ISOTOPE COMPOSITION OF WINE AS INDICATOR OF TERROIR SPATIAL VARIABILITY

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Abstract

The goal of this work was to determine the spatial variability of *terroir* using the isotope composition of wine. Carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope composition was measured in wines from Tempranillo (*Vitis vinifera* L.) vineyard, located in Rioja Appellation (Spain). Stable isotope composition, leaf area, vigour, yield components, grape and wine composition were determined in a grid of 85 geo-referenced points, that was drawn across the 5 ha vineyard area. Spatial variability of $\delta^{13}C$ and $\delta^{18}O$ of wine was studied and the vineyard area was divided into six sub-areas for each isotope. Spatial variability of wine isotope composition could be explained by variation in soil properties of the vineyard. Isotope composition of wine was related to vegetative growth and yield components. The wine water $\delta^{18}O$ was significantly correlated to lateral leaf area, total leaf area and vigour at harvest. Carbon isotope ($\delta^{13}C$) was an excellent indicator of yield per vine, cluster weight and berry weight. A significant correlation between $\delta^{13}C$ and total leaf area/yield ratio was also observed. Significant correlation was also observed between wine water $\delta^{18}O$ and the content of malic and tartaric acids in both grape and wine. Moreover, wine $\delta^{13}C$ and $\delta^{18}O$ were significantly correlated with the anthocyanins and total phenols content in grape. Colour density of wine was significantly related to wine water $\delta^{18}O$. Our results suggest that carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) records in wines are useful tools to study spatial variability of terroir in viticulture.

Key words: $\delta^{13}C$, $\delta^{18}O$, GIS, Tempranillo, grapevine, *Vitis vinifera*

1. INTRODUCTION

The wine isotope composition may be a good indicator of the spatial variability of *terroir*, since the variety, rootstock, soil and cultural practices had a strong impact on the stable isotopes of grapes (Tardaguila *et al.* 1997; van Leeuwen *et al.*, 2001; Gomez-Alonso and Garcia-Romero, 2010). Moreover, the influence of water status on the grapes and wine isotope composition has also been demonstrated (Tardaguila *et al.* 1997; van Leeuwen *et al.* 2001; de Souza *et al.* 2005). In fact, the wine isotopic composition has been used to characterize and authenticate a wine´s origin (Martin *et al.* 1988; Day *et al.* 1995; West *et al.* 2007).

The use of geostatistical techniques is widely spread on the scope of precision viticulture (Proffitt *et al.* 2004; Arnó *et al.* 2009). These techniques have been also employed to study spatial variability in winegrape vineyards (Bramley 2005). Reynolds *et al.* (2007) have used GIS (geographic information systems) to study the spatial variation of terroir. A novel GIS model of wine water $\delta^{18}O$ was used to predict regional origin and vintage of wine (West *et al.* 2007).

The main goal of this study was to assess the spatial variability of $\delta^{13}C$ and $\delta^{18}O$ in wines from Tempranillo (*Vitis vinifera* L.) of a commercial vineyard and to explore the relationship between the wine isotope composition and the vegetative growth, yield components and grape and wine composition.

2. MATERIALS AND METHODS

2.1. Vineyard and experimental design

The study was conducted in a commercial Tempranillo (*Vitis vinifera* L.) dry-farmed vineyard situated in Logroño (42º28'44''N, 2º29'35''W), in the Rioja wine growing region (Spain), during the 2008 season. Vine spacing was 2.8 m between rows and 1.2 m in the row. The vines were trained to a vertically shoot positioned trellis system (VSP) on a double cordon Royat and spur pruned to 10-12 buds per vine. No irrigation was applied. All parameters were determined in a grid of eighty five geo-referenced points (3 vines per point) that was drawn across the 5 ha vineyard area (Fig. 1).

2.2. Growth and yield components

Main, lateral and total leaf area were determined at veraison and harvest on each one of the 85 vine blocks using the method proposed by Smart and Robinson (1991). The total shoot length was determined in 2 shoots per vine to characterize the vine vigour as reported by Martinez de Toda *et al*. (2007). For yield assessment at harvest, all clusters per vine were separately counted and weighed. Yield per vine and cluster weight were determined. In the laboratory, a 100 berry sample per crop vine was weighed to determine berry weight.

2.3. Grape and wine analysis

For each vine, the total soluble solid concentration (ºBrix) was measured using a temperature-compensating digital refractometer (Atago, Japan) and titratable acidity, pH, tartaric and malic acid concentrations were determined according to the OIV methods (OIV 2009). Anthocyanin and phenolic concentrations were assessed according to the method of Iland *et al* (2004).

The grapes of 3 vines of each sampling point were blended and transported to the winery of the University of Rioja. A total number of 85 wine fermentations were conducted according to the microscale fermentation set-up proposed by Sampaio *et al* (2007). Alcohol concentration, titratable acidity, pH, malic acid, colour density and hue were determined according to the OIV methods (OIV 2009). Total polyphenol index was calculated by the absorbance reading at 280 nm as described by the EEC method (1990).

2.4. Isotope analysis.

The isotopic ratio $^{13}C/^{12}C$ was measured in ethanol distilled from wine, whereas the $^{18}O/^{16}O$ was determined in wine water. Both measurements were carried out using an Isotope Ratio Mass Spectrometer (IRMS SIRA II, VG FISONS, Cheshire, United Kingdom) coupled with an Elemental Analyser (NA 1500, Carlo Erba, Milan, Italy) and a CO_2 equilibration system (Isoprep 18 VG ISOGAS, Cheshire, United Kingdom) respectively. The procedures are described in the official methods OIV M-F-AS-312-06-ETHANO and OIV MA-F-AS2-09-MOUO18. The isotopic values of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ were expressed in δ‰ versus international reference materials: V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}C$, and V-SMOW (Vienna – Standard Mean Ocean Water) for $\delta^{18}O$, according to the following formula: [(Rs-Rstd)/Rstd] x 1000, where Rs is the isotope ratio measured for the sample and Rstd is the isotope ratio of the international standard. The uncertainty of the measurement (2 standard deviations) was 0.3‰ for $\delta^{13}C$ and $\delta^{18}O$ isotopes.

2.5. Mapping and spatial analysis

Vineyard spatial variability maps of the wines $\delta^{13}C$ and $\delta^{18}O$ were obtained from 85 geo-referenced sampling points, using GIS software (ESRI, Redlands, CA). Data were originally collected as point data with the spatial attribute for each location recorded along its coordinates. A continuous surface map was created by applying the mathematical approach of Ordinary Kriging and classified by k-means method (Bramley, 2005). The last step was to extract the mean for each cluster from the rest of the variables to make the regressions.

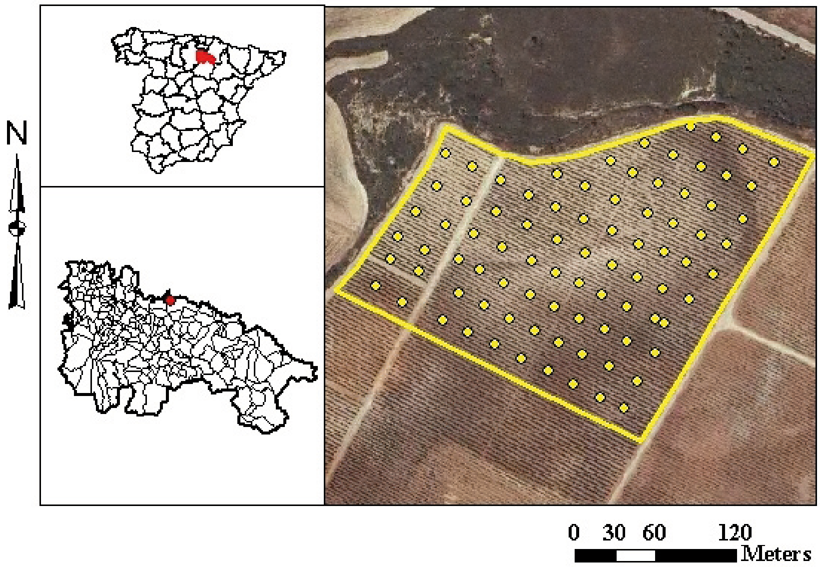

Fig. 1. Localization of the Tempranillo vineyard area (Logroño, Spain) with the 85 sampling points marked.

3. RESULTS AND DISCUSSION

Maps of spatial variability of wine $\delta^{13}C$ and $\delta^{18}O$ are presented in figure 2 and figure 3, respectively. The vineyard area was divided into six sub-areas or classes for each isotope. The $\delta^{13}C$ showed the lowest values in the west and south-centre of the vineyard, whereas the highest values were obtained in the centre and south of the Tempranillo vineyard. The spatial variability of $\delta^{18}O$ was very different from that of $\delta^{13}C$; in fact, the lowest values of wine water $\delta^{18}O$ were reached in the north and east areas of the vineyard.

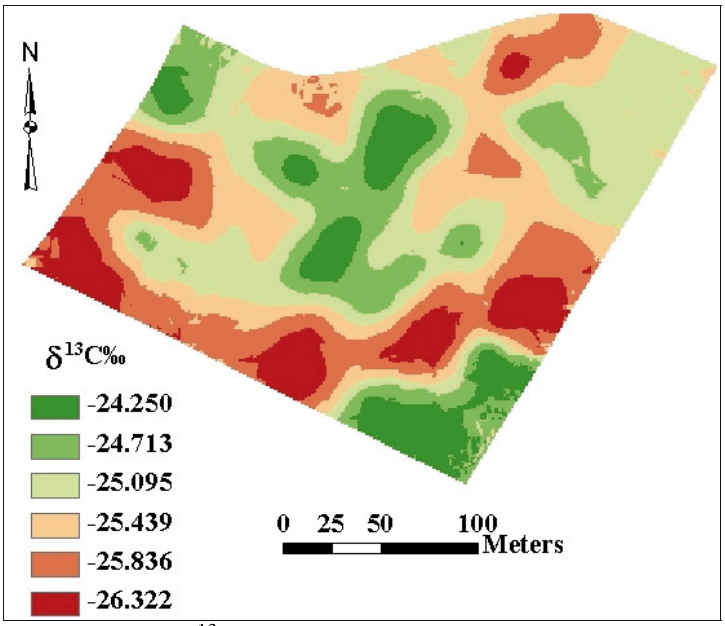

Fig. 2. Spatial variability of $\delta^{13}C$ in wine ethanol (6 classes) of the vineyard using 85 sampling points, with the mean for each class.

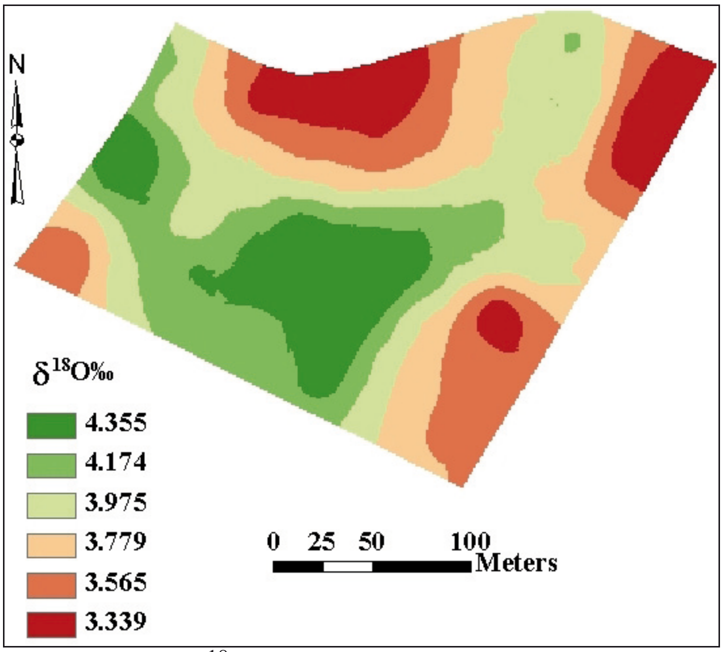

Fig. 3. Spatial variability of $\delta^{18}O$ in wine water (6 classes) of the vineyard using 85 sampling points, with the mean for each class.

Both stable isotopes were not correlated with each other (data not shown). The soil on the top and shoulder slopes in this vineyard supported plants that tended to have shallower rooting systems, smaller clay fraction, and weaker water holding capacity (Oliveira *et al.* 2010). A good relationship between grape sugar $\delta^{13}C$ and vine water status was observed (van Leeuwen *et al.* 2001; Gaudillere *et al.* 2002; de Souza *et al.* 2005). In fact, carbon isotope composition was proposed as integrated indicator of vineyard water status (Gaudillere *et al.* 2002). Our results suggest that the spatial variability of wine isotope composition could be explained by the variation in the water holding capacity of the soil.

Isotope composition of wine was related to vegetative growth (Tab. 1). The ethanol $\delta^{13}C$ was associated to main leaf area at veraison and harvest, but was not related to vigour (total shoot length). On the other hand, the wine water $\delta^{18}O$ was significantly correlated to lateral leaf area, total leaf area and vigour at harvest. In the same Tempranillo vineyard, Oliveira et al. (2010) have observed a relationship between the soil variability and the vegetative development and vigour of the vines. The influence of vineyard water status on both wine isotopic composition and vegetative growth could explain these results

Tab. 1. Regressions obtained between carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes with vegetative growth, where R^2 and p-values are shown.

Vegetative growth	$\delta^{13}C$ ‰		$\delta^{18}O$ ‰	
	R^2	p-value	R^2	p-value
Main leaf area at veraison (m^2/vine)	0.634	0.0462	0.041	0.7013
Main leaf area at harvest (m^2/vine)	0.630	0.0495	0.345	0.2204
Lateral leaf area at veraison (m^2/vine)	0.017	0.8030	0.216	0.3535
Lateral leaf area at harvest (m^2/vine)	0.002	0.9393	0.915	0.0028
Total leaf area at veraison (m^2/vine)	0.302	0.2588	0.308	0.2530
Total leaf area at harvest (m^2/vine)	0.048	0.6765	0.970	0.0003
Total shoot length at veraison (cm)	0.017	0.8030	0.416	0.1000
Total shoot length at harvest (cm)	0.268	0.2929	0.834	0.0109

Stable isotope composition of wine was also linked to yield components (Tab. 2). The ethanol $\delta^{13}C$ was correlated to yield, cluster weight and berry weight, whereas $\delta^{18}O$ was only related to cluster weight. Furthermore, a significant relationship between $\delta^{13}C$ and total leaf area/yield was observed in our study. The leaf to fruit ratio has been used for assessing winegrape quality in the vineyard (Smart and Robinson 1991; Tardaguila and Martinez de Toda, 2008).

Tab. 2. Regressions obtained between carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes with yield components, where R^2 and p-values are shown.

Yield components	**$\delta^{13}C$ ‰**		**$\delta^{18}O$ ‰**	
	R^2	**p-value**	**R^2**	**p-value**
Berry weight (g)	0.922	0.0023	0.042	0.6984
Cluster weight (g)	0.912	0.0030	0.697	0.0388
Yield (kg/vine)	0.734	0.0294	0.417	0.1659
Total leaf area/Yield (m^2/kg)	0.662	0.0450	0.277	0.2831

Grape and wine composition were related to carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes (Tab 3 and 4). The $\delta^{18}O$ values were significantly correlated with the malic and tartaric acid content both in grape and wine. Tartaric acid showed a poorer correlation, probably due to the effect of natural precipitation of bitartrate. Both isotopes were not related to sugar neither to alcohol content. However, ethanol $\delta^{13}C$ and wine water $\delta^{18}O$ were correlated with the concentration of anthocyanins and phenols. These results could be explained by the differences in vineyard water status and the associated canopy temperature. Water deficit during the growth period was demonstrated to have beneficial effects on the concentration of anthocyanins and total phenols in grapes (Koundouras *et al.* 2006). These results indicate an effect of soil on isotope composition of wine. Soil type was associated with the vine vigour and have a measurable effect on phenolic components of grape and wine (Cortell *et al.* 2005). However, Reynolds *et al.* (2007) used GIS to study the spatial variation in soil texture, yield components, grape and wine composition over four years, but they observed no consistent soil texture or vine size effects on berry or wine composition.

Tab. 3. Regressions obtained between carbon (δ13C) and oxygen (δ18O) isotopes with grape composition, where R2 and p-values are shown.

Grape composition	**$\delta^{13}C$ ‰**		**$\delta^{18}O$ ‰**	
	R^2	**p-value**	**R^2**	**p-value**
Soluble solids (ºBrix)	0.055	0.6539	0.138	0.4683
Titratable acidity (g/l)	0.484	0.1249	0.978	0.0002
pH	0.981	0.0001	0.951	0.0009
Malic acid (g/l)	0.430	0.1571	0.987	0.0001
Tartaric acid (g/l)	0.149	0.4503	0.726	0.0311
Anthocyanins (mg anth/berry)	0.550	0.0494	0.759	0.0239
Anthocyanins (mg anth/g berry)	0.892	0.0046	0.656	0.0510
Total phenols (AU/berry)	0.487	0.1239	0.862	0.0075
Total phenols (AU/g berry)	0.908	0.0032	0.720	0.0326

Tab. 4. Regressions obtained between carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes with wine composition, where R^2 and p-values are shown

Wine composition	$\delta^{13}C$ ‰		$\delta^{18}O$ ‰	
	R^2	p-value	R^2	p-value
Alcohol (% v/v)	0.093	0.5573	0.363	0.2059
Titratable acidity (g/l)	0.888	0.0049	0.096	0.5498
pH	0.252	0.3106	0.000	0.9843
Malic acid (g/l)	0.004	0.9113	0.945	0.0011
Tartaric acid (g/l)	0.149	0.4503	0.726	0.0311
Colour density	0.011	0.8445	0.538	0.0469
Hue	0.249	0.3133	0.133	0.4766
Total phenol index (TPI)	0.599	0.0379	0.202	0.3715

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