temperatures during the green berry growth stage. Warmer sites and normal rainfall produce more intense berry aroma characteristics (Carey et al., 2008b). Climate, and particularly insolation, has a significant impact on vine photosynthesis and, consequently, on the synthesis of phenolic compounds. Temperature also influences the accumulation of colour in berry skins. Rainwater availability affects vine vigour, production and indirectly affects the accumulation of sugars, acids and phenolic compounds in berries (Zamora, 2003).

The aim of this research was to evaluate varietal and temporal evolution for four grape varieties, by comparing measuring 18 variables (related with must quality, grape production and vigour of the grapevines) during the years 2007–2010, and studying the associations between these variables and climatic data and phenology events.

## 2. Materials and methods

### 2.1. Study site and experimental layout

The research was conducted in vineyards located in Cacabelos in the PDO area of Bierzo (León, Spain). Four vineyard blocks owned by the Ribas del Cúa Winery were selected with the following coordinate boundaries: 4720400 (North), 4719500 (South), 687600 (West) and 688800 (East) (Coordinate Reference System: ETRS89/UTM zone 29N). The mean altitude is 585 m and the mean slope is 5%. The soil to the first metre of depth has 11% gravel and a loamy texture (36% sand, 45% silt and 19% clay). Fertilization, tillage, and pest and weed management were uniformly applied in years 2007–2010.

All the vineyards were planted in 1997 with 1.1 m × 2.8 m vine by row spacing. The training system is a bilateral cordon and vines are vertical shoot-positioned with two pairs of shoot-positioning wires. The rootstock is 1103 Paulsen (*Vitis berlandieri* × *V. rupestris* cv 1103P) and each block has a different surface area and grape variety: Cabernet Sauvignon, Mencía, Merlot and Tempranillo.

The vineyards were sampled from 2007–2010 to characterize quality, production and vigour variables. A total of 162 vines were selected for measurement. A regular grid pattern of 20 m × 29 m was defined, corresponding to 14 vines/ha (one vine in 20 in each line and one line in 10 in each variety block). Each data vine was geo-referenced using a Topcon Hiper+GPS receiver (Topcon Corporation, Tokyo, Japan) with real time kinematic correction (centimetre precision).

### 2.2. Quality and production parameters

Data were collected one week before harvest (on 14 September in 2007, 11 September in 2008, 14 September in 2009 and 12 September in 2010). One hundred fifty berries per vine were randomly sampled to determine must quality. The berries were analyzed on the same day they were collected to ensure that their characteristics were preserved. Each grape sample was weighted in order to calculate the mean berry weight (BW, ×10<sup>-3</sup> kg); the grapes were immediately crushed and the juice was filtered through cheesecloth to determine TSS, pH, (TA), (TPC), (CI) and (CT). In order to avoid errors in the analytical data, must sampling was done three times. The wet chemical analyses were performed following regulated methods (European Commission Regulation (EC) No 2676/90).

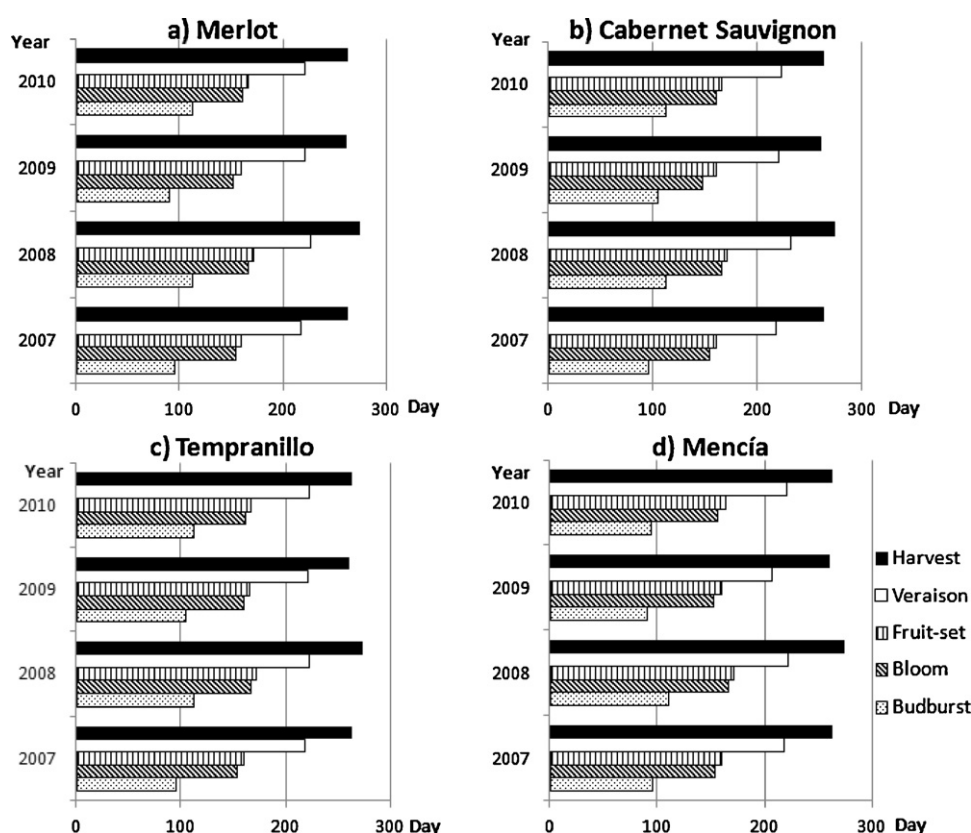
TSS (°Brix) was determined at 20 °C using a digital refractometer Atago PR1 (Atago Co., Tokyo, Japan). pH was measured using an electronic pH-metre Crison GLP21 (Crison Instruments, S.A., Alella, Barcelona, Spain). TA was measured as tartaric acid (g/L) by titration of grape juice with sodium hydroxide (0.1 N) to an endpoint pH of 7.

The CI and CT properties and TPC were estimated by absorbance using a UV-vis Spectrophotometer SP-2000 (Ningbo Hinotek Technology Co., Ningbo, China). The CI was obtained by summing juice absorbance (in a 10 mm glass cuvette) at 420 nm, 520 nm and 620 nm (CI = A<sub>420</sub> + A<sub>520</sub> + A<sub>620</sub>) and CT was calculated as the ratio between juice absorbance at 420 nm and at 520 nm (A<sub>420</sub>/A<sub>520</sub>). To calculate TPC (A<sub>280</sub>) the must was centrifuged (5000 rpm for 5 min) diluted with water (1/100 dilution) and spectrophotometer-measured to absorbance at 280 nm (in a 10 mm quartz cuvette), following Zamora (2003) and Pérez-Lamela et al. (2007).

At harvest time, all the clusters were collected and weighed. The variables calculated for the clusters from each data vine were: the total weight of the clusters or yield (Y, ×10<sup>-3</sup> kg), the number of clusters (NC) and the mean cluster weight (CW, ×10<sup>-3</sup> kg).

### 2.3. Vigour indicators

In December 2007, 2008 and 2009 (during dormant periods), the vigour parameters were also measured. All data vines were pruned and the shoot pruning weight (PW, ×10<sup>-3</sup> kg) and the number of shoots (NS) per vine were measured, from which were calculated the Ravaz index (RI; the ratio of yield to shoot pruning weight (Ravaz, 1911)) and the mean weight of shoots (WS, ×10<sup>-3</sup> g). The length of shoots per vine were measured using a tape measure in order to calculate the mean length of shoots (LS, ×10<sup>-2</sup> m), the mean number of buds per shoot (NB) and the mean length of the internode (LI, ×10<sup>-2</sup> m). Finally the mean shoot diameter (DS, ×10<sup>-3</sup> m) was measured at the base of the shoot using a vernier calliper.



**Fig. 1.** Time series of the major phenological events for the four studied varieties: (a) Merlot, (b) Cabernet Sauvignon, (c) Tempranillo and (d) Mencía. The initial day is 1 January.

#### 2.4. Phenological stages and climate data

The phenological events were reported between March and September to determine the data for budburst, bloom (floraison), fruit set (setting), veraison and harvest. Each phenological event was noted once 50% of the buds, flowers or berries had achieved the corresponding stage of growth. Fig. 1 shows the date (day from 1 January) for each grapevine stage for the four varieties. Climate data were collected from the nearest meteorological station, located at Carracedelo (4.5 km from Cacabelos) and the Winkler index values were calculated (Amerine and Winkler, 1944). Table 1 summarizes temperatures, rainfall and Winkler index data for each year.

#### 2.5. Statistical analysis

The data were explored to detect potential outliers, which were deleted. All data were analyzed for variance using SPSS v.17.0 (SPSS Inc., Chicago, Illinois, USA). The Tukey test was used to compare means ( $P < 0.05$ ) (Steel et al., 1985) and Pearson correlations were also calculated. Principal component analysis (PCA) without rotation was applied to the quality, production and vigour values.

**Table 1**  
Summary of climate characteristics for the four studied harvests (2007, 2008, 2009 and 2010).

Year	Rainfall (mm)	Winkler index	Average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)
2007	418.4	1176.2	11.4	37.0	−10.3
2008	444.2	1161.9	11.6	36.3	−6.6
2009	551.2	1353.5	12.3	35.0	−10.3
2010	870.2	1379.8	11.9	36.7	−7.5

Source: Meteorological Station of Carracedelo, León (Spain).

### 3. Results

#### 3.1. Statistical analysis of the grape samples

Table 2 shows the mean values and the results for ANOVA tests for variety and year. Vigour indicators showed higher inter-annual variations than must values. The NS values for Merlot 2007 were detected as outliers and were eliminated from the analysis. Merlot showed higher TSS values for all years but also showed lower values for BW and CW. Cabernet showed higher TA and NC but lower pH values. Mencía showed higher production parameters and Tempranillo showed higher vigour values, with the exception of RI due to the low production of this variety.

Significant differences ( $P < 0.05$ ) were observed between the four vineyard varieties for all parameters referring to the must, production and vigour indicators.

The effect of variety and of year on must parameters (Table 2) show that at harvest, TSS was highest (22.86) for Merlot; pH was lowest (3.14) for Cabernet and highest for Mencía and Tempranillo; TA was highest for Cabernet (10.80) and lowest for Mencía (6.82); TPC was highest for Mencía (14.27) and Tempranillo (14.73); CI values was lowest for Cabernet and CT values was lowest for Merlot.

**Table 2**  
ANOVA results for grape variety and year for physical and chemical parameters.

Variety	Must parameters				Production parameters							Vigour parameters							
	TSS	pH	TA	TPC	CI	CT	BW	Y	NC	CW	PW	RI	NS	WS	LS	NB	LI	DS	
Cabernet	21.41b	3.14c	10.80a	11.69b	2.44c	1.45b	1.20c	1720.62b	20.22a	86.22c	603.24b	3.26b	7.68b	61.90b	138.42b	18.26a	7.59b	8.47b	
Mencia	21.31b	3.29ab	6.82d	14.27a	3.96a	1.55a	1.79a	2856.26a	14.34c	203.11a	188.85d	16.38a	7.43b	25.43d	78.42d	16.43bc	4.58c	7.91c	
Merlot	22.86a	3.25b	8.69b	11.74b	4.22a	1.04d	1.17c	1167.68c	17.43b	66.85d	475.83c	2.57bc	11.17a	42.93c	115.20c	15.37c	7.33b	8.13bc	
Tempranillo	21.75b	3.33a	8.08c	14.73a	2.84b	1.27c	1.59b	857.53d	8.85d	112.31b	751.76a	1.35c	6.99b	79.51a	166.57a	17.08ab	9.52a	9.05a	
Year																			
2007	22.32b	3.07c	8.14c	14.05b	3.53a	1.21c	1.26c	1672.22ab	13.56b	146.38a	645.62a	6.16a	4.82c	63.02a	135.48a	20.01a	6.42b	8.46a	
2008	19.10c	3.21b	10.80a	11.81c	2.87b	1.37b	1.46b	1494.52b	14.05b	107.26b	387.34b	7.58a	8.66b	46.78b	117.46b	15.37b	7.59a	8.53a	
2009	23.24a	3.37a	8.77b	16.56a	3.11ab	1.34b	1.78a	1825.23a	15.41b	131.76a	454.64b	5.51a	9.62a	48.29b	120.63b	15.28b	7.66a	8.25a	
2010	22.21b	3.35a	6.78d	10.61d	3.70a	1.53a	1.35c	1873.01a	18.32a	106.70b									

Means with the same letter are not significantly different. Post hoc Tukey test.  $P < 0.05$ . Must parameters: TSS: total soluble solids ( $^{\circ}$ Brix); TA: titratable acidity (g/L); TPC: total phenolic content ( $A_{280}$ ); CI: colour intensity ( $A_{420} + A_{520} + A_{620}$ ); CT: colour tonality ( $A_{420}/A_{520}$ ). Production parameters: BW: mean berry weight ( $\times 10^{-3}$  kg); Y: yield ( $\times 10^{-3}$  kg); NC: number of clusters; CW: mean cluster weight ( $\times 10^{-3}$  kg); Vigour parameters (not calculated for 2010): PW: shoot pruning weight ( $\times 10^{-3}$  kg); RI: Ravaz index; NS: number of shoots; WS: mean weight of shoots ( $\times 10^{-3}$  kg); LS: mean length of shoots ( $\times 10^{-2}$  m); NB: mean number of buds per shoot; LI: mean length of internodes ( $\times 10^{-2}$  m); DS: mean diameter of shoots ( $\times 10^{-3}$  m).

Regarding years, TSS, pH and TPC were highest in 2009. TA was highest and TSS was lowest in 2008. CI and CT were highest and TPC was lowest in 2010.

Table 2 also summarizes the effect of variety and of year on production parameters. Thus BW, Y and CW were highest for Mencia while NC was highest for Cabernet (20.22). Tempranillo had the lowest Y and NC values, whereas Merlot had the lowest BW and CW values. BW was highest in 2009, Y and NC were highest in 2010 and CW was highest in 2007. BW and NC were lowest in 2007, Y was lowest in 2008 and CW was lowest in 2010.

The effect of variety and of year on vigour indicators (Table 2) shows that PW, WS, LS, LI and DS were highest for Tempranillo, RI was highest for Mencia and NS was highest for Merlot. Merlot had low values for the remaining vigour indicators. NS and LI were lowest whereas PW, WS, LS and NB were highest in 2007. In 2008 PW was lowest. RI and DS showed no significant differences between the years. This would indicate that climate does not affect these variables.

Table 3 shows the results of the ANOVA test for the variety–year effect on must, showing differences in all the must parameters for variety–year interactions. TSS and pH were highest for all the varieties in 2009, with Merlot obtaining the highest values for each year. The lowest TSS values were in 2008. The lowest pH values were achieved in 2007 for Cabernet. The highest TA and TPC values for all the varieties were obtained in 2008, with Cabernet obtaining the highest TA value each year. The lowest TA occurred for Mencia in 2010. The highest CI values were achieved in 2010 for three of the varieties and the highest CT values were obtained in 2010 for all the varieties. There was no apparent trend between varieties for TPC, CI and CT.

The variety–year effect on production parameters (Table 3) shows that the highest BW values were achieved in 2009 for all the varieties, with Mencia achieving the highest value each year. The lowest BW values were achieved in 2007 for Merlot. Y values were quite uniform over the four years, with Mencia achieving the highest values in 2008, 2009 and 2010. The highest NC values were achieved for all the varieties in 2010, with Cabernet achieving the highest value each year. The highest CW values were achieved in 2007, with Mencia again achieving the highest value each year. Merlot had the lowest values for CW each year.

Table 3 also summarizes the variety–year effect on vigour indicators. Tempranillo had the highest values for PW, WS, LS, LI and DS and the lowest values for RI and NS (explained by this variety having smaller numbers of long, thick shoots). The highest NB values were achieved for Cabernet. Merlot and Mencia had the lowest vigour indicator values. The highest vigour indicator values occurred in 2007 and 2009, but no trend was evident regarding low values.

Fig. 1 depicts the chronological classification of grapevine phenology, expressed according to day of the year (number of days after 1 January). Phenology processes for all the varieties developed earliest in 2007 and latest in 2008, and those for 2009 occurred earlier than for 2010.

### 3.2. Principal component analysis

PCA was conducted on the correlation matrix produced from the 18 parameters for four grape varieties over four years (Fig. 2). The PCA plot gives a visual overview of how different parameters were influenced by varieties and by years. Principal components (PC) 1, 2, 3, 4 and 5 had eigenvalues that were greater than 1.0. The first three PCs explained 58.31% of the total variance (PC 1: 31.46%; PC 2: 14.77%; PC 3: 12.08%). PC 4 and PC 5 explained an additional 9.49% and 7.93% of the variance. Furthermore, loading for PC 1 and PC 2 indicated that some parameters described the same variation among samples. Additionally, Fig. 2 shows the PC 1 and PC 2 values for each year and variety. These PC values were transformed to



**Table 3**  
ANOVA results for grape variety × year for physical and chemical parameters.

VP	Variety	Years			
		2007	2008	2009	2010
PW	Mencía	144.56bC	152.42bC	261.47aC	
	Cabernet	740.43aB	495.66bAB	546.16bA	
	Tempranillo	1085.81aA	518.94bA	590.96bA	
	Merlot	614.63aB	397.36bB	398.08bB	
RI	Mencía	15.60bA	21.64aA	12.99bA	
	Cabernet	3.68aB	2.53bB	3.41abB	
	Tempranillo	1.14aB	1.57aB	1.40aC	
	Merlot	2.56abB	1.96bB	3.14aBC	
NS	Mencía	5.02bB	8.69aAB	8.80aB	
	Cabernet	5.19bB	9.03aA	9.17aB	
	Tempranillo	4.17cB	7.16bB	9.56aB	
	Merlot	10.68aA	10.23aA	11.96aA	
WS	Mencía	25.33aC	18.79bD	30.70aB	
	Cabernet	68.44aB	54.31bB	61.02abA	
	Tempranillo	101.23aA	74.26bA	61.69bA	
	Merlot	55.24aB	39.41bC	33.59bB	
LS	Mencía	74.38bD	73.01bC	86.59aD	
	Cabernet	150.83aB	129.34bB	133.03bB	
	Tempranillo	193.56aA	153.96bA	150.85bA	
	Merlot	122.41aC	116.19abB	107.14bC	
NB	Mencía	19.02aB	14.94bA	15.18bAB	
	Cabernet	22.06aA	16.11bA	16.04bA	
	Tempranillo	20.64aAB	15.19bA	15.21bAB	
	Merlot	16.80aC	15.14abA	14.19bB	
LI	Mencía	3.64cD	4.68bC	5.42aD	
	Cabernet	6.65bC	8.14aB	8.12aB	
	Tempranillo	8.93bA	10.04aA	9.68aA	
	Merlot	7.05bB	7.63aB	7.35abC	
DS	Mencía	7.47cC	8.41aB	7.95bB	
	Cabernet	8.26bB	8.26bB	8.85aA	
	Tempranillo	9.36aA	9.31aA	8.56bA	
	Merlot	9.12aA	8.03bB	7.22cC	
MP	Variety	Years			
		2007	2008	2009	2010
TSS	Mencía	21.96bB	18.08cC	23.37aB	21.84bB
	Cabernet	22.27abB	18.95cB	22.51aC	21.91bB
	Tempranillo	22.08bB	19.41cB	23.12aCB	22.42abAB
	Merlot	23.36bA	20.56cA	24.49aA	23.03bA
pH	Mencía	3.12cA	3.23bB	3.41aB	3.40aA
	Cabernet	3.00cB	3.11bC	3.24aD	3.23aB
	Tempranillo	3.09dA	3.30cA	3.52aA	3.41bA
	Merlot	3.08dA	3.21cB	3.31bC	3.39aA
TA	Mencía	6.52bD	8.54aD	6.97bD	5.21cD
	Cabernet	9.56cA	14.03aA	11.03bA	8.58dA
	Tempranillo	8.58bB	9.73aC	7.66cC	6.35dC
	Merlot	7.67cC	10.65aB	9.56bB	6.90dB
TPC	Mencía	16.84bA	9.07 dB	18.90aA	12.20cB
	Cabernet	9.97bC	13.29aA	13.76aB	9.77bC
	Tempranillo	13.55bB	12.33bA	19.52aA	13.59bA
	Merlot	17.29aA	12.95bA	11.80bC	4.95cD
CI	Mencía	4.10bB	2.95cB	4.93aA	3.86bB
	Cabernet	2.21bC	3.01aB	1.60cD	2.96aD
	Tempranillo	2.58bcC	2.11cC	3.14abB	3.34aC
	Merlot	5.25aA	3.71bA	2.63cC	5.23aA
CT	Mencía	1.50bA	1.57abA	1.49bA	1.63aA
	Cabernet	1.05cB	1.52bA	1.56abA	1.68aA
	Tempranillo	0.94cB	1.20bB	1.22bB	1.48aB
	Merlot	1.11abB	1.04bC	0.88cC	1.16aC
PP	Variety	Years			
		2007	2008	2009	2010
BW	Mencía	1.52cA	1.77bA	2.15aA	1.70bA
	Cabernet	1.09cC	1.18bC	1.44aB	1.10cC

Table 3 (Continued)

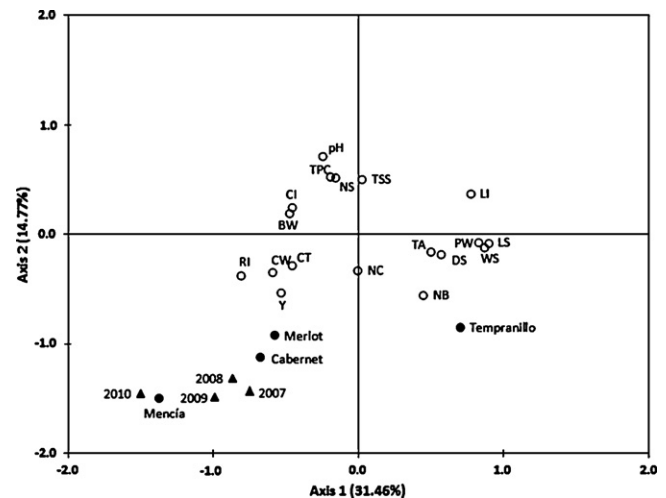
PP	Variety	Years			
		2007	2008	2009	2010
Y	Tempranillo	1.33cB	1.63bB	2.01aA	1.39cB
	Merlot	0.98cC	1.14bC	1.43aB	1.12bC
Y	Mencía	1840.29bB	2956.78aA	3289.06aA	3338.93aA
	Cabernet	2222.20aA	1164.15cB	1698.39bB	1808.39bB
	Tempranillo	1065.35aC	807.37aBC	757.08aC	798.00aD
	Merlot	1421.63aC	726.86bC	1267.87aBC	1254.37aC
NC	Mencía	8.13cC	15.67bB	15.53bA	18.04aB
	Cabernet	20.74abA	18.74bA	18.72bA	22.68aA
	Tempranillo	8.82abC	7.35bC	8.10bB	11.10aC
	Merlot	16.96bB	13.85bB	17.30bA	21.59aA
CW	Mencía	227.13aA	190.69bA	209.43abA	185.17bA
	Cabernet	109.64aC	62.73dC	92.37bC	80.14cB
	Tempranillo	140.43aB	103.21bB	129.80aB	81.14bB
	Merlot	84.13aD	52.14bC	72.99aC	58.14bC

Means in a column followed by different capital letters or in a row followed by different small letters are significantly different. Post hoc Tukey test.  $P < 0.05$ . Vigour parameters (VP) (not calculated for 2010). PW: shoot pruning weight ( $\times 10^{-3}$  kg); RI: Ravaz index; NS: number of shoots; WS: mean weight of shoots ( $\times 10^{-3}$  kg); LS: mean length of shoots ( $\times 10^{-2}$  m); NB: mean number of buds per shoot; LI: mean length of internodes ( $\times 10^{-2}$  m); DS: mean diameter of shoots ( $\times 10^{-3}$  m). Must parameters (MP). TSS: total soluble solids ( $^{\circ}$ Brix); TA: titratable acidity (g/l); TPC: total phenolic content ( $A_{280}$ ); CI: colour intensity ( $A_{420} + A_{520} + A_{620}$ ); CT: colour tonality ( $A_{420}/A_{520}$ ). Production parameters (PP). BW: mean berry weight ( $\times 10^{-3}$  kg); Y: yield ( $\times 10^{-3}$  kg); NC: number of clusters; CW: mean cluster weight ( $\times 10^{-3}$  kg).

values that ranged from 0.5 to 2.0 in order to plot all the values in a single plot.

The underlying dimension for PC 1 was production and vigour indicators, with positive loading for LS (0.90), WS (0.87), PW (0.82), LI (0.77) and DS (0.56) but negative loading for RI (−0.81), CW (−0.59) and Y (−0.54), on the left side of the plot (Fig. 2). For PC 2, which referred to must parameters, loading was positive for pH (0.71), TPC (0.52) and TSS (0.50) and was negative for NB (−0.55). PC 3 was loaded negatively for NS (−0.54). This indicates that the variables related to production parameters and vigour parameters are related to PC 1, while the must parameters are related to PC 2.

Including in the analysis all the parameters for all the varieties and all the years enables us to determine the stability of the parameters;



**Fig. 2.** Principal components (PC) extracted by factorial analysis of all the studied parameters. The main components were calculated for must, production and vigour parameters measured in 162 vines representing the four varieties (Cabernet Sauvignon, Mencía, Merlot and Tempranillo) for the four studied harvests (2007, 2008, 2009 and 2010). The PC values for years and varieties are indicated with black triangles and black circles, respectively.

the relationships between the parameters can be studied, given that the influence of the environment and variety is removed.

Tempranillo and Mencía were located at a considerable distance from each other, whereas Merlot and Cabernet were located very close to each other (Fig. 2). By introducing the means of the variables for each variety for all the years, the environmental factor is eliminated and also the effect of any disease that may only affect one class of parameter (e.g., botrytis affects must quality but not vigour parameters).

The PC values for the four years visually depict how the different varieties behaved throughout the study period (Fig. 2). Those for the years 2007, 2008 and 2009 were close to each other, indicating that there was little difference between the studied variables, in contrast with 2010, which had a rather different harvest. In this way we can study the relationship between variables in different years and eliminate the influence of variety, since as shown in Table 3, there are variables dependent on variety, such as TSS, always higher for Merlot.

#### 4. Discussion

Mencía is the variety best suited to the Bierzo growing area, as it had the lowest TA and TSS but also the highest TPC, CI, CT and production values, indicating good quality and quantity wines. Mencía also had low vigour values and very high RI values. RI is highly dependent on environmental and genotypic conditions; Kliewer and Dokoozlian (2005), furthermore, have indicated that a vineyard with RI values between 4 and 10 is balanced. On the basis of this observation, Mencía production is unbalanced, so it is advisable to thin out bunches to balance RI values.

Kodur (2011) reported a high juice pH to be undesirable for the production of quality wines as it results in reduced colour stability and a poor taste. pH indicates the strength of the acids present in the must (Blouin and Guimberteau, 2003). In a study of nitrogen fertilization, Brunetto et al. (2009) drew a similar conclusion regarding Cabernet-Sauvignon, with high TA, low pH and high chromatic values; hence, a good quality wine can be produced with this variety in the Bierzo but in small quantities, as output is low, with lots of bunches and high vigour values.

Tempranillo is the variety least adapted to the Bierzo growing conditions according to previous studies in the same area by González-Fernández et al., 2010. It had the highest TSS, indicating that it produces wines with high alcohol content, but also had high pH and low TA values, both important for grape juice stability and commonly used as indicators of quality (Esteban et al., 2002). The concentration of organic acids not only contributes to the acid taste of the must but also influences subsequent wine colour and microbiological stability (Blouin and Guimberteau, 2003). High pH values indicate wines with low colour stability. According to Alburquerque et al. (2007), increased TSS in Tempranillo is correlated with lower production and results in poorer quality wines. Tempranillo had the highest vigour values of the varieties included in our study.

Merlot had the highest TSS and lowest vigour values, corroborating the inverse relationship between quality and vigour (Cortell et al., 2005, 2008). It also had high CI, TA and TPC values but a low CT value. This is a good variety for producing quality wines, but in small quantities, given that production values are low.

The study of varieties reveals that Merlot and Cabernet were very similar. Tempranillo stood apart from the rest, with behaviour directly opposite to that of Mencía (Fig. 2).

Generally speaking there were no differences in production and vigour indicators in the different years of the study, indicating that weather conditions affect these variables less than they do wine quality. Referring to the years, 2007 was the driest and coldest year, with normal TSS, pH, TA and TPC values and high chromatic,

production and vigour values. 2008 had the highest TA and lowest TSS values and average values for the remaining must parameters. It also had high production values and the lowest vigour indicators, other than the RI, which was high for all the varieties. Wines produced in this year were of standard quality and high quantity. Rainfall did not differ greatly from other years. The warmest year was 2009, which had the highest TSS and pH values. TA, chromatic characteristics and production values were high, indicating wine of good quality and quantity. 2010 was the wettest year and had the highest Winkler index; it had the highest pH, which is undesirable for the production of quality wine, and also had the lowest TA and TPC values and the highest CI, CT and production values, indicating standard quality wine produced in high quantities. The RI for each variety over the four years of the study indicates that the vines are not balanced (Kliewer and Dokoozlian, 2005); further studies are necessary in order to detect and correct the causes of the imbalance.

Comparing the years, behaviour in 2008 and 2009 was very similar, whereas in 2007 and 2010 it was quite different (Fig. 2). This fits with the data on rainfall and on the Winkler index (Table 1): the fact that 2010 was the wettest year and 2007 the driest and coldest year would account for the differences in PC values.

The lowest minimum temperature was recorded in 2007, the year in which the phenology processes for all the varieties commenced earliest. The highest minimum temperature was recorded in 2008, which was also the year in which the phenology processes commenced latest for all the varieties. Phenology processes commenced earlier in 2009 than in 2010 and 2009 had a lower minimum temperature than 2010, indicating that phenology was directly related to climate (Spellman, 1999) and especially to minimum temperatures, as the lowest minimum temperature was recorded before the onset of the phenology processes.

As for the must parameters, 2007 had the lowest and 2010 had the highest CI and CT values, indicating that the minimum temperature, rainfall and commencement of phenology processes had a bearing on the synthesis of phenolic compounds and the accumulation of colour in berry skins (Zamora, 2003). The highest and lowest CW values occurred in 2007 and 2010, respectively, indicating that climate has a bearing on production parameters.

The fact that the variables related to production and vigour are related to PC 1 indicate that the vigour and production are directly related. A vine with good vigour will have a high diameter, number, weight and length of shoots and will also produce a high weight and number of clusters and a good yield (Cortell et al., 2005)

Parameters related to the must, such as pH, TPC and TSS, are related to PC 2. This is a useful indicator for the wineries that only perform a few tests to determine wine quality (e.g., TSS and pH) and forego more complicated and expensive analyses (like TPC). However, as Downey et al. (2006) conclude, TPC is crucial to producing high quality wine. According to Baluja (2012), PCA identifies parameters that determine must quality and predicts potential barrel-aged production, without the need for complex and costly analytical techniques

#### 5. Conclusions

The Mencía samples had the best quality must, so this variety is excellent for the production of high quality wines and is suitable for ageing in barrels. Because this variety has high grape production, it is ideally suited to the growing area and is the preferential variety for wines made in the Bierzo PDO. Tempranillo produces poorer quality wines and smaller quantities than the other varieties, indicating that it is poorly adapted to the growing conditions. Cabernet Sauvignon and Merlot, with intermediate levels for the studied parameters, are suitable for producing wines of

standard quality and quantity. The temporal evolution trend is not as sharp as the varietal evolution trend, given that the former is influenced by yearly climate variations that determine the grapevine growth stages and the grape compound synthesis.

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