A viability study of C-O isotope fingerprint for different geographical provenances of Spanish wine vinegars

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Abstract

Wine vinegar is an increasingly appreciated product in Europe and some high-quality vinegars have been certified with "Protected Designation of Origin" (PDO) in order to preserve and control their production methods. Spain has three of the five PDO wine vinegars existing in Europe. A tentative study was carried out in order to assess the utility of stable isotope analysis (δ^{13}C and δ^{18}O) for the characterization of those Spanish wine vinegars and if the values obtained could be used as a fingerprint of their geographical origin as compared with other regions. A total of 35 wine vinegar samples, belonging to the three Southern Spain PDOs and three Northern Spain non-PDO regions, were analyzed for their isotopic composition. Our analysis revealed that most of the Spanish vinegars presented δ^{13}C values that were in agreement with some other isotopic studies about Mediterranean vinegars, since all the samples were under -20‰ and the vast majority of them were below -24‰. On the other hand, the δ^{18}O analysis confirmed its utility for discriminating all the Spanish vinegars according to the three geographical coordinates (latitude, longitude

and altitude). Positive mean values were associated to Southern vinegars (2.16 ± 1.59‰) and negative $\delta^{18}O$ values were related with Northern vinegars (-2.93 ± 2.82‰). We also found a highly significant ($p < 0.001$) correlation of $\delta^{18}O$ with altitude ($R^2 = -0.48$) and longitude ($R^2 = 0.36$), which allowed us to conclude that $\delta^{18}O$ analysis could be used as a fingerprint of the geographical origin in Spanish wine vinegars.

Keywords: wine vinegar, Protected Designation of Origin, stable isotopes ratio, ^{13}C, ^{18}O, geographical origin.

1. Introduction

Vinegar is a food product consumed worldwide as a condiment and food-preserving agent, which can be produced by different methods and raw materials (such as malt, apple, rice, etc.). The legal definitions of vinegar vary from one country to another. The term "vinegar" can be described a product of a double fermentation (alcoholic followed by acetous fermentation) from substances of agricultural origin [1]. Within the wide range of vinegar types, wine vinegar is the most commonly produced and consumed vinegar in Mediterranean countries and Central Europe [2]. This product is the result of the conversion of must sugars into ethanol by the action of yeasts, and the subsequent ethanol oxidation by acetic acid bacteria [3]. In fact, in accordance with [4] (Annex VII, Part II), an authentic wine vinegar cannot contain synthetic acetic acid or acetic acid from the fermentation of sugars that are not derived from grapes (e.g., derived from beet or cane). Furthermore, authentic wine vinegars cannot be produced from dried grapes diluted with water [5,6]. In that sense, the main producers of wine vinegars as Italy or Spain consider vinegar as a product obtained from acetous fermentation of wine [1].

For many years, wine vinegar has been considered as a low-cost secondary product spontaneously derived from wine production. However, in recent years, wine vinegar has become a highly-appreciated food commodity in gastronomy [7]. As a result, the demand for high-quality wine vinegars has significantly increased over the last years. In this context, Spain is one of the major producers of high-quality wine vinegars, including three of the five types of vinegar registered in Europe [8] with a "Protected Designation of Origin" (PDO): "Vinagre de Jerez" (also known as "Sherry wine vinegar"), "Vinagre de Montilla-Moriles" and "Vinagre de Condado de Huelva". The production of these high-quality PDO wine vinegars in Spain is centered in Andalusia, a Southern Spanish region traditionally associated to wine culture, and each of them is produced using the corresponding protected wines ("Vino de Jerez", "Vino de Montilla-Moriles" and "Vino de Condado de Huelva"), which provide singular and specific characteristics. These vinegars have high prices in the market due to their high quality, the long aging time in wooden butts and the high cost of their production. Their high price increases the vulnerability of these products to fraud [9]. For this reason, impartial tools are required to fight against mislabeling or even falsification and better systems must be established to define their quality, authenticity and geographical origin. In many cases, an irrefutable conclusion about the authenticity of a sample using conventional methods is not possible and more accurate information is needed for obtaining a high guarantee of authentication. With this purpose,

the analysis of isotope ratios of some bioelements is being evaluated to provide a geographical profiling of food products that can be applied in geographical identification [1]. In this regard, the analysis of stable isotope ratios of carbon ($^{13}C/^{12}C$; expressed as $\delta^{13}C$) and oxygen ($^{18}O/^{16}O$; expressed as $\delta^{18}O$), among others, has already been introduced as an officially accepted method in food authenticity and origin determination [10]. Since 1991 the addition of water and exogenous sugars (from beet or sugarcane), the most common adulterations of wine, has been detected in wine by analyzing the isotopic ratios of hydrogen (D/H) and carbon ($^{13}C/^{12}C$) in ethanol and of oxygen ($^{18}O/^{16}O$) in water. OIV (International Organization of Vine and Wine) methods are currently adopted: OIV-MA-AS311-05 for site-specific analysis of the D/H ratio using ^{2}H-site-specific natural isotope fractionation NMR (^{2}H-SNIF-NMR), OIV-MA-AS312-06 for analysis of the $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$ ‰) using isotope ratio mass spectrometry (IRMS), OIV-MA-AS2-12 for analysis of the $^{18}O/^{16}O$ ratio (expressed as $\delta^{18}O$ ‰) using IRMS. Very recently, isotopic methods have been recognized by the European Committee for Standardization (CEN) and in part by OIV as a means of detecting the presence of exogenous acetic acid and tap water in wine vinegar [5,6].

The $\delta^{13}C$ of the acetic acid can indicate if the source of the acetic acid and the grape sugars is truly grape (wine) ethanol or wine must, or other ethanol made from fermentation of some other agricultural products such as cereal, potato starch, beetroot or sugarcane. Concerning photosynthetic pathway, some of those plants are C_4-type (Hatch-Slack, C4-dicarboxylic acid pathway), whose $\delta^{13}C$ values commonly range from -9‰ to -19‰. By contrast, these values usually range from -20‰ to -35‰ in vine and the rest of C_3-type plants, in which the atmospheric CO_2 is fixed through the reductive pentose phosphate pathway [11]. Since ^{13}C analysis by isotope ratio mass spectrometry (IRMS) has shown a strong capability to identify synthetic vinegars and distinguish and detect photosynthetic C_3-type (as grape) and C_4-type (as sugarcane or maize) derived products in mixtures, it could be used as a tool for the detection of adulterated vinegars [1]. Although the photosynthetic pathway defines the carbon isotope ratios of plant in its organic matter [12,13], some environmental factors or conditions, as water stress, can also cause these values to increase or decrease during the growing cycle, resulting in detectable differences even within the same genotype [14]. Furthermore, it must be noted that the isotopic values of acetic acid are not affected when the methods for transforming wine to vinegar are applied [6]. Regarding $\delta^{18}O$, the value for this isotope is primarily affected by the source of plant-available water. Chiocchini et al. [15] reported that lower values of $\delta^{18}O$ in extra-virgin olive oils were related to water from regions with high elevation, inland location and cool climate, whereas higher values were related to areas with low elevation, coastal location and warmer

weather. Similar studies carried out in wine also found that its isotopic composition is significantly determined by the climate conditions during the pre-vintage period [16,17]. In general terms, the ^{18}O content of the water in grape products has shown to depend on the environment -natural or anthropogenic- from which it originates. Thus, the isotopic ^{18}O analysis has also shown to be able to detect the addition of external water, not coming from the grapes. The possible dilutions with tap water in order to reduce the acetic degree in the resulting wine vinegar could be detected since this practice leads to significant changes in the ^{18}O isotopic ratio [1]. Thereby, Thomas & Jamin [18] demonstrated the potential of the oxygen stable isotope analysis of water to distinguish wine vinegar from vinegars made from dried grapes, where the ^{18}O isotopic content is influenced by the tap water used in production. Furthermore, Camin et al. [5] proved experimentally that $\delta^{18}O$ analysis of beverage water, officially used to detect the watering of wine and rehydration of concentrated fruit juice, can also be applied to vinegar to detect this kind of fraud. They established minimum values for the $\delta^{18}O$ of water in -2‰ and -5‰ for raw and diluted vinegars respectively. $\delta^{18}O$ values lower than -5‰ in wine vinegar products therefore could indicate an anomalous and excessive water addition.

The fact that water source affects $\delta^{18}O$ values makes the latter eligible to be selected as potential geographical markers of vinegars. Thus, the legal limits established for wine, which are based on the wine isotope databank, have been used as reference for $\delta^{18}O$ analysis to detect the geographical origin authenticity of wine vinegar [6]. Raco et al. [11] also confirmed the efficacy of $\delta^{18}O$ analysis, together with the determination of the deuterium (^2H) isotope content, for detecting the geographical origin of wine. However, since the ^2H and ^{18}O isotopic compositions of those products showed a high correlation in that research, δ^2H value could be considered a dependent value from the $\delta^{18}O$ value.

Despite the promising results, much work is needed since these types of studies are still scarce and had not previously been made with Spanish PDO. Therefore, it would be interesting to continue with the isotopic characterization of certified high quality wine vinegars, in order to develop specific applications. The aim of this work was to characterize Spanish wine vinegars from different regions with respect to their oxygen and carbon isotopic ratio and to assess the applicability of these values to determine their geographical origin. This tentative study also aimed to provide a better understanding of the isotopic composition of wine vinegars and its correlation with climatic conditions, water content of wine, production process and origin.

2. Material and Methods

2.1. Wine vinegar samples

27 samples provided by the Regulatory Councils of the three Andalusian PDOs "Vinagre de Condado de Huelva" (11), "Vinagre de Jerez" (11) and "Vinagre de Montilla-Moriles" (5) were selected and analyzed. Furthermore, eight samples belonging to commercial vinegars from Northern Spain - Galicia (2), Catalonia (3) and La Rioja (3) - with a guaranteed provenance were included. Further information about grape variety, year of harvest, acetic acid degree and geographical location can be found in Table 1.

The number of samples per group is not well balanced, being the Northern group considerably smaller than the Southern group of samples, although it was in accordance to the market availability of vinegars in Spain. Thus, the rate of production and therefore commercialization of wine vinegars in Spain is higher for those with PDO due to their high quality provided by their certification. Hence, the total number of wine vinegar samples collected from Southern Spain, registered under a PDO, has been higher than those samples from Northern regions due to the high demand, and therefore high presence of the former. Moreover, to ensure the provenance of the samples without a PDO (Northern wine vinegars), only wine vinegars produced from wines of high quality made with the typical varieties of geographical area were selected. This led to reduce the number of northern samples available but guaranteed its origin and quality. The year of harvest of the grape used to set up the vinegars of this set of samples was the same (2014) in order to compare samples of the same vintage and to avoid changes in the ^{18}O ratio due to this factor. Furthermore, the time of aging in wooden butts was also considered in the criteria selection of these samples, due to it is a factor that could have an isotopic fractionation effect. For this reason, all samples of this first set were aged for a short and similar aging period (between 6 and 12 months).

In addition, in order to test the possible variation of ^{18}O ratio between years of harvest and among different aging times, a second set of Spanish PDO vinegars were analyzed: 9 "Vinagre de Condado de Huelva" samples (6 from 2015: 3 with more than 12 months of aging and 3 with less than 12 months; and 3 from 2014 with more than 12 months), 11 "Vinagre de Jerez" samples (7 from 2015: 4 with more than 12 months of aging and 3 with less than 12 months; and 4 from 2014 with more than 12 months) and 4 "Vinagre Montilla-Moriles" samples (2 from 2015: one with more than 12 months of aging and other with

less than 12 months; and 2 from 2014 with more than 12 months) (Table 2).Samples were analyzed in triplicate.

2.2. Isotopic analysis

The $^{13}C/^{12}C$ and $^{18}O/^{16}O$ results are expressed in standard delta notation (δ) as per mil (‰) deviation from the standards VPDB (Vienna-Pee Dee Belemnite, IAEA, Vienna) normalized by assigning consensus values [19], and V-SMOW (Vienna-Standard Mean Ocean Water) normalized to the VSMOW−SLAP (Standard Light Antarctic Precipitation) scale, respectively, according to the equation:

$$\delta\ (^{i/j}\text{E})\ ‰ = \frac{^{i/j}R_p - {^{i/j}R_{Ref}}}{^{i/j}R_{Ref}} \times 1000$$

where $^{i/j}E$ denotes the higher (superscript i) the lower (superscript j) atomic mass number of element E and R is the ratio of the heavy to light stable isotope in the sample (R_p) and the international reference material (R_{ref})= $^{18}O/^{16}O$ or $^{13}C/^{12}C$ ratios.

$\delta^{13}C$ determination was performed by combusting the sample at 1020 °C in a Carlo Erba 1108 elemental analyzer coupled in continuous flow mode to an IRMS (Isotope Ratio Mass Spectrometer) VG Isochrom. The main standards used were NBS-22, IAEA CH6 and IAEA 600. The analytical precision, based on the repeated analysis of internal standard waters, was 0.1‰.

$\delta^{18}O$ determination was conducted by a process of equilibration with a mixture of gases He-CO_2 for 18 hours and further analysis of CO_2 in a GasBench coupled in continuous flow mode to an IRMS (Isotope Ratio Mass Spectrometer) Delta V Advantage, Bremen (Germany). The main standards used for correction were V-SMOW2 and SLAP2. Measurement precision (on the standards used) was 0.2‰.

All measurements were carried out against laboratory standards that are periodically calibrated against international standards recommended by the International Atomic Energy Agency (IAEA).

2.3. Statistical analysis

The isotopic data were analyzed using Infostat software (Grupo InfoStat, Argentina). Analysis of Variance (ANOVA) and Least Significant Difference (LSD) Fisher test were carried out on the data set to identify differences between groups of samples from certain geographical Spanish origin. The

Pearson correlation coefficient (R) and p-value were used to show the linear dependence and their significance between the studied variables. Probability values of $p < 0.05$ were adopted as the criterion for significant differences.

3. Results and discussion

3.1. $\delta^{13}C$ and $\delta^{18}O$ isotopic values in different Spanish wine vinegars

Isotopic ^{18}O and ^{13}C ratios vs. each corresponding standard obtained in the analysis of the different wine vinegar samples were shown in Table 1 and 2. Regarding those results, $\delta^{13}C$ values ranged from -26.4 (corresponding to a sample from "Vinagre de Condado de Huelva" PDO) to -22.2‰ (corresponding to a sample from "Galicia"), whereas $\delta^{18}O$ ranged from -6.2 (corresponding to a sample from La Rioja) to 6.8‰ (corresponding to a sample from "Vinagre de Condado de Huelva" PDO). With respect to ^{13}C isotope, several researches have demonstrated the association between $\delta^{13}C$ values and wine and wine vinegar adulterations due to the relationship of these values with the photosynthetic pathway. According to that, Raco et al. [11] claimed that the $\delta^{13}C$ (vs. VPDB) values from wine ethanol, that has been found to be not different from that of acetic acid after extraction from vinegars, should range from -20‰ to -35‰. By contrast, some other authors [1,6,20] established that values between -24‰ and -20‰ in temperate European regions have shown to be related to vinegars with acetic acid coming from C_4 plants or from grapes exposed to severe water stress. In regards to the results obtained in this study (Table 1 and 2), we found that all our samples presented values less than -20‰ and that the vast majority of them were below the threshold of -24‰.

With respect to the determination of stable oxygen isotope ratio, Camin et al. [5] established the minimum value of the $\delta^{18}O$ for raw vinegars (-2‰) and diluted vinegars (-5‰), which was also confirmed by Werner & Roßmann [1]. According to that, our results showed that most of the vinegar samples were in agreement with those authors' findings, with only two samples below -2‰ and three below -5‰ (Table 1 and 2). Besides, almost all of the samples belonging to the three Andalusian PDOs presented positive values of $\delta^{18}O$.

As a visual summary of the aforementioned results, Fig. 1 depicts the isotopic $\delta^{13}C$ and $\delta^{18}O$ values of all the samples considered in the present study, together with the aforementioned thresholds, separating samples according to their isotopic ^{13}C and ^{18}O content. As shown in the Fig. 1, the vinegars from the three Andalusian PDOs are placed together in the same area of the graph, regardless of the year of harvest and the aging time. In general terms, it can be observed that vinegar samples from "Vinagre de Jerez" PDO had

the most homogeneous isotopic values (SD values of $\pm$ 0.54 and $\pm$ 1.11 for $\delta^{13}C$ and $\delta^{18}O$, respectively), followed by "Vinagre de Condado de Huelva" ($\pm$ 0.95 and $\pm$ 1.45), possibly due to the fact that these PDOs are highly consolidated. Samples from "Vinagre de Montilla-Moriles" PDO, however, presented a higher dispersion ($\pm$ 1.07 and $\pm$ 2.62) within their isotopic values (Table 3). This recent PDO was registered in 2015 and that fact, together with the difficulty in obtaining a higher number of samples in the present study, could explain the obtained results [21,22]. Wine vinegars with a PDO indication are exposed to an exhaustive routine checked by the Regulatory Councils that largely prevent the occurrence of possible production frauds and errors. This is a quality guarantee confirmed by our results.

3.2. Isotopic differentiation of geographical origin

In order to determine whether the $\delta^{13}C$ and $\delta^{18}O$ change according to the geographical origin, two sets of ANOVA$_s$ and LSD Fisher tests were performed for each isotope. One set consisted on six different regions of Spain where wine vinegars are produced (Condado de Huelva, Jerez, Montilla-Moriles, Galicia, La Rioja and Catalonia) and the second set included those Spanish regions grouped in two classes according to latitude: Northern and Southern Spain. ANOVA results are shown in Table 3. With regard to $\delta^{13}C$, significant differences ($p < 0.05$) according to geographical origin and latitudes North and South were observed. The significant differences between North and South (-24.51 $\pm$ 1.19‰ for Northern vinegars and -24.92 $\pm$ 0.81‰ for Southern vinegars) were not very evident. Moreover, this parameter is more related to adulteration detection and no clear differentiation of the PDO zone is achieved. The level of discrimination was higher within $\delta^{18}O$ values. In fact, mean values of $\delta^{18}O$ obtained for the North (-2.93 $\pm$ 2.82‰) and South locations (2.16 $\pm$ 1.59‰) showed greater significant differences than $\delta^{13}C$ mean values. Furthermore, with respect to $\delta^{18}O$ results, positive mean values were associated to Southern vinegars ("Vinagre de Condado de Huelva", "Vinagre de Jerez" and "Vinagre de Montilla-Moriles" in this order) and negative $\delta^{18}O$ values were related with Northern vinegars (Table 3). The significant variability observed within $\delta^{18}O$ values reflected the effect of geographical origin and its associated meteorological conditions on the isotopic composition of wine vinegars, separating the considered samples according to Southern and Northern regions. Hence, the isotopic results obtained in this study were in agreement with other researches that demonstrated the utility of isotopic analysis in the determination of geographical origin of butter [23], the discrimination of milks produced at different altitudes [24] and the influence of climatic conditions and grape variety, highly related to geographical origin, in vinegars and wines [6,11].

With respect to samples from PDOs, the $\delta^{18}O$ mean value for "Vinagre de Condado de Huelva" samples were the highest (2.67 ± 1.45 ‰), while these values were significantly lower for "Vinagre de Montilla-Moriles" wine vinegars (1.60 ± 2.62 ‰). The values of "Vinagre de Jerez" samples were between these two extremes (1.91 ± 1.11 ‰). These intermediate values may be associated to the geographical origin of these samples, since the production area of "Vinagre de Jerez" PDO wine vinegars is located between "Vinagre de Condado de Huelva" and "Vinagre de Montilla-Moriles" PDOs (all of them in Southern Spain), being the land type and the latitude and longitude coordinates very similar (Table 1). These characteristics make their differentiation with only $\delta^{18}O$ isotopic analysis difficult to accomplish.

On the other hand, ANOVA was also performed in order to determine whether the $\delta^{13}C$ and $\delta^{18}O$ change according to time of aging in wood barrels and the year of harvest. Regarding the aging time factor, any significant differences were not observed for $\delta^{13}C$ isotopes. However, with regards to $\delta^{18}O$ isotope, it was possible to observe for each PDO that samples aged more than 12 months had higher values, incrementing around 1.5 times with respect to less aged samples (Table 2). In spite of this, the $\delta^{18}O$ mean value for "Vinagre de Condado de Huelva" samples aged for more than 12 months was the highest (3.69 ± 2.08‰) as it was observed before, "Vinagre de Jerez" aged samples had also intermediate values (3.18 ± 1.06‰) and the $\delta^{18}O$ mean values for "Vinagre de Montilla-Moriles" more aged vinegars were again significantly lower (2.82 ± 1.27‰). Furthermore, these values were even more significantly different from those of Northern vinegars due to $\delta^{18}O$ mean values for this group was negative (-2.93 ± 2.82‰). Regarding the year of harvest, and taking into account only samples for the same category (<12 months) to avoid the variance showed with the aging factor, $\delta^{13}C$ and $\delta^{18}O$ mean values did not show significant differences between the two years of harvest considered for the three PDOs, mainly due to the complex production process of these vinegars, that had many processes between the harvest of the grapes and the final product, including, among them, their aging in wood barrels. Thus, $\delta^{13}C$ mean values in 2014 and 2015 for this isotope were around -25.0 ± 0.16‰ and -25.20 ± 0.70‰, respectively, as well as mean values in 2014 and 2015 for $\delta^{18}O$ ratio were around 2.00 ± 0.5‰ 1.94 ± 0.70‰, respectively Hence, these results showed that even with different years of harvest or aging periods, the ^{13}C and ^{18}O ratios were mainly dependent of the geographical origin.

Finally, a study of correlations between the isotopic ratios and some vinegar parameters described in Table 1 was carried out. The Pearson's correlation coefficients, shown in Table 4, showed that there is a

highly significant ($p < 0.001$) correlation of the isotopic [18]O ratio with geographical location expressed as altitude (samples from regions with different elevation above sea level), longitude (samples from Eastern and Western regions) and latitude (samples from Southern and Northern regions). From these three geographical variables, the higher correlation was observed in latitude (-0.77). This result was in agreement with the conclusions of Renou et al. [24] on their research about milk and with those from Chiocchini et al. [15] for extra-virgin olive oils, who claimed that lower values of $\delta^{18}O$ were related to waters from regions with high elevation, inland location and cool climate. Consequently, and according to the obtained results, the $\delta^{18}O$ determination showed to be more useful for the discrimination of wine vinegar according to its origin than the $\delta^{13}C$, since [18]O ratio was correlated with the three geographical coordinates or parameters, whereas $\delta^{13}C$ was only correlated with the latitude ($p < 0.01$) and with a lower correlation coefficient (0.27 for $\delta^{13}C$ vs. -0.77 for $\delta^{18}O$). This Pearson's correlation analysis was in agreement with the results obtained in the previous ANOVAs (Table 2) and reinforced the conclusions of the present study.

4. Conclusions

The results of the present study indicated that $\delta^{18}O$ analysis can be used as a valid tool to distinguish among some Spanish wine vinegars from different latitude, North and South (negative and positive $\delta^{18}O$ values respectively), and even among regions with similar latitude. "Vinagre de Condado de Huelva" PDO wine vinegars showed the highest $\delta^{18}O$ values, followed by "Vinagre de Jerez" and "Vinagre de Montilla-Moriles" PDO samples. In fact, the results showed that mean values of $\delta^{18}O$ between 2.67‰ and 1.60‰ could be established for Southern Spanish PDO wine vinegars. This range could be used as a fingerprint of these high-quality wine vinegars. Furthermore, the $\delta^{18}O$ isotopic values showed a significant correlation with different altitude, latitude and longitude.

The carbon stable isotope analysis revealed that most of the Spanish vinegars presented $\delta^{13}C$ values that were in agreement with some other isotopic studies about European wine vinegars.

According to the results, although the time of aging in wood barrels seems to increase the $\delta^{18}O$ isotope of PDO vinegars, the same the isotopic relationship and differences was maintained among the different vinegars. Therefore, we could conclude that the ^{13}C and [18]O ratios were mainly dependent of the geographical origin, even with different years of harvest or aging periods.

In conclusion, the results of this study allowed confirming that stable isotope ^{18}O and ^{13}C analysis, especially the first one, could be considered as a useful analytical method for regulatory authorities in order to identify or assess the geographical origin of Spanish wine vinegars and verify the correct labeling of geographical denomination. Further studies with higher number of samples, and some controlled samples would contribute to define better ranges and limits of isotope ratios for each area.

Conflict of Interest

All Authors certify that they participated in the conception of the work and make public their responsibility for its content and that they did not omit any connections or funding agreements among the Authors and Companies that may have an interest in the publication of this article, and they approve the final manuscript which is submitting to *European Food Research and Technology*. All of them approve the authorship criteria and give to this Journal the exclusive rights to edit, publish, reproduce, distribute copies, make ready derivative works in paper, electronic or multimedia and include the article in indexes or national and international databases.

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TABLES

Table 1. Characteristics of the samples according to grape variety, geographical location and isotopic [18]O and ^{13}C ratios.

P.D.O. / Origin	Nº	Grape variety	Acetic degree	Spatial characteristics			$\delta^{13}C‰$ vs. VPDB	$\delta^{18}O‰$ vs. VSMOW
				Latitude	Longitude	Altitude (m)		
Condado de Huelva	1	Zalema	8.0	37° 22' 01" N	06° 32' 29" W	192	-26.2	1.8
	2		8.0	37° 22' 01" N	06° 32' 29" W	192	-25.2	1.5
	3		8.0	37° 22' 01" N	06° 32' 29" W	192	-25.0	2.4
	4		7.0	37° 22' 01" N	06° 32' 29" W	192	-25.2	2.4
	5		8.0	37° 22' 01" N	06° 32' 29" W	192	-25.2	6.8
	6		8.0	37° 22' 01" N	06° 32' 29" W	192	-22.3	2.3
	7		8.0	37° 22' 01" N	06° 32' 29" W	192	-25.1	2.0
	8		8.0	37° 22' 01" N	06° 32' 29" W	192	-24.5	1.9
	9		8.0	37° 22' 01" N	06° 32' 29" W	192	-25.1	2.3
	10		8.0	37° 22' 01" N	06° 32' 29" W	192	-24.4	2.8
	11		7.0	37° 22' 01" N	06° 32' 29" W	192	-24.9	3.1
Jerez	1	Palomino	7.0	36° 36' 58" N	06° 09' 08" W	20	-24.4	0.3
	2		7.0	36° 42′ 00″ N	06° 07′ 00″ W	56	-24.4	0.6
	3		7.0	36° 42′ 00″ N	06° 07′ 00″ W	56	-25.6	2.4
	4		7.0	36° 43' 08" N	06° 19' 48" W	32	-24.6	1.3
	5		7.0	36° 36' 58" N	06° 09' 08" W	20	-24.8	2.3
	6		7.0	36° 43' 08" N	06° 19' 48" W	32	-25.3	3.2
	7		7.0	36° 36' 58" N	06° 09' 08" W	20	-25.1	0.7
	8		7.0	36° 42′ 00″ N	06° 07′ 00″ W	56	-25.6	3.5
	9		7.0	36° 42′ 00″ N	06° 07′ 00″ W	56	-25.0	2.8
	10		7.0	36° 42′ 00″ N	06° 07′ 00″ W	56	-26.1	1.3
	11		7.0	36° 36' 58" N	06° 09' 08" W	20	-25.0	2.5
Montilla-Moriles	1	Pedro Ximénez	8.0	37° 36' 10" N	04° 38' 03" W	207	-24.2	-0.5
	2		8.0	37° 36' 10" N	04° 38' 03" W	207	-26.1	2.4
	3		9.7	37° 36' 10" N	04° 38' 03" W	207	-25.4	5.8
	4		7.0	37° 36' 10" N	04° 38' 03" W	207	-24.9	-0.1
	5		6.0	37° 29' 53" N	04° 25' 51" W	547	-23.3	0.3
Galicia	1	Albariño	6.0	42° 07′ 59” N	08° 15′ 47” W	187	-25.3	-2.2
	2		6.0	42° 07′ 59” N	08° 15′ 47” W	187	-22.2	-3.6
Catalonia	1	Chardonnay Blanc	6.0	41° 22' 50″ N	01° 36' 39″ E	257	-25.6	-1.4
	2		6.5	41° 22' 50″ N	01° 36' 39″ E	257	-24.7	1.9
	3		6.0	41° 22' 50″ N	01° 36' 39″ E	257	-25.5	-0.8
La Rioja	1	Viura	6.0	42° 28' 12″ N	02° 26' 44″ W	465	-23.9	-5.9
	2		6.0	42° 28' 12″ N	02° 26' 44″ W	465	-25.3	-5.2
	3		6.0	42° 28' 12″ N	02° 26' 44″ W	465	-23.7	-6.2

Note* All samples had the same year of harvest (2014) and were aged less than 12 months.

Table 2. Isotopic ^{18}O and ^{13}C ratios of a new set of PDO wine vinegars including different aging time and different years of harvest.

P.D.O. / Origin	Nº	Grape variety	Acetic acid	Year	Latitude	Longitude	Altitude	Aging (months)	δ^{13}C‰ vs. VPDB	δ^{18}O‰ vs. VSMOW
						Spatial characteristics				
Condado de Huelva	12	Zalema	10	2015	37° 22' 01" N	06° 32' 29" W	192	<12	-26.4	2.5
	13		7	2015	37° 22' 01" N	06° 32' 29" W	192	<12	-26.2	2.2
	14		8	2015	37° 22' 01" N	06° 32' 29" W	192	<12	-25.5	3.7
	15		8	2014	37° 22' 01" N	06° 32' 29" W	192	>12	-25.4	5.9
	16		8	2014	37° 22' 01" N	06° 32' 29" W	192	>12	-25.2	1.7
	17		8	2015	37° 22' 01" N	06° 32' 29" W	192	>12	-24.5	2.3
	18		6	2015	37° 22' 01" N	06° 32' 29" W	192	>12	-25.4	4.7
	19		8	2015	37° 22' 01" N	06° 32' 29" W	192	>12	-25.2	1.5
	20		6	2014	37° 22' 01" N	06° 32' 29" W	192	>12	-24.4	5.9
Jerez	12	Palomino	7	2015	36° 36' 58" N	06° 09' 08" W	20	<12	-24.9	0.6
	13		7	2015	36° 42' 00" N	06° 07' 00" W	56	<12	-24.9	2.1
	14		7	2015	36° 36' 58" N	06° 09' 08" W	20	<12	-25.1	3.0
	15		7	2015	36° 40' 43" N	06° 09' 13" W	20	<12	-24.9	0.7
	16		8	2014	36° 43' 08" N	06° 19' 48" W	32	>12	-25.1	3.7
	17		7	2015	36° 36' 58"N	06° 09' 08" W	20	>12	-24.9	3.7
	18		8.3	2014	36° 42' 00" N	06° 07' 00" W	56	>12	-25.8	3.9
	19		7	2014	36° 42' 00" N	06° 07' 00" W	56	>12	-25.7	2.1
	20		7	2015	36° 42' 00" N	06° 07' 00" W	56	>12	-24.3	2.2
	21		7	2014	36° 40' 43" N	06° 09' 13" W	20	>12	-25.2	2.0
	22		9.5	2015	36° 40' 43" N	06° 09' 13" W	20	>12	-24.9	4.6
Montilla-Moriles	6	Pedro Ximénez	8	2015	37° 36' 10" N	04° 38' 03" W	207	<12	-24.7	1.4
	7		8	2014	37° 36' 10" N	04° 38' 03" W	207	>12	-25.0	3.7
	8		8	2014	37° 36' 10" N	04° 38' 03" W	207	>12	-24.4	1.4
	9		8	2015	37° 36' 10" N	04° 38' 03" W	207	>12	-25.7	3.4

Table 3. Mean values ± standard deviation (SD) and ANOVA results of isotopic ^{13}C and ^{18}O ratios (‰) according to geographical origin.

Isotopic ratios	Geographical provenance	Mean value ± SD (‰)*
δ^{13}C (‰) vs. VPDB	Galicia	-23.74 ± 2.23 [A]
	La Rioja	-24.30 ± 0.86 [A.B]
	Montilla-Moriles	-24.79 ± 1.07 [B.C]
	Condado de Huelva	-24.82 ± 0.95 [B.C]
	Jerez	-25.08 ± 0.54 [C]
	Catalonia	-25.24 ± 0.50 [C]
δ^{18}O (‰) vs. VSMOW	Condado de Huelva	2.67 ± 1.45 [A]
	Jerez	1.91 ± 1.11 [A.B]
	Montilla-Moriles	1.60 ± 2.62 [B]
	Catalonia	-0.12 ± 1.75 [C]
	Galicia	-2.89 ± 0.99 [D]
	La Rioja	-5.77 ± 0.53 [E]

*Letters mean significant differences ($p < 0.05$) according to LSD test.

Table 4. Pearson's correlation coefficients among the different variables studied.

Variables	Latitude	Longitude	Altitude	$\delta^{13}C$
Latitude				
Longitude	-0.52***			
Altitude	0.54***	-0.55***		
$\delta^{13}C$	0.27**	0.10^{NS}	0.17^{NS}	
$\delta^{18}O$	-0.77***	0.36***	-0.48***	-0.35***

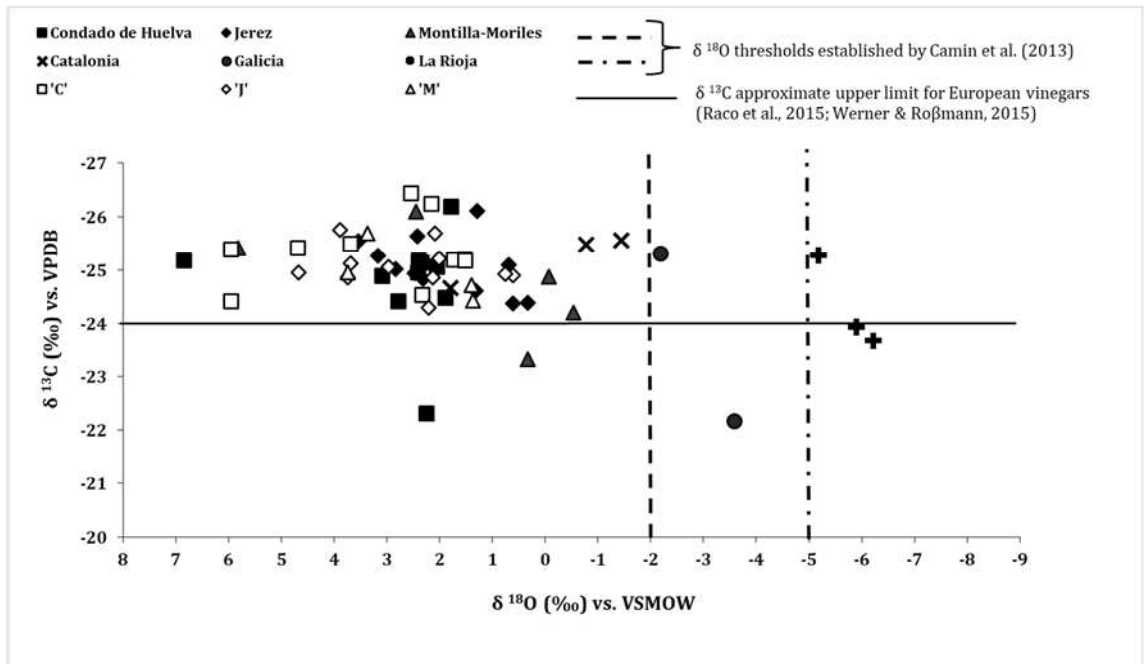*, **, ***: Significant at 5, 1 and 0.1% levels, respectively; NS: Non-significant.

FIGURES

Fig.1

Figure Captions

Fig. 1. $\delta^{13}C$ values and $\delta^{18}O$ values from different Spanish wine vinegars. Note: "C" "J" and "M": Condado de Huelva, Vinagre de Jerez and Montilla Moriles vinegars from 2015 and more than 12 months of aging, respectively (Table 2)