Journal Pre-proofs

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PII: S0026-265X(20)30698-6
DOI: https://doi.org/10.1016/j.microc.2020.105044
Reference: MICROC 105044

To appear in: *Microchemical Journal*

Received Date: 2 March 2020
Revised Date: 14 April 2020
Accepted Date: 15 May 2020

Please cite this article as: N.T. Jiménez-Morillo, V. Palma, R. Garcia, J. Alberto Pereira, C. Barrocas Dias, M. João Cabrita, acMultivariate geostatistical analysis of stable isotopes in Portuguese varietal extra virgin olive oils, *Microchemical Journal* (2020), doi: https://doi.org/10.1016/j.microc.2020.105044

Multivariate geostatistical analysis of stable isotopes in Portuguese varietal extra virgin olive oils

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Abstract

Stable isotope contents of carbon, hydrogen and oxygen are known to reflect the geo-climatic conditions under which olives grown. This study aims to unravel the correlation between some of the main geographic variables and the isotopic composition of different Portuguese varietal extra virgin olive oil (EVOO) samples. Thus, the isotopic composition ($\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$) of 38 EVOO samples from 11 olive varieties from 2 Portuguese regions (Alentejo and Trás-os-Montes) was studied using an elemental analyzer coupled to an isotope ratio mass spectrometry. Multivariate analysis indicated that bulk $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ values were enough to significantly ($P < 0.05$) predict altitude, latitude, longitude, temperature, rainfall, and sea distance. This work showed that the assessment of EVOO isotopic composition give information not only on the geographic origin, but also on the environmental conditions. To the best of our knowledge, this is the first report on bulk isotopic composition of Portuguese EVOOs.

Keywords: Extra virgin olive oil, Geographic origin, Multi linear regression, Stable isotopes, Statistical analysis

1. Introduction

Olive oil is unique among other vegetable oils owing to the health benefits, nutritional properties and peculiar organoleptic characteristics. Since they are only mechanically extracted and consumed without any further refining process, the natural compounds are preserved contributing to its higher nutritional value [1]. Recent studies have described potential health benefits to some compounds that compose olive oil matrix, being considered as a functional food, which arouses a huge interest for its consumption [2]. Extra virgin olive oil (EVOO) is considered the top grade of olive oil, and with the commercial value of EVOO and the recent introduction on the market of high-quality monovarietal olive oils, it can lead to an increase of fraudulent practices, namely those related with the botanical and geographical origin of the olive oil [3]. Therefore, an especial attention has been given to EVOO authenticity, particularly on ascertain its geographic and varietal origin. The noticeable influence that geoclimatic conditions have on the organoleptic quality of olive oils [4], prompted the attempt to differentiate EVOO based on their geographic origin using suitable analytical methodologies. EVOO geographic denomination is not determined by the commonly used physico-chemical parameters [5] since they are not sufficiently accurate to enable this differentiation. Therefore, it is an imperative need the development of appropriate analytical tools combined with statistical methods to guarantee the authenticity and traceability of olive oils, preventing illicit practices and protecting both the producers and consumers. Nowadays, the evaluation of the stable isotope composition of the main bioelements (C, N, H and O) by isotope ratio mass spectrometry (IRMS) seems to be a powerful approach to ensure geographical origin of foodstuffs [6]. Stable isotope analysis of fatty acids was introduced in the 1970s to study the pathways of lipid biosynthesis [7], but it was recently that IRMS technique has been applied to the authentication of olive oils. Since stable

isotopes can provide valuable information of geoclimatic characteristics of the production areas, as well as of the agricultural practices [8], they can be used as key markers of geographical origin, becoming a valuable approach in such kind of studies. Particularly, the C isotope composition ($\delta^{13}C$) of plant components (fruits, roots, leaves, etc.) is mainly related to the type of photosystem (C_3 and C_4), as well as to several environmental and edaphic factors, like humidity, temperature, precipitation, salinity of soil, water stress, etc. [9]. On the other hand, the isotopic composition of H and O are indicators of the water uptake and evapotranspiration of the growing plant, which, in turn, are very sensitive to the geoclimatic conditions (latitude, longitude, altitude, temperature, etc.) of production areas [10, 11]. Furthermore, $\delta^{2}H$ and $\delta^{18}O$ values are determined by the possible fractionation associated with the plant tissues biosynthesis pathways [12]. Owing to the conspicuous complexity of the data obtained with the different isotope analysis techniques, the use of multivariate statistical analyses, such as Principal Component Analysis (PCA), Partial Least Squares regression, Multi Linear Regression (MLR) and Discriminant Analysis, for both the interpretation of these data and the determination of geographical origin of olive oils [13,14] is needed.

This study aimed to evaluate the suitability of stable isotope composition of the main bioelements (C, H and O) for discriminating Portuguese varietal EVOO samples according to their geoclimatic conditions using multivariate statistical methods, such as PCA and MLR, for data evaluation.

2. Materials and Methods

2.1. Samples and experimental design

A total of 38 well-catalogued EVOO (*Olea europaea* L.) samples from 11 different olive varieties (Arbequina, Blanqueta, Carrasquenha, Cobrançosa, Cordovil de Serpa, Galega

Vulgar, Madural, Verdeal, Negrinha do Freixo, Picual and Verdeal Alentejana) produced in 12 different locations belonging to two well-differentiate Portuguese regions, Alentejo (Elvas, Mourão, Vidigueira, Serpa, Évora, Ferreira do Alentejo) and Trás-os-Montes (Freixo de Espada à Cinta, Alfandega da Fé, Macedo de Cavaleiros, Mirandela, Valpaços, and Vila Flor) (26 and 12 olive oil samples, respectively), were collected in November and December of 2016. For most of the samples, approximately 5 kg of each olive varieties were processed separately in an Abencor® system within 24 hours from harvesting. Fruits were crushed with a hammer mill and the olive paste was malaxed at 25° C for 30 min, in an olive paste mixer, finally the olive oil was separated by centrifugation. Other samples were taken directly from olive oil mills to avoid possible undeclared mixtures with olive oils from other cultivars and geographical origins before bottling. They were stored in dark-brown glass bottles at 20ºC in the dark. Each EVOO sample was geo-referenced, with data of latitude (UTM), longitude (UTM), altitude (m a.s.l.), sea distance (km), mean annual temperature (ºC) and mean annual rainfall (mm) respectively assigned (Table 1).

2.4. Stable isotope analysis

The carbon, hydrogen and oxygen isotope composition ratios ($^{13}C/^{12}C$, $^{2}H/^{1}H$ and $^{18}O/^{16}O$, respectively) of EVOO samples were determined by elemental analysis/isotope ratio mass spectrometry (EA/IRMS). The EA/IRMS system consisted of a Flash 2000 HT elemental analyzer (Thermo Scientific, Bremen, Germany) with two reactors: i) Combustion (C, N and S), and ii) Pyrolysis (H and O). The elemental analyzer is coupled by a ConFlo IV (Thermo Scientific) continuous flow open split interface to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific). Carbon isotope analysis used helium as carrier gas at a flow rate of 80 mL/min, while for hydrogen isotope analysis the helium flow was set at 120 mL/min. EVOO samples (0.5 to 1 mg) were

weighed in cups (IVA Analysentechnik GmbH & Co. KG, Meerbusch, Germany) made of tin for carbon and of silver for hydrogen and oxygen analysis. The cups were closed, folded, pressed to a small size and loaded in a MAS 200R (Thermo Scientific) automatic sampling carrousel. Appropriate calibration standards were prepared in the same manner and placed within batches of samples.

For carbon isotope analysis the cups were flush-combusted and flush-reduced concurrently under a helium carrier steam and oxygen pulse at 1020 ^{o}C in a quartz reactor filled with chromium oxide (Cr_2O_3), silvered cobaltous-cobaltic oxide (Ag (Co_3O_4)) and reduced copper (Cu). The gases were dried through a 10 cm long glass column filled with anhydrous magnesium perchlorate (Mg (ClO_4)$_2$), and then directed through a 3 m long and 4 mm i.d. stainless steel gas chromatography column packed with Porapak stationary phase at 40 ^{o}C for the separation of CO_2, which was analysed for its isotopic composition in the Delta V Advantage isotope ratio mass spectrometer. Pure CO_2 gas was inserted into the He carrier flow as pulses of the reference gas (250 mL/min).

For hydrogen and oxygen isotope ratios the samples were analysed using the pyrolysis reactor. This consists of an outer ceramic (Al_2O_3) tube and an inner glassy carbon reactor tube filled with high-purity glassy carbon granulates, and wool of silver and quartz. The silver cups were dropped sequentially under a steam of helium into the reactor tube held at 1450 ^{o}C. The produced pyrolysis gasses were passed through a 10 cm long glass column filled with a mixture of anhydrous magnesium perchlorate ($Mg(ClO_4)_2$), to dry the gas, and Carbosorb, to trap CO_2 generated during the pyrolysis reaction. The dry pyrolysis gasses were directed through a 3 m long and 4 mm i.d. stainless steel gas chromatography column packed with Porapack stationary phase at 70 ^{o}C for the separation of H_2 and CO. Hydrogen and oxygen were analysed for its isotopic

composition on the Delta V Advantage isotope ratio mass spectrometer. Pure H_2 and CO gas were inserted into the He carrier flow as pulses of the reference gas (250 mL/min).

The stable isotope abundances are reported in the delta (δ) notation ($\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$) in variations relative to an international standard. The isotope value was defined by Coplen in 2011 [15], according to equation 1:

$$\delta^{i}E_{sample} = \frac{R\left({}^{i}E/{}^{j}E\right)_{sample}}{R\left({}^{i}E/{}^{j}E\right)_{standard}} - 1 \quad \text{1)}$$

Where "*R"* is the molar ratio of the heavy (^{i}E) to light (^{j}E) most abundant isotope of chemical element "*E*" ($^{13}C/^{12}C$, $^{2}H/^{1}H$ and $^{18}O/^{16}O$). The "δ" values are reported in milliurey (mUr). The stable isotope standard for carbon is the Vienna Pee Dee Belemnite limestone (VPDB), while for hydrogen and oxygen is the Vienna Standard Mean Ocean Water (VSMOW). The standards used were those recognized by the International Atomic Energy Agency (IAEA). The standard deviation of bulk $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ were ± 0.1, 1.0 and 0.5 mUr, respectively. Each sample was measured in duplicated (n=2) to obtain its average and standard deviation.

2.5. Statistical analysis

Multivariate data treatments were carried out with the software Statgraphics Centurion XV, using the stable isotope values of carbon, hydrogen and oxygen of EVOO samples (n= 38) as independent variables. Principal component analysis (PCA) was used for simultaneous ordination of different geographic and climatic dependent variables and the $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ values (independent variables), illustrating their mutual relationships. Multiple linear regression (MLR) was applied considering the stable isotope composition

as independent variables and the geographic and climatic factors as dependent variables. Spurious models due to overfitting were detected and discarded after repeating MLR models with fully randomized dependent variables. This work has not used training models. Because, to our knowledge, this is the first study in which the isotope compositions of the Portuguese EVOOs are determined. Therefore, no previous data were available to evaluate training models. In addition, the predictions were made directly from all the experimental data acquired in this study, validating the predicted values by means of the leave-on-out, cross-validation method.

3. Results and Discussion

3.1. Stable isotope analysis of EVOO samples

The $\delta^{13}C$ values (Figure 1A) of EVOO samples, both from Alentejo and Trás-os-Montes regions, displayed a typical behavior of plants with C_3 photosystem [16]. The ranges of $\delta^{13}C$ values were different between the two main Portuguese regions, i.e., 3.9 mUr for Alentejo (from -27.9 mUr to -31.8 mUr) and 2.4 mUr for Trás-os-Montes (from -28.1 mUr to -30.5 mUr). Nevertheless, their mean values ($\delta^{13}C_{Alentejo}$ = -29.6 ± 0.6 mUr, $\delta^{13}C_{Trás\text{-}os\text{-}Montes}$ = -29.3 ± 0.6 mUr) showed no significant differences ($P > 0.05$). Although there was not a significant difference between regions, it was observed a little ^{13}C-enrichment in Trás-os-Montes samples. This fact may be due either to the differences in the carbon isotopic composition between the subregions or to the variations in the olive varieties [17]. In fact, we can observe figure 2 (A and B) that displays the $\delta^{13}C$ values of EVOO samples both for different subregions and olive varieties. With respect to subregions, it is observed that EVOO samples cultivated in Vila Flor (Trás-os-Montes) were isotopically heavier than the rest one, while Ferreira do Alentejo (Alentejo) olive

oils showed the lowest $\delta^{13}C$ value. The $\delta^{13}C$ values of plant cultured under irrigation proceedings display a more negative value (^{13}C-depletion) than that cultivated under drought conditions [18]. This idea may be related to the isotopic value of EVOO from Ferreira do Alentejo. However, we do not have this information. Concerning the varieties, Picual, collected in Alentejo, was isotopically lighter than the rest of varieties. Cobrançosa variety presents similar isotopic C values either in Alentejo or Trás-os-Montes. Therefore, the little difference in carbon composition between these regions seems to be mainly related to the location of the olive tree cultivations and/or agricultural procedures (irrigation).

Similarly, to carbon isotope composition, the mean $\delta^{18}O$ value (Alentejo = 23.0 ± 0.7 mUr and Trás-os-Montes = 22.7 ± 0.7 mUr) showed no significant difference (P> 0.05) between the two regions studied (Fig. 1B). However, their ranges of values were different, i.e., 3.2 mUr for Alentejo (from 24.5 to 21.3 mUr) and 2.1 for Trás-os-Montes (from 23.5 to 21.4 mUr). This difference may be linked with the subregion and/or olive variety. In this case, the fact that EVOO samples cultivated in Trás-os-Montes show a $\delta^{18}O$ value slightly lighter than that in Alentejo (Fig. 1B) may be due to the fact that the EVOOs produced in Valpaços, Macedo de Cavaleiros and Mirandela (Trás-os-Montes) displayed a lighter isotopic composition of oxygen than the others (Fig. 2C). On the other hand, some olive varieties cultivated in Alentejo showed an isotopic composition heavier than that were cultivated in Trás-os-Montes (Fig. 2D and Table 1).

On the other hand, the mean $\delta^{2}H$ value of the two regions of Portugal ($\delta^{2}H_{Alentejo}$ = -137.2 ± 2.2 mUr, $\delta^{2}H_{Trás\text{-}os\text{-}Montes}$ = -149.3 ± 2.8 mUr) showed significant difference (P < 0.05), being Trás-os-Montes region more ^{2}H-depleted than Alentejo one ($\delta^{2}H_{Trás\text{-}os\text{-}Montes}$: from -152.3 to -148.4 mUr, $\delta^{2}H_{Alentejo}$: from -146.6 to -130.7 mUr). The ^{2}H composition, which reflects the type of water uptake by the plants during its growth, is highly linked

(negatively and/or positively) both to the climatic conditions of the production area, as well as to its geographical characteristics [19]. In our case, Trás-os-Montes region shows higher elevation and rainfall amount than Alentejo one (Table 1). Nevertheless, Trás-os-Montes shows low seasonal temperatures (Table 1). The combination of low rainfall amount and high temperature causes a high evapotranspiration of the plants [20, 21], which is translated in an increase in the elimination of the light hydrogen isotope. Therefore, EVOO samples from Alentejo showed a heavier hydrogen composition. This result was also observed when it is studied the δ^2H value of the different subregions (Fig. 2E, Table 1), since the EVOO samples cultivated in subregions located into Trás-os-Montes were isotopically lighter than that are cultivated in subregions of Alentejo. Concerning olive varieties, there are significant difference among varieties (Fig. 2F). However, this difference may be directly related to culture location, because all olive varieties cultivated in Trás-os-Montes regions (Cobrançosa, Madural, Negrinha do Freixo and Verdeal) showed lower ^{2}H composition than that cultured in Alentejo. For instance, the Cobrançosa variety is present in both regions; however, those cultivated in Trás-os-Montes showed a significant ^{2}H-depletion compared with those from Alentejo. This may support the idea that the culture location is directly related to the isotopic value.

3.2. Chemometric analysis

Principal components analysis (PCA) was used to identify the possible positive and inverse correlation (angle of 0º or 180º, respectively) between the stable isotope (carbon, hydrogen and oxygen) values and geographical and climatic variables (Figure 3). Up to 75% of the total variance can be explained by two first components (component 1: 55.52 % and component 2: 20.41 %). The scatterplot of the loadings of PC-1 *vs* PC-2 showed that δ^2H values were strongly positive correlated with climatic conditions, mainly temperature (0º), as well as the geographic longitude of the production area. Nevertheless,

these are inversely correlated (180º) with latitude, altitude and, rainfall. With respect to sea distance, δ^2H values showed no correlation (90º). Several researchers observed the same trend between hydrogen isotope and environmental factors. For example, Chiocchini et al., in 2016 [22] found that high altitude produced the ^{2}H-depletion of organic samples. The positive correlation with temperature has recently been observed by Jiménez-Morillo et al. [23] using pyrolysis compound-specific isotope analysis. With respect to carbon isotope, it is highly correlated with sea distance (close to 0º) of the production area. However, the correlation with its altitude and latitude was not strong (higher than 45º). and rainfall. Furthermore, δ^{13}C values were negatively correlated with longitude (180º). These correlations were also established by Camin et al. in 2010 [24]. The temperature and rainfall were not correlated (90º) with the carbon isotope composition of the EVOO samples. Oxygen isotope composition of EVOO samples was directly correlated with longitude (close to 0º) but inversely with sea distance (180º). This correlation was also observed by other researchers [21, 25, 26], who observed that oxygen was linked with longitude (UTM) and distance from the sea (km). These correlations were corroborated using multi linear regression (MLR). The MLR, exclusively using the stable isotope values (independent variables) of EVOO samples (n= 38) led to significant ($P <$ 0.05) forecasting models for all geoclimatic (dependent) variables (i.e., latitude, longitude, altitude, rainfall, temperature and sea distance) from the total analysed samples. Fig. 4 plots the observed *vs* predicted values of each models: a) latitude, b) longitude, c) altitude, d) rainfall, e) temperature, and f) sea distance. The model validation was confirmed by a strict criterion based on the comparing the MLR cross-validation tests (observed vs predicted) with the alternative model computed from the fully randomized latent variables (latitude, longitude, altitude, rainfall, temperature and sea distance) from the 38 EVOO samples studied in this work. These models displayed a poor correlation (P

> 0.05) with isotopic data (Figure S1 in supplementary information). Therefore, it is proved that a significant forecasting model is only possible with the real (experimental) values [23, 27, 28]. The MLR analysis of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ values of different EVOO samples allowed to obtain the equations 2-7 of the prediction model:

$$\underline{\textbf{Latitude}} = 23.63 + 0.36 \times \delta^{13}C - 0.17 \times \delta^{2}H \quad (2)$$

$$\underline{\textbf{Longitude}} = 1.37 - 0.24 \times \delta^{13}C + 0.02 \times \delta^{2}H + 0.07 \times \delta^{18}O \quad (3)$$

$$\underline{\textbf{Altitude}} = 423.61 + 61.70 \times \delta^{13}C - 11.96 \times \delta^{2}H \quad (4)$$

$$\underline{\textbf{Rainfall}} = -2948.33 - 26.65 \times \delta^{2}H \quad (5)$$

$$\underline{\textbf{Temperature}} = 69.42 + 0.32 \times \delta^{2}H - 0.33 \times \delta^{18}O \quad (6)$$

$$\underline{\textbf{Sea distance}} = 1335.06 + 29.78 \times \delta^{13}C - 14.18 \times \delta^{18}O \quad (7)$$

Only those independent variables whose value of P has been less than 0.05 have been taken into account for the creation of the models. Therefore, there are geoclimatic variables that displayed in their model one or two independent variables (stable isotope composition).

The existence of correlation ($P < 0.05$) between stable isotope values and dependent variables (climatic and geographical) may explain why there was a significant difference between two Portuguese regions. On the other hand, it could say that the combined use of ^{2}H, ^{13}C and ^{18}O isotopic composition gives a valuable and precise information for the assessing of geographic origin of Portuguese EVOO samples, which is a very powerful tool, which could help to fight against food-fraud suffered by EVOO sector in Portugal. As far as authors are aware, this work is the first one that use, in combination, bulk isotopic composition ($\delta^{2}H$, $\delta^{13}C$ and $\delta^{18}O$) and multivariate statistical analysis (PCA and MLR), to assess the geographic origin of Portuguese EVOOs.

279

280 **Abbreviations Used**

281 C, carbon; DA, discriminant analysis; EA, elemental analysis; EVOO, extra virgin olive
282 oil; H, hydrogen; IAEA, international atomic energy agency; IRMS, isotope ratio mass
283 spectrometry; MLR, multi linear regression; N, nitrogen; O, oxygen; PCA, principal
284 component analysis; VPDB, Vienna pee dee belemnite; VSMOW, Vienna standard
285 mean ocean water.

286

287 **Acknowledgments**

288 This work was funded by European Regional Development Fund (FEDER) and National
289 Funds through Foundation for Science and Technology (FCT) under Project "Por3O -
290 Portuguese Olive Oil Omics for traceability and authenticity -
291 PTDC/AGRPRO/2003/2014, and by National Funds through FCT - Foundation for
292 Science and Technology under the Projects UIDB/05183/2020 and UID/AGR/00690/2019.
293 Pedro N. Jiménez-Morillo is acknowledged for statistical assistance.

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Conflict of Interest

In the name of all my co-workers I declare that there is no conflict of interest.

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Highlights:

- Isotopic composition ($\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$) of Portuguese varietal olive oils
- Bulk $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ values can be used to predict geographic variables
- Extra virgin olive oils from Alentejo and Trás-os-Montes
- First report on bulk isotopic composition of Portuguese EVOOs

Figure captions

Figure 1. Boxplots of the bulk carbon ($\delta^{13}C_{bulk}$ values, A), oxygen ($\delta^{18}O_{bulk}$ values, B) and hydrogen ($\delta^{2}H_{bulk}$ values, C) isotope composition of two Portuguese region (Alentejo and Trás-os-Montes). Boxplots display the ranges, lower and upper quartiles (Q1, Q3), and the median (Q2).

Figure 2. Boxplots of the Portuguese subregions and olive varieties of bulk carbon ($\delta^{13}C_{bulk}$ values A and B, respectively), oxygen ($\delta^{18}O_{bulk}$ values C and D, respectively) and hydrogen ($\delta^{2}H_{bulk}$ values E and F, respectively). Boxplots display the ranges, lower and upper quartiles (Q1, Q3), and the median (Q2).

Figure 3. Plot of components weights for the geographical variables and the stable isotope composition of carbon, hydrogen and oxygen ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, respectively) of EVOO samples.

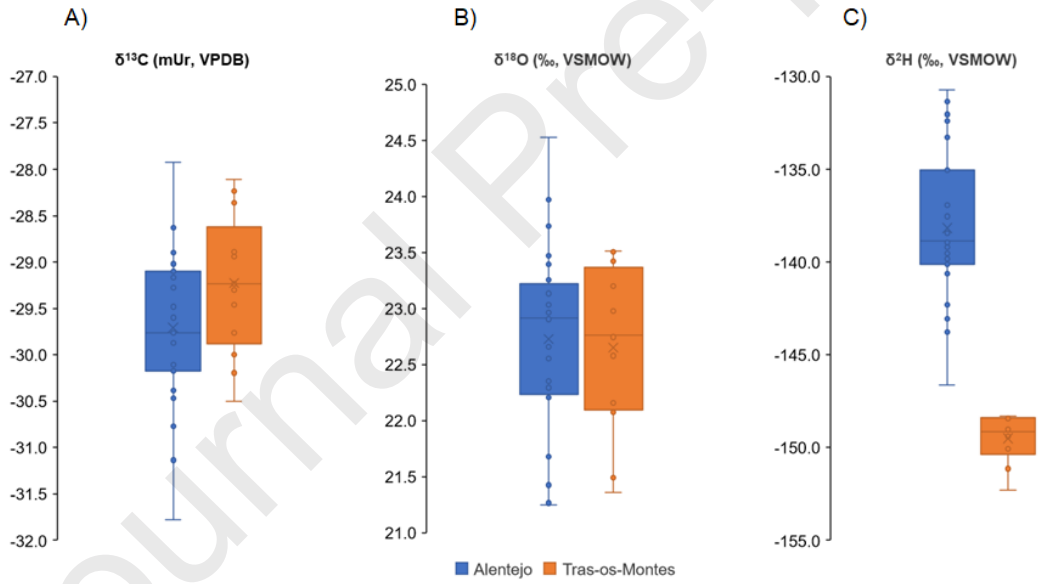
Fig 4. Observed *vs*. predicted values for geographical and climatic variables: A) latitude, B) longitude, C) altitude, D) rainfall, E) temperature, and F) Sea distance, calculated by PLS regression using stable isotope values of EVOOs as predictors.

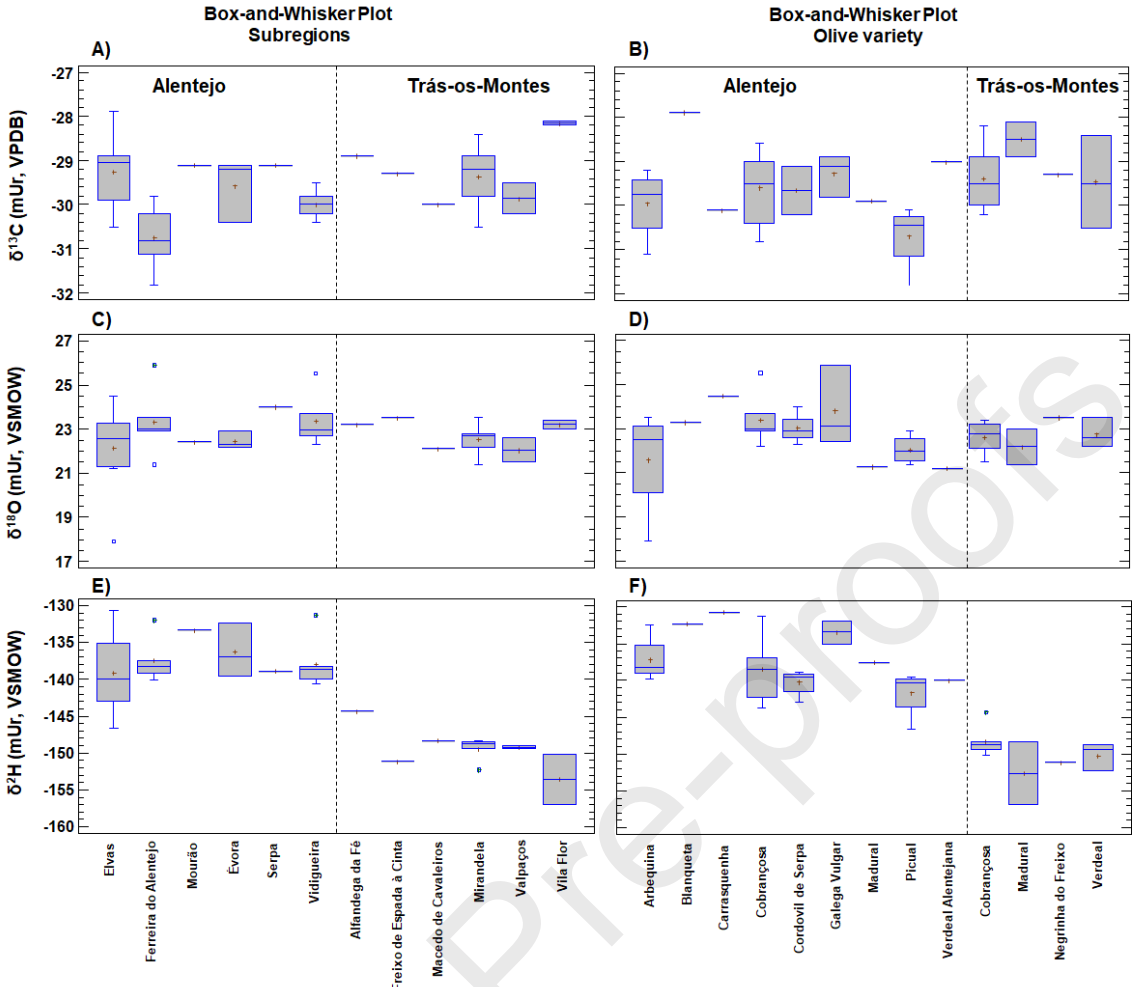
Table 1. Varieties, origin, region, geo-climatic information and stable isotope composition of EVOO samples.

Code	Varieties	Origen	Region *	Latitude (UTM)	Longitude (UTM)	Altitude (m.a.s.l)	Rainfall (mm)	Temperature (ºC)	Sea distance (km)	$\delta^{13}C$ (mUr, VPDB)	$\delta^{2}H$ (mUr, VSMOW)	$\delta^{18}O$ (mUr, VSMOW)
AArb1	Arbequina	Elvas	AL	38.88	7.16	328	679.3	18.01	197.1	-29.6	-139.8	17.9
AArb3	Arbequina	Évora	AL	38.41	7.72	190	738.1	18.17	96.27	-29.2	-132.4	22.3
AArb4	Arbequina	Vidigueira	AL	38.17	7.72	163	679.3	18.01	92.76	-29.9	-138.2	22.7
AArb5	Arbequina	Ferreira do Alentejo	AL	38.06	8.11	140	679.3	17.6	61.99	-31.1	-138.2	23.5
ABla1	Blanqueta	Elvas	AL	38.88	7.16	328	679.3	18.01	197.1	-27.9	-132.3	23.3
ACar1	Carrasquenha	Elvas	AL	38.88	7.16	328	679.3	17.6	197.1	-30.1	-130.7	24.5
ACob1	Cobrançosa	Vidigueira	AL	38.17	7.72	163	679.3	17.6	92.76	-29.8	-138.5	23.7
ACob3	Cobrançosa	Ferreira do Alentejo	AL	38.06	8.11	140	679.3	18.01	61.99	-30.8	-137.5	23.0
ACob4	Cobrançosa	Vidigueira	AL	38.17	7.72	163	738.1	18.17	92.76	-30.4	-138.9	23.0
ACob5	Cobrançosa	Elvas	AL	38.88	7.16	328	625.3	18.41	197.1	-29.0	-142.3	23.4
ACob6	Cobrançosa	Vidigueira	AL	38.17	7.72	163	738.1	18.17	92.76	-29.5	-131.3	25.5
ACob7	Cobrançosa	Évora	AL	38.41	7.72	190	679.3	17.99	96.27	-29.1	-136.9	22.9
ACob8	Cobrançosa	Elvas	AL	38.88	7.16	328	679.3	17.6	197.1	-28.6	-143.8	22.2
ACor1	Cordovil de Serpa	Elvas	AL	38.88	7.16	328	679.3	17.6	197.1	-29.1	-143.0	22.9
ACor2	Cordovil de Serpa	Vidigueira	AL	38.17	7.72	163	679.3	17.6	92.76	-30.2	-140.0	22.3
ACor3	Cordovil de Serpa	Ferreira do Alentejo	AL	38.06	8.11	140	679.3	17.6	61.99	-30.2	-139.2	22.9
ACor4	Cordovil de Serpa	Serpa	AL	37.94	7.60	218	679.3	17.6	114.17	-29.1	-138.9	24.0
AGal1	Galega Vulgar	Ferreira do Alentejo	AL	38.06	8.11	140	679.3	18.01	61.99	-29.8	-132.0	25.9
AGal2	Galega Vulgar	Mourão	AL	38.36	7.29	186	679.3	18.01	132.69	-29.1	-133.3	22.4
AGal4	Galega Vulgar	Elvas	AL	38.88	7.16	328	738.1	18.17	197.1	-28.9	-135.1	23.1
AMad1	Madural	Elvas	AL	38.88	7.16	328	679.3	17.6	197.1	-29.9	-137.5	21.3
APic 1	Picual	Elvas	AL	38.88	7.16	328	679.3	17.6	197.1	-30.5	-146.6	21.7
APic 3	Picual	Évora	AL	38.41	7.72	190	679.3	17.6	96.27	-30.4	-139.5	22.2
APic 4	Picual	Vidigueira	AL	38.17	7.72	163	679.3	18.01	92.76	-30.1	-140.6	22.9
APic 5	Picual	Ferreira do Alentejo	AL	38.06	8.11	140	679.3	18.01	61.99	-31.8	-140.1	21.4

AVer 1	Verdeal Alentejana	Elvas	AL	38.88	7.16	328	738.1	18.17	197.1	-29.0	-140.0	21.2
TM Cob 1	Cobrançosa	Macedo de Cavaleiros	TM	41.53	6.95	655	865.4	12.56	153.56	-30.0	-148.3	22.1
TM Cob 2	Cobrançosa	Mirandela	TM	41.48	7.18	254	121 2.5	12.75	134.78	-29.2	-149.3	22.8
TM Cob 3	Cobrançosa	Alfandega da Fé	TM	41.34	6.96	549	865.4	13.58	150.71	-28.9	-144.3	23.2
TM Cob 4	Cobrançosa	Mirandela	TM	41.49	7.26	359	121 2.5	12.75	127.3	-29.8	-148.4	22.7
TM Cob 6	Cobrançosa	Valpaços	TM	41.61	7.31	428	121 2.5	12.75	126.27	-30.2	-149.0	21.5
TM Cob 7	Cobrançosa	Vila Flor	TM	41.31	7.15	550	121 2.5	12.51	133.27	-28.2	-150.1	23.4
TM Mad 1	Madural	Vila Flor	TM	41.30	7.16	555	121 2.5	12.75	133.27	-28.1	-156.9	23.0
TM Mad 2	Madural	Mirandela	TM	41.49	7.18	228	121 2.5	12.75	134.78	-28.9	-148.3	21.4
TM Neg 3	Negrinha do Freixo	Freixo de Espada à Cinta	TM	41.11	6.84	605	121 2.5	12.51	153.63	-29.3	-151.1	23.5
TM Ver 1	Verdeal	Mirandela	TM	41.49	7.26	349	121 2.5	12.75	127.3	-28.4	-148.8	23.5
TM Ver 2	Verdeal	Valpaços	TM	41.61	7.30	416	865.4	13.58	126.27	-29.5	-149.4	22.6
TM Ver 3	Verdeal	Mirandela	TM	41.49	7.17	234	121 2.5	12.75	134.78	-30.5	-152.3	22.2

* AL = Alentejo; TM = Trás-os-Montes





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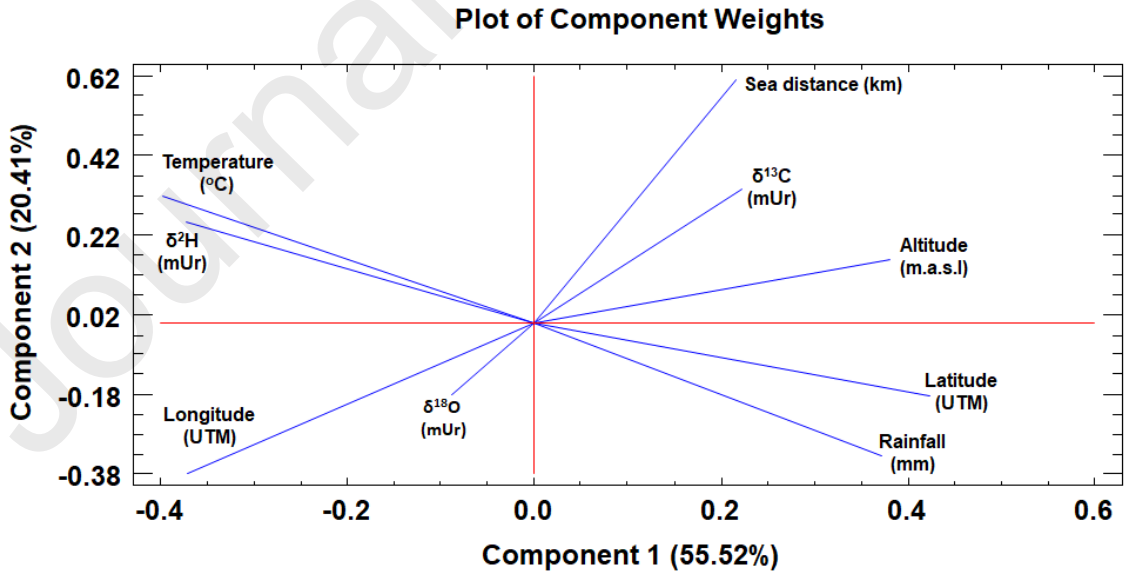

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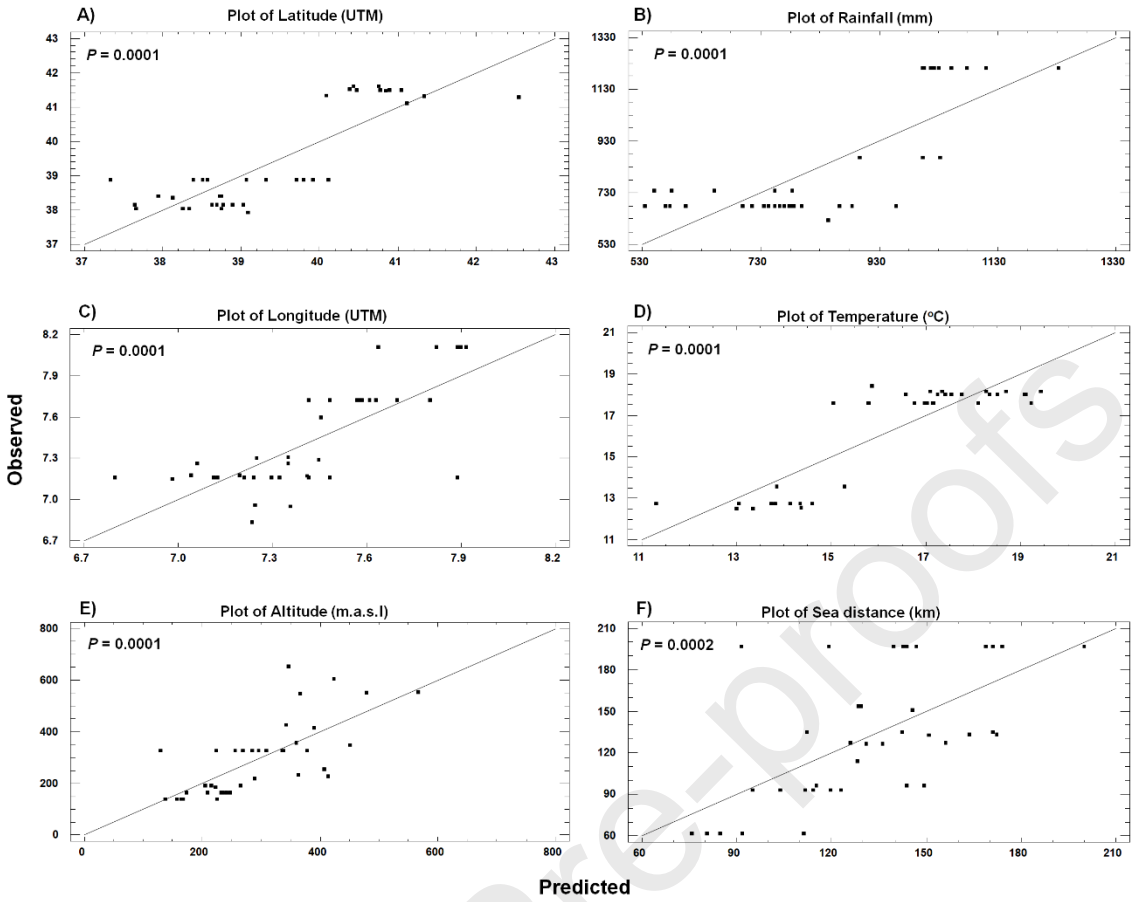

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