Food Control 110 (2020) 107023

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Pyrolysis-compound-specific hydrogen isotope analysis (δ^2H Py-CSIA) of Mediterranean olive oils

Nicasio T. Jiménez-Morillo[a,b], María João Cabrita[a,c,*], Cristina Barrocas Dias[b,d], Francisco J. González-Vila[e], José A. González-Pérez[e]

[a] ICAAM, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554, Évora, Portugal
[b] HERCULES, Universidade de Évora, Palácio do Vimioso, 7000-089, Évora, Portugal
[c] Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554, Évora, Portugal
[d] Departamento de Química, Escola de Ciências e Tecnologia, Universidade de Évora, CLAV, R. Romão Ramalho 59, 7000-671, Évora, Portugal
[e] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), MOSS Group. Reina Mercedes Av., 10, 41012, Seville, Spain

ARTICLE INFO

Keywords:
Analytical pyrolysis
Deuterium/hydrogen isotope analysis
Extra virgin olive oil
Geographical origin
Py-CSIA

ABSTRACT

The analysis of the hydrogen stable isotope composition (δ^2H) of organic compounds provides information about its geographical origin. In this work, δ^2H composition of specific compounds released by direct analytical pyrolysis (Py-CSIA) of extra virgin olive oils EVOOs was studied avoiding the use of any chemical and/or physical treatments, derivatization or previous separation steps. A collection of EVOOs from Mediterranean countries (Portugal, Spain, France, Tunisia and Turkey) was used for authentication of the olive oil samples. The δ^2H value for 9 pyrolysis compounds present in all EVOOs, ranged between −112 and −267 mUr. These compounds were selected as possible surrogate descriptors linked to the olive oil geographic origin. Principal Component Analysis showed that δ^2H was highly correlated with geographical longitude and annual temperature. Multiple Linear Regression analysis revealed that δ^2H value of pyrolysis compounds can significantly ($P < 0.05$) predicts longitude, mean annual temperature and distance to the sea. The results suggest that the methodology used has a high potential to assess EVOOs geographic origin. This is the first report that evaluates δ^2H directly from the pyrolysis products of olive oil using Py-CSIA. The approach used represents an innovative, fast, reproducible and reliable authentication technique.

1. Introduction

Olive is one of the most rewarding cultivated species in the arid and semiarid regions of the world and in particular in the Euro-Mediterranean region. The interest in the olive and its products responds not only to agronomic or economic reasons, but also to recognition of extra virgin olive oil (EVOO) superior organoleptic characteristics (pleasant, rather delicate flavor and aroma) and their potential health benefits, namely the lower incidence of several pathologies, including cardiovascular diseases and some neurological disorders, associated with its consumption (Fernández & Moreno, 1999). Global demand and consumption of EVOO have increased significantly since the 1990s (Milli, 1999). Part of that demand has been reflected in the exigency of high-quality olive oils (EVOO) and the appearance in the market of olive oils elaborated with specific characteristics, including oils of certain regions with well-known specific characteristics (protected designation of origin), or with singular olive composition (monovarietal or coupages). Owing to the high market value of EVOO, its authenticity assessment is becoming increasingly relevant and generally motivated by an increase in benefits. Therefore, it is becoming mandatory to establish EVOO authenticity and detect possible adulterations, which allows the protection of i) the producers in view of certification (ensuring quality and authenticity) and ii) the consumer (fraud detection and product safety). The EVOO authenticity depends mainly on the provenance: producers, regulators or consumers. Nevertheless, it is consensual that adulteration, geographic origin, production system and variety are the main authenticity issues associated with the EVOO, being particularly relevant the geographic origin and variety if genuineness of olive oils is intended to be protected (Council Regulations (EC) 510/2006). The European Union (EU) Member States have increased legislation to protect the rights of consumers and producers, establishing a framework for the development of geographical indications through the "protected designation of origin" (PDO), the "protected geographical indication" (PGI) and the

* Corresponding author. ICAAM, Universidade de Évora, Núcleo da Mitra, Ap. 94, 7006-554, Évora, Portugal.
E-mail address: mjbc@uevora.pt (M.J. Cabrita).

https://doi.org/10.1016/j.foodcont.2019.107023
Received 21 August 2019; Received in revised form 30 October 2019; Accepted 26 November 2019
Available online 27 November 2019
0956-7135/

"traditional specialty guaranteed" (TSG) labels (ECC, 2006). The geographical characterization of EVOO is a current problem, due to the high influence that environmental conditions and cultivate practices have on olive oil quality (Aby-Reidah, Yasin, Sevili & Montedoro, 2013). In addition, the geographic declaration of EVOO, is never controlled by physico-chemical parameters being vulnerable to fraud (Aparicio, Morales, Aparicio-Ruiz, Tena, & García-González, 2013). Therefore, the development of appropriate analytical and statistical methods is necessary to guarantee authenticity and traceability of olive oils and to prevent illicit practices in the sector.

Techniques used to determine botanical and geographical origin of EVOO include more or less conventional chromatography (HPLC, GC/MS) and spectroscopy techniques (FT-IR, Raman) (Gómez-Caravaca, Maggio, & Cerretani, 2016; Martins et al., 2020).

Nowadays, the assessment of the stable isotope composition of light elements by isotope ratio mass spectroscopy (IRMS) is a helpful approach to differentiate production areas and to authenticate the origin of fruits and vegetables (Camin et al., 2017). Single-isotope or multi-isotope ratios analysis of most abundant light elements in food ($\delta^{13}C$, $\delta^{2}H$, and $\delta^{18}O$) can provide a fingerprint that reflects both geo-climatic characteristics of the production area, as well as information about local agricultural practices. Carbon isotope composition ($\delta^{13}C$) is informative of certain growth conditions of the crop like salinity, water stress and water use efficiency (WUE) (Hartman & Danin, 2010). On the other hand, the isotopic composition of H and O are informative about the water consumption and evapotranspiration of the growing plant, which in turn are very sensitive proxies for geographic location (Brooks, Barnard, Coulombe, & McDonnell, 2010).

A more accurate approach to the usual isotope assessment in bulk samples, is the analysis of $\delta^{13}C$ in discrete oil components using a compound-specific isotope analysis (CSIA) approach i.e. fatty acids (FAs), Spangenber, 2016), sterols or aliphatic alcohols (Angerosa, Basti, & Vito, 1999), glycerol (Camin et al., 2010) or *n*-alkanes (Mihailova, Abbado, Kelly, & Pedentchouk, 2015). Nevertheless, to date, only one recent study has focused in hydrogen/deuterium ($\delta^{2}H$) isotope ratio composition of specific compounds (FAs) in olive oils (Paolini, Bontempo, & Camin, 2017). This compound specific hydrogen stable isotope analysis provides a more in-depth information on the geoclimatic characteristics and agricultural practices employed in the olive production of foodstuffs, improving geographical discrimination (traceability) (e.g. micro-climate, soil and water availability, sea influence) (Paolini et al., 2017; van Leeuwen, Prenzler, Ryan, & Camin, 2014).

Usually olive oil CSIA analysis involves a sample pretreatment, which consists of an extraction with organic solvents (harmful to the environment), purification and derivatization of specific compounds (Paolini et al., 2017). Recently, an analytical technique, which combines analytical pyrolysis and isotope ratio mass spectrometry (Py-GC-C/TC-IRMS, Py-CSIA), is being used to study foodstuffs and additives in food packaging (González-Pérez, Jiménez-Morillo, De la Rosa, Almendros & González-Vila, 2016; Llana-Ruíz et al., 2015, 2016). This is an innovative, fast, reproducible and robust method, used for the isotopic and chemical identification of organic compounds from complex matrices (De la Rosa, Jiménez-González, Jiménez-Morillo, Knicker, & Almendros, 2019; Miller et al., 2016). Furthermore, it avoids the extraction, purification and derivatization of non-volatile samples (González-Pérez et al., 2016; Miller et al., 2016). It is known that derivatization is a pretreatment commonly used for increasing the detection sensitivity of high polarity compounds (carboxyl and hydroxyl groups), such as FAs and alkylphenols, when conducting GC analysis (Igo, Honda, Lu, Kamiya, & Miyakoshi, 2015). Derivatization has also been used in combination with pyrolysis in the technique known as thermochemolysis (Challinor, 1991). In fact, in a previous study (Miller et al., 2016), we performed a derivatization step on speleothem samples from a volcanic cave in Easter Island for increasing the detection of FAs preserved in coralloid stalactites by thermally assisted hydrolysis-methylation (THM-Py-GC/MS). The authors were able to identify nine FAs derived from microorganisms and plant waxes in a speleothem layer. However, the use of a derivatization step in the Py-CSIA analysis will result in an addition of extra carbon and hydrogen to the specific compounds in olive oils, with an effect in their isotope signature, as described by Paolini et al. (2017) for conventional CSIA.

To our knowledge, less than 10 papers have been published so far on the use of Py-CSIA (e.g. González-Pérez et al., 2016; Llana-Ruíz-Cabello et al., 2015; Miller et al., 2016), due to the cost, resources (supply of gases and reference standards, etc) and expertise needed to operate the equipment. Moreover, as the isotope data obtained is complex and not always with a straight forward interpretation, the use of multivariate statistical analyses have been recently applied in several studies dealing with EVOO characterization (Gómez-Caravaca et al., 2016; Kritioti, Menexes, & Drouza, 2018). Such statistical treatments were essential for the data interpretation and in resolving isotopic markers surrogated to varietal and/or geographical origin of EVOO. In these studies, it was successfully proved that the geographical and varietal origin of EVOO samples could be related not only to the isotope composition of light bio-element, but also to the isotope composition of specific chemical compounds. However, there are restrictions in applying these methods that require the treatment of abundant data (isotope composition, classes of compounds, etc.) that are dependent on other factors related to the raw material (olive cultivar, climate, ripening and fruit quality) as well as to technological parameters (fruit harvest time and conditions, storage, transport, oil extraction process, and its preservation) (Gómez-Caravaca et al., 2016). Therefore, the identification of objective analytical parameters capable of providing useful elements for oil characterization and specifically of its geographical origin, is a complex issue.

In this study a multivariate statistical chemometrics strategy is applied to evaluate the suitability of $\delta^{2}H$ Py-CSIA for the categorization of EVOO samples produced in the Mediterranean basin, according to their geographic origin.

2. Materials and methods

2.1. Samples and experimental design

A total of fifteen EVOO (*Olea european* L.) samples produced in 5 different geographical regions from the Mediterranean basin (Portugal (Alentejo and Trás-os-Montes, AL and TM, respectively), France, Tunisia, Turkey and Spain, Fig. 1 SM and Table 1) were studied. All samples were from the , 2016 harvest. The samples were stored in dark-brown glass bottles at constant temperature of 20 °C until analysis. Each EVOO sample was geo-referenced, obtaining data of latitude (UTM), longitude (UTM), elevation (m a.s.l), distance to the sea (km), mean annual temperature (°C) and mean annual rainfall (mm) (Table 1).

2.2. Analytical pyrolysis (Py-GC/MS)

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i) attached to a gas chromatograph (Agilent 6890N). EVOO sample (10 μL) were poured on ultra-clean glass wool discs and introduced in small pyrolysis capsules (Frontier Laboratory). The capsules were introduced into a pre-heated furnace at 400 °C for 1 min and the evolved gases directly injected into the GC/MS for analysis. The GC was equipped with an ultra-inert fused silica (5% phenyl-methylpolysiloxane) capillary column HP-5ms-UI (Agilent J&W) of 30 m × 250 μm × 0.25 μm film thickness. The oven temperature was held at 50 °C for 1 min and then increased to 100 °C at 30 °C min^{-1}, from 100 °C to 300 °C at 10 °C min^{-1}, and stabilized at 300 °C for 10 min. The carrier gas was helium at a controlled flow of 1 mL min^{-1}. The detector was a mass selective detector (Agilent 5973 MSD) and mass spectra were acquired with a 70 eV ionizing energy. Compound assignment was achieved via comparison with published and stored

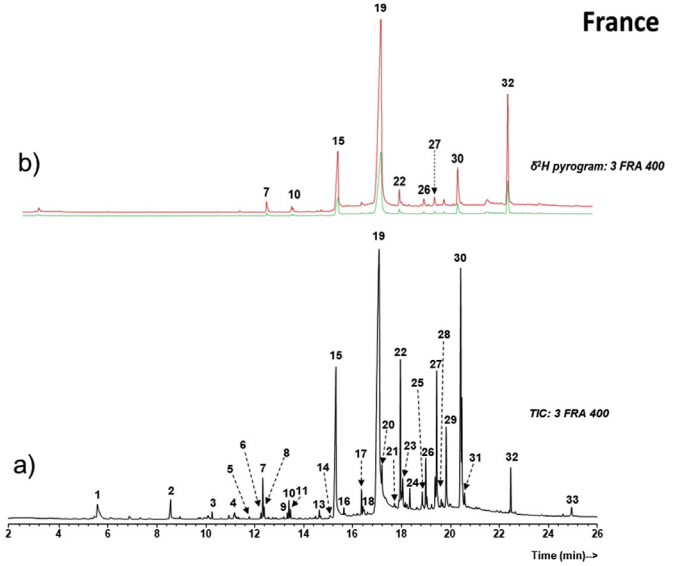

Fig. 1. a) Conventional EVOO Py-GC/MS and b) δ^2H Py-CSIA chromatogram run under the same chromatographic conditions and its correspondence to obtain both, isotopic and structural information (French EVOO as example).

(NIST and Wiley libraries) data. From the analysis of non-volatile samples, a total of 33 different compounds, without the use of derivatization, were identified, which were clustered in 11 groups: unsaturated fatty acid (UFA); unsaturated aliphatic hydrocarbon (UAH); saturated fatty acid (SFA); glycidyl ester of fatty acids (GEFA); fatty acid alkyl esters (FAAE); saturated aliphatic hydrocarbon (SAH); monoesters (MEST); aldehydes (ALD); steranes (STR); polysaccharide derived (PS) and isoprenoids (ISOP). Semi quantification analysis of the total ion chromatogram (TIC) was based on the peak area of each product released by pyrolysis, excluding minor compounds (area < 0.2% of total area). For each EVOO sample, the peak area of each identified organic compounds were calculated as percentages of the total chromatographic area.

2.3. Pyrolysis compound-specific deuterium isotope analysis (Py-CSIA)

The EVOO hydrogen isotope composition (δ^2H) of individual compounds were determined by direct Py-CSIA. This was performed using a double-shot pyrolyzer (Frontier Laboratory, model 3030D) attached to a Trace GC Ultra system (Thermo Scientific), which is connected to a GC-IsoLink II system (Thermo Scientific). This GC interface is equipped with two micro-reactors, one for thermal conversion (TC) set at 1420 °C, and another for combustion (C), set at 1020 °C. The GC system is coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific) via a ConFlo IV universal interface (Thermo Scientific). Aliquots of each EVOO sample (20 μL) were poured on ultra-clean glass wool discs and introduced into small pyrolysis capsules (Frontier Laboratory). The capsules, with the samples without derivatization, were introduced into a pre-heated furnace at 400 °C, with an interface at 250 °C, for 1 min and the evolved gases directly injected into the GC/IRMS for analysis.

Structural features of specific chromatographic compounds (peaks) were inferred by comparing and matching the mass spectra obtained by conventional Py-GC/MS with the Py-GC/TC-IRMS chromatograms obtained using the same chromatographic conditions (i.e., carrier flow, inlet temperature, column, etc).

For δ^2H measurement, each chromatographic compound passing through the pyrolysis micro-reactor, is gasified including the analyte of interest H_2 gas. Pure H_2 gas is mixed into the He carrier flow as pulses of reference gas. Before measuring the $^2H/^1H$ ratio, the $[H_3]^+$ factor is verified to be < 10 ppm/nA before every run. The variation of the $[H_3]^+$ factor during one run and between days is regularly checked to ensure a standard deviation lower than the unit for the whole measuring period.

The stable isotope abundances are reported in the delta (δ) notation (e.g. δ^2H) in variations relative to an international measurement standard. The isotope value is defined by Coplen (2011) (Eq. (1))

$$\delta^i E_{sample} = \frac{R(^iE/^jE)_{sample}}{R(^iE/^jE)_{s\ \tan dard}} - 1 \quad (1)$$

Where R is the molar ratio of the heavy (^iE) to light (^jE) most abundant isotope of chemical element E (e.g. $^2H/^1H$). The δ values are reported in "milli Urey" (mUr). The δ^2H values of EVOO samples were calibrated against saturated *n*-alkanes mixture (Fig. 2 SM) using the reference substance A7 (Biogeochemical Laboratories, Indiana University, U.S.A). The linear correlation between standard and measured (IRMS) δ^2H values from the A7 mixture (Fig. 3 SM) was used to derive sample δ^2H values relative to the Vienna Standard Mean Ocean Water (VSMOW) scale (Schimmelman et al., 2016). The repeatability and reproducibility of the method were calculated by a crossed Gage R&R study. The standard deviation of repeatability and reproducibility of δ^2H were ± 0.08 and 0.23 mUr, respectively. Background subtractions, as well as isotopic hydrogen composition are calculated using the ISODAT 3.0 software (Thermo Scientific, Bremen, Germany).

2.4. Statistical analysis

Univariate (one-way analysis of variance, one-way ANOVA) and

Table 1
Geographic and climatic information of EVOO samples.

EVOO sample	Latitude (UTM)	Longitude (UTM)	Altitude (m a.s.l)	Sea distance (km)	Annual temperature (°C)	Annual rainfall (mm)
1. Portugal (TM)	41.06	−6.83	586	154	14	865
	41.49	−7.17	234	135	13	1213
2. Portugal (AL)	38.88	−7.15	273	201	18	679
	38.17	−7.72	163	95	18	679
3. France	43.23	2.65	64	40	16	822
	43.08	2.23	180	69	15	882
	44.37	5.10	400	108	13	789
4. Tunisia	35.50	11.05	87	3	21	518
	35.04	9.48	332	131	17	377
	34.74	10.76	4	2	21	228
5. Turkey	36.78	31.45	9	3	24	807
	36.10	37.98	416	177	18	262
	36.70	37.10	709	80	17	262
6. Spain	37.34	−6.52	133	33	19	759
	37.40	−5.75	161	83	18	696

TM – Trás-os Montes; AL - Alentejo.

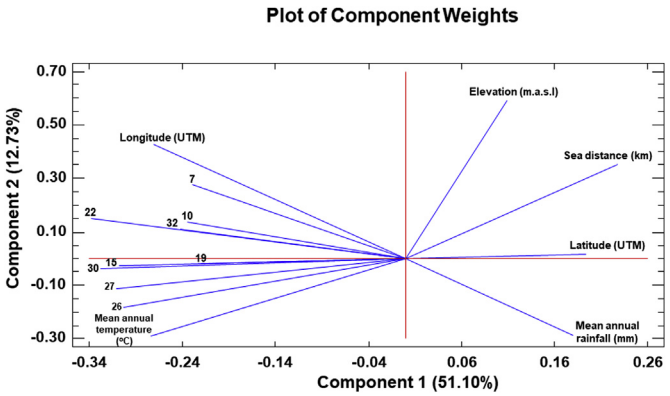

Fig. 2. Plot of components weights for the geographical variables and the δ^2H of the 9 identified pyrolysis compounds: 8-heptadecene (peak 7), 3-octadecene (peak 10), *n*-hexadecanoic acid (peak 15), *cis*-9-octadecenoic acid (peak 19), 9-tricosene (peak 22), 1-tetracosene (peak 26), oleic acid, 3-hydroxypropyl ester (peak 27), 1,13-hexacosadiene (peak 30), squalene (peak 32).

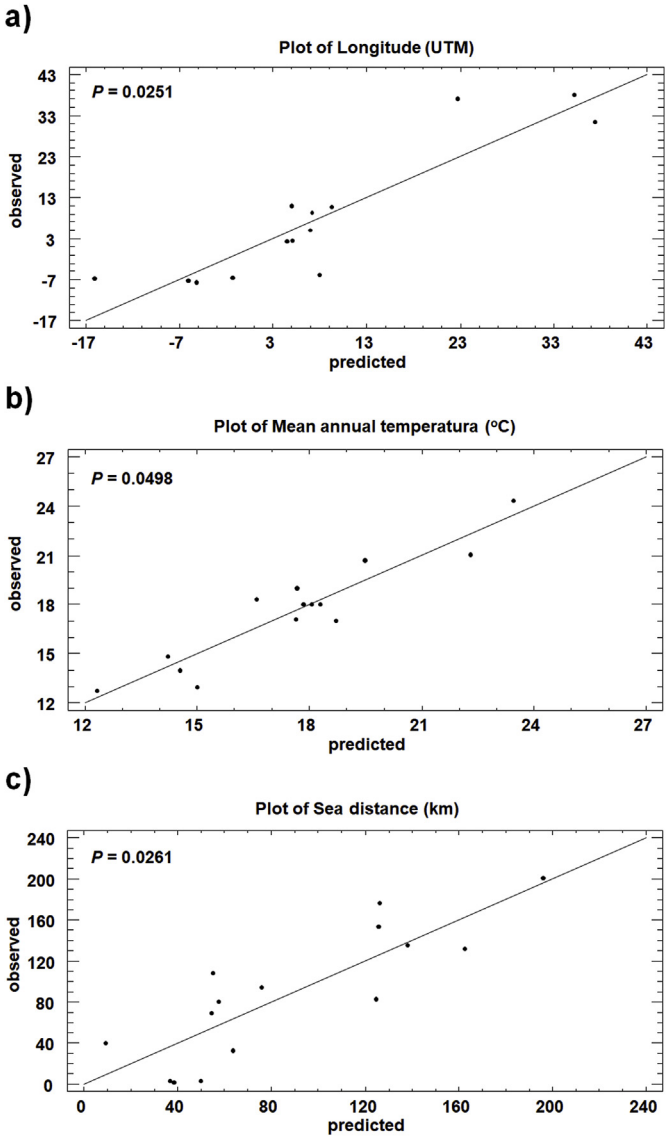

Fig. 3. Observed *vs*. predicted values for geographical variables: a) longitude, b) mean annual temperature and c) sea distance, calculated by MLR using δ^2H of identified pyrolysis compounds as predictors.

multivariate (principal component analysis, PCA and multiple linear regression, MLR) data treatments were performed using Statgraphics Centurion XV software. The δ^2H values of the 9 identified peaks from the EVOO samples—independent variables—were: 8-heptadecene (peak 7), 3-octadecene (peak 10), *n*-hexadecanoic acid (peak 15), *cis*-9-octadecenoic acid (peak 19), 9-tricosene (peak 22), 1-tetracosene (peak 26), oleic acid, 3-hydroxypropyl ester (peak 27), 1,13-hexacosadiene (peak 30), squalene (peak 32). One-way ANOVA was used to assess group differences. PCA was used for simultaneous ordination of different geographic and climatic dependent variables and the δ^2H values of identified pyrolysis compounds (independent variables), illustrating their mutual relationships. In addition, MLR was applied considering the identified pyrolysis compounds 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.

3. Results and discussion

3.1. Analytical pyrolysis (Py-GC/MS) of extra virgin olive oils

The results of the conventional analytical pyrolysis of EVOO was found well in line with what would be expected for olive oil (Boskou, Blekas, & Tsimidou, 2006) and is summarized in Fig. 1a. The peaks identification and respective compound family are reported in Table 2. The main families of compounds detected, which accounted for 91% of the total chromatogram area, were (Table 1 SM): unsaturated fatty

Table 2
List of compounds released by analytical pyrolysis. Reference numbers correspond to peak number in the pyrogram (Fig. 2).

Ref.	Rt (min)	Compound	Family[a]
1	5.64	2(3*H*)-Furanone, 5-methyl	PS
2	8.91	*n*-Decanoic acid (C10:0)	SFA
3	10.33	Pentadecane	SAH
4	11.02	*n*-Nonylcyclohexane	SAH
5	11.26	1,7-Hexadecadiene	UAH
6	12.30	6,9-Heptadecadiene	UAH
7	12.40	8-Heptadecene	UAH
8	12.60	Heptadecane	SAH
9	13.37	1,13-Octadecadiene	UAH
10	13.46	3-Octadecene	UAH
11	13.52	9-Octadecene	UAH
12	14.09	Neophytadiene	ISOP
13	14.74	8-Hexadecenal, 14-methyl	ALD
14	15.17	Palmitoleic acid (C16:1)	UFA
15	15.51	*n*-Hexadecanoic acid (C16:0)	SFA
16	15.70	9-Octadecenal	ALD
17	16.42	Hexadecanoic acid allyl ester	FAAE
18	16.49	(*Z*)-18-Octadec-9-enolide	MEST
19	17.18	*cis*-9-Octadecenoic acid (18:1 *cis*-9)	UFA
20	17.37	*n*-Octadecanoic acid (C18:0)	SFA
21	17.78	*trans*-9-Octadecenoic acid (18:1 trans-9)	UFA
22	17.99	9-Tricosene	UAH
23	18.03	1,12-Tricosadiene	UAH
24	18.41	Glycidyl palmitate	GEFA
25	18.91	1,13-Tetracosadiene	UAH
26	19.06	1-Tetracosene	UAH
27	19.50	Oleic acid, 3-hydroxypropyl ester	FAAE
28	19.69	1,13-Pentacosadiene	UAH
29	19.89	Glycidyl oleate	GEFA
30	20.52	1,13-Hexacosadiene	UAH
31	20.60	1-Hexacosene	UAH
32	22.51	Squalene	UAH
33	25.01	Stigmastan-3,5-diene	STR

[a] Polysaccharide derived (PS); saturated fatty acid (SFA); saturated aliphatic hydrocarbon (SAH); unsaturated aliphatic hydrocarbon (UAH); isoprenoids (ISOP); aldehydes (ALD); unsaturated fatty acid (UFA); fatty acid alkyl esters (FAAE); monoesters (MEST); glycidyl ester of fatty acids (GEFA) and steranes (STR).

acids (UFA), mainly oleic acid (*cis*-9-octadecenoic acid over 30%), unsaturated aliphatic hydrocarbons (UAH), incl. Squalene and saturated fatty acid (SFA), mainly palmitic acid (*n*-hexadecanoic acid 12.1 ± 3.8%). Among minor compounds, that accounted for c. 9% of total chromatogram, were polysaccharide derived compounds (PS), glycidyl ester of FAs (GEFA incl. palmitate and mainly oleate); fatty acid alkyl esters (FAAE); steranes (STR mainly stigmasta-3,5-diene); aldehydes (ALD); saturated aliphatic hydrocarbon (SAH), isoprenoids (ISOP, incl. phytol) and one monoester. These results were in agreement with the Codex Alimentarius Commision (REP15/CAC, 2015). The dominant compounds detected in all chromatogram profiles were *cis*-9-octadecenoic acid (C18:1) and *n*-hexadecanoic acid (C16:0) (UFA and SFA, respectively). Nevertheless, there were small differences among regions. It is known that fatty acid composition may differ among samples, depending on the zone of production (Boskou et al., 2006). For example, Spanish EVOO showed a high percentage of *cis*-9-octadecenoic acid (38.83%) but the lowest percentage of *n*-hexadecanoic acid (11.38%). This result was in line with the results reported in Tsimidou, Macrae, and Wilson (1987), who observed the same tendency for the studied EVOO produced in Spain, while the ones from Tunisia showed reverse results. Here, Tunisian EVOO did not follow that trend, showing the highest percentage of *cis*-9-octadecenoic acid. In particular, it is known that fatty acid content in vegetable oils differ due to several factors such as botanical origin (Ollivier, Artaud, Pinatel, Durbec, & Guerere, 2006), pedoclimatic conditions, soil characteristics, olive maturity (Guitérrez, Jiménez, Ruiz & Albi, 1999), altitude, etc. (Karabagias et al., 2013). One example was the difference between the two regions in Portugal (TM and AL). The TM EVOO sample showed the lowest percentage of *cis*-9-octadecenoic acid (24.94%), while, its percentage of *n*-hexadecanoic acid was higher than that in the AL sample (14.98 and 12.94%, respectively). Cetinkaya Kulak, Ozkan, Celik and Sekeroglu (2017) indicate that there is a moderate positive correlation between altitude and the percentage of *n*-hexadecanoic acid. Therefore, the high percentage of *n*-hexadecanoic acid in TM Portugal region may be related to the higher altitude of production of the oil. However, the rest of EVOO samples did not show this trend, meaning that other factors may be involved. Concerning to *cis*-9-octadecenoic acid content, the results showed that there is a gradual decline from high to low altitude. This result was in line with Ouni, Taamalli, Guerfel, Abdelly, Zarroul and Flamini (2012) findings. However, even though there were compositional differences between EVOO samples, these were in general small and the study of fatty acid composition obtained by Py-GC/MS was not able to give a clear differentiation among the geographic origin of the oils.

3.2. Pyrolysis-compound-specific hydrogen isotope analysis (δ2H Py-CSIA) of extra virgin olive oils

The pyrochromatograms of the compound-specific isotope (δ^2H) analysis (Py-CSIA) obtained could be matched with the Py-GC/MS chromatograms when using the same column type and chromatographic conditions (Fig. 1b and 4 SM). It was possible to assess δ^2H composition for 9 major pyrolysis compounds that varied between −112 and −267 mUr (Table 3). Hydrogen isotope composition of plant lipids are known to be directly related to the water (meteoric) uptake by plant, as well as to evapotranspiration process (Sachse et al., 2012; and references therein). Therefore, geographic and climatic factors in the area of production are known to be closely linked with δ^2H values (Clark & Fritz, 1997). In our case, Portugal EVOO samples (TM and AL) showed the lowest average δ^2H value (−177 mUr), while Turkish EVOO sample displays the highest one (−148 mUr). The lowest average ^{2}H composition of Portugal EVOOs may be related to local geoclimatic conditions. It is known that there is a negative correlation between δ^2H value of water uptake by plant and altitude, precipitation, temperature and oceanic distance (Fry, 2006). The geoclimatic factors that were correlated (positively and negatively) deuterium/hydrogen

Table 3
δ^2H values (mUr, VSMOW) of specific compounds of EVOO samples released by analytical pyrolysis. Maximum, minimum and mean δ^2H value for each geographical region, the average of fatty acids (FA) and α-alkene/di-alkene (Alk) δ^2H value. Average (± standard deviation) δ^2H value of specific compounds, with one-way ANOVA.

Compounds	Peak	Rt (s)[a]	Portugal (TM)		Portugal (AL)		France		Tunisia		Turkey		Spain		Average
			δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H	δ^2H (± SD) [b]
8-Heptadecene	7	807.6	−232.3	−168.4	−255.4	−174.4	−174.3	−167.9	−172.5	−174.1	−180.5	−162.0	−140.5	−148.5	−147.8	−220.4	−172.9	−178.3 ± 28.7 ab
3-Octadecene	10	870.1	−233.8	−174.0	−267.0	−171.7	−158.4	−174.8	−183.8	−170.0	−210.1	−169.9	−148.0	−180.7	−160.5	−222.1	−193.0	−187.2 ± 27.8 a
n-Hexadecanoic acid (C16:0)	15	984.8	−162.2	−162.2	−155.2	−162.3	−138.9	−158.4	−162.8	−146.5	−167.2	−154.9	−148.1	−150.4	−153.8	−165.6	−154.2	−156.2 ± 6.9 de
Oleic acid (C18:1)	19	1092.2	−138.0	−141.3	−142.0	−149.5	−112.4	−135.0	−143.1	−126.2	−141.1	−140.1	−135.6	−132.8	−136.8	−139.4	−133.4	−136.6 ± 7.3 f
9-Tricosene	22	1138.2	−169.7	−168.7	−164.0	−157.6	−119.4	−141.4	−152.8	−135.5	−160.7	−143.1	−133.8	−132.9	−131.0	−157.9	−141.1	−147.1 ± 14.2 e
1-Tetracosene	26	1199.0	−184.3	−199.1	−164.6	−177.3	n.d.	−162.4	−177.5	−133.1	−212.0	−164.0	−145.4	−157.0	−155.3	−161.7	−171.1	−168.9 ± 18.2 bc
Oleic acid, 3-OH-propyl ester	27	1225.6	−176.1	−192.1	−163.7	−172.4	−140.9	−153.0	−168.2	−138.6	−205.4	−158.7	−132.7	−153.3	−142.8	−158.1	−165.8	−161.4 ± 17.8 cd
1,13-Hexacosadiene	30	1283.3	−182.6	−191.0	−172.1	−167.7	−146.5	−163.6	−160.6	−151.9	−191.1	−145.2	−138.5	−155.4	−151.5	−172.0	−167.0	−163.2 ± 14.7 cd
Squalene	32	1408.2	−176.9	−168.9	−166.0	−162.5	−157.4	−168.1	−175.2	−164.8	−172.3	−175.3	−153.0	−161.5	−156.5	−167.6	−169.4	−166.6 ± 6.6 c
		Max	−138.3	−141.3	−142.0	−149.5	−112.4	−135.0	−143.1	−126.2	−141.1	−140.1	−132.7	−132.8	−131.0	−139.4	−133.4	
		Min	−233.8	−199.1	−267.0	−177.3	−174.3	−174.8	−183.8	−174.1	−212.0	−175.3	−153.0	−180.7	−160.5	−222.1	−193.0	
		Mean	−184.0	−174.0	−183.3	−166.2	−143.5	−158.3	−166.3	−149.0	−182.2	−157.0	−141.7	−152.5	−148.4	−173.9	−163.1	
		FA	−158.8	−165.2	−153.6	−161.4	−130.7	−148.8	−158.0	−137.1	−171.2	−151.2	−138.8	−145.5	−144.5	−154.4	−151.1	
		Alk	−196.6	−178.3	−198.2	−168.5	−151.2	−163.0	−170.4	−154.9	−187.8	−159.9	−143.2	−156.0	−150.4	−183.6	−169.1	

[a] Rt: retention time. TM – Trás-os-Montes; AL - Alentejo.
[b] One-way ANOVA. The different letters indicate significant ($P < 0.05$) differences between compounds according to the Tukey test.

isotope composition of EVOO samples will be discussed in the next section (section 3.3). Concerning the conspicuous isotopic difference found among the 9 major pyrolysis compounds, this may be attributed to the different biosynthetic pathways of compounds and the respective isotopic fractionation during synthesis. The average ^2H composition of FAs was higher than that of α-alkene/di-alkene (Alk) compounds (Table 3). Estep and Hoering (1980) found that fatty acid compounds are ^2H-enriched compared with hydrocarbons (*n*-alkanes/α-alkenes). The same trend was observed here. The differences of hydrogen isotope between families may be due to changes in the isotopic composition of starting material (biosynthetic precursors) or isotope effects (including exchanges of organic H with H_2O) related to biosynthetic reactions (Martin, Zhang, Naulet, & Martin, 1986) or to the isotopic composition of hydrogen added (mainly NADPH) during biosynthesis (Luo, Sternberg, Suda, Kumazawa, & Mitsui, 1991), or both (Sessions, Burgoyne, Schimmelmann & Hayes, 1986). The biosynthesis of alkane compounds is based on the reduction of long chain FAs to aldehydes, with the intervention of Acyl-CoA reductase enzyme and NADPH, and the subsequent aldehyde decarbonylation by aldehyde decarbonylase enzyme, producing carbon monoxide (Samuels, Kunst, & Jetter, 2008). On the other hand, Sessions et al. (1999) observed that in *Daucus*, there was a light trend toward ^2H-enrichment with long-chain FAs. The same pattern was observed here for all of EVOO samples ($\Delta^2H_{\text{Oleic acid vs palmitic acid}} \approx 20$ mUr). Concerning the sterane, its δ^2H value cannot be compared with that of the other compounds (FA and Alk) because its biosynthetic pathway is completely different. Sterane compounds are originated from the mevalonic acid, while the others are acetogenins and are based on the polymerization of acetyl CoA (Nes, 2011). Significant ($P = 0.0001$) differences in δ^2H values for specific compounds among EVOO samples were observed (Table 3). Therefore, specific compounds could be used as discriminant factors (independent variables) for different geographic origins of olive oil.

3.3. Statistical analysis

Traditionally, it has been proven that *n*-hexadecanoic and *cis*-9-octadecenoic acids (peaks 15 and 19, respectively) have good discriminant value to classify EVOO samples (D'Imperio, Dugo, Alfa & Mannina, 2007). Therefore, these together with the other identified compounds: 8-heptadecene (peak 7), 3-octadecene (peak 10), 9-tricosene (peak 22), 1-tetracosene (peak 26), oleic acid, 3-hydroxypropyl ester (peak 27), 1,13-hexacosadiene (peak 30), squalene (peak 32), were chosen here as possible markers surrogated to olive oil geographic origin.

Principal components analysis (PCA) was used to easily identify associations between the δ^2H value of specific compounds and geographical and climatic variables (Fig. 2). Up to 63% of the total variance can be explained with the two first components (component 1: 51.10% and component 2: 12.73%). The scatterplot of the loadings of PC-1 *vs* PC-2 showed that δ^2H values of the organic compounds were highly correlated with the geographic longitude of the production area, as well as its mean annual temperature. Nonetheless, these are also inversely correlated with elevation, latitude, mean annual precipitation and sea distance. Several researchers have observed the same trend between hydrogen isotope composition of plants and the environmental factors where these thrive. For example, Chiocchini, Portanera, Ciolfi, Brugnoli and Lauteri (2016) found that high altitude and sea distance produced a ^2H-depletion of organic samples. These correlations were corroborated using MLR. The MLR, exclusively using the δ^2H value of organic compounds as independent variables, has obtained significant ($P < 0.05$) forecasting models for longitude, mean annual temperature and sea distance. Plots of observed *vs* predicted values of each models: a) longitude, b) mean annual temperature, and c) sea distance, are depicted in Fig. 3. The model validation was confirmed by comparison with an alternative model computed from the fully randomized dependent variable, having not correlation ($P > 0.05$) with isotopic data (data not shown). The MLR analysis of δ^2H values of organic compounds released by analytical pyrolysis allowed to obtain the following equations of the prediction model:

$$\underline{\text{Longitude}} = 311.86 + 1.15 \times \delta^2H_{\text{8-Heptadecene}} - 0.95 \times \delta^2H_{\text{3-Octadecene}} - 1.10 \times \delta^2H_{\text{Palmitic a.}} + 1.60 \times \delta^2H_{\text{Oleic a.}} - 1.09 \times \delta^2H_{\text{9-Tricosene}} + 1.17 \times \delta^2H_{\text{1,13-Hexacosadiene}} + 1.20 \times \delta^2H_{\text{Squalene}} \quad (2)$$

$$\underline{\text{Mean annual temperature}} = 192.48–0.25 \times \delta^2H_{\text{8-Heptadecene}} + 0.14 \times \delta^2H_{\text{3-Octadecene}} - 0.41 \times \delta^2H_{\text{Palmitic a.}} + 0.82 \times \delta^2H_{\text{Oleic a.}} + 0.05 \times \delta^2H_{\text{1-Tetracosene}} - 0.61 \times \delta^2H_{\text{Oleic acid, 3-hydroxypropyl ester}} + 0.72 \times \delta^2H_{\text{1,13-Hexacosadiene}} + 0.69 \times \delta^2H_{\text{Squalene}} \quad (3)$$

$$\underline{\text{Sea distance}} = 76.18 + 1.72 \times \delta^2H_{\text{8-Heptadecene}} - 2.31 \times \delta^2H_{\text{3-Octadecene}} + 7.57 \times \delta^2H_{\text{Palmitic a.}} - 3.85 \times \delta^2H_{\text{Oleic a.}} - 3.32 \times \delta^2H_{\text{1,13-Hexacosadiene}} \quad (4)$$

The existence of correlation ($P < 0.05$) between δ^2H values and geoclimatic variables (longitude, mean annual temperature and sea distance) may explain why Portuguese EVOO samples (TM and AL) are isotopically lighter than the other EVOOs.

4. Conclusions

To the best of our knowledge, this is the first report that records and evaluates hydrogen stable isotope composition directly from the pyrolysis products of olive oil using Py-CSIA (Py-GC-TC/IRMS). The results suggested that δ^2H Py-CSIA is powerful and have high potential to assess geographic origin of EVOOs. More work is underway for tuning and validation of the technique that include EVOO from many other locations from the Mediterranean Basin.

Declaration of competing interest

All authors have participated in this research, either by conception and design, or analysis and interpretation of the data; drafting the article or revising it critically for important intellectual content; and approval of the final version.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript. Therefore, there is none declarations of interest.

Acknowledgments

The authors thanks to Project "MedOOmics – Mediterranean Extra Virgin Olive Oil Omics: profiling and fingerprinting- Arimnet2/0001/2015" funding by National Funds through Fundação para a Ciência e a Tecnologia (FCT); Project "Por3O - Portuguese Olive Oil Omics for traceability and authenticity - PTDC/AGRPRO/2003/2014, funding by European Regional Development Fund (FEDER) and National Funds through Fundação para a Ciência e a Tecnologia (FCT) and INTERCARBON (CGL2016-78937-R) project. We thank Dra. M.V. Ruíz-Méndez (IG-CSIC) and Basilippo enterprise for providing Spanish EVOO samples. V. Palma, D. Monís and A. Carmona are acknowledged for technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2019.107023.

References

Abu-Reidah, I. M., Yasin, M., Urbani, S., Sevili, M., & Montedoro, G. (2013). Study and characterization of Palestinian monovarietal Nabali virgine olive oils from northern west Bank of Palestine. *Food Research International, 54*, 1959–1964. https://doi.org/10.1016/j.foodres.2013.09.004.

Angerosa, F., Basti, C., & Vito, R. (1999). Virgin olive oil volatile compounds from

lipoxygenase pathway and characterization of some Italian cultivars. *Journal of Agricultural and Food Chemistry, 47*, 836–839. https://doi.org/10.1021/jf980911g.
Aparicio, R., Morales, M. T., Aparicio-Ruiz, R., Tena, N., & García-González, D. L. (2013). Authenticity of olive oil: Mapping and comparing official methods and promising alternatives. *Food Research International, 54*, 2025–2038. https://doi.org/10.1016/j.foodres.2013.07.039.
Boskou, D., Blekas, G., & Tsimidou, M. (2006). Olive oil composition. In D. Boskou (Ed.). *Olive oil* (pp. 41–72). New York: AOCS Publishing.
Brooks, R. J., Barnard, H. R., Coulombe, R., & McDonnell, J. J. (2010). Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature Geoscience, 3*, 100–104. https://doi.org/10.1038/ngeo722.
Camin, F., Larcher, R., Perini, M., Bontempo, L., Bertoldi, D., Gagliano, G., et al. (2010). Characterisation of authentic Italian extra-virgin olive oils by stable isotope ratios of C, O and H and mineral composition. *Food Chemistry, 118*, 901–909. https://doi.org/10.1016/j.foodchem.2008.04.059.
Camin, F., Boner, M., Bontempo, L., Fauhl-Hassek, C., Kelly, S. D., Riedl, J., et al. (2017). Stable isotope techniques for verifying the declared geographical origin of food in legal cases. *Trends in Food Science & Technology, 61*, 176–187. https://doi.org/10.1016/j.tifs.2016.12.007.
Cetinkaya, H., Kulak, M., Ozkan, A., Celik, M. A., & Sekeroglu, N. (2017). Influence of geographical factors on the fatty acid profile and oil yield of *Olea europaea* L. *Scientific Papers. Series A. Agronomy, 40*, 468–474.
Challinor, J. M. (1991). The scope of pyrolysis methylation reactions. *Journal of Analytical and Applied Pyrolysis, 20*, 15–24. https://doi.org/10.1016/0165-2370(91)80059-H.
Chiocchini, F., Portarena, S., Ciolfi, M., Brugnoli, E., & Lauteri, M. (2016). Isoscapes of carbon and oxygen stable isotope compositions in tracing authenticity and geographical origin of Italian extra-virgin olive oils. *Food Chemistry, 202*, 291–301. https://doi.org/10.1016/j.foodchem.2016.01.146.
Clark, I., & Fritz, P. (1997). *Environmental isotopes in hydrogeology*. Boca Raton, New York: Lewis Publishers.
Codex Alimentarius Commission (2015). REP15/CAP. In J. Fao, W. H. O. Food, S. Programme, C. Alimentarius, & C. T. Session (Eds.). *No Title, (July)*.
Coplen, T. B. (2011). Guidelines and recommended terms of expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry, 25*, 2538–2560. https://doi.org/10.1002/rcm.5129.
Council Regulation (EC) (2006). No 510/2006 of 20 March 2006 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Orkesterjournalen L, 93*(31.3), 12–25.
De la Rosa, J. M., Jiménez-González, M. A., Jiménez-Morillo, N. T., Knicker, H., & Almendros, G. (2019). Quantitative forecasting black (pyrogenic) carbon in soils by chemometric analysis of infrared spectra. *Journal of Environmental Management, 251*, 109567. https://doi.org/10.1016/j.jenvman.2019.109567.
D'Imperio, M., Dugo, G., Alfa, M., Mannina, L., & Segre, A. L. (2007). Statistical analysis on Sicilian olive oils. *Food Chemistry, 102*, 956–965. https://doi.org/10.1016/j.foodchem.2006.03.003.
Estep, M. F., & Hoering, T. C. (1980). Biogeochemistry of the stable hydrogen isotopes. *Geochimica et Cosmochimica Acta, 44*, 1197–1206. https://doi.org/10.1016/0016-7037(80)90073-3.
Fernández, J. E., & Moreno, F. (1999). Water use by olive tree. *Journal of Crop Production, 2*, 101–162. https://doi.org/10.1300/J144v02n02_05.
Fry, B. (2006). *Stable isotope ecology* (1st ed.). New York: Springer-Verlag.
Gómez-Caravaca, A. M., Maggio, R. M., & Cerretani, L. (2016). Chemometric applications to assess quality and critical parameters of virgin and extra-virgin olive oil. A review. *Analytica Chimica Acta, 913*, 1–21. https://doi.org/10.1016/j.aca.2016.01.025.
González-Pérez, J. A., Jiménez-Morillo, N. T., De la Rosa, J. M., Almendros, G., & González-Vila, F. J. (2016). Compound-specific stable carbon isotopic signature of carbohydrate pyrolysis products from C3 and C4 plants. *Journal of the Science of Food and Agriculture, 96*, 948–953. https://doi.org/10.1002/jsfa.7169.
Gutiérrez, F., Jiménez, B., Ruiz, A., & Albi, M. A. (1999). Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry, 47*, 121–127. https://doi.org/10.1021/jf980684i.
Hartman, G., & Danin, A. (2010). Isotopic values of plants in relation to water availability in the Eastern Mediterranean region. *Oecologia, 162*, 837–852. https://doi.org/10.1007/s00442-009-1514-7.
Igo, S., Honda, T., Lu, R., Kamiya, Y., & Miyakoshi, T. (2015). Application of derivatization pyrolysis gas chromatography/mass spectrometry to analysis of archaeological lacquerwares. *Journal of Analytical and Applied Pyrolysis, 114*, 302–307. https://doi.org/10.1016/j.jaap.2015.04.023.
Karabagias, I., Michos, C., Badeka, A., Kontakos, S., Stratis, I., & Kontominas, M. G. (2013). Classification of Western Greek virgin olive oils according to geographical origin based on chromatographic, spectroscopic, conventional and chemometric analyses. *Food Research International, 54*, 1950–1958. https://doi.org/10.1016/j.foodres.2013.09.023.
Kritioti, A., Menexes, G., & Drouza, C. (2018). Chemometric characterization of virgin olive oils of the two major Cypriot cultivars based on their fatty acid composition. *Food Research International, 103*, 426–437. https://doi.org/10.1016/j.foodres.2017.10.064.
Llana-Ruíz-Cabello, M., Pichardo, S., Jiménez-Morillo, N. T., Abad, P., Guillamón, E., González-Vila, F. J., et al. (2015). Characterisation of a bio-based packaging containing a natural additive from *Allium* spp. using analytical pyrolysis and carbon stable isotopes. *Journal of Analytical and Applied Pyrolysis, 120*, 334–340. https://doi.org/10.1016/j.jaap.2016.05.021.
Llana-Ruíz-Cabello, M., Pichardo, S., Jiménez-Morillo, N. T., Bermúdez, J. M., Aucejo, S., González-Vila, F. J., et al. (2016). Molecular characterisation of a bio-based active packaging containing *Origanum vulgare* L. essential oil using pyrolysis gas chromatography–mass spectrometry. *Journal of the Science of Food and Agriculture, 96*, 3207–3212. https://doi.org/10.1002/jsfa.7502.
Luo, Y.-H., Sternberg, L. dS. L., Suda, S., Kumazawa, S., & Mitsui, A. (1991). Extremely low D/H ratios of photoproduced hydrogen by cyanobacteria. *Plant and Cell Physiology, 32*, 897–900. https://doi.org/10.1093/oxfordjournals.pcp.a078158.
Martin, G. J., Zhang, B. L., Naulet, N., & Martin, M. L. (1986). Deuterium transfer in the bioconversion of glucose to ethanol studied by specific isotope labeling at the natural abundance level. *Journal of the American Chemical Society, 108*, 5116–5122. https://doi.org/10.1021/ja00277a013.
Martins, N., Jiménez-Morillo, N. T., Freitas, F., Garcia, R., Gomes da Silva, M., & Cabrita, M. J. (2020). Revisiting 3D van Krevelen diagrams as a tool for the visualization of volatile profile of varietal olive oils from Alentejo region, Portugal. *Talanta, 207*, 120276. https://doi.org/10.1016/j.talanta.2019.120276.
Mihailova, A., Abbado, D., Kelly, S. D., & Pedentchouk, N. (2015). The impact of environmental factors on molecular and stable isotope compositions of n-alkanes in Mediterranean extra virgin olive oils. *Food Chemistry, 15*, 114–121. https://doi.org/10.1016/j.foodchem.2014.10.003.
Mili, S. (1999). The olive oil sector: International challenges and future scenarios. *Olivae, 75*, 8–16.
Miller, A. Z., De la Rosa, J. M., Jiménez-Morillo, N. T., Pereira, M. F. C., González-Pérez, J. A., Calaforra, J. M., et al. (2016). Analytical pyrolysis and stable isotope analyses reveal past environmental changes in coralloid speleothems from Easter Island (Chile). *Journal of Chromatography A, 1461*, 144–152. https://doi.org/10.1016/j.chroma.2016.07.038.
Nes, W. D. (2011). Biosynthesis of cholesterol and other sterols. *Chemical Reviews, 111*, 6423–6451. https://doi.org/10.1021/cr200021m.
Ollivier, D., Artaud, J., Pinatel, C., Durbec, J. P., & Guerere, M. (2006). Differentiation of French virgin olive oil RDOs by sensory characteristics, fatty acid and triacylglycerol compositions and chemometrics. *Food Chemistry, 97*, 382–393. https://doi.org/10.1016/j.foodchem.2005.04.024.
Ouni, Y., Taamalli, A., Guerfel, M., Abdelly, C., Zarrouk, M., & Flamini, G. (2012). The phenolic compounds and compositional quality of Chétoui virgin olive oil: Effect of altitude. *African Journal of Biotechnology, 11*, 11842–11850. https://doi.org/10.5897/AJB11.3033.
Paolini, M., Bontempo, L., & Camin, F. (2017). Compound-specific δ13C and δ2H analysis of olive oil fatty acids. *Talanta, 174*, 38–43. https://doi.org/10.1016/j.talanta.2017.05.080.
Sachse, D., Billault, I., Bowen, G. J., Chikaraishi, Y., Dawson, T. E., et al. (2012). Molecular paleohydrology: Interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annual Review of Earth and Planetary Sciences, 40*, 221–249. https://doi.org/10.1146/annurev-earth-042711-105535.
Samuels, L., Kunst, L., & Jetter, R. (2008). Sealing plant surfaces: Cuticular wax formation by epidermal cells. *Annual Review of Plant Biology, 59*, 683–707. https://doi.org/10.1146/annurev.arplant.59.103006.093219.
Schimmelman, A., Qi, H., Coplen, T. B., Brand, W. A., Fong, J., Meier-Augenstein, W., et al. (2016). Organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: Caffeines, n-alkanes, fatty acid methyl esters, glycines, L-valines, polyethylenes, and oils. *Analytical Chemistry, 88*, 4294–4302. https://doi.org/10.1021/acs.analchem.5b04392.
Sessions, A. L., Burgoyne, T. W., Schimmelmann, A., & Hayes, J. M. (1999). Fractionation of hydrogen isotopes in lipid biosynthesis. *Organic Geochemistry, 30*, 1193–1200. https://doi.org/10.1016/S0146-6380(99)00094-7.
Spangenber, J. E. (2016). Bulk C, H, O, and fatty acid C stable isotope analyses for purity assessment of vegetable oils from the southern and northern hemispheres. *Rapid Communications in Mass Spectrometry, 30*, 2447–2461. https://doi.org/10.1002/rcm.7734.
Tsimidou, M., Macrae, R., & Wilson, I. (1987). Authentication of virgin olive oils using principal component analysis of triglyceride and fatty acid profiles: Part 1—classification of Greek olive oils. *Food Chemistry, 25*, 227–239. https://doi.org/10.1016/0308-8146(87)90148-8.
van Leeuwen, K. A., Prenzler, P. D., Ryan, D., & Camin, F. (2014). Gas chromatography-combustion-isotope ratio mass spectrometry for traceability and authenticity in foods and beverages. *Comprehensive Reviews in Food Science and Food Safety, 13*, 814–837. https://doi.org/10.1111/1541-4337.12096.