# Sesquiterpene, Alkene, and Alkane Hydrocarbons in Virgin Olive Oils of Different Varieties and Geographical Origins

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The hydrocarbon fraction of 30 virgin olive oils was analyzed, focusing in particular on the sesquiterpenes. The oil samples were of different geographical origins and obtained from different olive varieties. The hydrocarbon fraction was isolated by silica gel column chromatography of the unsaponifiable fraction of the oils. The sesquiterpene hydrocarbons were then fractionated, on the basis of their degree of unsaturation, by $AgNO_3$ TLC and silica gel $AgNO_3$ column chromatography. The composition of the sesquiterpenes was more complex than previously reported. Among the 31 sesquiterpenes detected, 24 have been tentatively identified, by comparison of the linear retention indices on two capillary columns of different polarities and mass spectra with those reported in the literature. The total concentration of the sesquiterpenes in the oils analyzed ranged from about 2 to 37 ppm. Among the sesquiterpenes the more abundant were α-farnesene, α-copaene, eremophyllene, and α-muurolene. The alkenes present in the hydrocarbon fraction were isolated by TLC $AgNO_3$ and characterized by GC-MS of their dimethyl disulfide derivatives. The series of *n*-$\Delta^9$-alkenes from $C_{22}$ to $C_{27}$, 8-heptadecene, and 6,10-dimethyl-1-undecene were detected. Among the *n*-alkanes, those with an odd number of carbon atoms predominated in all of the analyzed oils, the most common being $C_{23}$, $C_{25}$, $C_{27}$, and $C_{29}$. The concentration of the *n*-alkenes ranged from about 0.5 to 2 ppm, whereas for the *n*-alkanes the range was from 30 to 177 ppm.

**Keywords:** *Virgin olive oil; hydrocarbons; sesquiterpenes; alkanes; alkenes*

## INTRODUCTION

The unsaponifiable fraction of virgin olive oils represents ∼1−2% of the oil. Some components of the unsaponifiable fraction, such as sterols, terpenic alcohols, and tocopherols, have been extensively studied due to their importance both for characterizing and for assessing the quality of the oils. On the contrary, hydrocarbons have attracted little attention, probably because of their supposed minor importance for the characterization of the oils.

Capella et al. (*1*) reported the presence in olive oils, besides squalene, of *n*-alkanes in the range of $C_{11}$−$C_{30}$ and the possible presence of *n*-alkenes. Eisner et al. (*2*) found that squalene, a triterpenoid hydrocarbon, represents ∼80−90% of the hydrocarbon fraction, the rest being composed of a homologous series of normal chain and possibly iso, anteiso, and multiply branched hydrocarbons in the range of $C_{16}$−$C_{36}$. Jacini et al. (*3*) reported the presence of squalene and the series of *n*-alkanes from $C_{11}$ to $C_{30}$, iso and probably anteiso from $C_{13}$ to $C_{21}$. Bastic et al. (*4*) reported the presence, in virgin olive oils, of *n*-paraffins, isoprenoidal polyolefins, and squalene, with the latter compounds comprising >90% of the hydrocarbon fraction. The same authors suggested, moreover, that the compositions of the hydrocarbon fractions of the oils could be used for their characterization. McGill et al. (*5*) analyzed the hydrocarbon fraction of different oils in order to detect any eventual contamination by hydrocarbons from lubricating oils. They reported that in olive oils the most significant *n*-alkanes were $C_{23}$, $C_{25}$, and $C_{27}$ and suggested that the *n*-alkane compositions could be used to characterize specific plant seed oil. More recently, Lanzon et al. (*6*) studied extensively the hydrocarbon components of Spanish virgin olive oils. They also identified, besides the series of *n*-alkanes from $C_{14}$ to $C_{35}$ and squalene, two alkenes, 6,10-dimethyl-1-undecene and *n*-8-heptadecene. In oils obtained from olives of the Arbequina variety they also found the *n*-$\Delta^9$-alkenes from $C_{22}$ to $C_{25}$. The same authors tentatively identified seven sesquiterpene hydrocarbons in Spanish oils obtained from different olive varieties, namely, α-copaene, calarene, eremophyllene, muurolene, (*E*),(*E*)-α-farnesene, (*Z*)2,(*E*)4,(*E*)6-allofarnesene, and (*E*)2,(*E*)4,(*E*)6-allofarnesene. Koprivnjak et al. (*7*) used the hydrocarbon compositions to characterize virgin olive oils from Istria (Croatia), reporting the presence also in these oils of the *n*-alkenes from $C_{23}$ to $C_{25}$. Guinda et al. (*8*) used the differences in hydrocarbon compositions to distinguish virgin olive oils from different Spanish olive varieties.

In this work, the hydrocarbon fraction and in particular the sesquiterpenes of virgin olive oils from different olive varieties and from different geographical regions were studied by means of argentation thin-layer chromatography ($AgNO_3$-TLC), silica gel $AgNO_3$ column chromatography, capillary gas chromatography, and gas chromatography−mass spectrometry (GC-MS).

## MATERIALS AND METHODS

**Materials and Reagents.** All solvents and reagents were of analytical grade. Silica gel 60, 70−230 mesh ASTM, and the TLC silica gel plates, 0.25 mm thickness, were provided by Merck (Bracco, Milan, Italy). The silica gel was equilibrated with 2% (w/w) of water before use. Valencene standard was purchased from Extrasynthese (Genay, France). Hydrocarbon

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**Table 1. Sample Number, Origin, and Variety of Virgin Olive Oils**

| sample | origin | variety |
|---|---|---|
| 1 | Abruzzo (Italy) | Leccino |
| 2 | Abruzzo (Italy) | Leccino |
| 3 | Puglia (Italy) | Coratina |
| 4 | Puglia (Italy) | Coratina |
| 5 | Puglia (Italy) | Cima di Mola |
| 6 | Puglia (Italy) | Oleurola G. |
| 7 | Puglia (Italy) | Paranzana |
| 8 | Puglia (Italy) | Coratina |
| 9 | Romagna (Italy) | Ghiacciola |
| 10 | Romagna (Italy) | Nostrana di Brisighella |
| 11 | Veneto (Italy) | Grignano |
| 12 | Veneto (Italy) | Favarol |
| 13 | Veneto (Italy) | Radar |
| 14 | Veneto (Italy) | Leccino |
| 15 | Veneto (Italy) | Pendolino |
| 16 | Veneto (Italy) | Frantoio |
| 17 | Spain | Arbequina |
| 18 | Spain | Arbequina |
| 19 | Spain | Arbequina |
| 20 | Spain | Picual |
| 21 | Spain | vu[a] |
| 22 | Greece | vu |
| 23 | Greece | vu |
| 24 | Greece | vu |
| 25 | Greece | vu |
| 26 | Greece | Koroneiki |
| 27 | Greece | Hatinolia |
| 28 | Crete (Greece) | Koroneiki |
| 29 | Tunisia | Koroneiki |
| 30 | Tunisia | vu |

[a] vu, variety unknown; these oils could also be mixtures of oils of different varieties.

mixture from *n*-$C_8$ to *n*-$C_{32}$, "retention index standard", the hydrocarbon standards *n*-$C_{33}$, *n*-$C_{34}$, *n*-$C_{35}$, and *n*-$C_{36}$, and palladium 10% (w/w) on activated carbon and dimethyl disulfide (DMDS) were obtained from Sigma (Sigma-Aldrich, Milan, Italy). *n*-Eicosane was from Fluka (Buchs, Switzerland).

**Oil Samples.** Samples of virgin olive oils (30), the origin and variety of which are reported in Table 1, have been analyzed: 16 samples were from Italy; 5 samples were from Spain; 7 samples were from Greece; and 2 samples were from Tunisia.

**Isolation of the Hydrocarbon Fraction.** The oil (20 g), after the addition of 1 mL of the internal standard (*n*-eicosane) solution (0.120 mg/mL), was saponified with a 10% ethanolic potassium hydroxide solution, and the unsaponifiable fraction was extracted with two 100 mL aliquots of *n*-hexane according to the method of Guinda et al. (*8*). The hydrocarbon fraction was then isolated from the unsaponifiable matter by column chromatography (40 cm × 1.5 cm i.d.). The column was filled with 15 g of silica gel previously equilibrated with 2% (w/w) of water. The mobile phase was *n*-hexane at a flow rate of ~1 mL/min, and the first fraction of 100 mL was collected. The solvent was evaporated under a nitrogen stream, and the sample was redissolved in ~0.5 mL of *n*-hexane prior to the GC-MS analysis. To check for the presence of interfering hydrocarbon in the solvent, a blank (without the unsaponifiable fraction) column chromatography was carried out using the same procedure. The GC-MS analysis did not show any interfering peak.

**Fractionation of the Hydrocarbons.** Ten hydrocarbon fractions were combined in order to have a larger amount of alkenes and sesquiterpenes. The alkenes were separated from the hydrocarbon fraction by $AgNO_3$ TLC. The $AgNO_3$ TLC plate was prepared by dipping the plate for 1 min in a mixture of 10% (w/v) $AgNO_3$/water solution and ethyl alcohol in 1:1 (v/v) ratio. The plate was then dried in an oven at 70 °C for 20 min. The elution was carried out with *n*-hexane, and the bands were visualized under UV light (254 nm) after spraying with a 0.2% (w/v) ethanolic solution of 2,7-dichlorofluorescein. The bands were scraped off and extracted with $CH_2Cl_2$.

The sesquiterpenes were separated from the hydrocarbon fraction and fractionated on the basis of their degree of unsaturation by $AgNO_3$ TLC and silica gel $AgNO_3$ column chromatography. The silica gel $AgNO_3$ for the column chromatography was prepared by suspending 10 g of silica gel in 20 mL of a 10% (w/v) $AgNO_3$/water solution. The silica gel was then dried in an oven at 70 °C, and 5 g was used to prepare the column (30 cm × 1 cm i.d.). The mobile phase was *n*-hexane, and 10 fractions of 2 mL each were collected.

**Preparation of the DMDS Derivatives of the Alkenes.** The DMDS derivatives of the alkenes were prepared according to the method of Carlson et al. (*9*). The alkenes isolated from the $AgNO_3$ TLC were dissolved in 200 μL of *n*-hexane, and then 200 μL of neat DMDS and 100 μL of a solution of iodine in diethyl ether (60 mg/mL) were added. The mixture was held for 4 h or overnight at 40 °C. After cooling, the mixture was diluted with 0.5 mL of *n*-hexane and treated with a 0.5% (w/v) solution of sodium thiosulfate until the iodine color disappeared. The organic phase was separated and dried over anhydrous sodium sulfate, and then the solvent was evaporated under a nitrogen stream and the sample was redissolved in ~0.5 mL of *n*-hexane prior to the GC-MS analysis.

**GC and GC-MS.** A Carlo Erba Mega 5160 gas chromatograph equipped with an on-column injector and a flame ionization detector was used. The fused silica capillary column was an SPB5 (5% phenyl-methylpolysiloxane), 30 m × 0.32 mm i.d., 0.25 μm film thickness (Supelco, Bellefonte, PA). The column temperature was programmed at 60 °C for 1 min, then to 120 °C at a rate of 3 °C/min, and finally to 300 °C at 7 °C/min. The detector temperature was 300 °C and the carrier gas (helium) flow rate 1.3 mL/min. About 0.5−1 μL of sample solution was injected.

For the GC-MS analysis a Varian 3400 gas chromatograph coupled to a Varian Saturn ion trap detector was used. Two fused silica capillary columns were used: an apolar column DB-5 (5% phenyl-methylpolysiloxane), 30 m × 0.25 mm i.d., 0.25 μm film thickness (J&W, Folsom, CA) and a polar column Carbowax 20 M, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Supelco). The temperature for the apolar column was programmed at 60 °C for 3 min and then to 300 °C at a rate of 5 °C/min. The temperature for the polar column was programmed at 60 °C for 3 min, then to 160 °C at a rate of 4 °C/min, and finally to 280 °C at 10 °C/min. The injection was in splitless mode with helium as carrier gas at a flow rate of 1 mL/min. The injector, transfer line, and ion trap temperatures were, respectively 300, 300, and 170 °C for the apolar column and 280, 280, and 170 °C for the polar column. The electron impact (70 eV) spectra were recorded at 1 s/scan with a filament emission current of 10 μA. About 0.5−1 μL of sample solution was injected.

## RESULTS AND DISCUSSION

In the first 100 mL of *n*-hexane eluate from the silica gel column of the unsaponifiable of oils were found the *n*-alkane, the *n*-alkene, and the sesquiterpene hydrocarbons. After these compounds, squalene, which was not considered in this research, eluted. The GC-MS chromatogram (apolar column) of the first 100 mL fraction of a virgin olive oil is shown in Figure 1.

Quantitative analysis was carried out by the internal standard (I.S.) method using *n*-eicosane as I.S. and assuming a relative response factor of 1 with respect to all of the hydrocarbons at the FID detector (*6*). The repeatability of the method was assessed by analyzing the same oil sample 10 times. The mean and the relative standard deviation (RSD) of the most abundant sesquiterpenes and the other hydrocarbons are reported in Table 2.

**Sesquiterpenes.** The GC-MS chromatograms, on the apolar and polar columns, of the hydrocarbon fraction of a virgin olive oil relative to the elution range of sesquiterpenes are shown in Figures 2 and 3, respec-

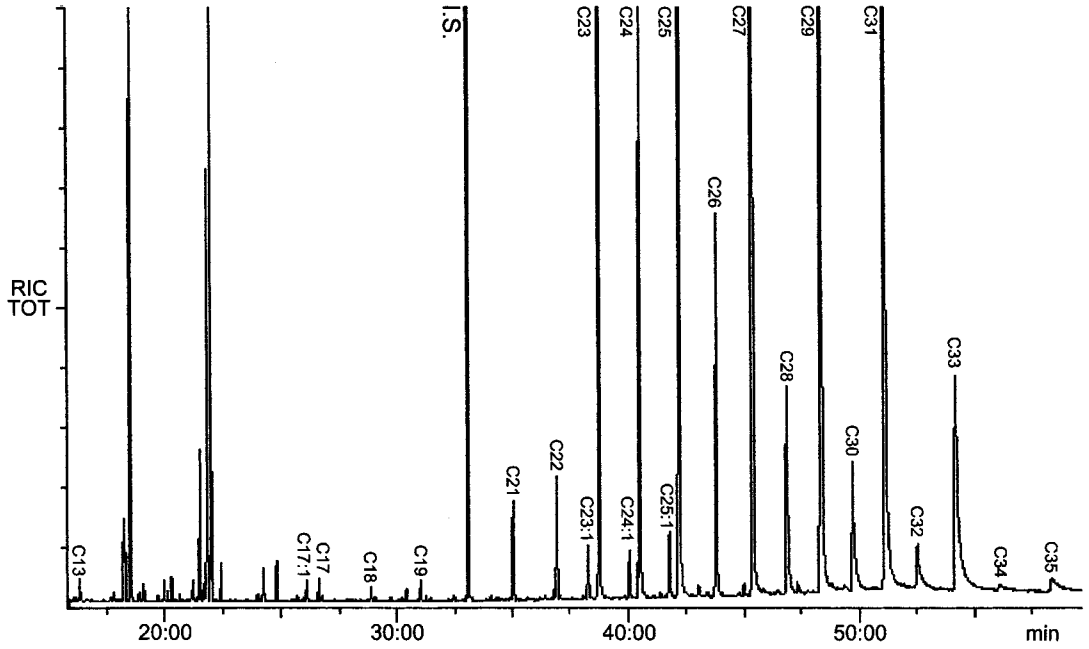


**Figure 1.** GC-MS chromatogram (apolar column) of the hydrocarbon fraction of a virgin olive oil. Internal standard was *n*-eicosane.

**Table 2. Repeatability Results of the Method**

| hydrocarbon | mean (ppm) | RSD (%) |
|---|---|---|
| α-copaene | 0.3 | 8.3 |
| eremophyllene | 0.4 | 7.0 |
| α-muurolene | 0.1 | 5.4 |
| α-farnesene | 3.8 | 7.4 |
| 6,10-dimethyl-1-undecene | 0.2 | 19.3 |
| C17:1 | 0.1 | 9.3 |
| C21 | 0.5 | 3.5 |
| C22 | 0.6 | 3.5 |
| C23:1 | 0.6 | 3.2 |
| C23 | 8.2 | 3.2 |
| C24:1 | 0.7 | 3.3 |
| C24 | 5.0 | 4.0 |
| C25:1 | 0.7 | 4.7 |
| C25 | 11.8 | 3.7 |
| C26 | 1.5 | 4.4 |
| C27 | 11.7 | 4.1 |
| C28 | 1.3 | 4.9 |
| C29 | 7.3 | 4.4 |
| C30 | 0.8 | 4.3 |
| C31 | 4.0 | 4.5 |
| C32 | 0.4 | 4.1 |
| C33 | 2.0 | 4.5 |
| C34 | 0.2 | 8.2 |
| C35 | 0.6 | 5.4 |

tively. The sesquiterpenes, molecular formula $C_{15}H_{24}$, were eluted between the *n*-alkanes $C_{13}$ and $C_{17}$. Among the 31 sesquiterpenes detected, 24 have been tentatively identified as reported in Table 3. The identification was based on the matching of mass spectra of the compounds with the reference mass spectra of two libraries (NIST90 and WILEY5) available with the software of the mass spectrometer and of a library specific for the analysis of essential oils, which contained reference spectra of many terpenoid compounds (*10*). This library reported also the linear retention indexes on an SE-52 type column. Because many sesquiterpenes have very similar mass spectra, the identification was also supported by comparison of the linear retention indexes, RI (*25*), calculated on two capillary columns of different polarities, with those reported in the literature. The results are reported in Table 3. Besides the mass spectral data and the retention indexes, some essential oils were used as standards for identification purpose. The sesquiterpenes, together with terpenes and their oxygenated derivatives, comprise the major part of the essential oils for which the composition has been extensively studied.

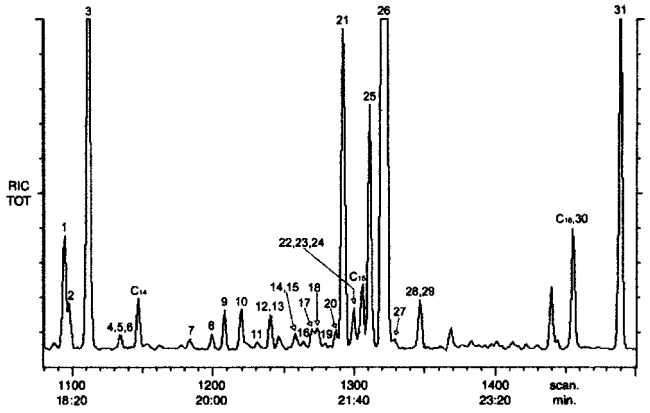


**Figure 2.** GC-MS chromatogram (apolar column) of the hydrocarbon fraction relative to the elution range of sesquiterpenes. For peak identification see Table 3.

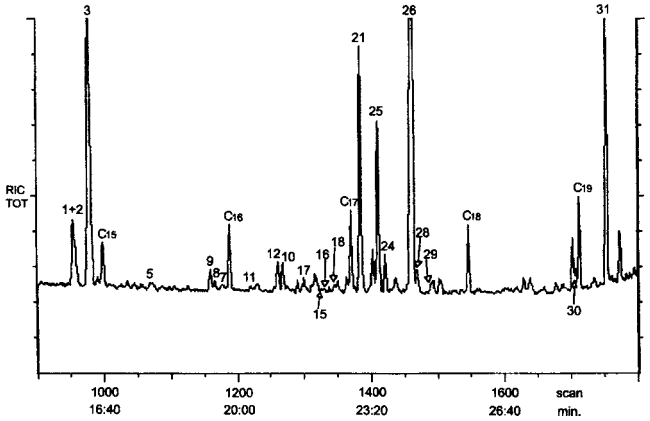


**Figure 3.** GC-MS chromatogram (polar column) of the hydrocarbon fraction relative to the elution range of sesquiterpenes. For peak identification see Table 3.

Only two sesquiterpenes, cyclosativene (peak 1) and longicyclene (peak 2), have a saturated structure and

**Table 3. Sesquiterpenes Tentatively Identified in the Hydrocarbon Fraction of Virgin Olive Oils**

| peak[a] | sesquiterpene | RI[b] | RI[b] (*lit.*) | RI[c] | RI[c] (*lit.*) | MS |
|---|---|---|---|---|---|---|
| 1 | cyclosativene | 1368 | 1368 (*10*), 1367 (*11*) | 1477 | 1469 (*11*) | (*10*) |
| 2 | longicyclene | 1371 | 1373 (*10*), 1371 (*12*) | 1480 | | (*10*) |
| 3 | α-copaene | 1380 | 1376 (*10*), 1375 (*13*) | 1488 | 1488 (*14*), 1488 (*15*) | (*10*)[d,e] |
| 4 | *β*-cubebene | 1392 | 1390 (*10*), 1385 (*16*) | 1521 | 1534 (11) | (*10*) |
| 5 | ni[f] | 1392 | | 1537 | | |
| 6 | *β*-elemene | 1395 | 1391 (*10*), 1391 (*18*) | 1589 | 1589 (*15*), 1590 (*17*) | (*10*)[e] |
| 7 | (*E*)-caryophyllene | 1423 | 1418 (*10*), 1418 (*19*) | 1594 | 1600 (*15*), 1587 (*13*) | (*10*)[d,e] |
| 8 | ni | 1433 | | 1589 | | |
| 9 | α-*trans*-bergamotene | 1439 | 1436 (*10*), 1435 (*20*) | 1585 | 1586 (*14*) | (*10*)[d,e] |
| 10 | (*Z*)-*β*-farnesene | 1446 | 1443 (*10*), 1447 (*19*) | 1644 | | (*10*)[d,e] |
| 11 | ni | 1453 | | 1618 | | |
| 12 | alloaromadendrene | 1459 | 1461 (*10*), 1455 (*11*) | 1640 | 1638 (*15*), 1642 (*11*) | (*10*)[d] |
| 13 | (*E*)-*β*-farnesene | 1459 | 1458 (*10*), 1449 (*21*) | 1668 | 1674 (*14*), 1660 (*11*) | (*10*)[d,e] |
| 14 | ni | 1471 | | 1697 | | |
| 15 | *β*-acoradiene | 1471 | 1466 (*10*) | 1674 | | (*10*) |
| 16 | drima-7,9(11)-diene | 1473 | 1469 (*10*) | 1677 | | (*10*) |
| 17 | ni | 1478 | | 1661 | | |
| 18 | *γ*-muurolene | 1480 | 1477 (*10*), 1473 (*16*) | 1686 | 1684 (*15*), 1683 (*11*) | (*10*)[d] |
| 19 | *γ*-curcumene | 1484 | 1480 (*10*), 1473 (*21*) | 1690 | | (*10*) |
| 20 | ni | 1488 | | 1682 | | |
| 21 | eremophyllene | 1492 | | 1707 | 1706 (*14*) | *d,e* |
| 22 | α-zingiberene | 1496 | 1495 (*10*), 1493 (*16*) | 1721 | 1705 (*16*) | (*10*) |
| 23 | α-selinene | 1496 | 1494 (*10*), 1494 (*20*) | 1721 | 1706 (*22*) | (*10*)[d,e] |
| 24 | ni | 1496 | | 1729 | | |
| 25 | α-muurolene | 1505 | 1499 (*10*), 1496 (*16*) | 1722 | 1725 (*15*), 1719 (*11*) | (*10*)[d,e] |
| 26 | (*E*),(*E*)-α-farnesene | 1510 | 1508 (*10*), 1515 (*23*) | 1751 | 1742 (*11*) | (*10*)[d,e] |
| 27 | *β*-curcumene | 1516 | 1512 (*10*) | 1741 | | (*10*) |
| 28 | *δ*-cadinene | 1528 | 1524 (*10*), 1526 (*26*) | 1757 | 1752 (*11*) | (*10*)[d,e] |
| 29 | *β*-sesquiphellandrene | 1528 | 1524 (*10*), 1521 (*16*) | 1771 | 1768 (*16*) | (*10*)[e] |
| 30 | (*Z*)2,(*E*)4,(*E*)6-allofarnesene | 1600 | | 1898 | | (*24*) |
| 31 | (*E*)2,(*Z*)4,(*E*)6-allofarnesene | 1631 | | 1932 | | (*24*) |

[a] Peak number refers to chromatogram of Figure 1. [b] Linear retention index on the apolar column (SE-52). [c] Linear retention index on the polar column (Carbowax). [d] NIST90 library. [e] WILEY5 library. [f] ni, not identified.

were eluted in $AgNO_3$ TLC with the band of *n*-alkanes. α-Copaene (peak 3) is a mono-unsaturated sesquiterpene that has already been detected in Spanish oils, mainly in those obtained from olives of the Hojiblanca variety (*8*). In our case α-copaene was present in all of the oils analyzed. *β*-Cubebene (peak 4), an unidentified sesquiterpene (peak 5), and *β*-elemene (peak 6) had very similar retention indices on the apolar column. Only the use of the polar column allowed a better separation and characterization. The compound corresponding to peak 5 was present mostly in Spanish oils, whereas in the other oils *β*-cubebene and *β*-elemene were present in different proportions.

A sesquiterpene (peak 8) had an RI value and mass spectrum in agreement with those of calarene or *β*-gurjonene. The presence of calarene in Spanish virgin olive oils was previously reported by Lanzon et al. (*6*), who used an apolar column (SE-52) for the separation. The RI value of the same compound on the polar column was distinctly different from that reported for calarene, therefore excluding the presence of this compound in our samples. Another sesquiterpene previously generically identified as muurolene (*6*) in our case had an RI value and a mass spectrum in agreement with the structure of α-muurolene (peak 25). *γ*-Muurolene (peak 18) was also detected.

(*E*),(*E*)-α-Farnesene (α-farnesene) (peak 26), a tetra-unsaturated acyclic sesquiterpene, was present in the largest amount with respect to the other sesquiterpenes in almost all of the oils analyzed. Besides α-farnesene, two other isomers with *β* configuration have been detected, namely, (*Z*)-*β*-farnesene (peak 10) and (*E*)-*β*-farnesene (peak 13). Two sesquiterpenes with higher RI values on both columns also belonged to the farnesene group. These compounds have been tentatively identified as (*Z*)2,(*E*)4,(*E*)6-allofarnesene (peak 30) and (*E*)2,(*Z*)4,(*E*)6-allofarnesene (peak 31) only by comparison of the mass spectra with literature data (*23*). The first coeluted with the $C_{16}$ hydrocarbon on the apolar column. The two *β*-farnesenes had very similar mass spectra, and the same was also true for the two allofarnesenes. Both pairs were, moreover, well separated both on the apolar column and on the polar column.

*δ*-Cadinene (peak 28) and *β*-sesquiphellandrene (peak 29) had practically the same RI values on the apolar column, but they were well separated (14 RI units) on the polar column. The same was valid also for the alloaromadendrene (peak 12) and (*E*)-*β*-farnesene (peak 13) pair.

The use of essential oils, as standard for some sesquiterpenes, was advantageous in the case of (*E*)-caryophyllene (peak 7), which is among the principal components of black pepper essential oil (*26*), α-*trans*-bergamotene (peak 9) present in the essential oil of carrot seeds (*27*), and α-zingiberene (peak 22), which is present in ginger oil (*18*). α-Zingiberene was eluted on the apolar column, together with α-selinene (peak 23) and another unidentified sesquiterpene (peak 24). The polar column allowed the separation of the unidentified compound from the other two, which were instead separated by silica gel $AgNO_3$ column chromatography, due to the presence of three double bonds in the structure of α-zingiberene with respect to α-selinene, which has two double bonds. In a similar way, *β*-curcumene (peak 27) was detected after separation by argentation chromatography from α-farnesene.

Eremophyllene (peak 21) had a mass spectrum and an RI value that were both very similar to those of valencene. The use of a pure standard of valencene allowed the exclusion of this compound and confirmation of eremophyllene.

**Table 4. Sesquiterpene Concentrations (Parts per Million) in the Samples of Virgin Olive Oils**

| sample[a] | α-copaene | eremophyllene | α-muurololene | α-farnesene |
|---|---|---|---|---|
| 1 | 1.4 | 0.3 | 1.0 | 2.1 |
| 2 | 1.9 | 0.4 | 1.2 | 1.8 |
| 3 | 3.8 | 0.3 | 2.2 | 0.4 |
| 4 | 4.2 | 0.3 | 2.3 | 0.5 |
| 5 | 0.5 | 0.7 | 0.3 | 20.3 |
| 6 | 0.6 | 0.4 | 0.3 | 31.1 |
| 7 | 3.4 | 0.8 | 2.0 | 28.0 |
| 8 | 5.2 | 0.1 | 2.7 | 0.3 |
| 9 | 0.3 | 0.2 | 0.1 | 5.0 |
| 10 | 4.2 | 0.3 | 2.3 | 0.5 |
| 11 | 0.8 | 0.7 | 0.2 | 13.9 |
| 12 | 0.2 | 0.6 | 0.1 | 6.2 |
| 13 | 0.2 | 0.3 | 0.1 | 3.0 |
| 14 | 0.2 | 0.3 | 0.1 | 2.1 |
| 15 | 0.2 | 0.3 | <0.1 | 2.0 |
| 16 | 0.2 | 0.2 | 0.1 | 2.4 |
| 17 | 0.4 | 0.5 | 0.1 | 3.1 |
| 18 | 0.3 | 0.4 | 0.1 | 3.8 |
| 19 | 0.3 | 0.4 | 0.1 | 3.3 |
| 20 | 0.4 | 0.6 | 0.2 | 7.1 |
| 21 | 0.2 | 0.6 | 0.1 | 5.3 |
| 22 | 0.4 | 0.5 | 0.2 | 2.3 |
| 23 | 0.5 | 0.5 | 0.2 | 1.3 |
| 24 | 0.5 | 0.4 | 0.2 | 1.2 |
| 25 | 0.7 | 0.3 | 0.4 | 2.7 |
| 26 | 0.4 | 0.4 | 0.2 | 1.3 |
| 27 | 0.1 | 0.2 | <0.1 | 1.7 |
| 28 | 0.5 | 0.2 | 0.2 | 0.5 |
| 29 | 0.4 | 0.3 | 0.2 | 1.2 |
| 30 | 0.1 | 0.6 | 0.1 | 6.4 |

[a] Sample numbers as reported in Table 1.

The composition of the sesquiterpene fraction, in the oils analyzed, was more complex than that previously reported (*6*). From the qualitative point of view most of the sesquiterpenes were more or less present in all of the oils analyzed, but from the quantitative point of view only four compounds were present in amounts >0.1−0.2 ppm, as reported in Table 4. The total concentration of the sesquiterpenes in the oils analyzed ranged from about 2 to 37 ppm. α-Farnesene was the more abundant sesquiterpene in almost all of the oils analyzed, amounting to 90% of the total in some samples. In particular, in four Italian oils (samples 5−7 and 11) α-farnesene was present in the range of 14−31 ppm. Besides α-farnesene, the most common sesquiterpenes were α-copaene, eremophyllene, and α-muurolene.

**Alkenes.** In the chromatogram shown in Figure 1 a series of peaks were present with mass spectra in agreement with mono-unsaturated hydrocarbon structures having 17, 23, 24, and 25 carbon atoms, respectively. These peaks disappeared after hydrogenation of the hydrocarbon fraction. The $AgNO_3$ TLC of the 10 combined hydrocarbon fractions permitted the isolation of only one band of alkenes. GC-MS analysis showed the presence, besides the $C_{17}$ hydrocarbon, of the series of *n*-alkenes from $C_{22}$ to $C_{27}$. The hydrogenation of this fraction resulted in a shift of the retention time with the new peaks being the same as the *n*-alkanes with the corresponding number of carbon atoms. The position of the double bond was determined by derivatization of the alkenes with DMDS. The mass spectra of the derivatives showed two intense diagnostic fragments due to the cleavage of the carbon−carbon bond between the carbon atoms carrying the methyl sulfide substituents (*9*, *28*).

The compounds identified were 8-heptadecene ($C_{17:1}$) and the series of *n*-alkenes from $C_{22:1}$ to $C_{27:1}$ with the double bond at position 9. The quantitative results are reported in Table 5.

**Table 5. Concentrations (Parts per Million) of *n*-Alkenes, *n*-Alkanes, and 6,10-Dimethyl-1-undecene in the Samples of Virgin Olive Oils**

| | | | *n*-alkanes | |
|---|---|---|---|---|
| sample[a] | 6,10-dimethyl-1-undecene | *n*-alkenes | even C no. | odd C no. |
| 1 | 0.1 | 1.0 | 10.1 | 52.1 |
| 2 | 0.2 | 0.8 | 10.2 | 53.7 |
| 3 | <0.1 | 1.0 | 8.7 | 37.4 |
| 4 | 0.2 | 1.1 | 9.2 | 43.2 |
| 5 | 0.7 | 0.5 | 10.5 | 50.3 |
| 6 | 1.4 | 0.7 | 12.3 | 53.7 |
| 7 | 2.3 | 1.4 | 10.5 | 56.0 |
| 8 | <0.1 | 0.8 | 7.0 | 33.8 |
| 9 | 1.5 | 0.6 | 4.9 | 25.6 |
| 10 | 0.7 | 0.6 | 10.3 | 35.6 |
| 11 | 0.1 | 0.8 | 9.0 | 51.9 |
| 12 | <0.1 | 0.3 | 8.2 | 41.5 |
| 13 | <0.1 | 0.2 | 7.7 | 39.7 |
| 14 | <0.1 | 0.3 | 11.1 | 44.9 |
| 15 | <0.1 | 0.5 | 10.6 | 50.0 |
| 16 | <0.1 | 0.3 | 7.1 | 34.5 |
| 17 | 0.3 | 2.1 | 10.2 | 48.5 |
| 18 | 0.2 | 2.1 | 9.7 | 45.9 |
| 19 | 0.2 | 2.1 | 10.0 | 46.8 |
| 20 | 2.5 | 0.6 | 11.5 | 53.6 |
| 21 | 0.1 | 1.4 | 8.4 | 44.1 |
| 22 | 3.0 | 0.4 | 35.3 | 139.0 |
| 23 | 2.2 | 0.5 | 30.7 | 126.7 |
| 24 | 2.4 | 0.5 | 32.6 | 113.2 |
| 25 | 2.2 | 0.5 | 29.2 | 113.4 |
| 26 | 1.4 | 0.5 | 34.9 | 133.0 |
| 27 | 0.4 | 0.2 | 18.4 | 69.7 |
| 28 | 1.8 | 0.2 | 33.7 | 124.1 |
| 29 | 2.0 | 0.4 | 36.5 | 140.7 |
| 30 | 0.4 | 1.5 | 9.9 | 48.7 |

[a] Sample numbers as reported in Table 1.

The $C_{22:1}$, $C_{26:1}$, and $C_{27:1}$ alkenes were present only at trace levels and were clearly recognizable only in the combined fractions. The presence of the $C_{26:1}$ and $C_{27:1}$ alkenes has not been previously reported.

In the alkene fraction another branched mono-unsaturated hydrocarbon was also present with the double bond at position 1 and a mass spectrum in agreement with that reported by Lanzon et al. (*6*) for 6,10-dimethyl-1-undecene ($C_{13:1}$). This hydrocarbon was present in all of the oils at levels <1 ppm with the exception of three Italian oils (samples 6, 7, and 9), a Spanish oil of variety Picual (sample 20), the Greek oils of variety Koroneiki (samples 26 and 28), and samples 22−25 and the Tunisian oil of variety Koroneiki (sample 29). The alkene $C_{17:1}$ was present at levels <1 ppm in all of the oils analyzed.

The Spanish oils of variety Arbequina (samples 17−19) were those with the highest concentrations of the *n*-alkenes $C_{23:1}$, $C_{24:1}$, and $C_{25:1}$, having values in the range of 0.6−0.8 ppm, followed by four Italian oils (samples 1−4) and a Tunisian oil (sample 30).

The only quantitative data reported in the literature regarding unsaturated hydrocarbons in virgin olive oils were for Spanish oils (*8*). Our results, relative to the Spanish oils analyzed, were in agreement with the literature data, in particular the presence of the higher concentration of *n*-alkenes $C_{23:1}$, $C_{24:1}$, and $C_{25:1}$ in the oils of Arbequina variety and a higher concentration of 6,10-dimethyl-1-undecene in the oil of Picual variety.

**Alkanes.** The concentrations of *n*-alkanes in the oils are reported in Table 5. The *n*-alkanes with an odd number of carbon atoms were predominant with respect to those with an even number of carbon atoms in all of the oils analyzed. These results are in agreement with

all of the data reported in the literature (*4*−*6*, *8*). The alkanes most represented were $C_{23}$, $C_{25}$, $C_{27}$, and $C_{29}$. This seems to be a characteristic of the virgin olive oils, because in the oils of sunflower, corn, peanut, and safflower the most common hydrocarbons are $C_{27}$, $C_{29}$, and $C_{31}$ (*5*). The concentration of *n*-alkanes varied over a wide range, from about 30 to 175 ppm. In particular, Italian and Spanish oils had lower amounts of *n*-alkanes, with a range between 30 and 65 ppm, compared to the Greek oils of variety Koroneiki (samples 26 and 28) and samples 22−25 and the Tunisian oil of variety Koroneiki (sample 29), which had a concentration range between 140 and 175 ppm.

## LITERATURE CITED


(1) Capella, P.; Fedeli, E.; Cirimele, H.; Jacini, G. Gli idrocarburi contenuti nella frazione insaponificabile di alcuni olii vegetali (Hydrocarbons contained in the unsaponifiable fraction of some vegetable oils). *Riv. Ital. Sostanze Grasse* **1963**, *40*, 603−606.

(2) Eisner, J.; Iverson, J. L.; Mozingo, A. K.; Firestone, D. Gas chromatography of unsaponifiable matter. III. Identification of hydrocarbons, aliphatic alcohols, tocopherols, triterpenoid alcohols and sterols present in olive oils. *J. Assoc. Off. Anal. Chem*. **1965**, *48*, 417−433.

(3) Jacini, G.; Fedeli, F. Neuere Untersuchungen der nichtglyceridischen Bestandteile von Pflanzenfetten. *Fette Seifen Anstrichm*. **1975**, *77*, 1−7.

(4) Bastic, M.; Bastic, L. J.; Jovanovic, J. A.; Spiteller, G. Hydrocarbons and other weakly polar unsaponifiables in some vegetable oils. *J. Am. Oil Chem. Soc*. **1978**, *55*, 886−891.

(5) McGill, Al. S.; Moffat, C. F.; Mackie, P. R.; Cruickshan, K. The composition and concentration of *n*-alkanes in retail samples of edible oils. *J. Sci. Food Agric*. **1993**, *61*, 357−362.

(6) Lanzon, A.; Albi, T.; Cert, A.; Gracian, J. The hydrocarbon fraction of virgin olive oil and changes resulting from refining. *J. Am. Oil Chem. Soc*. **1994**, *71*, 285−291.

(7) Koprivnjak, O.; Conte, L. S. Caratteristiche della frazione idrocarburica e composizione degli acidi grassi degli oli di oliva vergini provenienti dalla zona di Pola (Croazia). *Riv. Ital. Sostanze Grasse* **1996**, *73*, 317−320.

(8) Guinda, A.; Lanzon, A.; Albi, T. Differences in hydrocarbons of virgin olive oils obtained from several olive varieties. *J. Agric. Food Chem*. **1996**, *44*, 1723−1726.

(9) Carlson, D. A.; Roan, C.; Yost, R. A.; Hector, J. Dimethyl disulfide derivates of long chain alkenes, alkadienes, and alkatrienes for gas chromatography/mass spectrometry. *Anal. Chem*. **1989**, *61*, 1564−1571.

(10) Adams, R. P. In *Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 1995.

(11) Binder, R. G.; Flath, R. A. Volatile component of pineapple guava. *J. Agric. Food Chem*. **1989**, *37*, 734−736.

(12) Andersen, N. H. Identification of sesquiterpene hydrocarbon fraction from gas−liquid chromatography retention data. *J. Chromatogr*. **1969**, *44*, 52−59.

(13) Perry, N. B.; Baxter, A. J.; Brennan, N. J.; Van Klink, J. W.; McGimpsey, J.; Douglas, M. H.; Joulain, D. Dalmatian sage. Part 1. Differing oil yields and compositions from flowering and non-flowering accession. *Flavour Fragrance J*. **1996**, *11*, 231−238.

(14) Vernin, G.; Metzger, J. Analysis of basil oils by GC-MS data bank. *Perfum. Flavour*. **1984**, *9*, 71−86.

(15) Vernin, G.; Metzger, J.; Suon, K. N.; Fraisse, D.; Ghiglione, C.; Hamoud, A.; Pàrkànyi, C. GC-MS-SPECMA bank analysis of essential oils and aromas. *Lebensm. Wiss. Technol*. **1990**, *23*, 25−33.

(16) Kollmannsberger, H.; Nitz, S. Über die Aromastoffzusammensetzung von Hochdruckextracten: 2. Piment (*Pimenta dioica*). *Chem. Mikrobiol. Technol. Lebensm*. **1993**, *15*, 116−126.

(17) Njoroge, S. M.; Ukeda, H.; Kusunose, H.; Sawamura, M. Volatile components of japanese yuzu and lemon oils. *Flavour Fragrance J*. **1994**, *9*, 159−166.

(18) Bartley, J.; Foley, P. Supercritical fluid extraction of Australian-grown ginger (*Zingiber officinalis*). *J. Sci. Food Agric*. **1994**, *66*, 365−371.

(19) De Pooter, H. L.; Schamp, N. M.; Aboutabl, E. A.; El Tohamy, S. F.; Doss, S. L. Essential oils from the leaves of three *Pistacia* species grown in Egypt. *Flavour Fragrance J*. **1991**, *6*, 229−232.

(20) Jantan, I. B.; Ahmad, A. R.; Ahmad, A. S.; Mohd Ali, N. A. A comparative study of the essential oils of five *Piper* species from peninsular Malaysia. *Flavour Fragrance J*. **1994**, *9*, 339−342.

(21) Weyerstahl, P.; Splittgerber, U.; Marschall, H.; Kaul, V. Constituents of the leaf essential oil of *Hypericum perforatum* L. from India. *Flavour Fragrance J*. **1995**, *10*, 365−370.

(22) Gaydou, E. M.; Faure, R.; Bianchini, J. P.; Lamaty, G.; Rakotonirainy, O.; Randriamiharisoa, R. Sesquiterpene composition of basil oil. Assignment of the $^1$H and $^3$C NMR spectra of $\beta$-elemene with two-dimensional NMR. *J. Agric. Food Chem*. **1989**, *37*, 1032−1037.

(23) Kollmannsberger, H.; Nitz, S. Über die Aromastoffzusammensetzung von Hochdruckextracten: 3. Gewürznelken (*Syzygium aromaticum*). *Chem. Mikrobiol. Technol. Lebensm*. **1994**, *16*, 112−123.

(24) Williams, H. J.; Strand, M. R.; Vinson, S. B. Synthesis and purification of the allofarnesenes. *Tetrahedron* **1981**, *37*, 2763−2767.

(25) Sun, Y.; Zhang, R.; Wang, Q.; Xu, B. Programmed-temperature gas chromatographic retention index. *J. Chromatogr. A* **1993**, *657*, 1−15.

(26) Chacko, S.; Jayalekshmy, A.; Gopalakrishnan, M.; Narayanan, C. S. Roasting studies on black pepper (*Piper nigrum* L.). *Flavour Fragrance J*. **1996**, *11*, 305−310.

(27) Cu, J. Q.; Perineau, F.; Delmas, M.; Gaset, A. Comparison of the chemical composition of carrots seed essential oil extracted by different solvents. *Flavour Fragrance J*. **1989**, *4*, 225−231.

(28) Francis, G. W.; Veland K. Alkylthiolation for the determination of double-bond positions in linear alkenes. *J. Chromatogr*. **1981**, *219*, 379−384.


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