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Short communication

# Geographical discrimination of extra-virgin olive oils from the Italian coasts by combining stable isotope data and carotenoid content within a multivariate analysis

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ABSTRACT

We have determined the isotopic composition and the carotenoid contents of 38 extra-virgin olive oils (EVOOs) from seven regions along the Italian coasts, by means of isotope ratio mass spectrometry (IRMS) and resonant Raman spectroscopy (RRS), respectively. The application of linear discriminant analysis to our overall results demonstrated the combination of isotope and carotenoid data is a promising method to discriminate EVOOs from production sites that are impacted by similar geographical and climatic parameters. In particular, this dual approach allowed correct classification of 82% EVOO samples, while separate IRMS and RRS investigations were able to discriminate only samples from Sicily and Latium, respectively.



## 1. Introduction

Extra-Virgin Olive Oil (EVOO) is produced by physical extraction from the fruits of the *Olea europaea* tree. The geographical origin of EVOO is one of the most relevant factors that determine its quality and, thus, its commercial value, since the EVOO composition and nutraceutical values may depend on regional differences in climate, soil, agricultural practices and local cultivars. Thus, the European Union (EU) has set regulations for the protection of claims relating to geographic indications and origin designations for EVOOs (Woodcock, Downey, & O'Donnell, 2008), and products from defined high-quality oil-producing regions may use a Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) on their labels. For these reasons, food processors, retailers, enforcement agencies, and consumers require an independent mechanism to confirm that any given EVOO claiming PDO or PGI status actually complies with all relevant specifications.

The development of analytical methodologies for EVOO authentication is, thus, a challenging task, and several experimental techniques have been employed to assure olive oil traceability and authenticity. Chromatographic techniques, such as gas chromatography (Aparicio & Aparicio-Ruìz, 2000), gas chromatography mass spectrometry (Spangenberg & Ogrinc, 2001), and high performance liquid chromatography (Christopoulou, Lazaraki, Komaitis, & Kaselimis, 2004) have been employed to estimate the content of specific compounds (such as fatty acids, sterols, phenolics or hydrocarbons) as a function of olive oil geographical origin. Isotope ratios mass spectrometry (IRMS) has demonstrated that EVOOs from different production sites can be discriminated based on their stable isotopic composition (Angerosa et al., 1999; Camin et al., 2010). Nuclear magnetic resonance (Mannina, Patumi, Proietti, Bassi, & Segre, 2001), solid-phase micro-extraction gas chromatography-mass spectrometry (SPME-GC) (Young, Quinn, & Trumble, 2012), and spectroscopic techniques, such as near infrared (Casale, Casolino, Oliveri, & Forina, 2010), Fourier-transform infrared (Tapp, Defernez, & Kemsley, 2003) and Raman (Korifi, Le Dreau, Molinet, Artaud, & Dupuy, 2011) spectroscopies have allowed olive oil origin to be determined using fingerprinting approaches.

However, when a specific meteorological or geographical factor affects different geographical regions similarly, EVOOs may be difficult to distinguish if analysed using a technique that is sensitive to that parameter. In this case, an improved classification of food products can be achieved by combining the analytical data obtained using different techniques, within a multivariate approach (Casale et al., 2010; Chiavaro et al., 2011).

In particular, we have previously shown that the combination of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ data, as measured by IRMS, could provide

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information about the geographical origin of olive oil (Portarena, Gavrichkova, Lauteri, & Brugnoli, 2014; Portarena et al., 2015). However, the water cycle has a significant impact on carbon and oxygen isotope composition in plant tissues, which is mediated by the evaporative demand of the geographical origin, stomatal conductance, and photosynthetic capacity. Thus, stable isotope analysis may fail in discriminating plant materials coming from regions where the meteorological cycle of evaporation, condensation, and precipitation is impacted by similar climate parameters, as occurring along the Italian coasts (Longinelli & Selmo, 2003).

Resonant Raman spectroscopy (RRS) is able to excite resonantly the vibrational modes of carotenoid pigment molecules, allowing a comparative quantification of carotenoid content (El-Abassy, Donfack, & Materny, 2009). This may depend on climatic parameters, such as light exposition, distance from the sea, temperature, and amount of precipitations (Issaoui et al., 2010). However, the carotenoid content can be affected also by genetic factors, mainly related to the olive variety (Giuffrida, Salvo, Salvo, La Pera, & Dugo, 2007), agronomical aspects, such as nutrient availability and fruit ripening (Baccouri et al., 2008), and processing and storage conditions (Gimeno, Castellote, Lamuela, De la Torre, & Lopez-Sabater, 2002). Since all these aspects may be specific of the olive growing region, the carotenoid content could be a good candidate as EVOO geographical tracer.

In this preliminary work, we selected EVOO samples coming from PDO production areas along the Italian coasts, which is a macro-region characterized by similar climatic and geographical factors, and analysed isotopic composition and carotenoid content by means of IRMS and RRS, respectively. The geographical discrimination of our EVOO samples based on these two parameters is unsatisfactory if considered separately. However, by combining IRMS and RRS outputs within a multivariate statistical method, the analysis resulted in a prediction model that was able to classify correctly 82% of our EVOO samples.

Thus, the combination of isotope data and carotenoid content is a promising experimental approach to discriminate geographically EVOOs, even if their production sites are impacted by similar geographical and climatic parameters.

## 2. Materials and methods

### 2.1. Sampling sites

EVOO samples from 38 different sites located in seven different Italian regions (Apulia, Latium, Ligury, Molise, Sardinia, Siciliy, Tuscany) were collected during the 2011 harvest. Olives were harvested and milled by local farmers, and olive oil samples were then given to us by the Consorzio Olivicolo Italiano (UNAPROL). Sampling sites were selected along the Italian coasts, in a belt of 70 km, to reduce the so-called 'continental effect' (Angerosa et al., 1999), and they are shown in Fig. SM1 (see Supplementary material).

The olive growing areas were differentiated by latitude, longitude, altitude and climatic conditions. Geo-climatic information on the sampling sites are reported in Table SM2 (see Supplementary material). Colleagues from the Institute of Atmospheric Sciences and Climate of National Research Council (CNR-ISAC) provided temperature, total rainfall and relative humidity mean values. Latitude and longitude were taken from Google Earth (www.googleearth.com). The farmers provided data of harvest and altitude, based on their local knowledge.

### 2.2. Stable isotope analysis

Analyses of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ isotope content ratio were performed using an isotope ratio mass spectrometer (Isoprime, GV, Cheadle, UK) connected to an elemental analyser (NA1500, Carlo Erba, Milan, Italy) and to a pyrolysis system (Euro Pyr-OH, Euro Vector Instruments & Software, Milan, Italy). Isotopic compositions were scale normalized with IAEA international standards, on two contemporaneously analysed scale anchors, and are expressed as $\delta$ notation according to the expression: $\delta = (R_s - R_{std})/R_{std} \times 1000$, where $R_s$ is the isotope ratio of the sample and $R_{std}$ is the isotope ratio of the international standard. In particular NBS-22 fuel oil and IAEA-CH6 sucrose were used for scale normalization of measured $\delta^{13}C$ values to the VPDB scale; IAEA-601 and IAEA-602 were used for scale normalization of measured $\delta^{18}O$ values to the VSMOW scale.

The standard deviation of replicate measurements for each standard and sample was ±0.1‰ for $\delta^{13}C$, and ±0.3‰ for $\delta^{18}O$. Possible changes in analytical conditions within a run were corrected using NBS-22 fuel oil and IAEA-CH6 sucrose for $\delta^{13}C$ measurements and IAEA-601 (IAEA) and IAEA-CH6 sucrose ($\delta^{18}O$ = +36.4‰VSMOW) for $\delta^{18}O$. This latter reference was assigned since 2005 (Boschetti & Iacumin, 2005) and accepted in the European TRACE project (contract No. FP6-2003-FOOD-2-A 006942).

### 2.3. Raman spectroscopy analysis

Samples of selected EVOOs were dripped on to glass microscope slides and illuminated by the 514.5 nm line of an Argon laser with an excitation power of less than 5 mW on the sample. The laser was focused within the drop using a microscope equipped with a 10× objective. The scattered signal was then recorded at 180° backscattered geometry and dispersed by a single monochromator with a 1800 grooves/mm diffraction grating. The spectrometer (Super Labram, Jobin Yvon) was equipped with a liquid nitrogen cooled charge coupled device (CCD) detector with a resolution better than 5 $cm^{-1}$. Energy calibration was adjusted daily, by using a Si single crystal as a reference sample.

For each EVOO sample, RR spectra in the 900–1800 $cm^{-1}$ range were acquired from four different points on the droplet. The intensity variability among spectra from the same sample was less than 5%. Each spectrum was obtained by averaging five scans of 10 s each. The weak fluorescence background was subtracted from the spectra by an automatic procedure implemented in the LabSpec5 software (Horiba Scientific, Edison, New Jersey). The four spectra representative of each sample were averaged before the statistical analysis.

### 2.4. Statistical treatment of data

The data were statistically evaluated using Statistica v8 (StatSoft Italia srl, Padua, Italy). First, principal component analysis (PCA) was performed using as input variables the intensities of Raman bands listed in SM3 (see Supplementary material), properly normalized at the intensity of the peak located at 1440 $cm^{-1}$ (Zou et al., 2009). This allowed us to study the structure of the dataset, and to detect the most discriminating coordinates (principal components, PCs).

Linear discriminant analysis (LDA) classification model was then developed using as input variables the PCs obtained by PCA, together with the carbon and oxygen isotopic values, after suitable variable standardization. Canonical variates (CVs) were obtained from LDA as the vectors minimizing the Wilks' lambda parameter, which is calculated as the sum of squares of the distances between points belonging to the same category divided by the total sum of squares. Values of Wilks' lambda approaching zero are obtained with well resolved categories, whereas overlapped categories make Wilks' lambda to approach one.

To test the predictive discrimination power and the stability of the obtained classification model, this was further cross-validated