

# The potential of HPLC triglyceride profiles for the classification of Cretan olive oils

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One hundred and twenty authentic olive oil samples of the two major Cretan olive cultivars, Koroneiki and Mastoidis, collected at different maturity stages have been obtained from different producing areas and analysed by isocratic high-performance liquid chromatography according to the official EC method. Multivariate analysis, including principal component analysis and canonical discrimination analysis, were used to characterize the oils according to cultivar, location and sampling date. Using the triglyceride compositional data, the two varieties examined were sufficiently separated by means of discriminant analysis procedures. Within each variety the oils were grouped quite clearly according to their geographic origin. Samples that originated from neighbouring locations with no marked differences in geographic morphology, or samples from locations with some extremes in the climatic conditions, presented some discrepancies in classification. © 1997 Elsevier Science Ltd

## INTRODUCTION

It is well known that every oil or fat has a characteristic triglyceride profile which is unique to the type of oil and can be used in detecting adulteration (Synouris-Vrettakou *et al.*, 1984, 1995; Flor *et al.*, 1993). The need to determine the origin of an oil has become necessary after the introduction of the 'Appellation of Origin' on olive oils and involves a deep knowledge of the physical and chemical characteristics as well as properly defined cultivar names.

Researchers have tried recently, in several studies, to correlate the chemical composition of olive oil to (1) geographic origin (Leardi & Paganuzzi, 1987; Aparicio *et al.*, 1987; Ferreiro & Aparicio, 1992; Gigliotti *et al.*, 1993; Tsimidou *et al.*, 1987), (2) cultivar (Aparicio *et al.*, 1990; Boschelle *et al.*, 1994) or (3) year of harvest (Forina *et al.*, 1983; Tsimidou & Karakostas, 1993).

Sophisticated statistical methods for the classification of the oils have been applied to several chemical components, including sterols and some triterpenic alcohols and hydrocarbons (Ferreiro & Aparicio, 1992), sterols and fatty acids (Forina *et al.*, 1983), fatty acids and triglycerides (Tsimidou *et al.*, 1987).

Furthermore, comparative studies between extra virgin olive oils of different geographic origin (Gigliotti *et al.*, 1993) showed that some triglycerides present quan-

titative intervals which can be correlated with the geographic origin of the sample.

The island of Crete and especially the Chania region is well known for the quality of olive oil produced; however, only very limited work has been carried out on Cretan olive oil and only in the frame of datasets from wide geographic areas with large variations in sampling method, altitude and climatic parameters. Therefore, it is of utmost importance to have a reliable identification and classification of olive oils according to olive variety and/or geographic origin, based on samples certified for their geographic origin, cultivar and stage of maturity.

In this research work we studied the triglyceride fraction of 120 virgin olive oil samples of two different cultivars coming from different locations (characterizing different cultivated zones), at different maturity stages from the Chania region during one crop year.

## MATERIALS AND METHODS

### Materials

A total of 120 extra virgin olive oil samples were obtained in one harvesting period (1992–1993) from Koroneiki and Mastoides cultivars. These two cultivars cover the total cultivated area in the region under investigation. The sampling locations (Fig. 1) were chosen with respect to differences in altitude, distance

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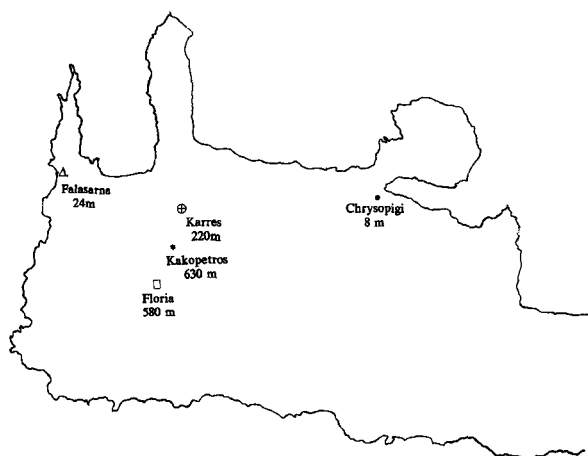


Fig. 1. Map of Chania with labels on the sampling locations.

from the sea and climate. For Koroneiki (cultivar 1, C1), five locations were sampled: Chrysopigi, (Ch); Falasarna, (Fa); Kakopetros, (Ka); Floria, (Fl) and Kares, (Kr). For Mastoides (cultivar 2, C2), Chrysopigi (Ch), Kakopetros (Ka) and Floria (Fl) were sampled, since this cultivar is not cultivated in the other two locations.

Samplings were carried out over a time period from the beginning of November to the end of January at three sampling dates (different stages of maturity) and including five trees (replications) in each case.

For each sampling, olive fruits were collected all around the tree from all the layers of the tree canopy. The above-mentioned factors (i.e. cultivar, location and sampling date) were combined to give 24 cases (Table 1), which were classified by discriminant analysis.

Oil was extracted using a laboratory-scale olive mill as follows. Olives were immediately washed, de-leaved and crushed with a hammer crusher operating at 3000 rpm. The resulting paste was mixed at  $28 \pm 2^\circ\text{C}$  for 30 min and pressed in a laboratory press at  $205 \text{ kg cm}^{-2}$ . After decanting, the oil was centrifuged and filtered.

## METHODS

### Triglyceride analysis by HPLC

The analytical separation of triglycerides was performed according to the official method of the EC (Anonymous, 1991). The apparatus consisted of a Jasco liquid chromatograph (Model PU 980) coupled with a refractive index detector (Jasco 830-RI) and a software interface for the processing of the acquired data.

Injection was by means of a Rheodyne injection valve (Model 7125) with  $20 \mu\text{l}$  fixed loop (Rheodyne, CA, USA). The chromatographic separation was achieved on a Kromasil 100 C18,  $5 \mu\text{m}$  column ( $250 \text{ mm} \times 4 \text{ mm}$  i.d.), obtained from MZ Analysentechnik (Mainz, Germany) at  $40^\circ\text{C}$ . Isocratic elution was carried out at a flow rate of  $0.7 \text{ ml min}^{-1}$  with a mixture of acetone:acetonitrile (60:40, v/v) as mobile phase.

### Triglyceride compositional data and statistical analysis

Twenty triglycerides [LLL (C18:2–C18:2–C18:2); OLLn (C18:1–C18:2–C18:3); PLLn (C16:0–C18:2–C18:3); OLL (C18:1–C18:2–C18:2); OOLn (C18:1–C18:1–C18:3); PLL

Table 1. Denomination of the samples according to cultivar, geographic origin and sampling date

Cultivar	Location	Sampling date <sup>a</sup>	Combination name
1(C1)	Chrysopigi (Ch)	1(S1)	C1ChS1
1(C1)	Chrysopigi (Ch)	2(S2)	C1ChS2
1(C1)	Chrysopigi (Ch)	3(S3)	C1ChS3
1(C1)	Falasarna (Fa)	1(S1)	C1FaS1
1(C1)	Falasarna (Fa)	2(S2)	C1FaS2
1(C1)	Falasarna (Fa)	3(S3)	C1FaS3
1(C1)	Kakopetros (Ka)	1(S1)	C1KaS1
1(C1)	Kakopetros (Ka)	2(S2)	C1KaS2
1(C1)	Kakopetros (Ka)	3(S3)	C1KaS3
1(C1)	Floria (Fl)	1(S1)	C1FlS1
1(C1)	Floria (Fl)	2(S2)	C1FlS2
1(C1)	Floria (Fl)	3(S3)	C1FlS3
1(C1)	Kares (Kr)	1(S1)	C1KrS1
1(C1)	Kares (Kr)	2(S2)	C1KrS2
1(C1)	Kares (Kr)	3(S3)	C1KrS3
2(C2)	Chrysopigi (Ch)	1(S1)	C2ChS1
2(C2)	Chrysopigi (Ch)	2(S2)	C2ChS2
2(C2)	Chrysopigi (Ch)	3(S3)	C2ChS3
2(C2)	Kakopetros (Ka)	1(S1)	C2KaS1
2(C2)	Kakopetros (Ka)	1(S2)	C2KaS2
2(C2)	Kakopetros (Ka)	3(S3)	C2KaS3
2(C2)	Floria (Fl)	1(S1)	C2FlS1
2(C2)	Floria (Fl)	2(S2)	C2FlS2
2(C2)	Floria (Fl)	3(S3)	C2FlS3

Cultivars: C1, Koroneiki; C2, Mastoides.

<sup>a</sup> $n = 5$  ( $n$  is number of replications per sampling date).

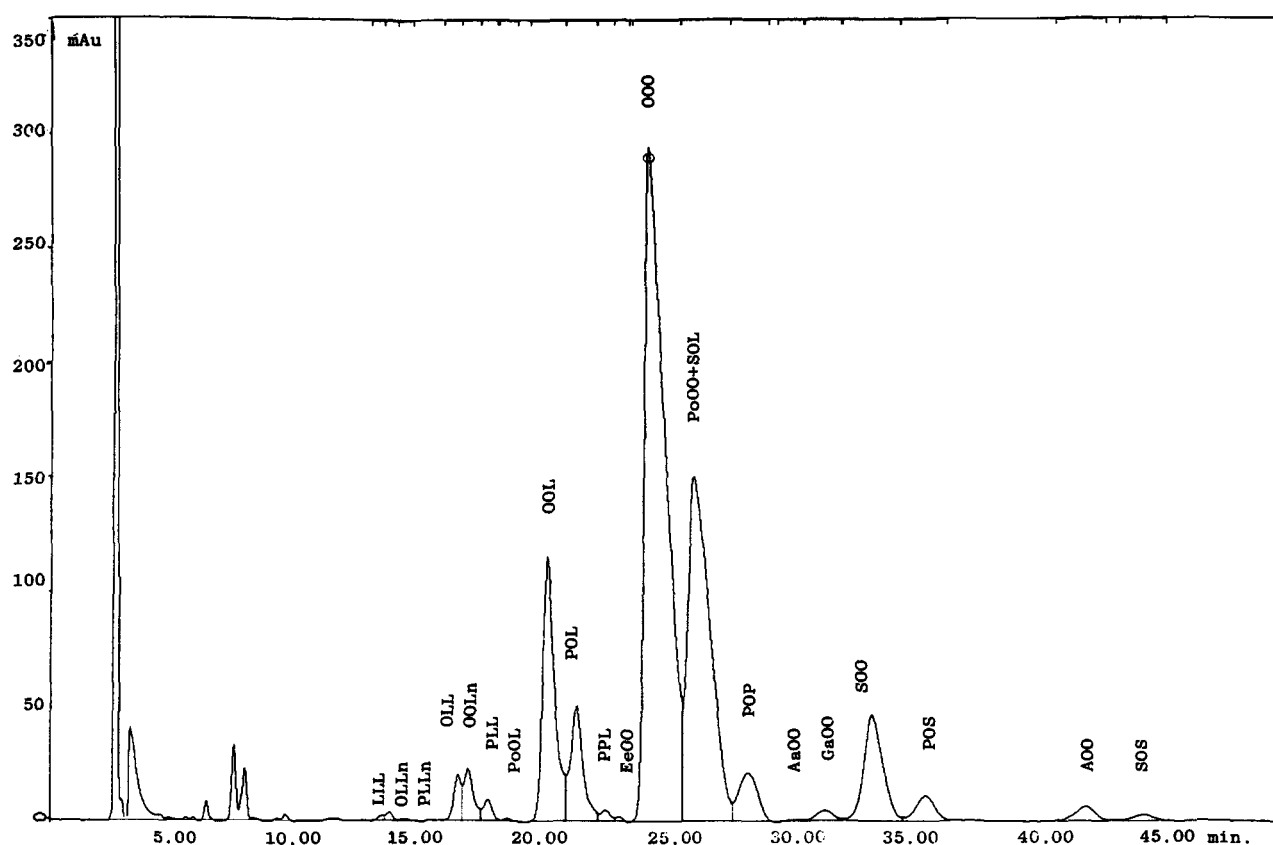


Fig. 2. Typical HPLC triglyceride elution profile of a virgin olive oil.

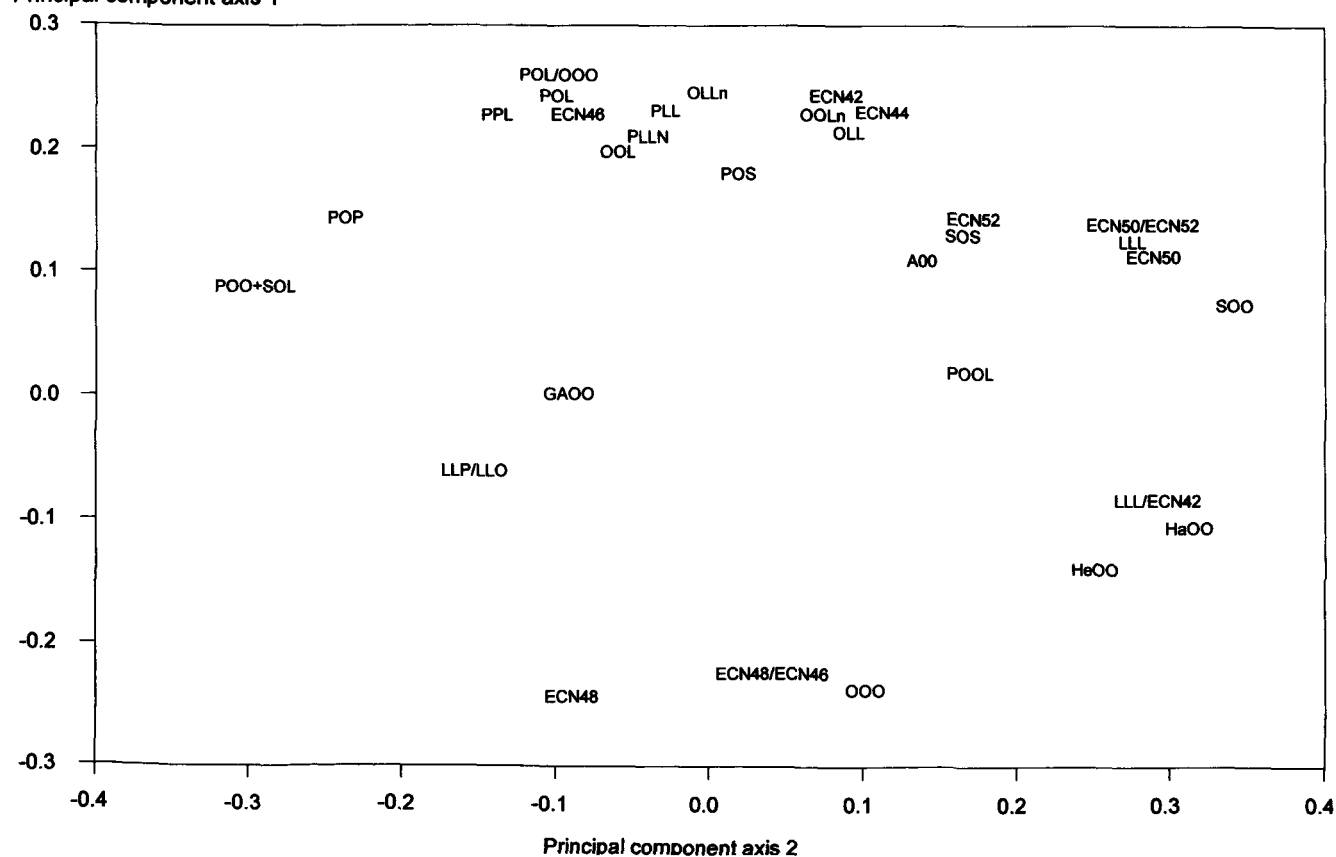
Table 2. Mean values  $\pm$  95% confidence interval of triglycerides of cultivar Koroneiki grown in different cultivated locations in Chania region during the crop year 1992–1993

	C1Ch	C1Fa	C1Fl	C1Ka	C1Kr
LLL	0.084 $\pm$ 0.007 b	0.100 $\pm$ 0.008 a	0.075 $\pm$ 0.004 bc	0.067 $\pm$ 0.007 c	0.096 $\pm$ 0.007 a
OLLn	0.181 $\pm$ 0.011 b	0.237 $\pm$ 0.009 a	0.143 $\pm$ 0.007 c	0.127 $\pm$ 0.009 d	0.194 $\pm$ 0.009 b
PLLn	0.072 $\pm$ 0.007 b	0.090 $\pm$ 0.006 a	0.053 $\pm$ 0.006 cd	0.044 $\pm$ 0.005 d	0.062 $\pm$ 0.007 bc
OLL	1.048 $\pm$ 0.075 b	1.41 $\pm$ 0.071 a	0.915 $\pm$ 0.050 c	0.906 $\pm$ 0.078 c	1.35 $\pm$ 0.087 a
OOLn	1.460 $\pm$ 0.028 b	1.68 $\pm$ 0.037 a	1.34 $\pm$ 0.017 c	1.23 $\pm$ 0.026 d	1.50 $\pm$ 0.033 b
PLL	0.542 $\pm$ 0.014 b	0.645 $\pm$ 0.013 a	0.465 $\pm$ 0.015 c	0.421 $\pm$ 0.015 d	0.525 $\pm$ 0.019 b
PoOL	0.071 $\pm$ 0.007 b	0.096 $\pm$ 0.011 a	0.073 $\pm$ 0.005 b	0.054 $\pm$ 0.007 c	0.073 $\pm$ 0.008 b
OOL	9.98 $\pm$ 0.318 b	11.4 $\pm$ 0.292 a	9.50 $\pm$ 0.294 b	9.71 $\pm$ 0.346 b	10.9 $\pm$ 0.482 a
POL	4.50 $\pm$ 0.145 b	5.73 $\pm$ 0.100 a	3.79 $\pm$ 0.104 c	3.91 $\pm$ 0.147 c	4.68 $\pm$ 0.169 b
PLL	0.321 $\pm$ 0.014 b	0.454 $\pm$ 0.018 a	0.264 $\pm$ 0.010 c	0.245 $\pm$ 0.013 c	0.310 $\pm$ 0.018 b
EeOO	0.134 $\pm$ 0.013 ab	0.126 $\pm$ 0.013 b	0.155 $\pm$ 0.013 a	0.117 $\pm$ 0.010 b	0.111 $\pm$ 0.017 b
OOO	45.3 $\pm$ 0.470 c	41.2 $\pm$ 0.364 d	50.06 $\pm$ 0.385 a	49.1 $\pm$ 0.813 b	45.5 $\pm$ 0.479 c
POO + SOL	24.5 $\pm$ 0.438 a	24.6 $\pm$ 0.428 a	23.3 $\pm$ 0.275 b	23.7 $\pm$ 0.446 b	23.1 $\pm$ 0.420 b
POP	2.74 $\pm$ 0.129 b	3.07 $\pm$ 0.120 a	2.33 $\pm$ 0.081 c	2.39 $\pm$ 0.115 c	2.38 $\pm$ 0.114 c
AaOO	0.101 $\pm$ 0.014 a	0.096 $\pm$ 0.012 a	0.109 $\pm$ 0.016 a	0.083 $\pm$ 0.015 a	0.101 $\pm$ 0.016 a
GaOO	0.543 $\pm$ 0.025 b	0.523 $\pm$ 0.022 b	0.597 $\pm$ 0.033 a	0.509 $\pm$ 0.038 b	0.496 $\pm$ 0.031 b
SOO	5.79 $\pm$ 0.094 ab	5.68 $\pm$ 0.084 b	4.79 $\pm$ 0.105 d	5.10 $\pm$ 0.171 c	5.97 $\pm$ 0.106 a
POS	1.30 $\pm$ 0.064 b	1.40 $\pm$ 0.043 a	0.949 $\pm$ 0.040 d	1.02 $\pm$ 0.093 d	1.20 $\pm$ 0.049 c
AOO	0.984 $\pm$ 0.024 abc	1.02 $\pm$ 0.041 ab	0.908 $\pm$ 0.025 c	0.945 $\pm$ 0.096 bc	1.05 $\pm$ 0.043 a
SOS	0.367 $\pm$ 0.021 bc	0.449 $\pm$ 0.033 a	0.289 $\pm$ 0.032 d	0.328 $\pm$ 0.027 cd	0.378 $\pm$ 0.030 b
ECN42	0.336 $\pm$ 0.020 b	0.426 $\pm$ 0.019 a	0.27 $\pm$ 0.013 c	0.237 $\pm$ 0.017 d	0.352 $\pm$ 0.015 b
ECN44	3.121 $\pm$ 0.091 c	3.83 $\pm$ 0.116 a	2.80 $\pm$ 0.058 d	2.61 $\pm$ 0.089 e	3.45 $\pm$ 0.109 b
ECN46	14.8 $\pm$ 0.458 c	17.6 $\pm$ 0.373 a	13.6 $\pm$ 0.387 d	13.9 $\pm$ 0.488 d	15.9 $\pm$ 0.642 b
ECN48	72.5 $\pm$ 0.509 b	68.9 $\pm$ 0.487 d	75.7 $\pm$ 0.323 a	75.2 $\pm$ 0.712 a	71.0 $\pm$ 0.589 c
ECN50	7.64 $\pm$ 0.164 a	7.61 $\pm$ 0.142 a	6.33 $\pm$ 0.157 c	6.63 $\pm$ 0.239 b	7.66 $\pm$ 0.148 a
ECN52	1.35 $\pm$ 0.037 bc	1.47 $\pm$ 0.064 a	1.20 $\pm$ 0.052 d	1.27 $\pm$ 0.099 dc	1.42 $\pm$ 0.071 ab

**Table 3. Mean values  $\pm$  95% confidence interval of triglycerides of cultivar Mastoidis grown in different cultivated locations in Chania region during the crop year 1992–1993**

	C2Ch	C2F1	C2Ka
LLL	0.055 $\pm$ 0.006 b	0.140 $\pm$ 0.015 a	0.064 $\pm$ 0.007 b
OLLn	0.115 $\pm$ 0.008 b	0.157 $\pm$ 0.012 a	0.096 $\pm$ 0.007 c
PLLn	0.038 $\pm$ 0.007 b	0.052 $\pm$ 0.007 a	0.034 $\pm$ 0.005 b
OLL	0.721 $\pm$ 0.029 b	1.19 $\pm$ 0.078 a	0.783 $\pm$ 0.041 b
OOLn	1.2 $\pm$ 0.034 b	1.52 $\pm$ 0.060 a	1.07 $\pm$ 0.036 c
PLL	0.401 $\pm$ 0.020 b	0.482 $\pm$ 0.015 a	0.346 $\pm$ 0.019 c
PoOL	0.094 $\pm$ 0.010 a	0.108 $\pm$ 0.009 a	0.091 $\pm$ 0.013 a
OOL	9.02 $\pm$ 0.211 a	9.36 $\pm$ 0.378 a	9.32 $\pm$ 0.199 a
POL	3.41 $\pm$ 0.084 ab	3.57 $\pm$ 0.150 a	3.29 $\pm$ 0.083 b
PPL	0.172 $\pm$ 0.023 a	0.190 $\pm$ 0.017 a	0.161 $\pm$ 0.010 a
EcOO	0.469 $\pm$ 0.023 a	0.495 $\pm$ 0.038 a	0.374 $\pm$ 0.020 b
OOO	50.3 $\pm$ 0.657 b	50.8 $\pm$ 0.699 b	52.0 $\pm$ 0.397 a
POO + SOL	23.1 $\pm$ 0.578 a	20.1 $\pm$ 0.554 c	21.7 $\pm$ 0.334 b
POP	2.28 $\pm$ 0.120 a	1.70 $\pm$ 0.159 c	1.99 $\pm$ 0.077 b
AaOO	0.305 $\pm$ 0.019 b	0.423 $\pm$ 0.037 a	0.246 $\pm$ 0.022 c
GaOO	0.532 $\pm$ 0.032 a	0.499 $\pm$ 0.025 ab	0.465 $\pm$ 0.020 b
SOO	5.46 $\pm$ 0.135 b	6.67 $\pm$ 0.135 a	5.71 $\pm$ 0.270 b
POS	1.08 $\pm$ 0.039 a	1.09 $\pm$ 0.029 a	1.00 $\pm$ 0.074 a
AOO	0.888 $\pm$ 0.048 b	1.04 $\pm$ 0.047 a	0.885 $\pm$ 0.037 b
SOS	0.348 $\pm$ 0.026 b	0.422 $\pm$ 0.028 a	0.315 $\pm$ 0.056 b
ECN42	0.207 $\pm$ 0.019 b	0.349 $\pm$ 0.024 a	0.194 $\pm$ 0.015 b
ECN44	2.43 $\pm$ 0.065 b	3.30 $\pm$ 0.145 a	2.29 $\pm$ 0.089 b
ECN46	12.60 $\pm$ 0.264 a	13.1 $\pm$ 0.530 a	12.8 $\pm$ 0.269 a
ECN48	75.7 $\pm$ 0.287 a	72.60 $\pm$ 0.745 b	75.8 $\pm$ 0.343 a
ECN50	7.07 $\pm$ 0.123 b	8.26 $\pm$ 0.151 a	7.18 $\pm$ 0.332 b
ECN52	1.24 $\pm$ 0.063 b	1.46 $\pm$ 0.072 a	1.20 $\pm$ 0.085 b

Principal component axis 1

**Fig. 3. PCA grouping of variables.**

(C16:0-C18:2-C18:2); PoOL (C16:1-C18:1-C18:2); OOL (C18:1-C18:1-C18:2); POL (C16:0-C18:1-C18:2); PPL (C16:0-C16:0-C18:2); EeOO (C17:1-C18:1-C18:1); OOO (C18:1-C18:1-C18:1); POO+ SOL (C16:0-C18:1-C18:1+C18:0-C18:1-C18:2); POP (C16:0-C18:1-C16:0); AaOO (C17:0-C18:1-C18:1); GaOO (C20:1-C18:1-C18:1); SOO (C18:0-C18:1-C18:1); POS (C16:0-C18:1-C18:0); AOO (C20:0-C18:1-C18:1); SOS (C18:0-C18:1-C18:0)] were identified by calculating the equivalent carbon numbers (ECN); data were expressed in percentage composition of the different triglycerides (Anonymous, 1991).

Also the values for ECN42, ECN44, ECN46, ECN48, ECN50, ECN52, as well as the ratios ECN48/ECN46, ECN50/ECN52, LLL/ECN42, LLP/LLO and LOP/OOO were calculated.

Comparison of the means, either between cultivars or between locations, was achieved using ANOVA and,

when significant, Duncan's test was performed to group the cultivars or locations, respectively. Multivariate analysis based on correlation, principal component canonical discrimination and clustering was applied to investigate the variables and to classify the combinations of cultivar, location and sampling date.

### RESULTS AND DISCUSSION

A typical HPLC elution profile of triglycerides is shown in Fig. 2. All the virgin olive oil samples studied presented qualitatively similar chromatographic profiles.

The mean values and the confidence intervals of the different triglycerides for the samples of Koroneiki and Mastoidis in the different locations studied are shown in Tables 2 and 3, respectively. In all samples analysed, the value of LLL did not exceed the maximum limit of

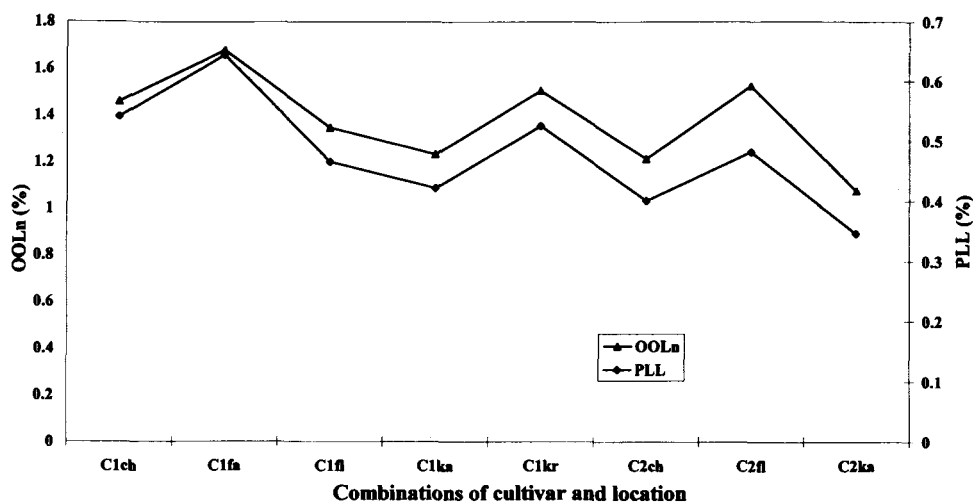


Fig. 4. Positive correlation between variables OOLn and PLL.

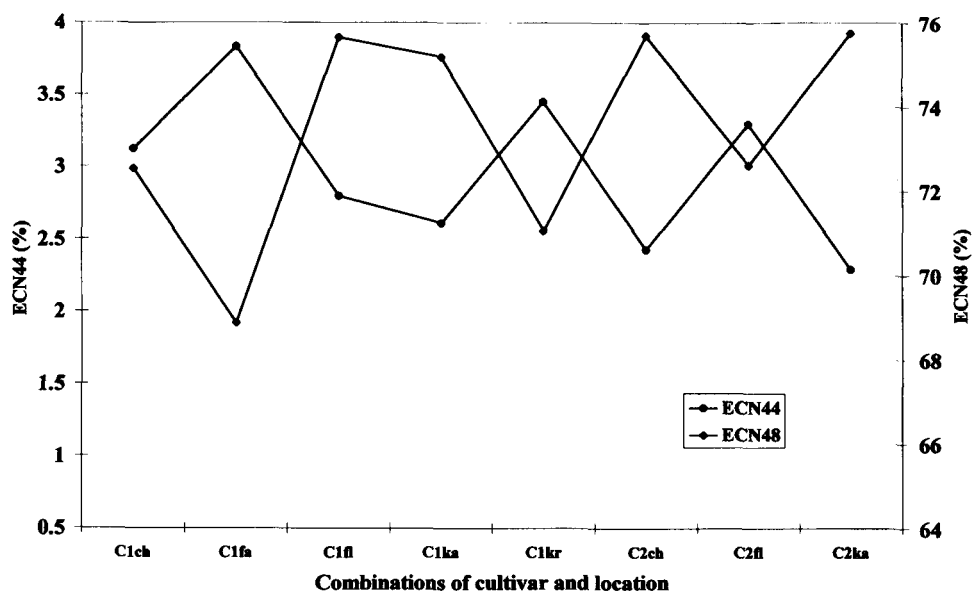


Fig. 5. Negative correlation between variables ECN44 and ECN48.

0.5% determined by the EC Regulation for different olive oil grades (Anonymous, 1991).

The values of the variables OLLn, OOLn, PLL and ECN44 for both cultivars differ significantly between

locations. No significant differences were found between locations, either in the variable AaOO in cultivar Koroneiki, or in the variable POS in cultivar Mastoidis. ECN46, ECN48, ECN52, LOP/OOO & LLP/LLO did

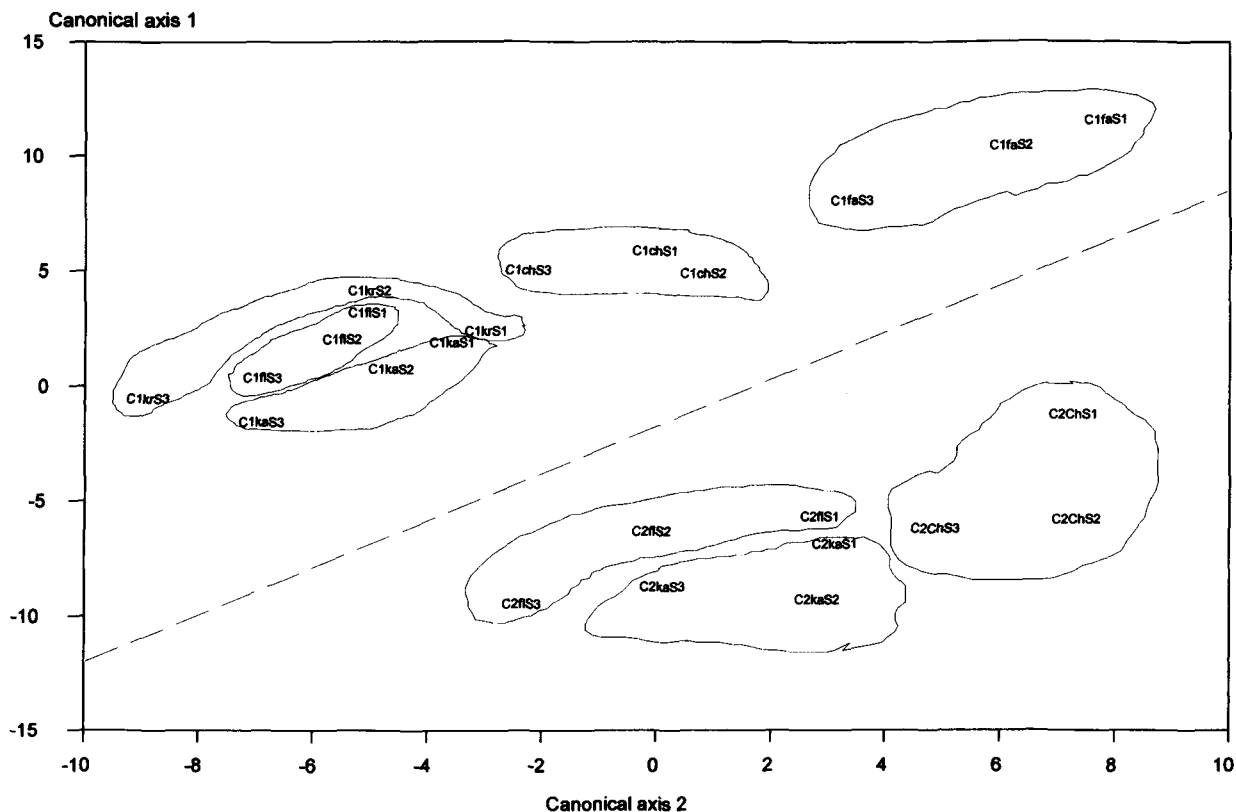


Fig. 6. Canonical representation for the discrimination of the samples according to cultivar, geographic origin and sampling date.

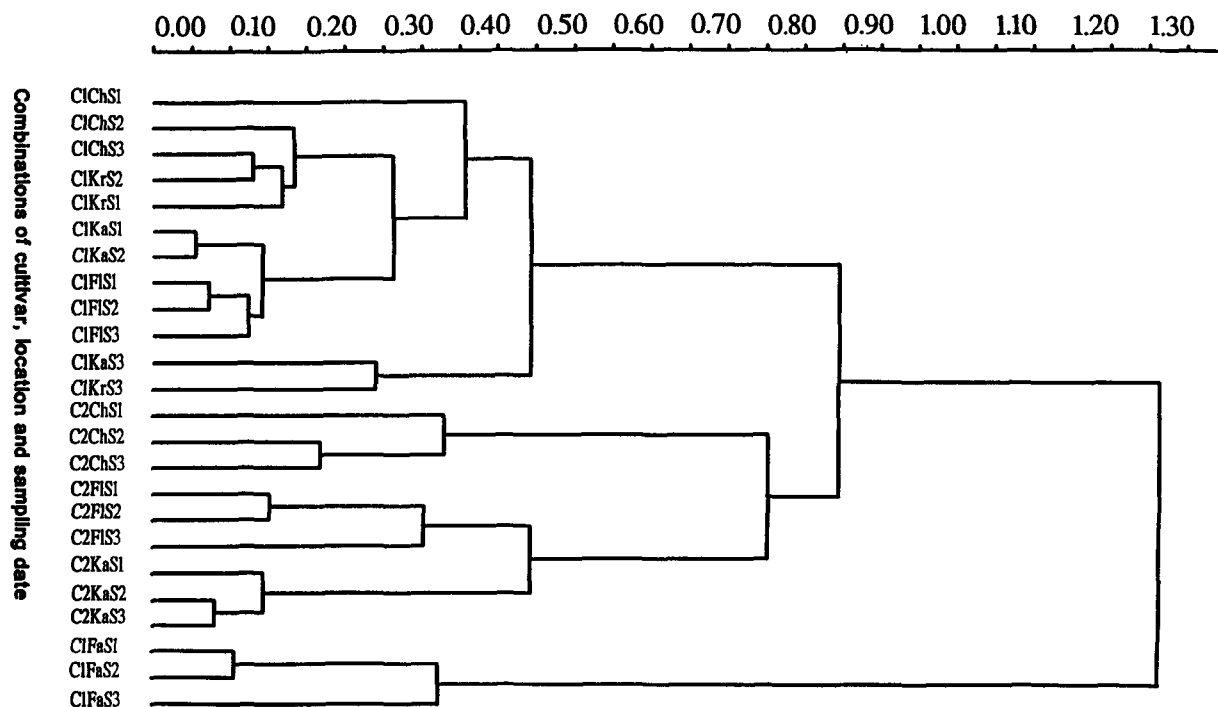


Fig. 7. Dendrogram of olive oils from Chania region.



not show significant differences between the locations Kakopetros and Floria.

The plot of the first two principal components is shown in Fig. 3. The distance of the variables across principal component 1 is inversely proportional to the correlation between them. For example, the variables OOLn and PLL have a strong positive correlation, as can be concluded from the correlation matrix ( $r = 0.90878$ ), the principal component analysis (PCA) graphic representation and Fig. 4. On the other hand, a strong negative correlation between ECN44 and ECN48 ( $r = -0.93698$ ) is evident from both the PCA plot and Fig. 5.

With no reduction in the original variables, canonical discriminant analysis was performed for the classification of the olive oil samples. A classical representation of the scores on canonicals 1 and 2 is shown in Fig. 6. The distribution of the samples seems to be affected by cultivar.

A strong correlation was observed between cultivar and a direction on the plane of the first two canonicals separating oils from the Koroneiki and Mastoidis cultivars. Within the first cultivar a satisfactory discrimination between locations was achieved. The locations Falasarna and Chrysopigi constitute two distinct groups, while the other three locations of cultivar Koroneiki constitute one group. This can be explained if we take into account the climate (humidity and rainfall) and the geographic position (altitude, latitude, distance from the sea) of each sampling site. The three locations—Kares (Kr), Kakopetros (Ka) and Floria (Fl)—that appear as one group in the canonical plot, belong to the same central mountainous area with similar climatic conditions and only small differences in altitude. Distance from the sea seems to affect the dispersion of the samples across the canonical 2 axis.

The Mahalanobis distances (above diagonal) and the probability of significance (below diagonal) between combinations are given in Table 4. The dendrogram constructed on the basis of those distances is shown in Fig. 7. In this dendrogram the two cultivars are well discriminated. The oils of Falasarna seem to behave as a separate case and are not included in the samples of cultivar 1. This discrepancy can be attributed to distinctive local climatic conditions. Falasarna has the lowest annual rainfall and the maximum evaporation, while the minimum relative humidity is higher in comparison with the other sampling sites.

In the present work, HPLC triglyceride compositional data combined with canonical discrimination analysis showed considerable potential for the classification of olive oil samples according to cultivar and geographic origin.

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## REFERENCES

- Anonymous, 1991. EEC Regulation N. 2568/91. *Off. J. Eur. Commun.*, No. L248, 34th Year, 5 September.
- Aparicio, R., Albi, T., Lanzon, A. & Navas, M. A. (1987). SEXIA, un sistema experto para la identificación de aceites: base de datos de zonas oliveras. *Grasas Aceites Seville*, **38**(1), 9–14.
- Aparicio, R., Ferreiro, L., Cert, A. & Lanzon, A. (1990). Caracterización de aceites de oliva vírgenes Andaluces. *Grasas Aceites Seville*, **41**(1), 23–39.
- Armanino, C., Leardi, R., Lanteri, S. & Modi, G. (1989). Chemometric analysis of Tuscan olive oils. *Chemometr. Intell. Lab. Syst.*, **5**, 343–354.
- Boschelle, O., Rogic, A., Kocjancic, D. & Conte, L. S. (1994). Caratteristiche compositiva della frazione lipidica di due cultivar di olivo dell'isola di Cherso (Croazia) in funzione della maturazione. *Riv. Ital. Sostanze Grasse*, **LXXI**, 341–346.
- Ferreiro, L. & Aparicio, R. (1992). Influencia de la altitud en la composición química de los aceites de oliva vírgenes de Andalucía. Ecuaciones matemáticas de clasificación. *Grasas Aceites Seville*, **43**(3), 149–156.
- Flor, R. V., Le Tiet Hecking & Martin, B. D. (1993). Development of high-performance liquid chromatography criteria for determination of grades of commercial olive oils. Part I. The normal ranges for the triacylglycerols. *J. Am. Oil Chem. Soc.*, **70**(2), 199–203.
- Forina, M., Armanino, C., Lanteri, S., Calcagno, C. & Tiscornia, E. (1983). Valutazione delle caratteristiche chimiche dell'olio di oliva in funzione dell'annata di produzione mediante metodi di classificazione multivariati. *Riv. Ital. Sostanze Grasse*, **LX**, 607–613.
- Gigliotti, C., Daghetta, A. & Sidoli, A. (1993). Indagine conoscitiva sul contenuto trigliceridico di oli extra vergini di oliva di varia provenienza. *Riv. Ital. Sostanze Grasse*, **LXX**, 483–489.
- Leardi, R. & Paganuzzi, V. (1987). Caratterizzazione dell'origine di oli di oliva extravergini mediante metodi chemiometrici applicati alla frazione sterolica. *Riv. Ital. Sostanze Grasse*, **LXIV**, 131–136.
- Synouri, S., Staphylakis, C., Kontou, S. & Tzamtzis, V. (1995). Study on the characteristics of Greek virgin olive oil. *OLIVAE*, **57**, 27–33.
- Synouri-Vrettakou, S., Komaitis, M. E. & Voudouris, E. C. (1984). Triglyceride composition of olive oil, cottonseed oil and their mixtures by low temperature crystallization and gas liquid chromatography. *J. Am. Oil Chem. Soc.*, **61**(6), 1051–1055.
- Tsimidou, M. & Karakostas, K. X. (1993). Geographical classification of Greek virgin olive oil by non-parametric multivariate evaluation of fatty acid composition. *J. Sci. Food Agric.*, **62**, 253–257.
- Tsimidou, M., Macrae, M. & Wilson, I. (1987). Authentication of virgin olive oils using principal component analysis of triglyceride and fatty acid profiles. Part I. Classification of Greek olive oils. *Food Chem.*, **25**, 227–239.