

# Differences in Hydrocarbons of Virgin Olive Oils Obtained from Several Olive Varieties

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An analytical method has been developed to study, as a whole, the hydrocarbon fraction—other than squalene—of virgin olive oils. This method was applied to the study of oils from representative olive varieties of Spanish olive groves: Arbequina, Cornicabra, Empeltre, Hojiblanca, and Picual. The composition profiles of some of the hydrocarbons of oils from the above mentioned varieties are shown in this paper. The ranges of concentration in milligram per kilogram of oil of these hydrocarbons are also shown. Significant differences in the content of some hydrocarbons of the five varieties analyzed were observed. These quantitative differences were used to distinguish between virgin olive oils from different olive varieties.

**Keywords:** *Virgin olive oil; hydrocarbons; olive varieties*

## INTRODUCTION

The hydrocarbons of the unsaponifiable fraction of olive oil have been studied by different authors. Eisner *et al.* (1965) stated that squalene makes up around 85–90% of the hydrocarbon fraction and established the presence of various homologous series of normal and iso- and/or antiisohydrocarbons and multiple branched chains in the range C16–C36. Capella *et al.* (1963) detected only one series of *n*-paraffins in the range C13–C30, in which alkanes with an odd number of carbon atoms predominated over those with an even number. Jacini and Fedeli (1975) concluded that this fraction is formed of normal-chain paraffins, together with small amounts of iso and probably anti-iso isomers and with olefins. Bastic *et al.* (1978), studying a sample of unrefined olive oil, reported that squalene makes up more than 90% of the hydrocarbon fraction, the rest being mainly isoprenoidal polyolefins and *n*-paraffins, and concluded that the composition of hydrocarbon fractions of vegetable oils showed differences which could be used for their characterization. McGill *et al.* (1993) suggested that the most significant *n*-alkanes in the olive oil were C23, C25, and C27. Lanzón *et al.* (1994) detected in numerous Spanish virgin olive oils 6,10-dimethyl-1-undecene, various sesquiterpenes, the series of *n*-alkanes from C14 to C35, *n*-8-heptadecene, and squalene; *n*-9-alkenes have also been found in oils from the olives of the Arbequina variety. We have developed an analytical procedure for characterizing the hydrocarbon fractions from different olive varieties. This procedure is based upon fractionation and quantification of hydrocarbons in virgin oils.

## MATERIALS AND METHODS

**Natural Material.** Industrial samples of extra virgin olive oils were obtained by means of horizontal centrifugation from different mills in several Spanish olive grove zones. The olives were harvested ripe. The number, origin, and varieties of olive oil samples analyzed are given in Table 1. The five varieties of olives tested represent 80% of Spanish virgin olive oil production.

**Reagents.** Hexane (alkane mixture), ACS reagent, bp 65–75 °C, and ethanol (96%, v/v) were distilled through a

**Table 1. Numbers, Origin, and Varieties of Olive Oil Samples Analyzed**

variety	no. of samples <sup>a</sup>	origin	
		province	country/town
Arbequina	2	Lérida	Borjas Blancas
	2		Sarroca
	2		Mayals
	2		Albages
	2		Llardecans
Cornicabra	2	Toledo	Mora
	2		Navahermosa
	2		Belvis de la Jara
	2		Los Navalmorales
	2		El Romeral
Empeltre	2	Teruel	Alcañiz
	2		Alcoriza
	2		Calenda
	2		La Fresneda
	2		Valjunquera
Hojiblanca	2	Málaga	Antequera
	2		Archidona
	2	Córdoba	Lucena
	2		Cabra
	2		Puente Genil
Picual	2	Jaen	Bailén
	2		Lopera
	2		Porcuna
	2		Arjona
	2		Menjibar

<sup>a</sup> Ten samples of each variety (five samples from two crops from each Spanish olive grove).

fractionation column. Silica gel 60 was used for column chromatography (90–230 mesh, Merck ref 7754; Dasmittadl, Germany). A standard solution of 0.125 mg/mL *n*-eicosane (Sigma, St. Louis, MO; >99% as tested by gas chromatography in hexane) was utilized. All other materials were analytical grade.

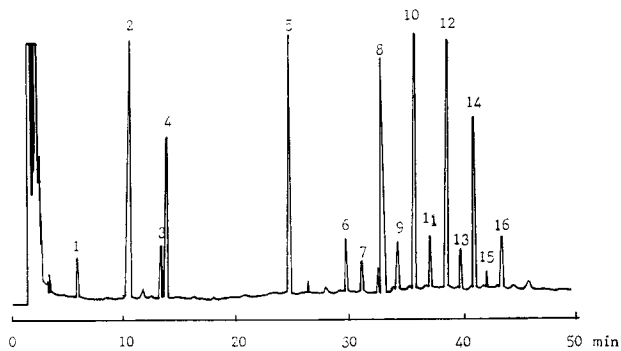
**Apparatus.** All GC analyses of hydrocarbons were performed with a HP-5890 (Hewlett-Packard, Avondale, PA) gas chromatograph equipped with a flame ionization detector and a wide bore capillary column of borosilicate glass [0.75 mm i.d. × 30 m long, coated with a 1 μm film thickness of liquid phase SPB-1 (chemically bonded methylsilicone)]. An injector for packed columns was used with an adapter for wide bore capillary columns. Data were collected and processed with an integrating recorder (HP-3390A).

**Extraction of the Unsaponifiable Matter.** To 20 g of oil was added 1 mL of the standard solution of *n*-eicosane. The

**Table 2. Percentage Distribution of Hydrocarbons in the First Fraction of 60 mL Eluted with Hexane of Samples Analyzed**

	Arbequina		Cornicabra		Empeltre		Hojiblanca		Picual	
	mean <sup>a</sup>	SD <sup>b</sup>	mean	SD	mean	SD	mean	SD	mean	SD
Terpenics										
C13:1 <sup>c</sup>	0.4	0.2	8.0	1.5	0.2	0.2	2.2	0.8	17.0	2.9
$\alpha$ -copaene	1.3	0.5	2.0	0.3	0.4	0.2	11.6	1.8	0.7	0.2
eremophylene	0.5	0.3	1.3	0.4	0.1	0.1	3.7	2.9	2.4	2.4
muurolene	0.3	0.1	0.2	0.2	0.1	0.1	5.2	1.9	traces	
<i>n</i> -Alkanes, <i>n</i> -Alkenes										
C17:1 <sup>d</sup>	0.1	0.1	0.9	0.1	0.1	0.1	0.2	0.2	0.8	0.2
C21	0.9	0.1	0.6	0.3	0.3	0.1	0.7	0.3	1.2	0.2
C22	1.1	0.1	traces		1.0	0.2	1.0	0.4	traces	
C23:1 <sup>e</sup>	1.7	0.4	traces		traces		traces		traces	
C23	9.4	0.9	3.2	0.4	17.7	1.2	2.9	0.3	5.1	0.7
C24:1 <sup>e</sup>	1.5	0.4	traces		traces		traces		traces	
C24	5.9	0.6	2.5	0.4	15.7	0.9	2.2	0.3	2.3	0.3
C25:1 <sup>e</sup>	1.7	0.2	traces		0.3	0.1	0.6	0.2	0.4	0.4
C25	18.1	0.8	11.8	1.3	31.0	1.1	10.7	0.5	12.1	1.0
C26	3.3	0.2	3.5	0.5	3.6	0.2	2.8	0.3	2.9	0.2
C27	21.3	0.8	15.9	1.6	11.0	0.8	19.9	1.6	16.4	1.3
C28	3.3	0.6	3.2	0.3	1.8	0.2	3.9	1.2	3.7	0.9
C29	15.3	0.8	19.4	0.6	9.8	0.7	16.6	2.0	13.3	0.9
C30	1.6	0.3	2.4	0.3	0.9	0.2	2.0	0.2	2.0	0.2
C31	7.8	0.6	14.0	1.5	3.8	0.5	8.5	1.4	11.4	2.0
C32	0.7	0.1	1.6	0.2	0.3	0.1	0.9	0.1	1.2	0.5
C33	2.9	0.5	7.1	1.0	1.5	0.2	3.3	0.6	5.4	1.7
C34	0.3	0.1	0.6	0.2	0.1	0.1	0.3	0.4	0.4	0.3
C35	0.6	0.1	1.8	0.3	0.3	0.2	0.8	0.3	1.3	0.5

<sup>a</sup> Mean of 10 samples. <sup>b</sup> standard deviation. <sup>c</sup> 6,10-Dimethyl-1-undecene. <sup>d</sup> Double bond in the 8 position. <sup>e</sup> Double bond in the 9 position.



**Figure 1.** Gas chromatogram of the first hydrocarbon fraction of virgin olive oil from Hojiblanca olive variety. For peak identification, see Table 4.

mixture was saponified for 30 min with 75 mL of 10% ethanolic potassium hydroxide. The solution was passed to a 500 mL decanting funnel, 100 mL distilled water was added, and the mixture was extracted twice with 100 mL portions of hexane. If any emulsion appeared, the solution was left for a short time to allow it to break up. The hexane solution was evaporated to dryness in a rotary evaporator at 30 °C under reduced pressure.

**Isolation of Hydrocarbons.** The hydrocarbons were separated from other components by column chromatography (column 1.5 cm i.d. and 50 cm long, with a Teflon stopcock, and filled with 15 g of silica gel slurred in hexane). The total unsaponifiable matter (0.2 g approximately) was dissolved in approximately 1 mL of hexane, and the solution was introduced onto the column head. The flask was rinsed two or three times with hexane, approximately 1 mL each time. These rinsings were added to the column head, waiting for the previous addition to pass completely onto the column. The sample was then eluted with hexane at a rate of about 1 mL/min.

Two fractions of 60 and 75 mL were collected and then concentrated to approximately 1 mL in the rotary evaporator at 30 °C under reduced pressure. Each was analyzed separately by gas chromatography.

**Gas Chromatography.** Nitrogen was used as carrier gas at a flow rate of 14 mL/min. The initial oven temperature was 110 °C for 60 min and then was programmed to rise at 5 °C/min to 300 °C; injector temperature, 300 °C; detector temperature, 320 °C. The components were identified in an earlier paper by Lanzon *et al.* (1994). The first fraction of 60 mL was quantified with *n*-eicosane as internal standard. This standard was used to quantify the terpenic hydrocarbons by assuming the same response factor. Although this is not absolutely accurate, it is acceptable enough for the purpose of this work. To quantify the components of the second fraction, 1 mL of the *n*-eicosane solution was added to each. This hydrocarbon was chosen as standard after checking that its concentration in the original chromatogram was negligible.

## RESULTS AND DISCUSSION

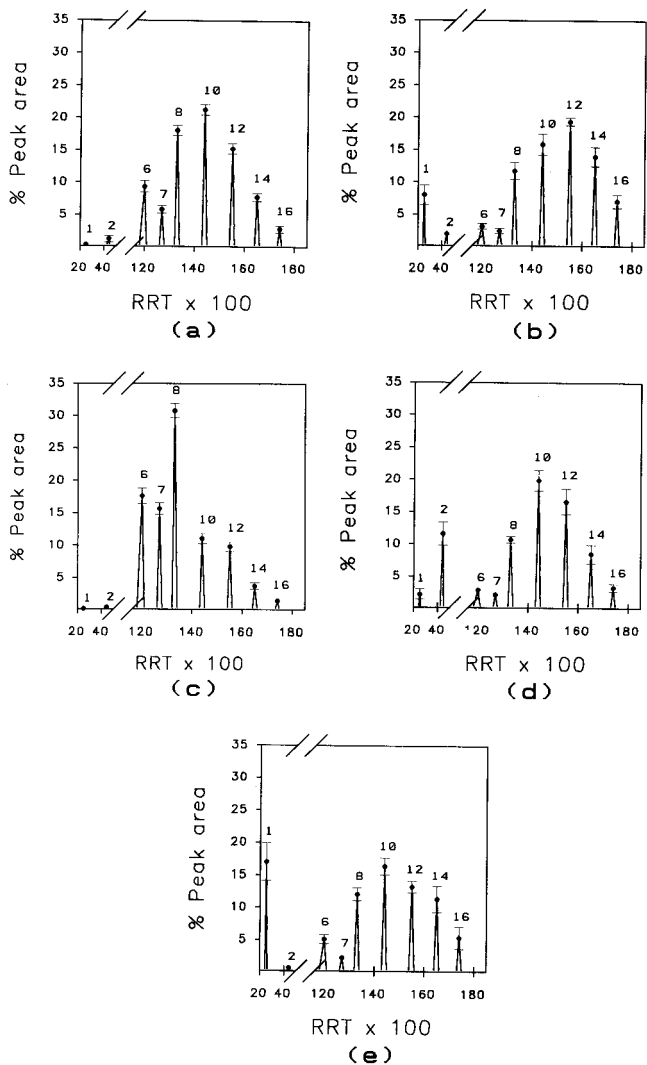
The first fractions eluted from the silica gel column of 10 samples of each of the five studied varieties were obtained, and each was analyzed by gas chromatography. Figure 1 shows the chromatogram of one of them, corresponding to a virgin olive oil from the Hojiblanca variety. In the first fraction the main hydrocarbons that appeared in the chromatogram were 6,10-dimethyl-1-undecene, cyclic sesquiterpenes, and saturated and unsaturated hydrocarbons between C17 and C35.

The hydrocarbon content measurements were determined from mean values of the 10 samples (five samples from each Spanish olive grove and from two crops). Table 2 shows the percentage distribution of *n*-alkanes and *n*-alkenes, as well as the terpenic hydrocarbons in the first fraction from the 50 virgin olive oils analyzed and their corresponding standard deviations. Hydrocarbons are omitted if they appeared to be present in only trace levels in all varieties. The 9-tricosene and 9-tetracosene were only found at significant concentrations in the Arbequina variety. In Table 2 significant ( $p \leq 0.05$ ) differences in the contents of some hydrocar-

**Table 3. Concentration Ranges of Terpenes, *n*-Alkanes, and *n*-Alkenes Found in 50 Samples of Virgin Olive Oils from Five Spanish Olive Varieties**

hydrocarbons	Arbequina (mg/kg)	Cornicabra (mg/kg)	Empeltre (mg/kg)	Hojiblanca (mg/kg)	Pical (mg/kg)
C13:1 <sup>a</sup>	nd-0.4	2.2-3.7	0.1-0.3	0.2-1.0	4.4-6.1
cyclic sesquiterpenes <sup>b</sup>	0.9-1.5	0.8-1.8	0.2-0.9	2.7-8.3	0.1-2.4
$\alpha$ -farnesene	0.3-2.7	0.7-5.2	0.2-1.4	1.8-11.7	8.8-32.6
C17:1 <sup>c</sup>	nd-0.2	0.3-0.4	nd-0.2	nd-0.2	0.2-0.3
<i>n</i> -alkenes <sup>d</sup>	1.2-3.4	nd	0.1-0.5	0.1-0.6	nd-0.3
<i>n</i> -alkanes (even carbon number)	6.1-9.5	4.1-6.2	16.8-21.0	3.4-8.7	3.0-4.9
<i>n</i> -alkanes (odd carbon number)	29.0-47.0	21.6-36.8	55.2-70.1	16.1-43.3	14.8-25.4

<sup>a</sup> 6,10-Dimethyl-1-undecene. <sup>b</sup>  $\alpha$ -Copaene, eremophylene, and muurolene. <sup>c</sup> 8-heptadecene. <sup>d</sup> Double bond in the 9 position: C23:1, C24:1, and C25:1. nd, not detected.



**Figure 2.** Hydrocarbon composition profiles (only some of the first fraction) of virgin olive oils from five olive varieties: (a) Arbequina, (b) Cornicabra, (c) Empeltre, (d) Hojiblanca, and (e) Pical; RRT, relative retention time. For peak identification, see Table 4.

bons of the first fraction can be observed. Of all the hydrocarbons with an odd number of carbon atoms in the chain, heptacosane in the Arbequina and Pical varieties, pentacosane in the Empeltre variety, and nonacosane in the Hojiblanca variety were the major compounds. Of the hydrocarbons with an even carbon number, octacosane in the Cornicabra, Hojiblanca, and Pical varieties and tetracosane in the Arbequina and Empeltre varieties were the major components. Of the cyclic sesquiterpenes,  $\alpha$ -copaene was only a minor

**Table 4. Hydrocarbons, Peak Number, and Relative Retention Time (RRT) of Compounds Reported in Figures 1 and 2**

peak no.	hydrocarbon	RRT
1	6,10-dimethyl-1-undecene	0.25
2	$\alpha$ -copaene	0.44
3	eremophylene <sup>a</sup>	0.55
4	muurolene <sup>a</sup>	0.56
5	eicosane <sup>b</sup>	1.00
6	tricosane	1.20
7	tetracosane	1.27
8	pentacosane	1.33
9	hexacosane	1.39
10	heptacosane	1.44
11	octacosane	1.50
12	nonacosane	1.55
13	triacontane	1.60
14	hentriacontane	1.65
15	dotriacontane	1.70
16	tritriacontane	1.74

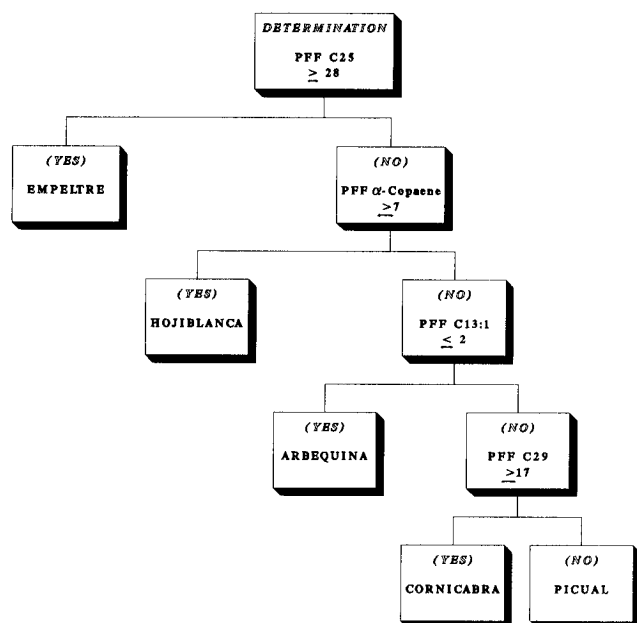
<sup>a</sup> Tentative assignment. <sup>b</sup> Internal standard.

compound of the hydrocarbons present in the Pical variety. In all the varieties studied, the total content of hydrocarbons with an odd number of carbons was higher than the total content of the hydrocarbons with an even carbon number (Table 2).

The values corresponding to the hydrocarbons 6,10-dimethyl-1-undecene,  $\alpha$ -copaene, tricosane, tetracosane, pentacosane, heptacosane, nonacosane, hentriacontane, and tritriacontane are those that present the greatest differences among the varieties studied.

The composition profiles of the five oils studied are shown in Figure 2. These profiles indicate mean values and their confidence limits. The peaks that appear in Figure 2 are identified in Table 4. It can be observed that each variety has a characteristic profile.

The only significant component that appears in the chromatogram of the second fraction eluted from the silica gel column for all samples analyzed was  $\alpha$ -farnesene. No chromatograms of the second fractions obtained are shown in this study. The concentration means for  $\alpha$ -farnesene are shown in Table 3. This table also shows the absolute concentration (mg/kg of oil) of several hydrocarbon groups of the 50 oils analyzed. Some of these concentrations are evidently different. The minimum concentration of total hydrocarbons, excluding squalene, in the Empeltre variety is higher than the maximum concentrations in all the other varieties. The Hojiblanca variety possesses a concentration greater than that of all the cyclic sesquiterpenes. In addition, the Arbequina variety concentration in *n*-alkenes is 10 times higher than the others. Finally the *n*-alkanes odd carbon number concentration is always higher than that of the *n*-alkanes even carbon number.



**Figure 3.** Diagram for easy identification of virgin olive oils from several olive varieties; PFF, percentage first fraction.

## CONCLUSIONS

The virgin olive oils from Arbequina, Cornicabra, Empeltre, Hojiblanca, and Picual varieties could be differentiated by studying their hydrocarbon fraction. Differences were apparent regardless of the grove from which the olives were obtained. Figure 3 presents a simple, general scheme of the process of identification of the five varieties studied.

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