



Università degli Studi Mediterranea di Reggio Calabria
Archivio Istituzionale dei prodotti della ricerca

The effect of cultivar and harvest season on the n-alkane and the n-alkene composition of virgin olive oil.

This is the peer reviewed version of the following article:

Original

The effect of cultivar and harvest season on the n-alkane and the n-alkene composition of virgin olive oil / Giuffrè, A.M.. - In: EUROPEAN FOOD RESEARCH AND TECHNOLOGY. - ISSN 1438-2385. - 247:1(2021), pp. 25-36. [10.1007/s00217-020-03604-x]

Availability:

This version is available at: <https://hdl.handle.net/20.500.12318/65476> since: 2024-11-25T11:00:29Z

Published

DOI: <http://doi.org/10.1007/s00217-020-03604-x>

The final published version is available online at: <https://link.springer.com/article/10.1007/s00217-020->

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website

Publisher copyright

This item was downloaded from IRIS Università Mediterranea di Reggio Calabria (<https://iris.unirc.it/>) When citing, please refer to the published version.

(Article begins on next page)

Dear Author,

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For fax submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/ corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style. Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL: [http://dx.doi.org/\[DOI\]](http://dx.doi.org/[DOI]).

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information go to: <http://www.link.springer.com>.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

ArticleTitle	The effect of cultivar and harvest season on the <i>n</i> -alkane and the <i>n</i> -alkene composition of virgin olive oil	
Article Sub-Title		
Article CopyRight	Springer-Verlag GmbH Germany, part of Springer Nature (This will be the copyright line in the final PDF)	
Journal Name	European Food Research and Technology	
Corresponding Author	Family Name	Giuffrè
	Particle	
	Given Name	Angelo Maria
	Suffix	
	Division	Dipartimento di Agricoltura
	Organization	Risorse forestali, Ambiente Risorse zootecniche, Ingegneria agraria, Alimenti, Università degli Studi Mediterranea di Reggio Calabria, AGRARIA
	Address	Contrada Melissari, 89124, Reggio Calabria, Italy
	Division	Dipartimento di Agraria
	Organization	Università degli Studi 'Mediterranea' di Reggio Calabria
	Address	Contrada Melissari, 89124, Reggio Calabria, Italy
	Phone	
	Fax	
	Email	amgiuffre@unirc.it
	URL	
	ORCID	
Schedule	Received	1 June 2020
	Revised	26 August 2020
	Accepted	29 August 2020
Abstract	<p>Linear hydrocarbons such as <i>n</i>-alkanes and <i>n</i>-alkenes are contained in the unsaponifiable fraction and are one of the less studied class of components in olive oil. This work was conducted in two subsequent harvest seasons (2016–2017 and 2017–2018) and attentioned the oils of nine olive cultivars: Cassanese, Coratina, Itrana, Leccino, Nociara, Ottobratica, Pendolino, Picholine and Sinopolese grown in the same geographical area (Rizziconi) of the region of Calabria (South Italy). Seven out of the nine cultivars were allochthonous for the geographical area where the experiment was conducted. Height <i>n</i>-alkanes with odd-carbon chain number, seven <i>n</i>-alkanes with even-carbon chain number and three <i>n</i>-alkenes were detected in the following elution order: heneicosane, docosane, tricosene, tricosane, tetracosene, tetracosane, pentacosene, pentacosane, hexacosane, heptacosane, octacosane, nonacosane, triacontane, entriacontane, dotriacontane, tritriacontane, tetratriacontane, pentatriacontane. The cultivar variable produced very high significant differences, this was particularly evident for Sinopolese oil showing a total <i>n</i>-alkanes and <i>n</i>-alkenes content of 260 and 290 mg/kg respectively for the first and the second harvest season, whereas Ottobratica and Picholine oils contained less than 100 mg/kg and Cassanese and Itrana oils contained less than 50 mg/kg. The highest <i>n</i>-alkene content was found in the oil of Ottobratica 1.97–161 mg/kg, Picholine 2.34–1.99 mg/kg and in Sinopolese 1.93–2.91 mg/kg. The odd/even ratio was less than 3 for Picholine and Sinopolese and less than 5 for all other cultivars. Docosane was 12.30 and 17.61 mg/kg for Sinopolese and less than 2 mg/kg for all other cultivars. Harvest season did not influence significantly the <i>n</i>-alkanes and <i>n</i>-alkenes content.</p>	

Keywords (separated by '-') EVOO - Aliphatic hydrocarbon - Endogenous - Harvest year - Linear hydrocarbon - Minor component - Unsaponifiable

Footnote Information



2 The effect of cultivar and harvest season on the *n*-alkane 3 and the *n*-alkene composition of virgin olive oil

4 Angelo Maria Giuffrè^{1,2}

5 Received: 1 June 2020 / Revised: 26 August 2020 / Accepted: 29 August 2020
6 © Springer-Verlag GmbH Germany, part of Springer Nature 2020

7 Abstract

8 Linear hydrocarbons such as *n*-alkanes and *n*-alkenes are contained in the unsaponifiable fraction and are one of the less
AQ1 studied class of components in olive oil. This work was conducted in two subsequent harvest seasons (2016–2017 and 2017–
10 2018) and attentioned the oils of nine olive cultivars: Cassanese, Coratina, Itrana, Leccino, Nociara, Ottobratica, Pendolino,
11 Picholine and Sinopolese grown in the same geographical area (Rizziconi) of the region of Calabria (South Italy). Seven out
AQ2 of the nine cultivars were allochthonous for the geographical area where the experiment was conducted. Height *n*-alkanes
13 with odd-carbon chain number, seven *n*-alkanes with even-carbon chain number and three *n*-alkenes were detected in the
14 following elution order: heneicosane, docosane, tricosene, tricosane, tetracosene, tetracosane, pentacosene, pentacosane,
AQ3 hexacosane, heptacosane, octacosane, nonacosane, triacontane, entriacontane, dotriacontane, tritriacontane, tetratriacontane,
16 pentatriacontane. The cultivar variable produced very high significant differences, this was particularly evident for Sinopolese
17 oil showing a total *n*-alkanes and *n*-alkenes content of 260 and 290 mg/kg respectively for the first and the second harvest
18 season, whereas Ottobratica and Picholine oils contained less than 100 mg/kg and Cassanese and Itrana oils contained less
19 than 50 mg/kg. The highest *n*-alkene content was found in the oil of Ottobratica 1.97–161 mg/kg, Picholine 2.34–1.99 mg/
20 kg and in Sinopolese 1.93–2.91 mg/kg. The odd/even ratio was less than 3 for Picholine and Sinopolese and less than 5 for
21 all other cultivars. Docosane was 12.30 and 17.61 mg/kg for Sinopolese and less than 2 mg/kg for all other cultivars. Harvest
22 season did not influence significantly the *n*-alkanes and *n*-alkenes content.

23 **Keywords** EVOO · Aliphatic hydrocarbon · Endogenous · Harvest year · Linear hydrocarbon · Minor component ·
24 Unsaponifiable

25 Introduction

26 Olive oil is constituted by more than two hundred compounds. Glycerides, partial glycerides, free fatty acids, phosphatides are the constituents of the major quantity of the oil (98.5%), whereas other components such as tocopherols, phenols, pigments, sterols, fatty alcohols, waxes are the so

called minor components because they represent about the 1.5% of the oil. *n*-Alkanes and *n*-alkenes are minor components of endogenous origin in the group of hydrocarbons and have a specific pattern which can contribute to distinguish the different vegetable oils such as: sesame seed oil, walnut seed oil, sunflower seed oil [1, 2]; crude palm kernel oil [3, 4]; peanut and sunflower seed oils [2]; avocado pulp oil [5]; linum seed oil [6]; tomato seed oil [7]. This is due to the specificity of the *n*-alkanes and *n*-alkenes carbon-chain length in each vegetable oil and the specificity of the relative quantity of each *n*-alkane and *n*-alkene in each oil. If the *n*-alkane composition in animal fat is considered, it can be observed a specific behaviour. Tejada et al. [8] in intramuscular lipids of Iberian fresh ham found that the carbon-chain length varied between C12 and C32 with an even to odd prevalence and with C12, C14, C16 and C18 as the major *n*-alkane. This is in contrast with the even to odd prevalence of *n*-alkanes of vegetable origin. Even if the major source

A1 ✉ Angelo Maria Giuffrè
A2 amgiuffre@unirc.it

A3 ¹ Dipartimento di Agricoltura, Risorse forestali, Ambiente
A4 Risorse zootecniche, Ingegneria agraria, Alimenti,
A5 Università degli Studi Mediterranea di Reggio Calabria,
A6 AGRARIA, Contrada Melissari, 89124 Reggio Calabria,
A7 Italy

A8 ² Dipartimento di Agraria, Università degli Studi
A9 'Mediterranea' di Reggio Calabria, Contrada Melissari,
A10 89124 Reggio Calabria, Italy

in animal tissue is from vegetables of the diet, Pétron et al. [9] found no relationship between the *n*-alkane composition of vegetables in animal feed and the *n*-alkane composition in intramuscular lipids of dry-cured Iberian ham, this was probably because hydrocarbons are also originated by oxidation of fatty acids [10].

Low quantity of *n*-alkanes is absorbed by the mammalian small intestine [11], more in detail, the longer the carbon-chain length the lower the absorption [12].

Olive oil can be contaminated by mineral oils of different origin such as: air pollution [13], environmental pollution [14], wrong storage condition [15], wrong processing systems [16], presence of lubricants, motor oils, pesticides [17], and olive fruit transport.

In previous works conducted in the same geographical area and with regards to the oil of the same olive cultivars considered in the present work, it was studied the effect of cultivar and harvest season on triglycerides [18], waxes [19], sterols [20] and fatty alcohols [21]. The aim of this work was to study the *n*-alkane and *n*-alkene composition in the oil of nine olive cultivars grown in the same geographical area of the Calabria region (South Italy), in addition, in this context was studied the effect of cultivar and harvest season on the *n*-alkane and *n*-alkene composition.

Materials and methods

Plant materials

Fruits were picked from olive trees grown in mono cultivar plantations, in the geographical area of Rizziconi, in the Gioia Tauro Plan at 115 m on the sea level (Calabria, South Italy).

A total of nine cultivars were chosen for this experiment, in detail: seven allochthonous (Cassanese, Coratina, Itrana, Leccino, Nociera, Pendolino and Picholine) and two autochthonous (Ottobratica and Sinopolese) for this geographical area. It has to be pointed out that Cassanese is a Calabrian cultivar but not from the specific geographical area where our experiment was conducted. For all the cultivars the same agronomic conditions were applied in a flat ground, of alluvial origin, with silt and sand. The trees were not irrigated because the climate is humid and temperate. The maximum rain fell was 165 mm in March 2016 and the minimum was 2 mm in June 2017. The temperature reached a maximum of 39.4 °C on August 2017 (in the morning) and a minimum of -3.0 °C on January 2017 (in the night). For each cultivar were chosen thirty plants 25–35-year old, healthy and uniform in size, grown along a line between two opposite corners of the orchard. Plants of all cultivars were own-rooted. The same fertilisation criterion was applied each year for all the studied cultivars (N, P and K in a ratio 20/10/10).

Pruning was conducted every two years but dead woods were removed each year. Pest treatments were conducted against *Bactrocera oleae*, *Spillocaea oleagina* and *Colletotrichum gloeosporioides*. The experiment was conducted for two subsequent harvest years (2016–2017 and 2017–2018) and sampling was conducted in the following harvest dates: 3 October, 18 October, 3 November, 17 November, 5 December, 19 December, 3 January. From each cultivar 2 kg olives/tree (for a total of 60 kg) were manually and randomly picked at each harvest date until drupes were found on trees. Oil extraction was conducted within 5 h from picking in a small mill “Mini 30” (AGRIMEC Valpesana, Calzaiolo, S. Casciano VP, Florence), with the following procedure: fruits were separated by leaves, stems and any solid material and a mild washing with water was made before crushing by a hammer-mill at room temperature. The olive paste was mixed (for 35 min at 18–20 °C) without water adding and placed in a pile of circular steel grids before to apply a continuous, slow and mild increase in pressure up to 200 bars. The final pressure was maintained for 20 min. Olive oil was separated by waste water by a laboratory centrifuge for 10 min at 3000 rpm and the supernatant (oil) was filtered in a paper filter before to be stored in a 100 mL amber glass bottles (15–20 °C) until analysis, i.e. within two days after oil extraction.

Chemicals

All reagents of analytical grade and chromatographic grade were purchased from Carlo Erba (Milan, Italy). Silica gel was purchased from Merck (Darmstadt, Germany). Pure standards of alkanes were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Analytical procedure

n-Alkane and *n*-alkene fraction was obtained by applying the method described for the determination of stigmastadienes in vegetable oils and proposed in the Annex XVII of the European regulation [22]. Shortly, *n*-hexacosane (as internal standard) was diluted in *n*-hexane and dosed in a 250-mL glass flask. The solvent was evaporated by a mild stream of nitrogen and olive oil and alcoholic potash at 10% were dosed in the glass flask. At this point the reflux condenser was started to heat the mixture to a slight boiling for 30 min until complete saponification reaction. Thereafter the unsaponifiable fraction was extracted by a glass funnel before to be carefully introduced in a silica-gel glass column with *n*-hexane as an eluent. The chromatographic elution was conducted with a flow rate of 1 mL/min, approximately. *n*-Alkanes and *n*-alkenes were contained in the first 35 mL of eluate. The eluted volume was concentrated by a Rotary evaporator before GC-on column analysis.

147 Gas chromatography

148 A Carlo Erba HRGC3000 instrument was used for gas-chromatographic analysis, equipped with an on-column injector, a FID detector and a capillary column SE-54 MEGA-Milano-Italy (column length 25 m, ID 0.32 mm and film thickness 0.25 μm). The oven temperature program was: 153 60 °C (1 min isotherm), a 5 °C/min increase up to 290 °C (40 min isotherm). The detector temperature was set at 155 310 °C. The identification of peaks was conducted by comparing their retention indices with those of pure standards and with data reported in the literature. 157

158 Statistical analysis

159 Five fruit samplings were conducted for each cultivar for each harvest season. For each fruit sampling were prepared two batches of 30 kg drupes each. One replicate was conducted on the oil of each batch, with a total of two replicates/cultivar/fruit sampling. 163

164 To calculate means and standard deviations of the 20 final replicates of each cultivar (2 replicates \times five samplings \times two harvest seasons) was used Excel 2010 as a software. The SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used to analyse the means by one-way ANOVA and Tukey's test, at 5% probability, with cultivar and the harvest season as variables. 170

171 Results and discussion

172 Eighteen peaks were detected in the gas-chromatogram, fifteen *n*-alkanes and three *n*-alkenes. *n*-Alkanes ranged between C21 and C35, with 8 odd-carbon chain and 7 even-carbon chain components. *n*-Alkenes ranged between C23:1 and C25:1. The same GC profile was obtained in olive oils from Croatia [23], and in olive oils from Greece, Spain, Tunisia and from different geographical areas of Italy [24]. 178

179 C21 was the first peak revealed by the gas-chromatogram and accounted for less than 1 mg/kg in all cultivars except for Sinopolese, in which it was 4.08 mg/kg in 2016–2017 and 6.52 in 2017–2018 harvest season. C21 was found in highest quantity in 2016–2017 harvest season in the oil of Coratina, Itrana, Leccino, Nociara and Pendolino whereas it was in highest quantity in 2017–2018 in the oil of Cassanese, Ottobratica and Sinopolese, i.e. the three Calabrian cultivars (Fig. 1). The two-way ANOVA analysis indicated that the cultivar and the interaction cv \times harvest season had a very high significant influence ($p \leq 0.001$) on the C21 content (Table 1). Moreda et al. [2] reported of studies on the *n*-alkane composition of five edible vegetable oils and described a GC profile with *n*-alkanes ranging between 15 and 35 carbon-chain atoms; in that context, C21 was

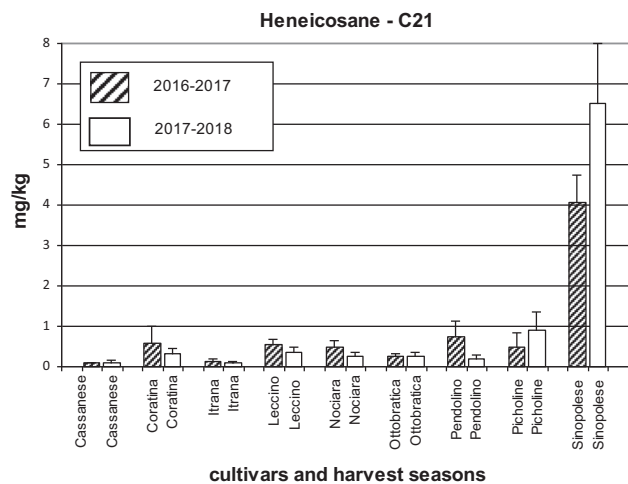


Fig. 1 Variation in the Heneicosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

quantified for 0.81 mg/kg in a virgin olive oil. A study on *n*-alkane content of intramuscular lipids of fresh ham found C12 as the first eluted *n*-alkane [8], namely a carbon chain with 9 methylene groups less than in olive oil and, in that case, C21 content ranged between 0.73 and 0.81 mg/kg [8]. In studies of other vegetable oils such as avocado pulp oil and tomato seed oil were found C21 [5] and C19 [7] as the first eluted *n*-alkane, this can consent to suppose that fat of animal origin contains *n*-alkanes with a shorter chain than vegetable oils. 203

C22 showed a similar pattern to C21 with Sinopolese and Picholine oils having the highest quantity: the former varied between 12.30 mg/kg (2016–2017) and 17.61 mg/kg (2017–2018), the latter varied between 14.6 mg/kg (2016–2017) and 1.78 mg/kg (2017–2018). From all other cultivars was extracted an oil accounting for less than 1 mg/kg (Fig. 2). Cultivar and cv \times harvest season influenced very significantly ($p \leq 0.001$) the C22 content (Table 1). Herchi et al. [6] studied the *n*-alkane composition of linum seed oil and found a C22 content ranging between 2.8 and 3.6 mg/kg, i.e. in a lower quantity than our Sinopolese oil but in a higher quantity than in the oil of all other olive cultivars (Fig. 2). Sakohou et al. [25] applied a GC–MS analytical method with a DB5 MS fused silica capillary column and found C22 and C36 respectively as the first and the last eluted *n*-alkanes in olive oil of Meski cv grown in north-east Tunisia, instead of C21 and C35 found in the oils of our study this could be one parameter useful to distinguish oils of different geographical origin and of different cultivar. 222

C23:1 was the first eluted *n*-alkene in the GC chromatogram. The GC column used in this work, containing 5% phenyl, 1% vinyl, 94% methyl polysiloxane and having a low polarity has separated components so that *n*-alkenes were 226

Table 1 Total *n*-alkanes; total odd-carbon chain *n*-alkanes; total even-carbon chain *n*-alkanes; total *n*-alkenes; total *n*-alkanes and *n*-alkenes

	Total <i>n</i> -alkanes (mg/kg)	Total odd-carbon chain <i>n</i> -alkanes (mg/kg)	Total even-carbon chain <i>n</i> -alkanes (mg/kg)	Total <i>n</i> -alkenes (mg/kg)	Total <i>n</i> -alkanes and <i>n</i> -alkenes (mg/kg)
Cassanese 2016–2017	21.13 ± 9.04	16.93 ± 7.41	4.20 ± 1.67	0.16 ± 0.03	21.29 ± 9.07
Cassanese 2017–2018	19.06 ± 6.37	14.92 ± 4.99	4.14 ± 1.39	0.14 ± 0.04	19.20 ± 6.38
Coratina 2016–2017	69.72 ± 5.98	54.80 ± 5.58	14.93 ± 4.19	1.28 ± 0.27	71.00 ± 6.24
Coratina 2017–2018	48.85 ± 9.78	36.42 ± 8.19	9.43 ± 2.35	0.55 ± 0.15	46.40 ± 9.91
Itrana 2016–2017	22.00 ± 6.63	17.15 ± 4.75	4.74 ± 1.92	0.11 ± 0.05	22.00 ± 6.63
Itrana 2017–2018	23.39 ± 4.67	18.93 ± 3.74	4.46 ± 0.94	0.11 ± 0.03	23.50 ± 4.65
Leccino 2016–2017	47.62 ± 27.64	37.61 ± 19.77	10.01 ± 7.88	0.13 ± 0.01	47.75 ± 27.66
Leccino 2017–2018	46.08 ± 17.90	34.85 ± 12.53	11.23 ± 5.41	0.12 ± 0.04	46.20 ± 17.91
Nociara 2016–2017	52.55 ± 12.13	39.90 ± 9.18	12.65 ± 3.07	0.28 ± 0.11	52.83 ± 12.22
Nociara 2017–2018	41.25 ± 11.22	31.34 ± 8.37	9.91 ± 2.89	0.15 ± 0.07	41.40 ± 11.28
Ottobratica 2016–2017	86.46 ± 30.57	69.92 ± 23.43	16.54 ± 7.36	1.97 ± 1.08	88.43 ± 31.60
Ottobratica 2017–2018	82.05 ± 32.34	66.04 ± 24.68	16.02 ± 7.71	1.61 ± 1.03	83.67 ± 33.31
Pendolino 2016–2017	54.45 ± 20.38	44.72 ± 17.24	9.73 ± 3.14	0.22 ± 0.23	54.67 ± 20.60
Pendolino 2017–2018	51.94 ± 16.70	43.13 ± 14.11	8.81 ± 2.64	0.06 ± 0.04	52.00 ± 16.73
Picholine 2016–2017	75.26 ± 10.13	55.11 ± 7.22	20.15 ± 2.95	2.34 ± 0.57	77.60 ± 10.55
Picholine 2017–2018	84.76 ± 2.89	62.37 ± 2.23	22.39 ± 0.73	1.99 ± 0.43	86.75 ± 3.30
Sinopolese 2016–2017	258.50 ± 32.86	192.13 ± 24.71	66.37 ± 8.56	1.93 ± 0.91	260.43 ± 33.52
Sinopolese 2017–2018	287.09 ± 44.47	210.36 ± 32.88	76.73 ± 11.77	2.91 ± 0.84	290.00 ± 45.20

Variations in the oils of the studied cultivars are considered in the two harvest seasons (2016–2017 and 2017–2018) and are expressed as mg/kg ± standard deviation

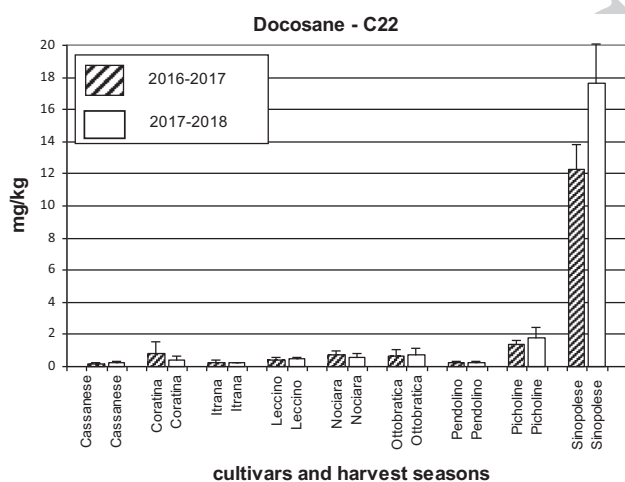


Fig. 2 Variation in the Docosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

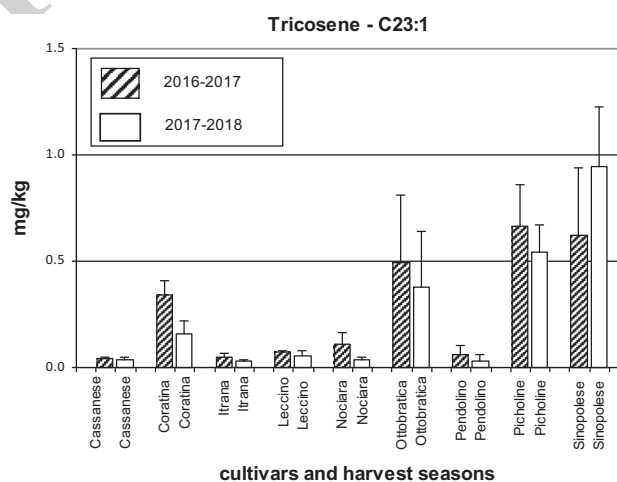


Fig. 3 Variation in the Tricosene content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

227 eluted before the homologous *n*-alkane with the same carbon
 228 chain number. In almost all cultivars the highest content was
 229 found in the 2016–2017 harvest season except for Sinopolese
 230 in which the highest content was found in 2017–2018. All
 231 cultivars showed a C23:1 quantity lower than 1 mg/kg. In
 232 detail, in Coratina, Ottobratica, Picholine and Sinopolese
 233 oils, the C23:1 content varied between 0.16 mg/kg (Coratina

2017–2018) and 0.95 mg/kg (Sinopolese 2017–2018),
 whereas in the oil of all other cultivars the C21 content
 was ≤ 0.11 mg/kg (Fig. 3).

The *n*-alkane and *n*-alkene profile showed a characteristic
 bell-shape profile, with C23 as the first of these hydrocarbons
 being in the initial part of the bell-shape, even if differently
 for each cultivar. In fact, C23 accounted for 75.24 and

241 89.90 mg/kg respectively for the first and the second harvest
 242 seasons in Sinopolese oil and between 18.61 and 20.19 mg/
 243 kg in Picholine oil. All other cultivars showed a C23 con-
 244 tent ≤ 10.66 mg/kg found in Nociara in 2016–2017, (Fig. 4).
 245 The C23 content was influenced ($p \leq 0.001$) by both culti-
 246 var and $cv \times$ harvest season (Table 1). El Antari et al. [26]
 247 studied the *n*-alkane composition of olive oils produced in
 248 different sites of Morocco and found a C23 content ranging
 249 between 3.54 and 9.24 mg/kg, also in this case C23 designed
 250 the initial part of the bell-shape for the *n*-alkane profile.
 251 Among the odd-numbered *n*-alkanes, C23 and C25 were
 252 found to predominate in the oils from northern and southern
 253 Italy, France, Croatia, Morocco and southern Greece [27],
 254 this is in accordance especially with our oils extracted from
 255 Sinopolese cv. (Fig. 1, 4, 8, 10, 12, 14, 16, 18).

256 C24:1 was the second detected *n*-alkene in the gas-chro-
 257 matographic profile. The highest absolute quantities were
 258 found in 2017–2018 harvest season in the Sinopolese oil
 259 (0.94 mg/kg) and in 2016–2017 harvest season in the Picho-
 260 line oil (0.68 mg/kg). Ottobratica produced an oil ranging
 261 between 0.53 and 0.45 mg/kg and Coratina remained above
 262 and below the 0.2 mg/kg level respectively in the 2016–2017
 263 and in the 2017–2018. In all other cases was found a C24:1
 264 content \leq than 0.06 mg/kg of Nociara oil (2016–2017),
 265 (Fig. 5).

266 C24 was the third major *n*-alkane. Its content was high-
 267 est in Sinopolese oil (43.32 and 48.80 mg/kg), followed
 268 by Picholine (14.60–15.60 mg/kg) and by Leccino, Noci-
 269 ara and Ottobratica oils in which C24 was closed between
 270 5.25 and 7.69 mg/kg. In all the other cultivars, in both the
 271 two harvest seasons, C24 was less or equal than 5.03 mg/
 272 kg (Coratina 2016–2017), (Fig. 6). The cultivar showed a
 273 very high significant effect on the C24 content ($p \leq 0.001$)

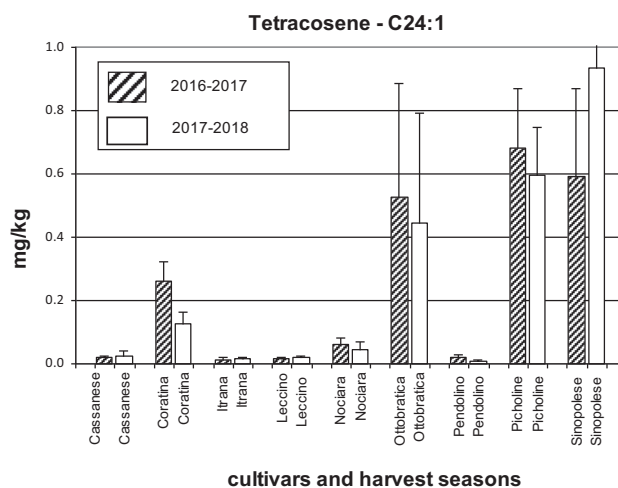


Fig. 5 Variation in the Tetracosene content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

274 instead of harvest season and the interaction of $cv \times$ harvest
 275 season (Table 1). Koprivnjak et al. [28] in olive oils from
 276 Istria (Croatia) found a C24 content 4 times lower in Bjelica
 277 cv oil than in Buza and Leccino oils.

278 C25:1 was the third and last *n*-alkene eluted in the GC
 279 chromatogram. The lowest quantities were found in Cas-
 280 sanese, Itrana, Leccino, Nociara and Pendolino oils,
 281 accounting for less than 0.2 mg/kg. Only in Sinopolese
 282 oil (2017–2018) was found a C25:1 content slightly more
 283 than 1 mg/kg (Fig. 7). Our results indicated that the interac-
 284 tion $cv \times$ harvest season had a very high significant effect
 285 ($p \leq 0.001$) on the C25:1 content (Table 1).

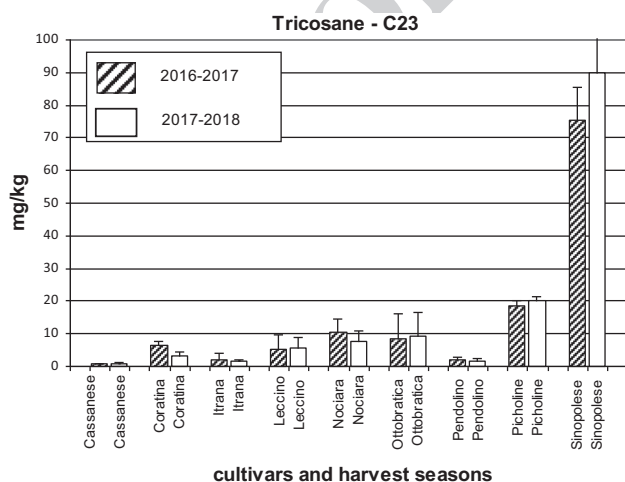


Fig. 4 Variation in the Tricosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

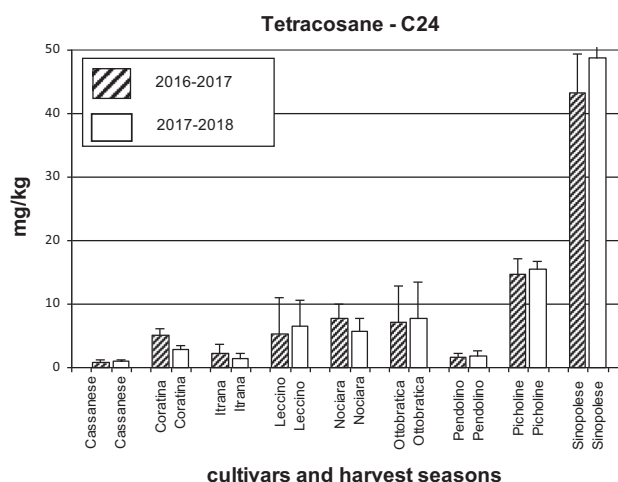


Fig. 6 Variation in the Tetracosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

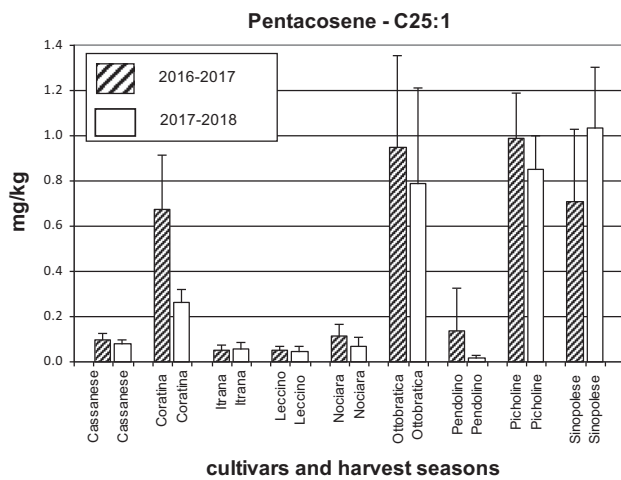


Fig. 7 Variation in the Pentacosene content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

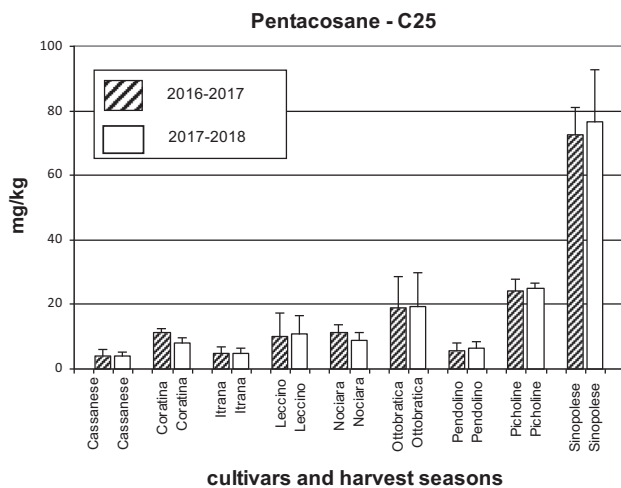


Fig. 8 Variation in the Pentacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

286 C25 was the second major *n*-alkane. The studied cultivars
 287 can be classified in 3 main groups: Cassanese,
 288 Coratina, Itrana, Leccino, Nociara and Pendolino oils
 289 showing less than 15 mg/kg; Ottobratica and Picholine oils
 290 ranging between 15 and 30 mg/kg and lastly Sinopolese
 291 oil accounting for more than 70 mg/kg (Fig. 8). Findings
 292 of other authors on Tunisian olive oil reported C25 as the
 293 major *n*-alkane which was quantified in 22.10 mg/kg [25].
 294 El Antari et al. [26] found C25 as one of the predominant
 295 *n*-alkanes in Moroccan olive oil ranging between 4.30 and
 296 10 mg/kg. Troya et al. [29] reported about the importance
 297 of C25, in fact they used the C21/C25 ratio as one of the

main parameters useful to predict the botanical origin of
 vegetable oil.

The C26 content in Sinopolese oil was found to be 6.39
 and 6.23 mg/kg respectively for the first and the second har-
 vest season. By and large it was double in quantity with
 respect to Coratina, Ottobratica and Sinopolese oils; triple
 with respect to Leccino, Nociara and Pendolino oils, and
 4–5 times higher than in Cassanese and Itrana oils. In the oil
 of the autochthonous cultivars the C26 content was higher
 in the 2016–2017 with respect to the 2017–2018 harvest
 season: 1.33–1.27 mg/kg for Cassanese, 3.27–3.10 mg/kg
 for Ottobratica and 6.39–6.23 mg/kg for Sinopolese (Fig. 9).

In the oil of the autochthonous cultivars, C27 con-
 tent was higher in the 2016–2017 with respect to the
 2017–2018 harvest season: 4.67–4.46 mg/kg for Cassanese,
 15.75–13.47 mg/kg for Ottobratica and 19.23–18.25 mg/
 kg for Sinopolese. This behaviour was also found in
 Coratina, Leccino and Nociara oils, in contrast to Itrana
 (4.08–6.06 mg/kg), Pendolino (11.90–12.38 mg/kg) and
 Picholine (5.22–7.95 mg/kg) (Fig. 10). In a study con-
 ducted in olive oils produced in Morocco, C27 was quanti-
 fied in a range of 2.87–40.85 mg/kg with a high variability
 between the studied samples [26].

C28 was higher in the 2016–2017 than in the 2017–2018
 harvest season, in the oil of all cultivars that exceeded 1 mg/
 kg in both the harvest seasons. Whereas, in the cultivars
 showing a border line content (slight higher or lower than
 1 mg/kg) only Itrana (0.65–0.87 mg/kg) and Picholine
 (0.77–1.09 mg/kg) oils showed and inverse rate. Ottobrat-
 ica and Pendolino oils predominated on all other cultivars
 for the C28 content, i.e. 3.0 mg/kg in both cultivars in the
 2016–2017 harvest season (Fig. 11).

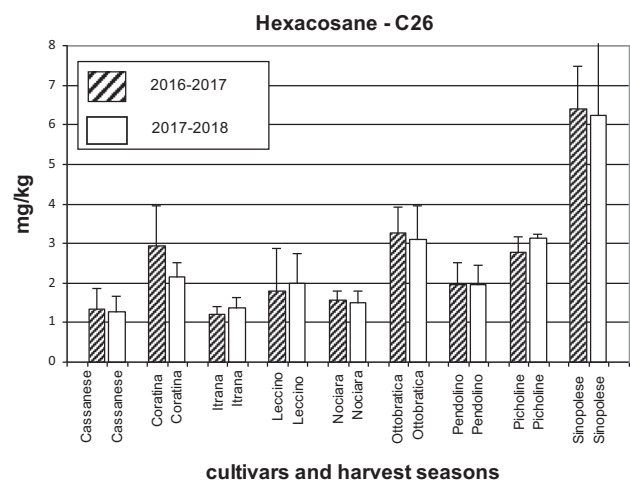


Fig. 9 Variation in the Hexacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

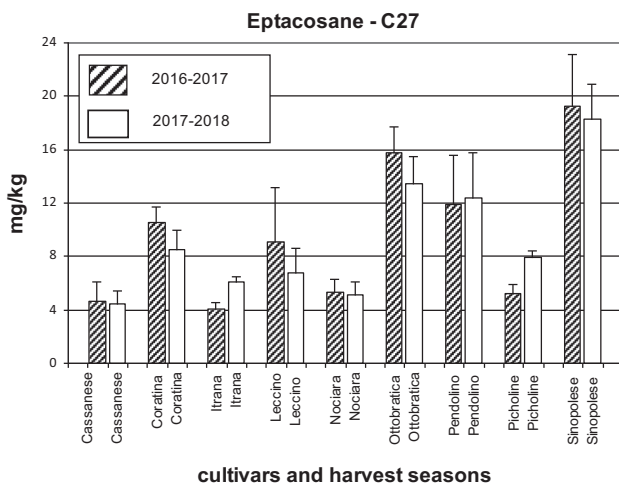


Fig. 10 Variation in the Eptacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

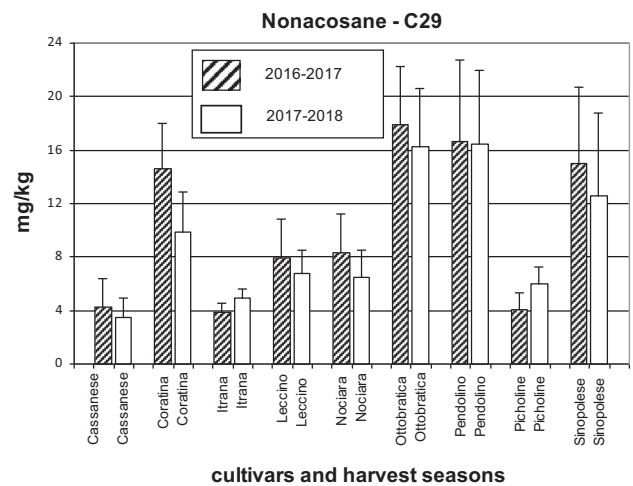


Fig. 12 Variation in the Nonacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

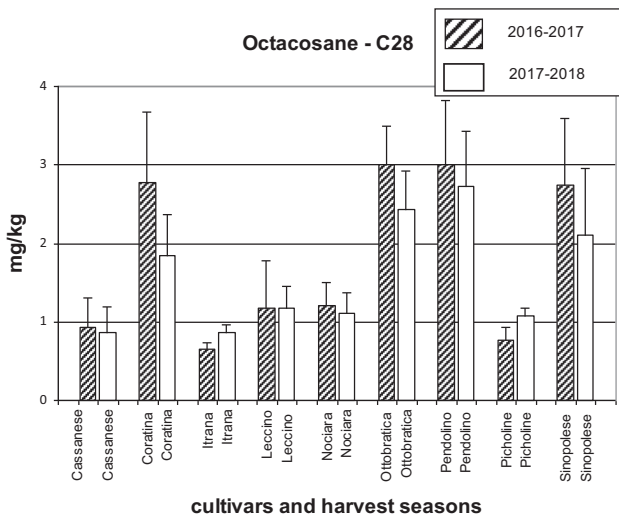


Fig. 11 Variation in the Octacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

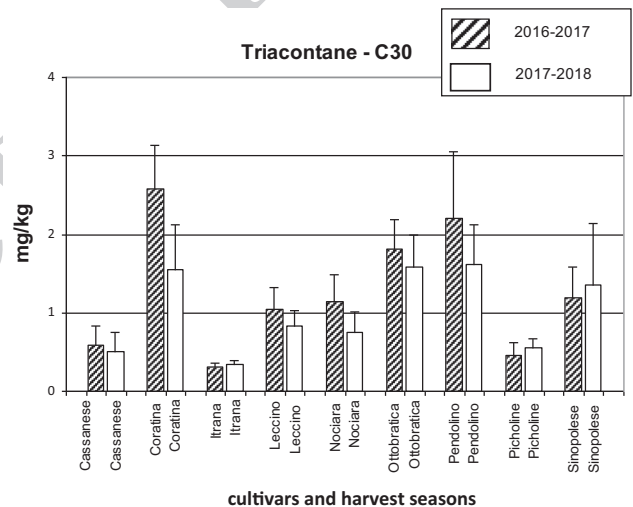


Fig. 13 Variation in the Triacotane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

330 C29 content was highest in the oil of the 2016–2017
 331 harvest season for all cultivars except than Itrana and
 332 Picholine. By Coratina, Ottobratica, Pendolino and Sino-
 333 polese it was obtained an oil with a C29 content ranging
 334 between 9.84 mg/kg (Coratina 2017–2018) and 19.92 mg/
 335 kg (Ottobratica 2016–2017). The oil of all other cultivars
 336 in both the studied harvest seasons showed a C29 con-
 337 tent ranging between 8.34 mg/kg (Nociara 2017–2018)
 338 and 3.51 mg/kg (Cassanese 2017–2018), (Fig. 12).
 339 Koprivnjak et al. [30] studied four olive oils of Croatian
 340 cultivars and found that C29 was the predominant linear

hydrocarbon in the oil of Debela, Rosulja and Slatka cul- 341
 tivars whereas in the Naska oil prevailed C25. 342

C30 was higher in the 2016–2017 than in the 2017–2018 343
 harvest season in the oil of almost all cultivars except for 344
 Itrana and Picholine (Fig. 13), following the same behav- 345
 iour of C28 (Fig. 11). The highest C30 content was found 346
 in Coratina, Pendolino and Ottobratica (2.57, 2.20 and 347
 1.89 mg/kg, respectively), whereas Itrana and Picholine 348
 showed the lowest values ranging between 0.32 mg/kg 349
 (Itrana, first harvest season) and 0.56 mg/kg (Picholine, 350
 second harvest season), (Fig. 13). Cultivar and the interac- 351
 tion cv \times harvest season had a high significant ($p \leq 0.01$) 352

353 and a significant ($p \leq 0.05$) effect (Table 1). The ratio C24/
354 C30 was the second main parameter found by Troya et al.
355 [29] useful to predict the botanical origin of vegetable oil,
356 hence the importance of knowing also the content of these
357 two compounds.

358 C31 was higher in the 2016–2017 than in the 2017–2018
359 harvest season in the oil of almost all the studied cultivars
360 except for Picholine oil (Fig. 14). Coratina oil showed the
361 highest C29 content in the first harvest season (8.75 mg/
362 kg), followed by Pendolino and Ottobratica oils (6.34 and
363 6.33 mg/kg respectively). Itrana and Picholine oils con-
364 tained less than 2.0 mg/kg C31 in both the harvest seasons
365 (Fig. 14). C31 value was affected by both cultivar ($p \leq 0.01$)
366 and harvest season ($p \leq 0.05$) but not by their interaction
367 ($p > 0.05$). In a study conducted on olive fruits and olive
368 leaves of Frantoio, Moraiolo and Leccino cvs, C31 was
369 found to be one of the predominant *n*-alkanes throughout
370 the ripening periods of fruits [31].

371 C32 content was higher in the second harvest season
372 than in the first for Picholine and Sinopolese, in contrast
373 with the tendency showed by all other cultivars. Coratina
374 oil contained the highest C32 content in both the harvest
375 seasons (0.62 and 0.56, respectively). As for C31, Itrana and
376 Picholine oils showed the lowest C32 content, i.e. less than
377 0.2 mg/kg in both the harvest seasons (Fig. 15). C32 values
378 of our oils were in agreement with findings of other authors
379 [32] in olive oils of seven cultivars grown in the Extrema-
380 dura region of Spain. C32 was the last eluted *n*-alkane found
381 in the tomato seed oil [7] this could allow to differentiate an
382 oil extracted from a fruit from an oil extracted from a seed.

383 C33 was higher in 2016–2017 than in 2017–2018 for
384 the oil of all cultivars except for Sinopolese oil. As for C31

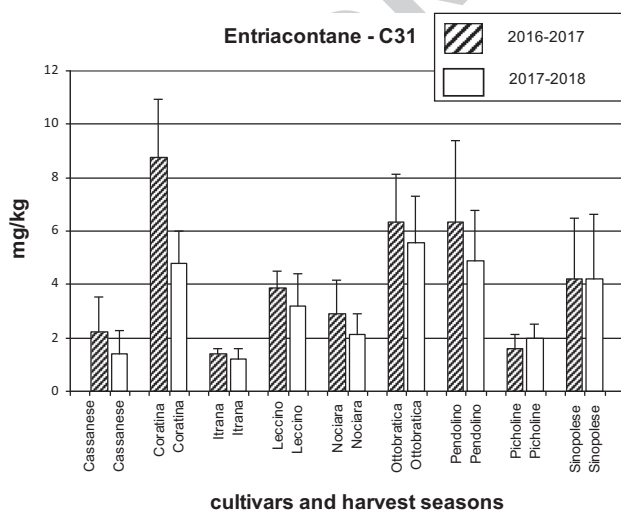


Fig. 14 Variation in the Entriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

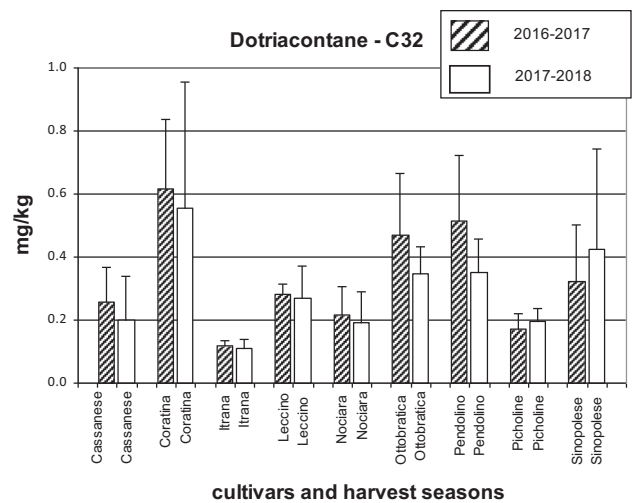


Fig. 15 Variation in the Dotriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

385 and C32, Itrana and Picholine oils showed the lowest C33
386 content, i.e. less than 0.5 mg/kg in both the harvest sea-
387 sons. In all cases the C33 content was lower than 2.40 mg/
388 kg (Fig. 16), whereas Sakouhi et al. [25] found 4.5 mg/kg in
389 olive oil of Meski cv.

390 C34 was the last even-carbon chain alkane eluted by
391 the GC analysis. No. C34 content of the studied cultivar
392 occurred as much as 0.2 mg/kg. Itrana, Leccino, Nociera
393 and Picholine oils occurred with less than 0.1 mg/kg in both
394 the studied harvest seasons. Coratina oil showed a C34 con-
395 tent double in the first harvest season compared with the
396 second one (0.20–0.10 mg/kg), on the contrary, Sinopolese

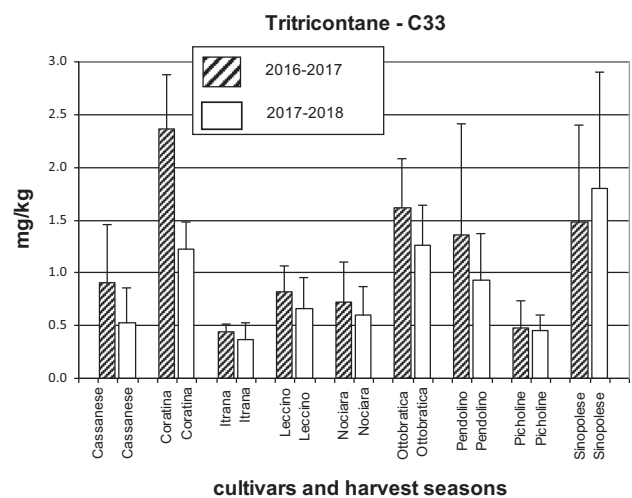


Fig. 16 Variation in the Tritriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

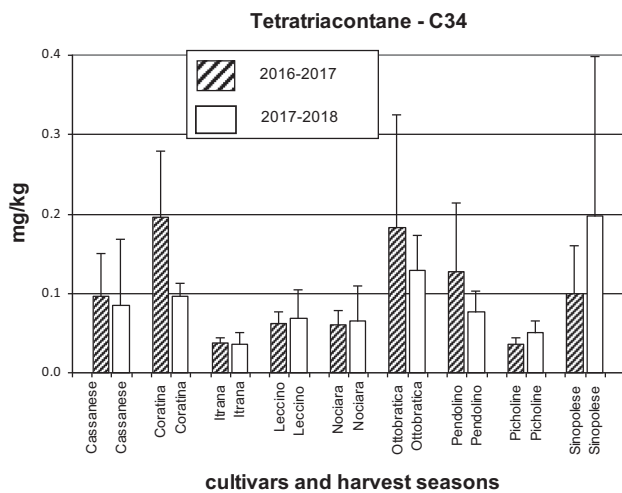


Fig. 17 Variation in the Tetratriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

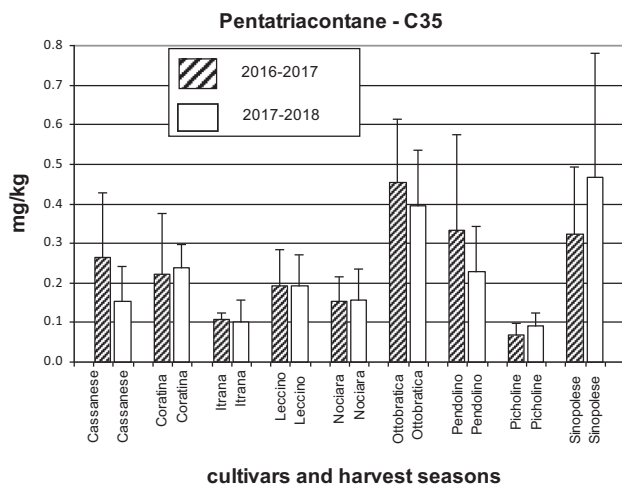


Fig. 18 Variation in the Pentatriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

oil showed a C34 content twice higher in the second harvest season with respect to the first one (0.10–0.20 mg/kg), (Fig. 17). C34 was the last eluted *n*-alkane found in the avocado pulp oil [7], i.e. with one methylene group less than in olive oils of the present study.

C35 was at the same time the last eluted odd-carbon chain alkane and also the last component revealed by the GC chromatogram. This was in accordance with findings of other authors with regards to studies on olive oil [23, 24, 30, 32, 33], and studies on olive fruits and olive leaves [31]. In our study, C35 predominated in the oil of Ottobratica (0.45–0.40 mg/kg) and Sinopolese (0.32–0.47 mg/kg), i.e. the two autochthonous cultivars for the geographical

area where the experiment was conducted. Picholine and Itrana oils accounted for the lowest C35 content: the former remained below the 0.1 mg/kg level in both the harvest seasons, the latter accounted for 0.11 and 0.10 mg/kg in the first and in the second harvest season, respectively (Fig. 18).

Total *n*-alkanes taken together herein are depicted in the Table 1. Sinopolese oil abundantly prevailed on other studied cultivars accounting for 258.50 and 287.09 mg/kg in the first and in the second harvest season, respectively. The second oil for *n*-alkane content was the one of Ottobratica, even if it accounted for 86.46 and 82.05 mg/kg, i.e. 2.99–3.50 times less than in Sinopolese oil. Coratina, Leccino, Nocciara and Pendolino oils sampled in 2017–2018 contained between 41.25 and 54.45 mg/kg respectively, whereas Cassanese and Itrana oils contained less than 30 mg/kg in both the harvest seasons. The total *n*-alkane content of the second harvest season was higher than in the first one for Itrana, Picholine and Sinopolese oils, in opposition to the behaviour of the oil of our other studied cultivars. Findings of other authors showed that the *n*-alkane content in olive oil from Central and South Italy and Croatia was higher than in oils of other Countries of the Mediterranean basin, such as Spain and Northern Greece [27]. *n*-Alkanes lack in functional groups, are very stable molecules and can survive in fossil records for millions of years [34] also for this reason they are important because can be used in studies of paleoecology and paleoclimatology [35]. In addition, they can be used to distinguish a virgin olive oil from a pomace olive oil and the virgin olive oil originated from first to fourth malaxation or pomace oil from first to third extraction [36]. Food can be contaminated with hydrocarbons (mineral oils) and with polyolefinic products (oligomers) [37] which are soluble in vegetable oils, hence it is necessary to know the endogenous *n*-alkane composition of a vegetable oil such as olive oil to better evaluate the endogenous and the hexogenous hydrocarbon composition.

The total odd-carbon chain *n*-alkanes (OCCA) content is quantified in the Table 1. Aside from Sinopolese oil, all other cultivars showed a total odd-carbon chain content lower than 70 mg/kg. In detail, Sinopolese contained 192.13–210.36 mg/kg, namely 2.75 to 3.19 times more than the oil of the second major cultivar: Ottobratica 69.92–66.04 mg/kg. Picholine contained 55.11–62.37 mg/kg, and lastly Cassanese (16.93–14.92 mg/kg). Itrana, Picholine and Sinopolese oils showed the total highest OCCA content in the 2017–2018 harvest season, in contrast with all other studied cultivars. Mihailova et al. [27] studied the olive oils of eight Mediterranean Countries and found odd-number homologues such as C23, C25, C27, C29 and C31 to be the most prevalent *n*-alkanes and found C23, C25, C27 and C29 prevailing in the Italian olive oils samples in northern, central and southern regions.

The absolute value of total even-carbon chain *n*-alkanes (ECCA) was lower than the total OCCA. Sinopolese oil contained one third ECCA than OCCA (Table 1). Picholine showed the second major ECCA content (20.15–22.39 mg/kg) and all other cultivars showed an ECCA content lower than 20 mg/kg in both the harvest seasons. As for OCCA also for ECCA, Cassanese oil showed the lowest content (4.20–4.14 mg/kg). In the 2017–2018 harvest season was revealed the highest ECCA content in the oils of Leccino, Picholine and Sinopolese in opposition to the results found for all other cultivars. Total ECCA content was found to be affected by cultivar ($p \leq 0.001$) and by the interaction $cv \times$ harvest season ($p \leq 0.05$), (Table 2). Findings of Koprivnjak et al. [28] on Leccino, Buza and Bjelica olive oils produced in restricted zone of Istria (Croatia) and sampled in three different fruit ripening stages showed ECCA always in lower quantity than OCCA.

The total *n*-alkene content was highest in Ottobratica, Picholine and Sinopolese oils ranging between 1.61 mg/kg

(Ottobratica 2017–2018) and 2.91 (Sinopolese 2017–2018). In the oil of all cultivars except than in Sinopolese, the *n*-alkene content of the first harvest season prevailed on the content of the second harvest season (Table 1). In the oil of Cassanese, Itrana, Leccino, Nociara and Pendolino, in both the harvest seasons, the total *n*-alkene content did not occur to as much as 0.28 mg/kg (Fig. 23). The two-way ANOVA analysis demonstrated that the total *n*-alkenes were influenced by cultivar ($p \leq 0.001$) and by the interaction $cv \times$ harvest season ($p \leq 0.05$), (Table 2). It is worthy of note that in this study was detected and quantified the *n*-alkene content, whereas in almost all the few studies published in this field it was quantified only the *n*-alkane fraction [2, 25–28, 31]. *n*-Alkenes can be used as a marker to distinguish natural oils from isomeric paraffins (*n*-alkanes) migrated from packaging to the food [13]. On the other hand, unsaturated hydrocarbons appear during the process of oil refining [2]. For this reason, it is fundamental to know the *n*-alkene content in natural edible oils and also the *n*-alkene content in the oil of each specie and of each cultivar grown in a specific geographic area.

As a direct consequence of the single fraction of *n*-alkanes and *n*-alkenes, the sum of the total *n*-alkane and *n*-alkene contents was highest in Sinopolese (260 mg/kg in 2016–2017 and 290 mg/kg in 2017–2018) and lowest in Cassanese and in Itrana accounting for less than 24 mg/kg in both the harvest seasons (Table 1).

In the Fig. 19 is described the OCCA/ECCA ratio. This value was highest in Pendolino (4.53–4.86) and Ottobratica (4.41–4.34) oils, in both the harvest seasons. Picholine (2.74–2.79) and Sinopolese (2.90–2.74) showed the lowest

Table 2 Analysis of the variance, two-way ANOVA

	Cultivar	Harvest season	Cultivar \times harvest season
C21	***	n.s	***
C22	***	n.s	***
C23:1	***	n.s	***
C23	***	n.s	***
C24:1	***	n.s	***
C24	***	n.s	n.s
C25:1	n.s	n.s	***
C25	***	n.s	n.s
C26	***	n.s	n.s
C27	***	n.s	n.s
C28	***	n.s	n.s
C29	***	n.s	n.s
C30	**	n.s	*
C31	**	*	n.s
C32	**	n.s	n.s
C33	**	n.s	n.s
C34	n.s	n.s	n.s
C35	**	n.s	n.s
Alkanes and alkenes Total	***	n.s	n.s
Total alkanes	***	n.s	n.s
Total alkenes	***	n.s	*
Alkanes/Alkenes	n.s	n.s	***
Even Alkanes	***	n.s	*
Odd Alkanes	***	n.s	n.s
Odd/Even ratio	***	n.s	n.s

Significance level for the variables: cultivar, harvest season and interaction of cultivar \times harvest season

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s., not significant; $p > 0.05$

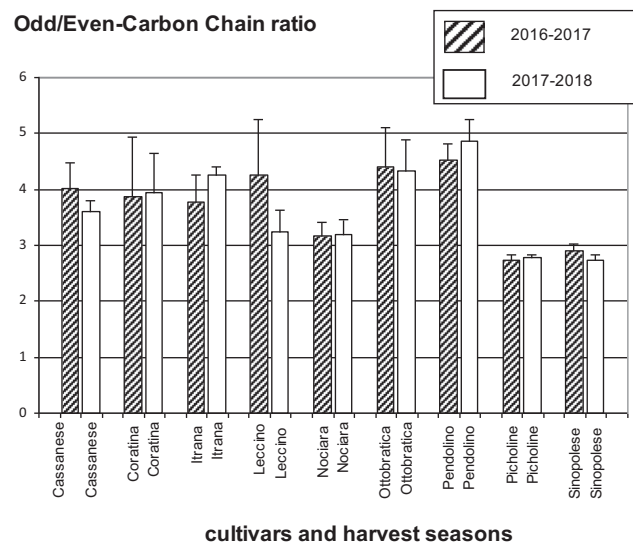


Fig. 19 Variation in the odd/even carbon chain *n*-alkane ratio for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

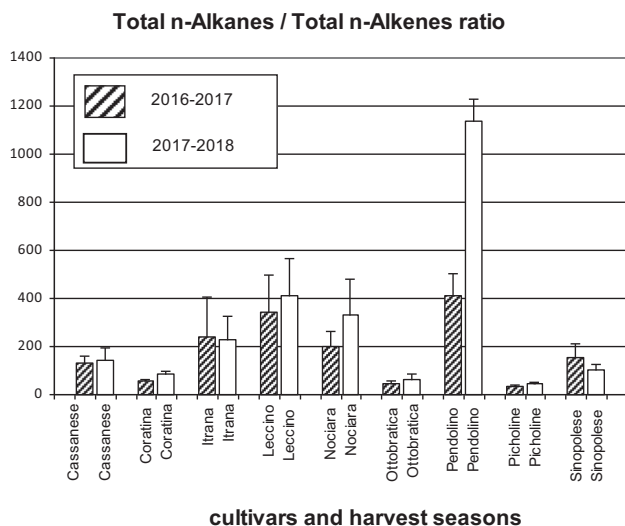


Fig. 20 Variation in the total *n*-alkanes/total *n*-alkenes ratio for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means \pm standard deviation

values. The OCCA/ECCA ratio was highest in the first harvest season for Cassanese, Leccino, Ottobratica and Sinopolese. The two-way analysis of variance showed a very high significant effect ($p \leq 0.001$) by the cv \times harvest season interaction (Fig. 19). This is in accordance with studies of Eglinton and Hamilton [38, 39] who found that that plants produce *n*-alkanes with a strong odd-over-even ratio.

The total *n*-alkanes/total *n*-alkenes ratio is depicted in the Fig. 20. A marked difference between harvest seasons was found in the Pendolino oil (408.70–1137.48). Leccino showed the second major ratio in both the harvest seasons (343.55–409.13). The lowest ratio was found in the oil of Picholine (33.15–43.97).

Conclusion

Hydrocarbons such as *n*-alkanes and *n*-alkenes are contained in the unsaponifiable fraction of an olive oil and are a group of components until now scarcely attentioned but many information can be obtained from their study to qualify an olive oil. Findings on the effect of cultivar and harvest season on *n*-alkane and *n*-alkene composition of nine olive cultivars in a two-year study, are here reported. Sinopolese oil predominated on all other cultivars in the *n*-alkane and *n*-alkene content when the carbon-chain length ranged between 21 and 27 and between 34 and 35; whereas Coratina oil predominated in the range C30–C33 carbon-chain length and Ottobratica and Pendolino oils predominated in the range C28–C29. The two-way ANOVA analysis has demonstrated that the interaction of cultivar \times harvest season had a very high

significant effect on short carbon-chain *n*-alkanes and *n*-alkenes, while almost for all components, cultivar had a high significant ($p \leq 0.01$) or a very high significant effect ($p \leq 0.001$). The relevant effect of cultivar could be additionally used to distinguish the monocultivar olive oils.

Acknowledgements This research was supported by: Distretto ad alta tecnologia agroindustriale della Calabria AGRIFOODTECH—PROGETTO PON03PE_00090_2. Sustainable models and new technologies for olives and olive oil.

Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

Ethics requirements This article does not contain any studies with human or animal subjects which require permission from ethics committees or other institutions.

References

- Mc Gills AS, Moffat CF, Mackie PR, Cruickshank P (1993) The composition and concentration of *n*-alkanes in retail samples of edible oils. *J Sci Food Agric* 61:357–362
- Moreda W, Pérez-Camino MC, Cert A (2001) Gas and liquid chromatography of hydrocarbons in edible vegetable oils. *J Chromatogr A* 936:159–171
- Tan YA, Kuntom A (1993) Gas chromatographic determination of hydrocarbons in crude palm kernel oil. *J AOAC Int* 76:371–376
- Tan YA, Kuntom A (1994) Hydrocarbons in crude palm kernel oil. *J AOAC Int* 77:67–73
- Giuffrè AM (2005) Changes in the *n*-alkane composition of avocado pulp oil (*Persea americana*, Miller) during fruit ripening. *Grasas Aceites* 56:75–78
- Herchi W, Harrabi S, Rochut S, Boukhchina S, Kallel H, Pepe C (2009) Characterization and quantification of the aliphatic hydrocarbon fraction during linseed development (*Linum usitatissimum* L.). *J Agr Food Chem* 57:5832–5836
- Giuffrè AM, Capocasale M (2016) *n*-Alkanes in tomato (*Solanum lycopersicum* L.) seed oil: the cultivar effect. *Int Food Res J* 23:979–985
- Tejeda JF, García C, Petró MJ, Andrés AI, Antequera T (2001) *n*-Alkane content of intramuscular lipids of Iberian fresh ham from different feeding systems and crossbreeding. *Meat Sci* 57:371–377
- Pétron MJ, Antequera T, Muriel E, Tejeda JF, Ventanas J (2004) Linear hydrocarbons content of intramuscular lipids of dry-cured Iberian ham. *Meat Sci* 66:295–300
- Shahidi F, Rubin LJ, D'Souza LA (1986) Meat flavour volatiles: a review of the composition technique of analysis and sensory evaluation. *CRC Crit Rev Food Sci* 24:219–227
- Tulliez JE, Bories GF (1975) Métabolisme des hydrocarbures paraffiniques et naphthéniques chez les animaux supérieurs. II. Accumulation et mobilisation chez le rat. *Annales de la Nutrition et de l'Alimentation* 29:213–221
- Mayer RW, Lamb CS (1984) The possible use of *n*-alkanes in herbage as indigestible faecal markers. *P Nutr Soc* 43:39A
- Neukon HP, Grob K, Biedermann M, Noti A (2002) Food contamination by C20–C50 mineral paraffins from the atmosphere. *Atmos Environ* 36:4839–4847

- 596 14. Grob K (2018) Mineral oil hydrocarbons in food: a review. *Food*
597 *Addit Contam A* 35:1845–1860. <https://doi.org/10.1080/19440>
598 [049.2018.1488185](https://doi.org/10.1080/19440049.2018.1488185)
- 599 15. Grob K, Bronz M (1994) Analytical problems in determining 3,5-
600 stigmastadiene and campestadiene in edible oils. *Riv Ital Sostanze*
601 *Gr* 71:291–295
- 602 16. Grob K, Artho A, Biedermann M, Egli J (1991) Food contamination
603 by hydrocarbons from lubricating oils and release agents:
604 determination by coupled LC-GC. *Food Addit Contam* 8:437–442
- 605 17. EFSA Panel on Contaminants in the food chain (CONTAM).
606 Scientific opinion on mineral oil hydrocarbons in food. *EFSA J*.
607 2012;10:2704.
- 608 18. Giuffrè AM (2013) Influence of cultivar and harvest year on
609 triglyceride composition of olive oils produced in Calabria
610 (Southern Italy). *Eur J Lipid Sci Tech* 115:928–934. [https://doi.](https://doi.org/10.1002/ejlt.201200390)
611 [org/10.1002/ejlt.201200390](https://doi.org/10.1002/ejlt.201200390)
- 612 19. Giuffrè AM (2013) Influence of harvest year and cultivar on wax
613 composition of olive oils. *Eur J Lipid Sci Technol* 115:549–555.
614 <https://doi.org/10.1002/ejlt.201200235>
- 615 20. Giuffrè AM, Louadj L (2013) Influence of crop season and cultivar
616 on sterol composition of monovarietal olive oils in Reggio
617 Calabria (Italy). *Czech J Food Sci* 31:256–263
- 618 21. Giuffrè AM (2014) The effects of cultivar and harvest year on the
619 fatty alcohol composition of olive oils from Southwest Calabria
620 (Italy). *Grasas Aceites* 65:e011. <https://doi.org/10.3989/gya.07391>
621 [3](https://doi.org/10.3989/gya.073913)
- 622 22. Consolidated Text on the characteristics of olive oil and
623 olive-residue oil and on the relevant methods of analysis.
624 01991R2568—IT—04.12.2016—031.005.
- 625 23. Koprivnjak O, Conte LS (1996) Caratterizzazione della frazione
626 idrocarburica e composizione degli acidi grassi degli oli d'oliva
627 vergini provenienti dalla zona di Pola (Croazia). *Riv Ital Sostanze*
628 *Gr* 73:317–320
- 629 24. Bortolomeazzi R, Berno P, Pizzale L, Conte LS (2001) Sesquit-
630 erpene, alkene and alkane hydrocarbons in virgin olive oils of
631 different varieties and geographical origins. *J Agr Food Chem*
632 49:3278–3283
- 633 25. Sakouhi F, Herchi W, Sbei K, Absalon C, Boukhchina S (2011)
634 Characterisation and accumulation of squalene and n-alkanes in
635 developing Tunisian *Olea europaea* L. fruits. *Int J Food Sci Tech*
636 46:2281–2286. <https://doi.org/10.1111/j.1365-2621.2011.02747>
637 [.x](https://doi.org/10.1111/j.1365-2621.2011.02747.x)
- 638 26. El Antari A, Hilal A, Boulouha B, El Moudni A (2000) Influence
639 of variety, environment and cultural techniques on the character-
640 istics of olive fruits and the chemical composition of extra virgin
641 olive oil of Morocco. *Olivae* 80:29–36
- 642 27. Mihailova A, Abbado D, Kelly SD, Pedentchouk N (2015) The
643 impact of environmental factors on molecular and stable isotope
644 compositions of n-alkanes in Mediterranean extra virgin olive
oils. *Food Chem* 173:114–121. <https://doi.org/10.1016/j.foodchem.2014.10.003> 645
- 646 28. Koprivnjak O, Moret S, Populin T, Lagazio C, Conte LS (2005)
647 Variety differentiation of virgin olive oil based on n-alkane pro-
648 file. *Food Chem* 90:603–608. <https://doi.org/10.1016/j.foodchem.2004.04.019> 649
- 650 29. Troya F, Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso
651 EF (2015) Classification of vegetable oils according to their
652 botanical origin using n-alkane profiles established by GC–MS.
653 *Food Chem* 167:36–39 654
- 655 30. Koprivnjak O, Procida G, Favretto L (1997) Determination of
656 endogenous aliphatic hydrocarbons of virgin olive oils of four
657 autochthonous cultivars from Krk Island (Croatia). *Food Technol*
658 35:125–131 659
- 660 31. Mihailova A, Abbado D, Pedentchouk N (2015) Differences in
661 n-alkane profiles between olives and olive leaves as potential indi-
662 cators for the assessment of olive leaf presence in virgin olive oils.
663 *Eur J Lipid Sci Technol* 117:1480–1485. [https://doi.org/10.1002/](https://doi.org/10.1002/ejlt.201400406)
664 [ejlt.201400406](https://doi.org/10.1002/ejlt.201400406) 665
- 666 32. Osorio Bueno E, Sánchez Casas J, Montaña García A, Gallardo
667 González L (2005) Discriminating power of the hydrocarbon
668 content from virgin olive oil of Extremadura cultivars. *J Am Oil*
669 *Chem Soc* 82:1–61 670
- 671 33. Gómez-Coca RB, del Carmen P-C, Moreda W (2016) Saturated
672 hydrocarbon content in olive fruits and crude olive pomace oils.
673 *Food Addit Contam A* 33:391–402. [https://doi.org/10.1080/19440](https://doi.org/10.1080/19440049.2015.1133934)
674 [049.2015.1133934](https://doi.org/10.1080/19440049.2015.1133934) 675
- 676 34. Eglinton G, Logan GA (1991) Molecular preservation. *Phil Trans*
677 *Roy Soc London B* 333:315–328 678
- 679 35. Bush RS, McInerney FA (2013) Leaf wax n-alkane distributions
680 in and across modern plants: implications for paleoecology and
681 chemotaxonomy. *Geochim Cosmochim Acta* 117:161–179 682
- 683 36. Pineda M, Rojas M, Gálvez-Valdivieso G, Aguilar M (2017) The
684 origin of aliphatic hydrocarbons in olive oil. *J Sci Food Agric*
685 97:4827–4834 686
- 687 37. Biedermann M, Grob K (2015) Comprehensive two-dimensional
688 gas chromatography for characterizing mineral oils in foods and
689 distinguishing them from synthetic hydrocarbons. *J Chromatogr*
690 *A* 1375:146–153. <https://doi.org/10.1016/j.chroma.2014.11.064> 691
- 692 38. Eglinton G, Hamilton RJ (1963) The distribution of alkanes. In:
693 Swain T (ed) *Chemical plant taxonomy*. Academic Press, London
694 156:1322–1335 695
- 696 39. Eglinton G, Hamilton RJ (1967) Leaf epicuticular waxes. *Science*
697 156:1322–1335 698

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. 699

Journal:	217
Article:	3604

Author Query Form

Please ensure you fill out your response to the queries raised below and return this form along with your corrections

Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

Query	Details Required	Author's Response
AQ1	Author names: Please confirm if the author names are presented accurately and in the correct sequence (given name, middle name/initial, family name). Given name: [Angelo Maria] Last name [Giuffrè].	
AQ2	Author details: Kindly check and confirm whether the corresponding author affiliation is correctly identified.	
AQ3	Affiliation: Kindly check and confirm the OD and ON details are correctly identified in Affiliation 1 and 2.	
AQ4	Figure: Figure 23 is cited in the manuscript; however corresponding figure and caption are not provided. Kindly check and advise us how to proceed here.	
AQ5	As References [13] and [38] are same, we have deleted the duplicate reference and renumbered accordingly. Please check and confirm	
AQ6	Reference: Kindly provide complete list of author names for the Ref. [17]	

Author Proof