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Abstract

Linear hydrocarbons such as *n*-alkanes and *n*-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 *n*-alkanes with odd-carbon chain number, seven *n*-alkanes with even-carbon chain number and three *n*-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 *n*-alkanes and *n*-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 *n*-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 *n*-alkanes and *n*-alkenes content.

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

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

Angelo Maria Giuffrè[1,2]

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Abstract

Linear hydrocarbons such as *n*-alkanes and *n*-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 *n*-alkanes with odd-carbon chain number, seven *n*-alkanes with even-carbon chain number and three *n*-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 *n*-alkanes and *n*-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 *n*-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 *n*-alkanes and *n*-alkenes content.

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

Introduction

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. Tejeda 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

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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, Nociara, 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 thirthy 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*, *Spilocaea 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 superrnatant (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.

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Gas chromatography

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: 60 °C (1 min isotherm), a 5 °C/min increase up to 290 °C (40 min isotherm). The detector temperature was set at 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.

Statistical analysis

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.

To calculate means and standard deviations of the 20 final replicates of each cultivar (2 replicates × five samplings × 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.

Results and discussion

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].

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 × 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 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.

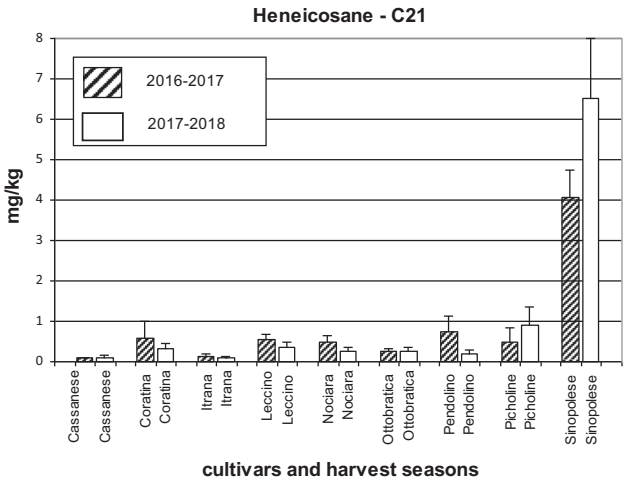

Fig. 1 Variation in the Heneicosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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 × 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). Sakohoui 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.

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

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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 *n*-alkanes (mg/kg)	Total odd-carbon chain *n*-alkanes (mg/kg)	Total even-carbon chain *n*-alkanes (mg/kg)	Total *n*-alkenes (mg/kg)	Total *n*-alkanes and *n*-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

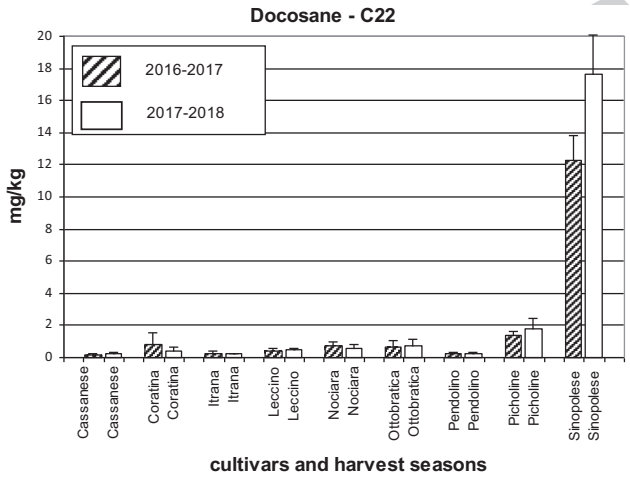

Fig. 2 Variation in the Docosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

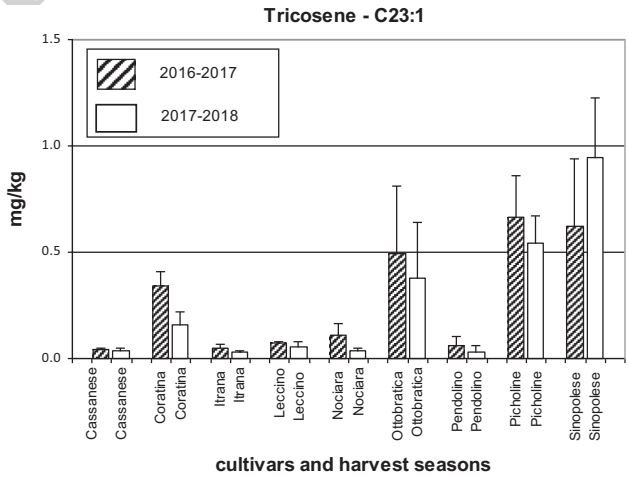

Fig. 3 Variation in the Tricosene content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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

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

C24:1 was the second detected *n*-alkene in the gas-chromatographic profile. The highest absolute quantities were found in 2017–2018 harvest season in the Sinopolese oil (0.94 mg/kg) and in 2016–2017 harvest season in the Picholine oil (0.68 mg/kg). Ottobratica produced an oil ranging between 0.53 and 0.45 mg/kg and Coratina remained above and below the 0.2 mg/kg level respectively in the 2016–2017 and in the 2017–2018. In all other cases was found a C24:1 content ≤ than 0.06 mg/kg of Nociara oil (2016–2017), (Fig. 5).

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

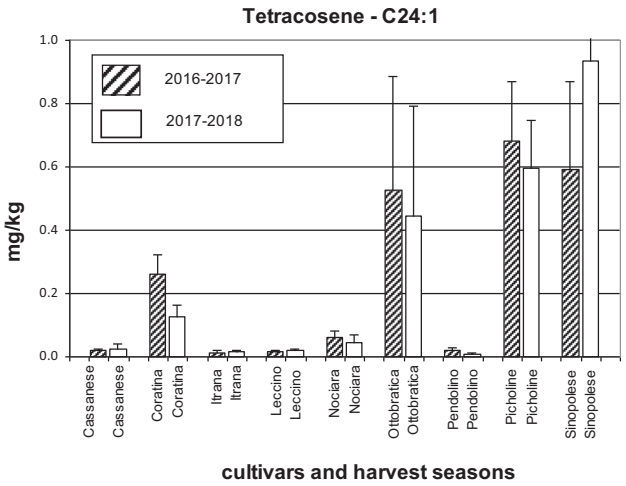

Fig. 5 Variation in the Tetracosene content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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

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

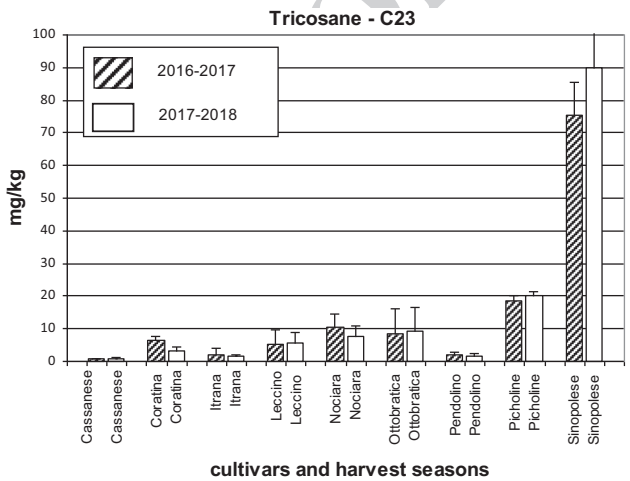

Fig. 4 Variation in the Tricosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

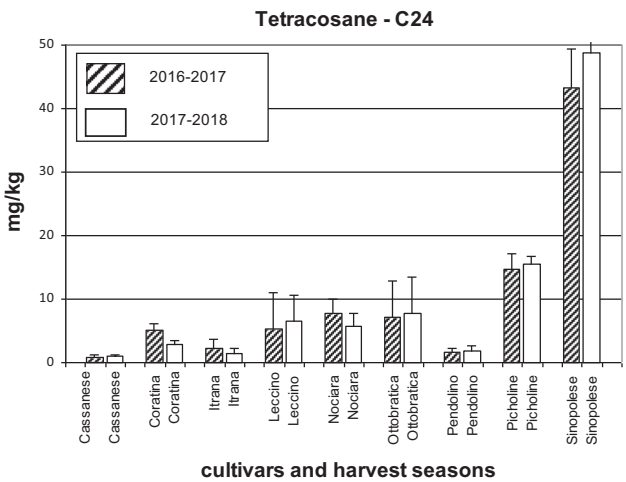

Fig. 6 Variation in the Tetracosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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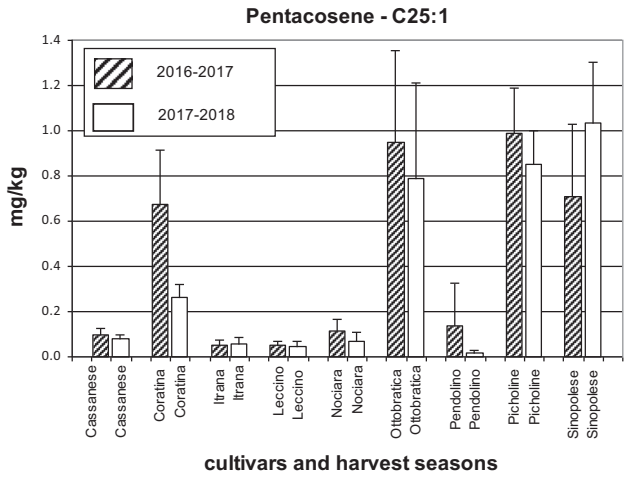

Fig. 7 Variation in the Pentacosene content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

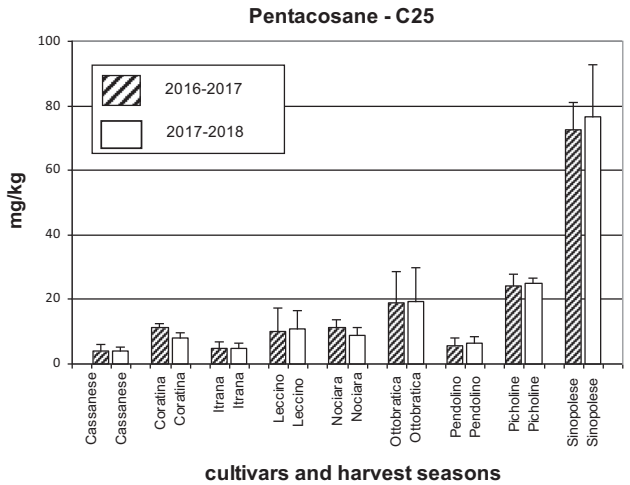

Fig. 8 Variation in the Pentacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

C25 was the second major *n*-alkane. The studied cultivars can be classified in 3 main groups: Cassanese, Coratina, Itrana, Leccino, Nociara and Pendolino oils showing less than 15 mg/kg; Ottobratica and Picholine oils ranging between 15 and 30 mg/kg and lastly Sinopolese oil accounting for more than 70 mg/kg (Fig. 8). Findings of other authors on Tunisian olive oil reported C25 as the major *n*-alkane which was quantified in 22.10 mg/kg [25]. El Antari et al. [26] found C25 as one of the predominant *n*-alkanes in Moroccan olive oil ranging between 4.30 and 10 mg/kg. Troya et al. [29] reported about the importance 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 harvest 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 content 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 conducted in olive oils produced in Morocco, C27 was quantified 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. Ottobratica 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).

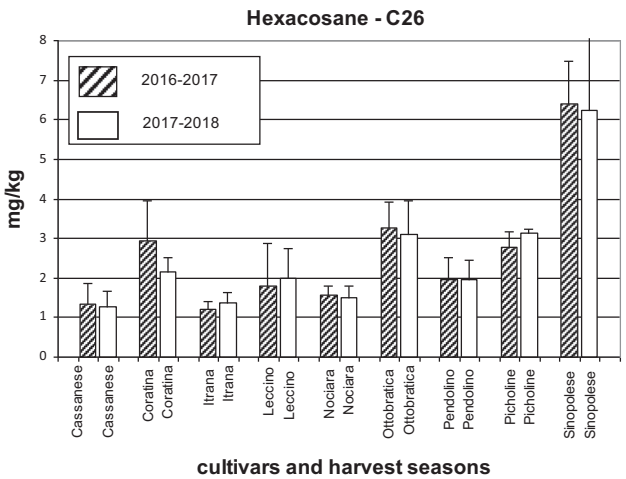

Fig. 9 Variation in the Hexacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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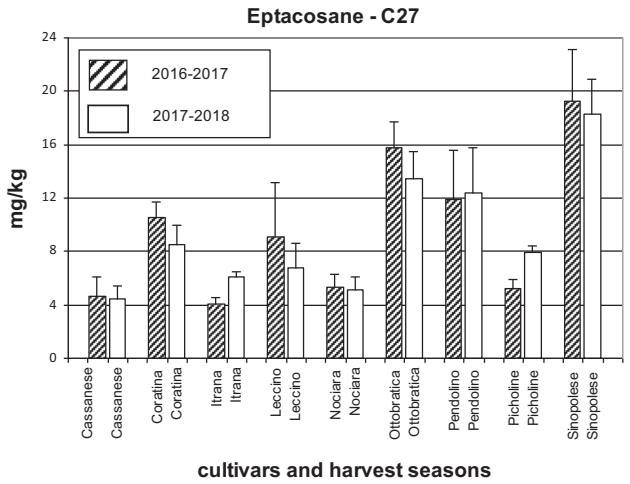

Fig. 10 Variation in the Eptacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

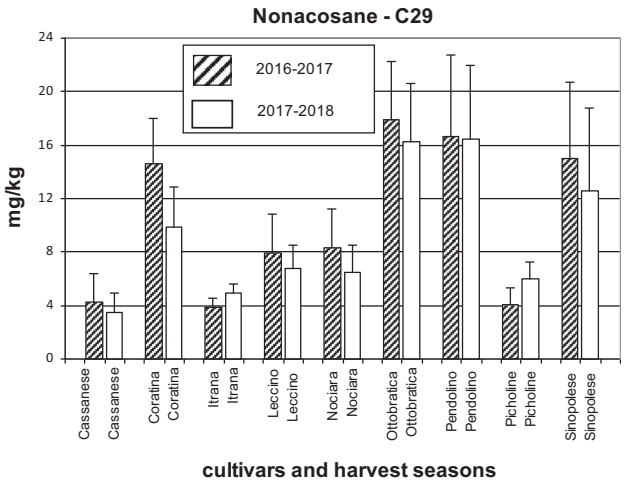

Fig. 12 Variation in the Nonacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

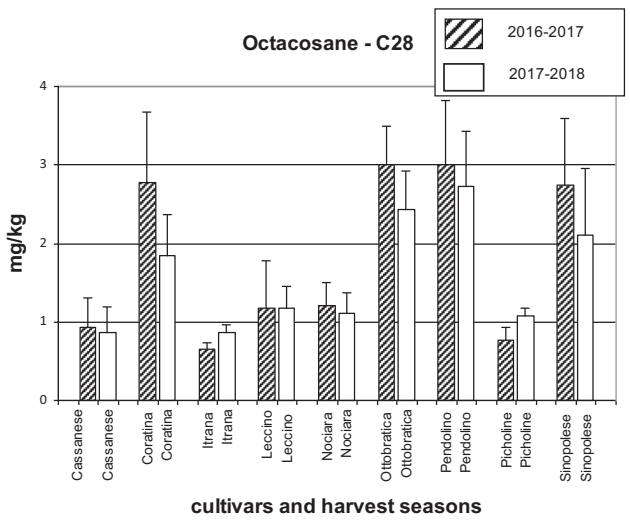

Fig. 11 Variation in the Octacosane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

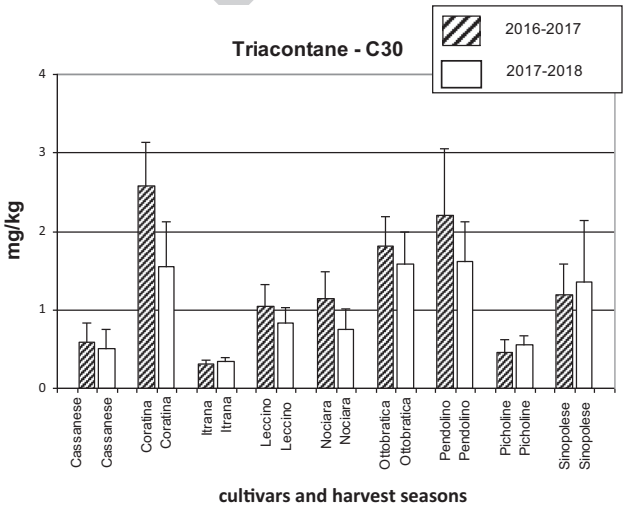

Fig. 13 Variation in the Triacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

C29 content was highest in the oil of the 2016–2017 harvest season for all cultivars except than Itrana and Picholine. By Coratina, Ottobratica, Pendolino and Sinopolese it was obtained an oil with a C29 content ranging between 9.84 mg/kg (Coratina 2017—2018) and 19.92 mg/kg (Ottobratica 2016–2017). The oil of all other cultivars in both the studied harvest seasons showed a C29 content ranging between 8.34 mg/kg (Nociara 2017–2018) and 3.51 mg/kg (Cassanese 2017—2018), (Fig. 12). Koprivnjak et al. [30] studied four olive oils of Croatian cultivars and found that C29 was the predominant linear hydrocarbon in the oil of Debela, Rosulja and Slatka cultivars whereas in the Naska oil prevailed C25.

C30 was higher in the 2016–2017 than in the 2017–2018 harvest season in the oil of almost all cultivars except for Itrana and Picholine (Fig. 13), following the same behaviour of C28 (Fig. 11). The highest C30 content was found in Coratina, Pendolino and Ottobratica (2.57, 2.20 and 1.89 mg/kg, respectively), whereas Itrana and Picholine showed the lowest values ranging between 0.32 mg/kg (Itrana, first harvest season) and 0.56 mg/kg (Picholine, second harvest season), (Fig. 13). Cultivar and the interaction cv × harvest season had a high significant ($p \leq 0.01$)

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

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

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

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

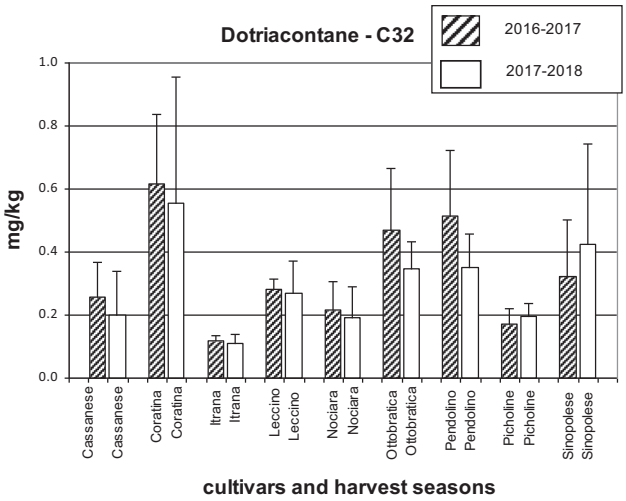

Fig. 15 Variation in the Dotriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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

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

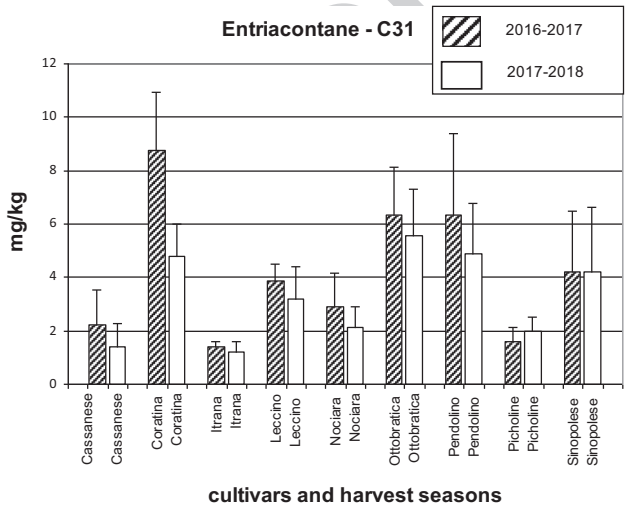

Fig. 14 Variation in the Entriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

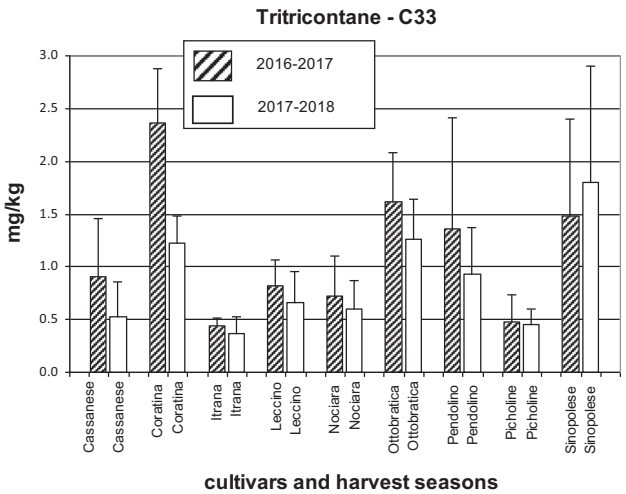

Fig. 16 Variation in the Tritriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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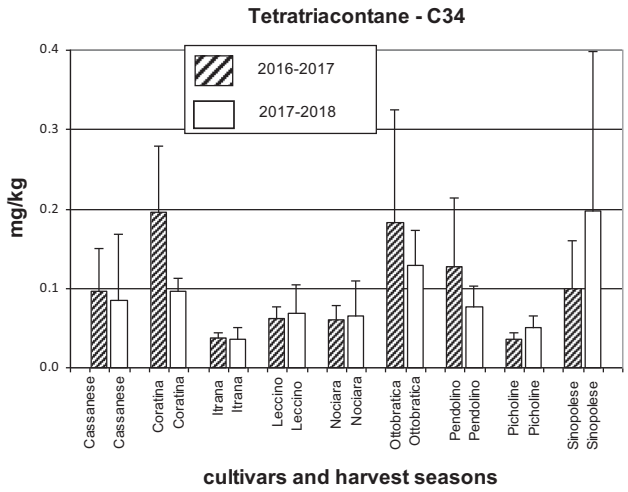

Fig. 17 Variation in the Tetratriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

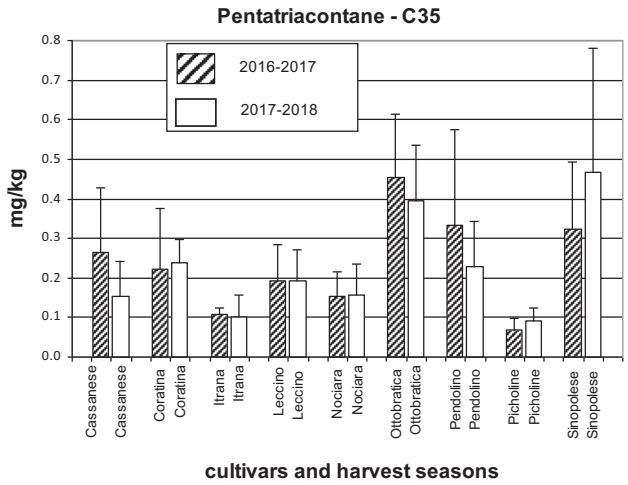

Fig. 18 Variation in the Pentatriacontane content for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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, Nociara 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.

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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 × 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 AQ4 analysis demonstrated that the total *n*-alkenes were influenced by cultivar ($p \leq 0.001$) and by the interaction cv × 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 × 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 × harvest season

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

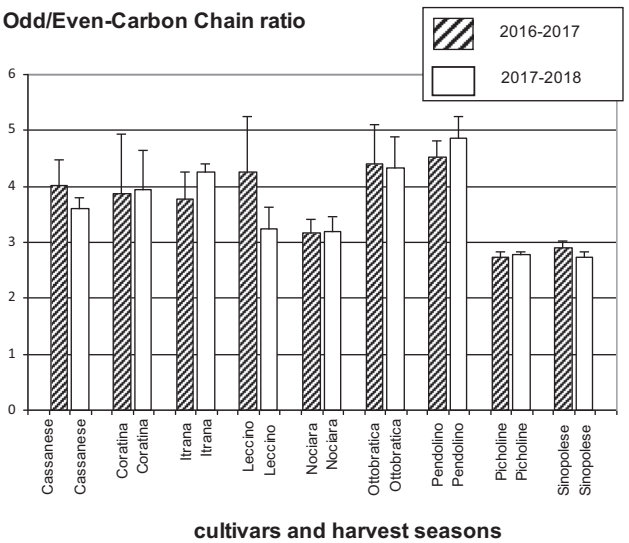

Fig. 19 Variation in the odd/even carbon chain *n*-alkane ratio for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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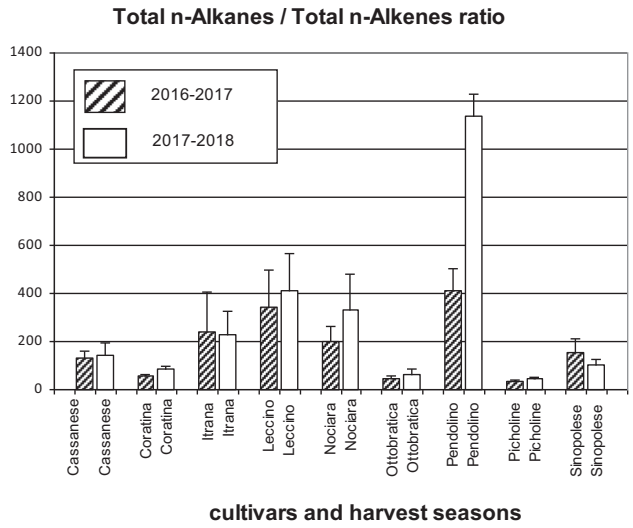

Fig. 20 Variation in the total *n*-alkanes/total *n*-alkenes ratio for two harvest seasons (2016–2017 and 2017–2018), in the oil of the studied cultivars. The values represent the means ± standard deviation

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 × 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 × 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.

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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

1. 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
2. Moreda W, Pérez-Camino MC, Cert A (2001) Gas and liquid chromatography of hydrocarbons in edible vegetable oils. J Chromatogr A 936:159–171
3. Tan YA, Kuntom A (1993) Gas chromatographic determination of hydrocarbons in crude pal kernel oil. J AOAC Int 76:371–376
4. Tan YA, Kuntom A (1994) Hydrocarbons in crude palm kernel oil. J AOAC Int 77:67–73
5. Giuffrè AM (2005) Changes in the n-alkane composition of avocado pulp oil (*Persea americana*, Miller) during fruit ripening. Grasas Aceites 56:75–78
6. 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
7. Giuffrè AM, Capocasale M (2016) *n*-Alkanes in tomato (*Solanum lycopersicum* L.) seed oil: the cultivar effect. Int Food Res J 23:979–985
8. Tejeda JF, García C, Petrón 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
9. 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
10. 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
11. Tulliez JE, Bories GF (1975) Métabolsme des hydrocarbures paraffiniques et naphténiques chez les animaux supérieurs. II. Accumulation et mobilisation chez le rat. Annales de la Nutrition et de l'Alimentation 29:213–221
12. Mayes RW, Lamb CS (1984) The possible use of *n*-alkanes in herbage as indigestible faecal markers. P Nutr Soc 43:39A
13. Neukon HP, Grob K, Biedermann M, Noti A (2002) Food contamination by C20–C50 mineral paraffins from the atmosphere. Atmos Environ 36:4839–4847

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14. Grob K (2018) Mineral oil hydrocarbons in food: a review. Food Addit Contam A 35:1845–1860. https://doi.org/10.1080/19440049.2018.1488185
15. Grob K, Bronz M (1994) Analytical problems in determining 3,5-stigmastadiene and campestadiene in edible oils. Riv Ital Sostanze Gr 71:291–295
16. Grob K, Artho A, Biedermann M, Egli J (1991) Food contamination by hydrocarbons from lubricating oils and release agents: determination by coupled LC-GC. Food Addit Contam 8:437–442
17. EFSA Panel on Contaminants in the food chain (CONTAM). Scientific opinion on mineral oil hydrocarbons in food. EFSA J. 2012;10:2704.
18. Giuffrè AM (2013) Influence of cultivar and harvest year on triglyceride composition of olive oils produced in Calabria (Southern Italy). Eur J Lipid Sci Tech 115:928–934. https://doi.org/10.1002/ejlt.201200390
19. Giuffrè AM (2013) Influence of harvest year and cultivar on wax composition of olive oils. Eur J Lipid Sci Technol 115:549–555. https://doi.org/10.1002/ejlt.201200235
20. Giuffrè AM, Louadj L (2013) Influence of crop season and cultivar on sterol composition of monovarietal olive oils in Reggio Calabria (Italy). Czech J Food Sci 31:256–263
21. Giuffrè AM (2014) The effects of cultivar and harvest year on the fatty alcohol composition of olive oils from Southwest Calabria (Italy). Grasas Aceites 65:e011. https://doi.org/10.3989/gya.073913
22. Consolidated Text on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. 01991R2568—IT—04.12.2016—031.005.
23. Koprivnjak O, Conte LS (1996) Caratterizzazione della frazione idrocarburica e composizione degli acidi grassi degli oli d'oliva vergini provenienti dalla zona di Pola (Croazia). Riv Ital Sostanze Gr 73:317–320
24. Bortolomeazzi R, Berno P, Pizzale L, Conte LS (2001) Sesquiterpene, alkene and alkane hydrocarbons in virgin olive ols of different varieties and geographical origins. J Agr Food Chem 49:3278–3283
25. Sakouhi F, Herchi W, Sbei K, Absalon C, Boukhchina S (2011) Characterisation and accumulation of squalene and n-alkanes in developing Tunisian *Olea europaea* L. fruits. Int J Food Sci Tech 46:2281–2286. https://doi.org/10.1111/j.1365-2621.2011.02747.x
26. El Antari A, Hilal A, Boulouha B, El Moudni A (2000) Influence of variety, environment and cultural techniques on the characteristics of olive fruits and the chemical composition of extra virgin olive oil of Morocco. Olivae 80:29–36
27. Mihailova A, Abbado D, Kelly SD, Pedentchouk N (2015) The impact of environmental factors on molecular and stable isotope 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
28. Koprivnjak O, Moret S, Populin T, Lagazio C, Conte LS (2005) Variety differentiation of virgin olive oil based on n-alkane profile. Food Chem 90:603–608. https://doi.org/10.1016/j.foodchem.2004.04.019
29. Troya F, Lerma-García MJ, Herrero-Martínez JM, Simó-Alfonso EF (2015) Classification of vegetable oils according to their botanical origin using *n*-alkane profiles established by GC–MS. Food Chem 167:36–39
30. Koprivnjak O, Procida G, Favretto L (1997) Determination of endogenous aliphatic hydrocarbons of virgin olive oils of four autochthonous cultivars from Krk Island (Croatia). Food Technol Biotech 35:125–131
31. Mihailova A, Abbado D, Pedentchouk N (2015) Differences in *n*-alkane profiles between olives and olive leaves as potential indicators for the assessment of olive leaf presence in virgin olive oils. Eur J Lipid Sci Technol 117:1480–1485. https://doi.org/10.1002/ejlt.201400406
32. Osorio Bueno E, Sánchez Casas J, Montaño García A, Gallardo González L (2005) Discriminating power of the hydrocarbon content from virgin olive oil of Extremadura cultivars. J Am Oil Chem Soc 82:1–61
33. Gómez-Coca RB, del Carmen P-C, Moreda W (2016) Saturated hydrocarbon content in olive fruits and crude olive pomace oils. Food Addit Contam A 33:391–402. https://doi.org/10.1080/19440049.2015.1133934
34. Eglinton G, Logan GA (1991) Molecular preservation. Phil Trans Roy Soc London B 333:315–328
35. Bush RS, McInerney FA (2013) Leaf wax n-alkane distributions in and across modern plants: implications for paleoecology and chemotaxonomy. Geochim Cosmochim Ac 117:161–179
36. Pineda M, Rojas M, Gálvez-Valdivieso G, Aguilar M (2017) The origin of aliphatic hydrocarbons in olive oil. J Sci Food Agric 97:4827–4834
37. Biedermann M, Grob K (2015) Comprehensive two-dimensional gas chromatography for characterizing mineral oils in foods and distinguishing them from synthetic hydrocarbons. J Chromatogr A 1375:146–153. https://doi.org/10.1016/j.chroma.2014.11.064
38. Eglinton G, Hamilton RJ (1963) The distribution of alkanes. In: Swain T (ed) Chemical plant taxonomy. Academic Press, London
39. Eglinton G, Hamilton RJ (1967) Leaf epicuticular waxes. Science 156:1322–1335

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