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original scientific paper

Determination of Endogenous Aliphatic Hydrocarbons of Virgin Olive Oils of Four Autochthonous Cultivars from Krk Island (Croatia)

Olivera Koprivnjak[1]*, *Giuseppe Procida*[2] *and Luciano Favretto*[2]

[1] Institute for Agriculture and Tourism – Poreč, University of Rijeka
C. Hugues 8, 52440 Poreč, Croatia

[2] Dipartimento di Economia e Merceologia delle Risorse Naturali e della Produzione, University of Trieste, via A. Valerio 6, 34127 Trieste, Italy

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Summary

Analytical processes that would enable simple and reliable control of food products protected by mark of origin have been increasingly studied. One of the series of parameters that can give the information about the origin of virgin olive oil is the composition of endogenous hydrocarbon fraction, which has been, according to the papers of some Spanish investigators, specific with respect to the cultivar of olive. In this paper the fraction of endogenous hydrocarbon of four autochthonous cultivars from Krk Island: Debela, Naška, Rosulja and Slatka, was determined. Cultivar Naška is noticeably different in regard of total content of alkanes (2.5 times higher than the other three cultivars), and it shows a specific profile of composition in which the components with 25, 23 and 24 carbon atoms in chain dominate. By testing the applied method a high recovery level of studied components (over 90%) and a good repeatability (the average of coefficient of variation for 15 components about 2.20%) were found.

Keywords: olive oil, aliphatic hydrocarbons, analytical methods

Introduction

The recent trends in the development of agriculture and food production are directed at the preservation of the typical products that are identifiable in regard of geographic origin. In this context, virgin olive oil represents a very interesting object of research. Besides precise knowledge of the parameters regarding the cultivars, the environment, the methods of cultivation and extraction of oil in a determined region, it is necessary to be acquainted with the quality and composition characteristics of oils that are produced in these circumstances. For these reasons, the search of analytical methods suitable for accurate characterisation of the oil origin is lively.

The most significant information of this kind comes from the unsaponifiable fraction. Recently, some papers in which the endogenous hydrocarbons of olive oil are the object of this type of research have been published *(1–4)*.

The endogenous aliphatic hydrocarbons are present in vegetable oils in rather small amounts; McGill *et al. (5)* reported that the range of this content is from 10 to 150 mg/kg. For this reason, in the same analytical methods by which this fraction is separated before gas-chromatographic analysis, a previous concentration by extraction of unsaponifiable part of oil is included *(2,6–9)*. Other authors performed a pre-separation combining the classical column chromatography and HPLC technique *(5,10)*. Grob *et al. (11)* carried out the analysis of hydrocarbons in vegetable oils and fats by automated on-line coupled LC-GC technique with loop-type interface.

In this paper, samples of virgin olive oils are analysed according by the method reported by Lanzón *et al. (2)*. We have evaluated this method by testing recovery and repeatability. The examined cultivars – Debela, Naška, Rosulja and Slatka are autochthonous varieties from Krk Island (Croatia). The quality characteristics of

* author to whom correspondence should be addressed

these oils, the composition of remaining part of unsaponifiable fraction and the sensory characteristics were studied by Procida *et al. (12)*, while in this paper we report the characteristics of endogenous aliphatic hydrocarbon fraction.

Materials and Methods

Preparation of Unsaponifiable Matter and Separation of Aliphatic Hydrocarbon Fraction

The preparation of unsaponifiable matter was carried out according to the official method of the European Community *(13)* – annex XVII, designed for the determination of stigmastadienes in vegetable oils.

The aliphatic hydrocarbon fraction was separated from unsaponifiable part by column chromatography on silica gel. The glass chromatography column (internal diameter 2.0 cm; length 50 cm) was prepared according to Lanzón *et al. (2)*. The flow rate of elution was maintained at 1 mL/min approximately. With a view to determine the volume of the fraction containing aliphatic hydrocarbons, we have collected 10 fractions of 10 mL each that were then analysed by gas chromatography.

Gas chromatography (GC) analyses were carried out on a Shimadzu GC 14A gas-chromatograph equipped with a split/splitless injector and a flame ionisation detector (FID) using a fused silica capillary column (30 m × 0.32 mm) coated with a 1.0 μm film of SPB 5 (Supelco). Integration of peak areas was performed by a Shimadzu C-R4A integrator. The chromatographic conditions were:

- temperature of the oven: 120 °C → (4 °C/min) → 280 °C (5 min) → (4 °C/min) → 305 °C (10 min);
- temperature of the injector and detector: 315 °C;
- split ratio: 1:20 (volume ratio);
- carrier gas (helium) flow rate: 2 mL/min

The peaks were identified on the basis of a comparison of their retention times with those of the available standards and with those reported in the bibliography *(1,2)*.

Evaluation of Recovery

With the aim of choosing an appropriate oil matrix for determination of recovery, we have analysed 5 samples of seed oil from market applying the method described above. On the basis of these results, we have chosen soybean oil, into which we have added 5 mg/kg of each of the following standards:

1-heptadecene (17:1), purity ≈ 99%, Fluka (Switzerland); n-eicosane (20:0), purity > 97%, Fluka (Switzerland); n-hexacosane (26:0), purity ≈ 99%, Sigma (USA); n-hexatriacontane (36:0), Sigma (USA).

The complete analysis had been repeated 5 times. For each single standard we have calculated the response factor and inserted it in the calculation of recovery. The recovered amounts were calculated by the following equation:

$$m_{\text{HYDRO.}} = \frac{m_{C_{20:0}}}{A_{C_{20:0}}} \times \frac{A_{\text{HYDRO.}}}{F_R}$$

$m_{\text{HYDRO.}}$= mass of single aliphatic hydrocarbon

$m_{C_{20:0}}$= mass of n-eicosane

$A_{\text{HYDRO.}}$ = peak area of single aliphatic hydrocarbon

$A_{C_{20:0}}$= peak area of n-eicosane

F_R = response factor of FID.

The recoveries were expressed as relative values with respect to the standard n-eicosane.

Evaluation of Repeatability of Method

The complete analysis of a sample of virgin olive oil was repeated 10 times. 6.25 mg/kg of n-eicosane was added as internal standard. The mean value, the standard deviation and the coefficient of variation were calculated for the total content of aliphatic hydrocarbon and for the composition of this fraction.

The repeatability of the method for six unidentified peaks in the part of chromatogram that belongs to the sesquiterpenes was evaluated on the basis of the ratio: peak area/n-eicosane area.

Preparation of Samples

The samples of virgin olive oil from four autochthonous and most commonly cultivated varieties in Krk Island were obtained by means of continued centrifugal system Pieralisi. Every one of these samples was analysed twice, adding 6.25 mg/kg of n-eicosane as internal standard.

Results and Discussion

The method applied in this paper includes a previous separation of the aliphatic hydrocarbon fraction from the other part of unsaponifiable matter by column chromatography on silica gel. The order of elution of components with a flow rate of hexane of 1 mL/min is given in Table 1. The aliphatic hydrocarbons were eluted from glass chromatography column from the tenth to fortieth mL of hexane, so we have decided to collect the first 50 mL of eluate.

The complete method was evaluated by carrying out a control of recovery and repeatability. For the evaluation of recovery it was suitable to dispose of an oil matrix with negligible amounts and with a narrow composition of aliphatic hydrocarbons. For this reason we have analysed five samples of seed oils from market: soybean oil, sunflower oil, grape seed oil, hazelnut oil *I* and hazelnut oil *II*.

Table 1. Order of elution of components from silica gel column

V(hexane)/mL	Components
10	/
20	aliphatic hydrocarbons
30	aliphatic hydrocarbons + peaks N° 1,2,3,4 *
40	aliphatic hydrocarbons + peaks N° 5 and 6 *
50	peaks N° 5 and 6 *
60	peaks N° 5 and 6 *
70	peaks N° 5 and 6 *
80	/
90	squalene
100	/

* peaks in the sesquiterpenes' part of gas-chromatogram trace

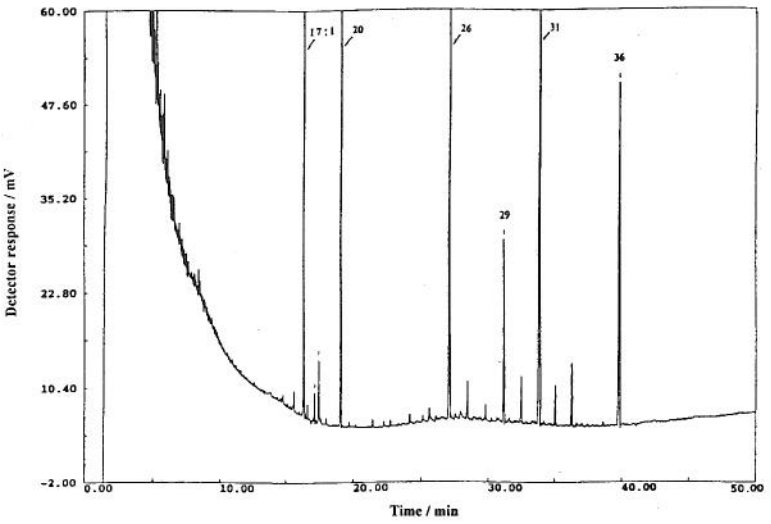

Fig. 1a. GC trace of aliphatic hydrocarbons: SOYA + STANDARDS

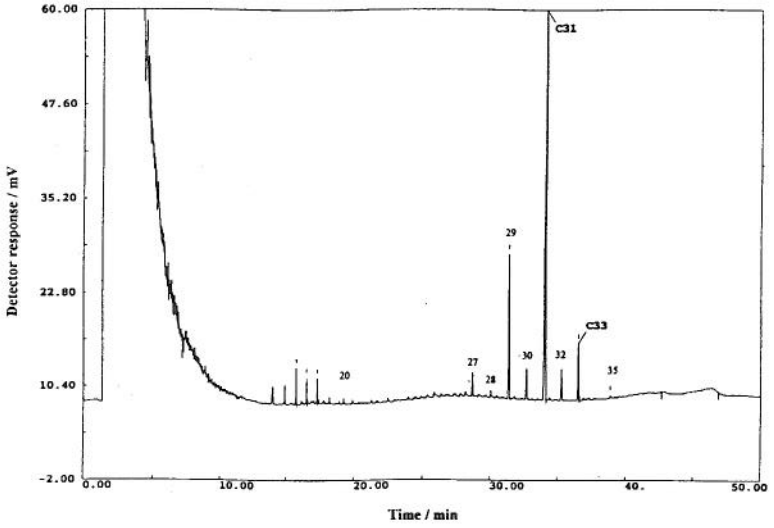

Fig. 1b. GC trace of aliphatic hydrocarbons: SOYBEAN OIL

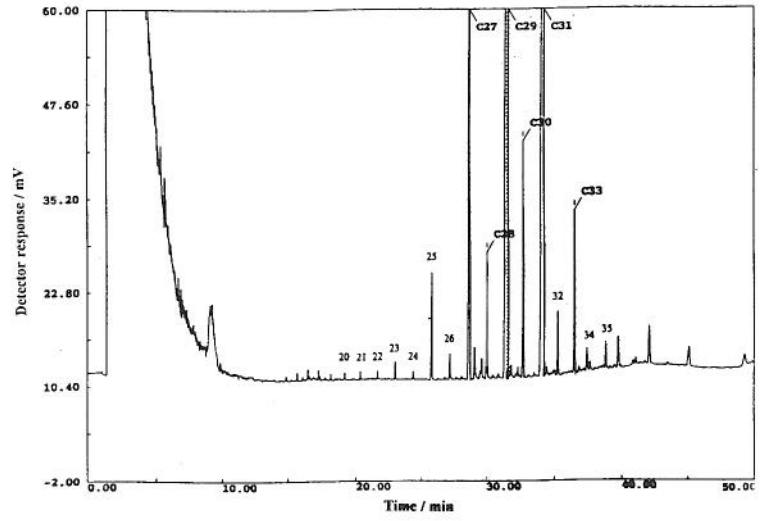

Fig. 1c. GC trace of aliphatic hydrocarbons: SUNFLOWER OIL

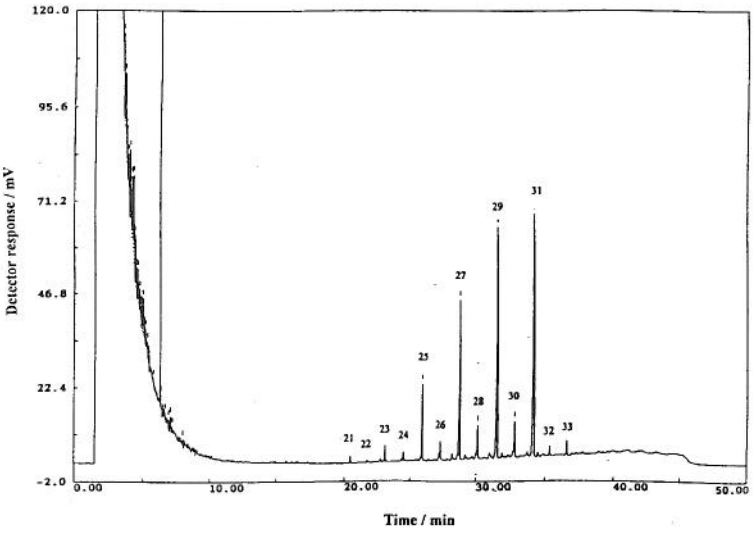

Fig. 1d. GC trace of aliphatic hydrocarbons: GRAPE SEED OIL

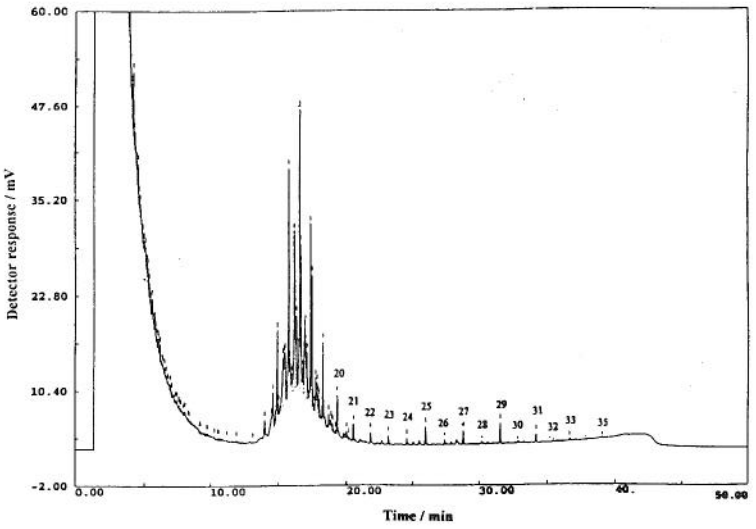

Fig. 1e. GC trace of aliphatic hydrocarbons: HAZELNUT OIL *I*

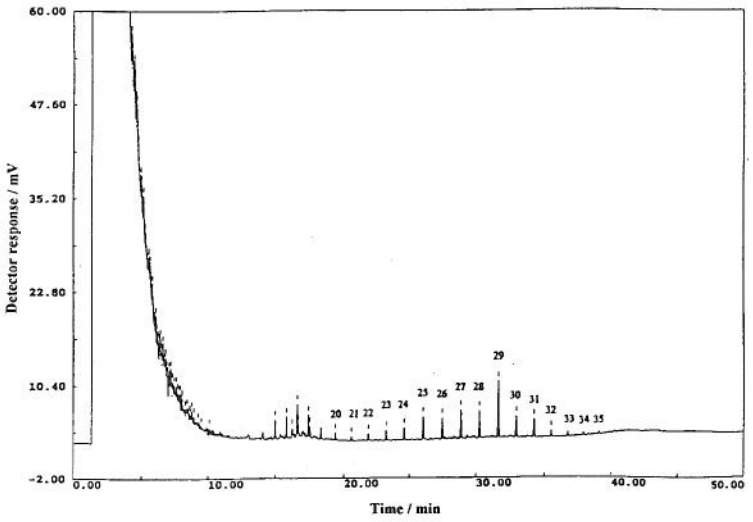

Fig. 1f. GC trace of aliphatic hydrocarbons: HAZELNUT OIL *II*

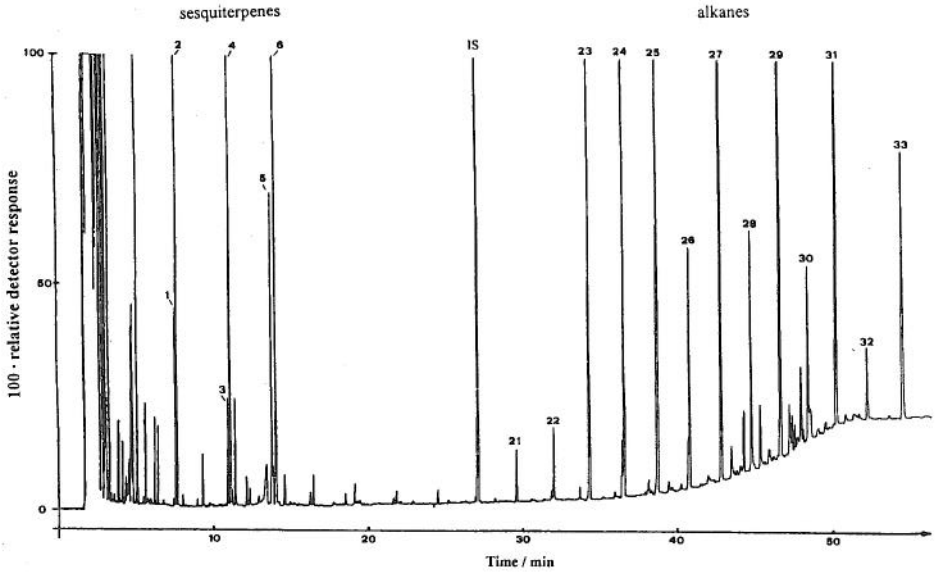

Fig. 1g. GC trace of aliphatic hydrocarbons: VIRGIN OLIVE OIL (Rosulja)

The GC traces of some of these oils and of the virgin olive oil from cultivar Rosulja are shown in Fig. 1a to 1g. Fig. 1e shows the GC trace of hazelnut oil *I* where »hump« characteristic for a contamination by mineral oil is visible *(11)*. This group of peaks of paraffins, unresolved by gas chromatography, is also present in the hazelnut oil *II* but in much lower amounts (Fig. 1f).

As it was expected, the content of aliphatic hydrocarbons in hazelnut oils was low, but all peaks in the range from $C_{20:0}$ to $C_{35:0}$ were present. Between the other three seed oils, the sample of soybean oil was more appropriate for the above mentioned purpose. The fraction of aliphatic hydrocarbons of this sample was concentrated into the range from $C_{27:0}$ to $C_{33:0}$. The standards for the recovery trials ($C_{17:1}$, $C_{26:0}$ and $C_{36:0}$) were chosen in a manner to cover the start, the middle and the end of the molecular mass range of the aliphatic hydrocarbon fraction present in virgin olive oils.

Table 2. shows the recovery data that present the mean values obtained from 5 replicates of a complete method. These values are over 90% for all of three standards, so we can conclude that the method enables to gain a high recovery level of studied components.

The data regarding the repeatability of the method are given in Table 3. The coefficient of variation for single components ranged between 0.31 and 6.79%, while

Table 2. Response of FID and recoveries

Hydrocarbon standard	Response factor	Recovery / % *
C17:1	1.00	101.4
C20:0	1.00	100.0
C26:0	1.12	91.5
C36:0	0.82	91.3

* mean of 5 analyses

Table 3. Repeatability of method – aliphatic hydrocarbons

Number of C atoms	Repetitions										Mean	St. dev.	CV / %
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10			
						w / %							
21	0.56	0.62	0.57	0.59	0.58	0.62	0.63	0.58	0.56	0.58	0.59	0.03	4.34
22	0.78	0.86	0.78	0.78	0.78	0.85	0.84	0.75	0.75	0.74	0.79	0.04	5.52
23	9.19	9.27	9.27	9.21	9.24	9.27	9.27	9.18	9.12	9.20	9.22	0.05	0.55
24	6.93	7.02	6.96	6.93	6.95	6.99	7.00	6.91	6.84	6.89	6.94	0.05	0.78
25	18.03	18.15	18.19	18.06	18.03	18.09	18.15	18.16	18.08	18.13	18.11	0.06	0.31
26	2.73	2.76	2.68	2.67	2.66	2.70	2.72	2.65	2.62	2.72	2.69	0.04	1.50
27	16.68	16.63	16.74	16.73	16.76	16.64	16.66	16.77	16.80	16.51	16.69	0.09	0.52
28	2.73	2.74	2.68	2.74	2.68	2.79	2.70	2.78	2.75	2.72	2.73	0.04	1.37
29	22.08	21.97	22.22	22.16	22.22	22.11	22.10	22.33	22.37	22.17	22.17	0.12	0.54
30	2.19	2.08	2.00	2.02	2.01	2.00	2.01	1.91	1.99	2.01	2.02	0.07	3.56
31	11.18	11.03	11.10	11.17	11.21	11.12	11.09	11.25	11.20	11.11	11.15	0.07	0.60
32	1.02	1.03	0.96	1.06	0.97	1.01	0.97	1.03	1.01	1.01	1.01	0.03	3.13
33	4.42	4.39	4.40	4.41	4.41	4.40	4.37	4.45	4.46	4.39	4.41	0.03	0.62
34	0.41	0.41	0.39	0.37	0.41	0.43	0.39	0.39	0.41	0.39	0.40	0.02	4.25
35	1.06	1.05	1.07	1.09	1.08	0.94	1.10	0.88	1.05	1.05	1.04	0.07	6.79
Σ (mg/kg)	46.10	46.15	46.71	46.02	46.30	46.58	46.28	46.75	46.62	46.35	46.39	0.26	0.57

Table 4. Repeatability of method – sesquiterpenes' part

Peak	Repetitions										Mean	St. dev.	CV / %
N°	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10			
	Peak area / n-eicosane peak area												
1	7.83	8.37	8.92	8.63	8.68	3.14	3.17	1.93	2.15	2.09	5.49	3.19	58.11
2	19.03	19.69	21.40	20.08	20.08	20.83	20.73	18.57	21.76	20.35	20.25	0.99	4.89
3	8.03	8.21	8.57	8.27	8.37	8.58	8.66	8.51	8.88	8.52	8.46	0.25	2.95
4	41.77	42.50	44.83	42.64	42.84	44.23	43.89	42.23	45.58	43.80	43.43	1.23	2.83
5	21.10	21.93	23.60	21.47	21.69	22.76	22.02	21.23	22.72	22.12	22.06	0.78	3.54
6	36.52	36.48	38.18	37.14	36.52	40.60	37.17	36.68	38.04	37.33	37.47	1.26	3.36
Σ	126.45	128.81	136.58	129.60	129.50	137.00	132.47	127.22	136.98	132.12	131.67	4.03	3.05

the average of the values for 15 components is 2.20%. Repeatability of the method changes for the worse when the components are present in low percentages (under 2.5% of total), but the coefficient of variation goes over the value of 5% only in two cases.

The researchers who have proposed this method *(2,4)* stated that substances eluted from the glass chromatography column together with the aliphatic hydrocarbons till 60 mL of hexane were sesquiterpenes. These substances have short retention time in the GC analysis and are placed on the first part of GC chromatogram. Despite the fact that in our case we have not eluted complete fraction of sesquiterpenes (it was collected the first 50 mL), we have considered interesting to evaluate the repeatability with the regard to this group of peaks. In this case the calculation was accomplished on the basis of the ratio: area single peak/ area n-eicosane peak; the results are shown in Table 4. A high coefficient of variation of peak N° 1 is observable, while those of the other components do not exceed 5%.

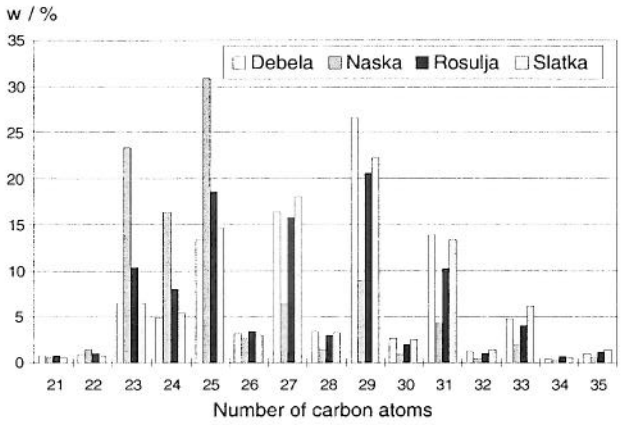

Fig. 2. Composition of endogenous aliphatic hydrocarbons of 4 cultivars

Fig. 2 represents the composition and content of aliphatic hydrocarbons in the samples of oils from Krk Island. The sample Naška has a total content of alkanes approximately 2.5 times higher than the other cultivars. Furthermore, this cultivar shows a noticeably different profile of composition. While for the other cultivars the main component in the composition of alkanes is $C_{29:0}$, the top of the composition profile of cultivar Naška is displaced toward alkanes with shorter chain – the main peaks are $C_{25:0}$, $C_{23:0}$ and $C_{24:0}$. In one and all of four cases, $C_{24:0}$ is the principal component with even number of carbon atom in a chain. Another particularity of cultivar Naška is the lowest sum of total of ratio: area peak sesquiterpene/ area peak n-eicosane. This sum total is from 2 to 5 times lower than the other three cultivars (Table 5). The comparison between the cultivars in regard of sesquiterpenes does not reveal any important differences, but this information must be taken with caution due to incomplete elution of these components discussed before.

Table 5. Peaks of sesquiterpenes of 4 cultivars on GC trace

Peak N°	Debela	Naška	Rosulja	Slatka
	Fraction of total area / %			
3	5.43	6.22	7.53	5.75
4	44.15	31.44	38.98	40.14
5	1.74	18.56	19.80	1.21
6	48.68	43.78	33.63	52.90
	Peak area / n-eicosane peak area			
Σ	83.30	22.52	111.42	73.72

Table 6. Main aliphatic hydrocarbons and total content – comparison of bibliography data

Cultivar	Main peaks	Reference	w / mg kg^{-1}
Arbequina	C27:0, C25:0, C29:0		36–60
Cornicabra	C29:0, C27:0, C31:0	Guinda	26–43
Empeltre	C25:0, C23:0, C24:0	*et al.* (3)	72–92
Hojiblanca	C27:0, C29:0, C25:0		20–53
Picual	C27:0, C29:0, C25:0		18–31
Leccino	C25:0, C27:0, C29:0		62
Bianchera	C25:0, C29:0, C27:0	Koprivnjak	48
Carbonazza	C25:0, C27:0, C23:0	*et al.* (4)	47
Busa	C29:0, C27:0, C31:0		40
Debela	C29:0, C27:0, C31:0		31
Naška	C25:0, C23:0, C24:0		104
Rosulja	C29:0, C25:0, C27:0		42
Slatka	C29:0, C27:0, C25:0		40

Conclusions

On the basis of these preliminary results, it is possible to conclude that the cultivar Naška has a particular composition of aliphatic hydrocarbon fraction, not only in comparison with the other three cultivars from Krk

Island, but also against the bibliographic data regarding the cultivars of different origin (Table 6) *(3,4)*. With regard to the endogenous aliphatic hydrocarbon fraction, only the Spanish cultivar Empeltre is similar to Naška. This cultivar has also high value of total content and the main peaks are also $C_{25:0}$, $C_{23:0}$ and $C_{24:0}$. With the aim of proposing an indicator of variety origin of olive oils from Krk Island, it will be interesting to verify in what measure these values depend on the ripeness of olives, and how they are influenced by meteorological variation in different years.

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Određivanje endogenih alifatskih ugljikovodika u prirodnom maslinovom ulju četiriju autohtonih sorti s otoka Krka

Sažetak

U posljednje se vrijeme sve više istražuju analitički postupci kojima bi se jednostavno i pouzdano kontrolirali prehrambeni proizvodi zaštićeni oznakom podrijetla. Sastav frakcije endogenih ugljikovodika, prema radovima nekih španjolskih istraživača, specifičan je s obzirom na sortu masline, čime ujedno daje informaciju o podrijetlu prirodnog maslinovog ulja. U radu je određena frakcija endogenih ugljikovodika četiriju autohtonih sorti maslina s otoka Krka: Debela, Naška, Rosulja i Slatka. Sorta Naška bitno se razlikuje od ostalih triju sorti po 2,5 puta većoj ukupnoj količini alkana, te po specifičnom sastavu u kojem prevladavaju alkani s 25, 23 i 24 ugljikova atoma u lancu. Ispitujući primijenjenu metodu utvrđen je visok stupanj točnosti za proučavane sastojke (preko 90%), te dobra ponovljivost (srednja je vrijednost koeficijenta varijacije za 15 sastojaka 2,20%).