Classification of Virgin Olive Oils of the Two Major Cretan Cultivars Based on Their Fatty Acid Composition

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ABSTRACT: Fatty acid composition was determined for 105 virgin olive oil samples of the two dominant Cretan olive cultivars, Koroneiki and Mastoides, harvested from different producing areas at different maturity stages. The oils of the Koroneiki cultivar were characterized by lower concentrations of oleic and decaheptanoic and higher concentrations of linoleic and palmitic acids. Oils obtained from high-altitude locations were rich in monounsaturated fatty acids, while oils obtained from low-altitude locations had higher content of saturated fatty acids. Palmitic and palmitoleic acids increased with increasing altitude in both cultivars examined. The statistical analysis of the compositional data showed significant potential for the classification of the samples according to cultivar and location of origin.

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KEY WORDS: Altitude, Cretan olive oil, cultivar, fatty acids, location, maturation stage.

It is well established by many authors that fatty acid composition of olive oil is strongly influenced by cultivar (1–3), maturation stage of fruit (4–8), and the zone of origin, characterized by certain pedoclimatic factors as well as other minor areal parameters (9–12). Some researchers have tried to verify the origin of certain oils using their fatty acid profile aided by sophisticated statistical methods (3,13,14).

Olive oil is the most important agricultural product in the island of Crete and the quality of oil from the Chania region is well known. However, a thorough study of the compositional characteristics for oils of this region has not been performed.

In a previous work (15), the triacylglycerol profile of olive oil samples combined with canonical discrimination analysis showed considerable potential for the classification of the oils according to cultivar and geographic origin. In this research work, we studied the fatty acid composition of virgin olive oil samples of two different varieties from different cultivated zones at three maturity stages from the Chania region.

MATERIALS AND METHODS

A total of 105 extra virgin olive oil samples was obtained in one harvesting period from Koroneiki and Mastoides cultivars. The choice of sampling locations was made on the basis of their altitude, distance from the sea, and climate. Chrysopigi is a low-altitude location (8 m). Falasarna is also a low-altitude location (24 m) very close to the sea with the lowest annual rainfall, the maximum evaporation, and highest relative humidity of the sampling locations. Kakopetros and Floria are high-altitude locations (630 m and 580 m, respectively). Both belong to the same central mountainous area with similar climatic conditions. Samplings were taken at three different stages of maturity from 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.

Oil was extracted using a laboratory-scale mill as follows: olives were immediately washed, deleafed, and crushed with a hammer crusher operating at 3,000 rpm. The resulting paste was mixed at 28 ± 2°C for 30 min and pressed in a laboratory press at 205 kg/cm^2. After decanting, the oil was centrifuged and filtered.

For oil content determination, 50 g of olive paste was dried in an oven at 105°C to constant weight. The dry paste was extracted with hexane using a Soxhlet apparatus. After 12 h of extraction, the solvent was evaporated and the oil content was determined.

For fatty acid analysis, standards of fatty acid methyl esters of high purity were obtained from Polyscience (Niles, IL).

Fatty acid methyl ester preparation. Fatty acid methyl esters were prepared from olive oil using a cold saponification method. Olive oil (0.25 g) was transferred into a test tube, 5 mL hexane and 0.5 mL of 2 N methanolic potassium hydroxide solution were added. The mixture was centrifuged at 3,500 rpm for 2 min. The upper layer (1 µL) was analyzed by a Hewlett-Packard (Palo Alto, CA) gas chromatograph (HP 6890 series) equipped with an autoinjector, a capillary column BPX70 (50 m length, 0.22 mm i.d., and 0.25 µm film thickness) (SGE Scientific Pty Ltd., Australia), and a flame ionization detector. Helium was used as carrier gas and nitrogen as makeup gas. The oven temperature was held at 165°C for 5 min and increased to 220°C at a rate of 2°C/min and held for 15 min. The inlet temperature was 250°C and the detector temperature was 280 °C. The flow rate was 0.8 mL/min and the split ratio was 1/80. Twelve fatty acids including C16:0; C16:1; C17:0; C17:1; C18:0; C18:1; C18:2; C20:0;

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C18:3; C20:1; C22:0, and C24:0 were identified from retention times similar to the reference standard mixture analyzed under the same operational conditions. The results were expressed as percentage of total fatty acids using the Hewlett-Packard ChemStation software. Comparison of the mean either between varieties, locations, or samplings was achieved using one-way analysis of variance, and Duncan's test was performed to show significant differences among treatments. Multivariate analysis of fatty acid data sets, based on factor and discriminant analysis, was performed to classify the oils.

RESULTS AND DISCUSSION

Olive fruit oil content by percent on a dry weight basis is presented in Table 1 for Koroneiki and Mastoides varieties. Oil content increased from November to January in both varieties. The lower oil content of Mastoides cultivar at Floria is attributed to the high crop load of the trees (16).

The mean values, the confidence intervals, and the least significant difference ($P \leq 0.05$) of the different fatty acids for the oils of Koroneiki and Mastoides in the different locations studied are presented in Tables 2 and 3. Generally, oils of Koroneiki are characterized by lower concentration of C18:1, C17:0, and C17:1 acids and higher concentration of C18:2 and C16:0 acids compared to the oils of Mastoides cultivar.

There were substantial differences in fatty acid composition between locations, being significant in the case of Koroneiki oils for C16:0, C16:1, C18:0, C18:1, and C18:2 and in the case of Mastoides oils for C16:0, C16:1, C17:0, C17:1, C18:0, C18:3, C20:1, and C22:0 acids. In both cases C16:1 decreased significantly with increasing altitude (11). Oil samples from Chrysopigi and Falasarna, low-altitude locations with high mean temperatures, had higher content of saturated fatty acids. It has been reported by other researchers (12) that

TABLE 1
Mean[a] Values ± Standard Deviation of Oil Content on Dry Weight Basis of Olive Fruits from Koroneiki and Mastoides Varieties During Maturation

Cultivation area	Harvesting date	Oil content (%) on dry wt. basis Koroneiki	Oil content (%) on dry wt. basis Mastoides
Chrysopigi	November	40.85 ± 1.45	50.01 ± 1.66
	December	42.45 ± 3.11	50.25 ± 2.21
	January	48.02 ± 1.98	54.62 ± 2.68
Falasarna	November	43.03 ± 1.55	
	December	43.95 ± 3.64	
	January	49.23 ± 3.78	
Kakopetros	November	45.23 ± 4.42	51.20 ± 4.99
	December	44.65 ± 3.04	52.90 ± 3.56
	January	48.14 ± 3.85	54.46 ± 4.17
Floria	November	42.97 ± 3.70	29.64 ± 3.42
	December	47.55 ± 4.18	33.59 ± 5.16
	January	47.91 ± 2.02	33.11 ± 5.30

[a]Mean values of three determinations.

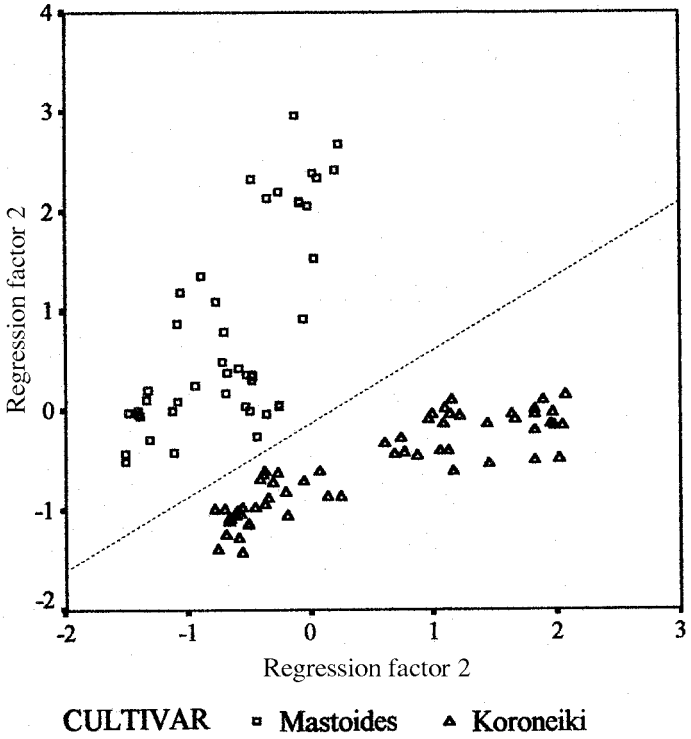

FIG. 1. Scatter plot of olive oil samples based on regression variables extracted from principal component analysis.

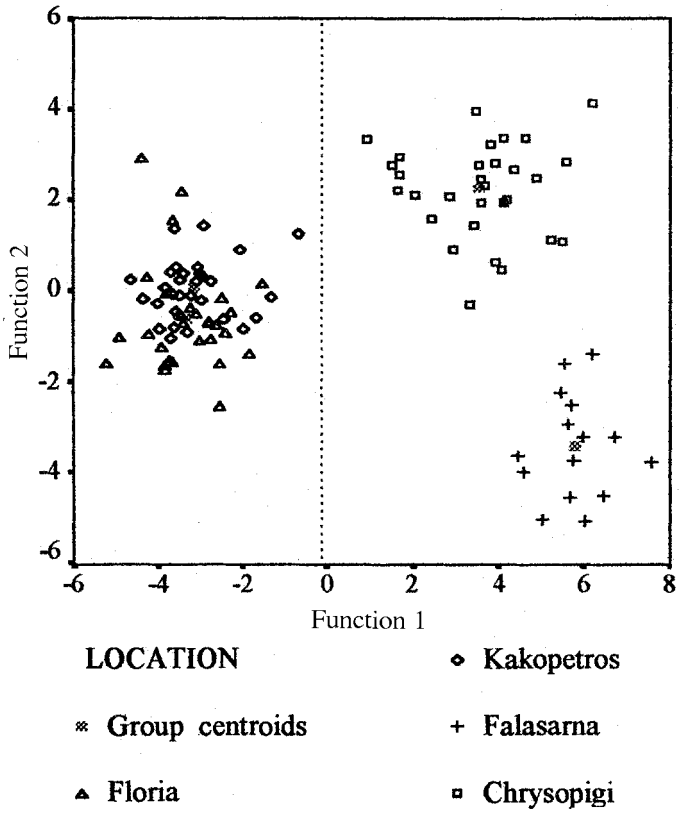

FIG. 2. Canonical discriminant analysis for the classification of oil samples according to geographic origin.

TABLE 2
Mean Values ± 95% Confidence Interval and Least Significant Difference (LSD, $P \leq 0.05$) of Fatty Acid Compositional Data of the Koroneiki Olive Variety from Different Cultivated Areas in Chania Region[a]

Fatty acid	Chrysopigi	Falasarna	Floria	Kakopetros	LSD
C14:0	0.01 ± 0.00[b]	0.01 ± 0.00[a]	0.01 ± 0.00[c]	0.01 ± 0.001[c]	0.001
C16:0	11.66 ± 0.26[b]	12.52 ± 0.35[a]	10.38 ± 0.18[d]	10.75 ± 0.22[c]	0.342
C16:1	0.73 ± 0.02[b]	0.78 ± 0.01[a]	0.60 ± 0.02[d]	0.64 ± 0.02[c]	0.025
C17:0	0.05 ± 0.01[a]	0.05 ± 0.00[a]	0.05 ± 0.003[a]	0.047 ± 0.01[a]	0.005
C17:1	0.07 ± 0.01[b]	0.07 ± 0.01[b]	0.08 ± 0.01[a]	0.07 ± 0.01[b]	0.009
C18:0	2.94 ± 0.05[b]	3.05 ± 0.08[a]	2.31 ± 0.05[d]	2.53 ± 0.11[c]	0.099
C18:1	77.27 ± 0.24[c]	74.66 ± 0.40[d]	79.94 ± 0.22[a]	79.34 ± 0.44[b]	0.441
C18:2	5.52 ± 0.29[b]	7.02 ± 0.27[a]	5.05 ± 0.21[c]	5.11 ± 0.30[c]	0.353
C18:3	0.68 ± 0.02[b]	0.76 ± 0.01[a]	0.58 ± 0.02[c]	0.55 ± 0.02[d]	0.022
C20:0	0.51 ± 0.01[a]	0.52 ± 0.01[a]	0.43 ± 0.01[b]	0.43 ± 0.01[b]	0.010
C20:1	0.28 ± 0.00[c]	0.29 ± 0.003[b]	0.31 ± 0.01[a]	0.29 ± 0.09[c]	0.007
C22:0	0.18 ± 0.01[a]	0.16 ± 0.002[b]	0.16 ± 0.01[b,c]	0.15 ± 0.01[c]	0.01
C24:0	0.06 ± 0.01[b]	0.07 ± 0.01[a]	0.05 ± 0.004[b,c]	0.05 ± 0.01[c]	0.008
Saturated	15.40 ± 0.27[b]	16.38 ± 0.39[a]	13.39 ± 0.22[d]	13.96 ± 0.27[c]	0.389
Monounsaturated	78.35 ± 0.24[c]	75.80 ± 0.39[d]	80.94 ± 0.21[a]	80.34 ± 0.44[b]	0.440

[a]Superscript letters (a–d) indicate significant differences.

TABLE 3
Mean Values ± 95% Confidence Interval and Least Significant Difference (LSD, $P \leq 0.05$) of Fatty Acid Compositional Data of the Mastoides Olive Variety from Different Cultivated Areas in Chania Region[a]

Fatty acid	Chrysopigi	Floria	Kakopetros	LSD
C16:0	10.61 ± 0.31[a]	8.88 ± 0.24[c]	9.66 ± 0.32[b]	0.389
C16:1	0.67 ± 0.03[a]	0.51 ± 0.02[c]	0.55 ± 0.02[b]	0.029
C17:0	0.17 ± 0.004[b]	0.22 ± 0.02[a]	0.14 ± 0.01[c]	0.017
C17:1	0.30 ± 0.01[b]	0.32 ± 0.03[a]	0.23 ± 0.01[c]	0.025
C18:0	2.64 ± 0.06[c]	3.39 ± 0.10[a]	2.84 ± 0.15[b]	0.146
C18:1	79.53 ± 0.26[b]	79.28 ± 0.46[b]	80.41 ± 0.35[a]	0.486
C18:2	4.53 ± 0.14[b]	5.53 ± 0.34[a]	4.76 ± 0.13[b]	0.298
C18:3	0.53 ± 0.01[b]	0.73 ± 0.04[a]	0.45 ± 0.02[c]	0.035
C20:0	0.44 ± 0.01[b]	0.51 ± 0.01[a]	0.43 ± 0.02[b]	0.015
C20:1	0.28 ± 0.005[b]	0.29 ± 0.01[a]	0.27 ± 0.004[c]	0.007
C22:0	0.14 ± 0.004[b]	0.16 ± 0.01[a]	0.12 ± 0.01[c]	0.007
C24:0	0.06 ± 0.004[a]	0.05 ± 0.01[b]	0.05 ± 0.004[b]	0.006
Saturated	14.07 ± 0.25[a]	13.21 ± 0.22[b]	13.24 ± 0.35[b]	0.368
Monounsaturated	80.78 ± 0.23[b]	80.40 ± 0.44[b]	81.46 ± 0.35[a]	0.464

[a]Superscript letters (a–d) indicate significant differences.

TABLE 4
Pearson's Correlation Coefficients Between the Different Fatty Acids

	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0
C14:0	1.000												
C16:0	0.369	1.000											
C16:1	0.476	**0.937**[a]	1.000										
C17:0	0.138	–0.695	–0.618	1.000									
C17:1	0.089	–0.681	–0.592	**0.985**[a]	1.000								
C18:0	0.602	–0.118	–0.110	0.493	0.378	1.000							
C18:1	–0.700	–0.788	–0.780	0.397	0.453	–0.408	1.000						
C18:2	0.572	0.421	0.456	–0.291	–0.368	0.441	**–0.841**[a]	1.000					
C18:3	0.740	0.325	0.341	–0.035	–0.148	0.616	**–0.741**[a]	**0.734**[a]	1.000				
C20:0	0.769	0.382	0.377	–0.011	–0.120	**0.811**[a]	**–0.761**[a]	0.686	**0.864**[a]	1.000			
C20:1	–0.101	–0.010	–0.047	–0.210	–0.233	–0.231	–0.001	0.132	0.184	–0.038	1.000		
C22:0	0.415	0.314	0.311	–0.331	–0.401	0.165	–0.448	0.412	0.614	0.579	0.279	1.000	
C24:0	0.334	0.509	0.487	–0.108	–0.102	0.098	–0.476	0.217	0.321	0.274	0.139	0.157	1.000

[a]Strong correlation coefficients ($P \leq 0.05$).

the percentage of C18:1 is negatively correlated with the relative humidity of the atmosphere. Falasarna had the highest relative humidity compared to the other locations studied, and samples from Falasarna had the lowest concentration of C18:1 (74.66%) and the minimum C18:1/C18:2 ratio (10.66). On the contrary, areas of high altitude such as Floria and Kakopetros (580 m and 630 m, respectively) had cooler climate. Olive oils from these locations had greater levels of monounsaturated fatty acids (6,9) (Tables 2 and 3).

Strong positive correlation coefficients ($P \leq 0.05$) in Table 4 were found between C16:0–C16:1 ($r = 0.937$), C17:0–C17:1 ($r = 0.985$), C18:3–C20:0 ($r = 0.864$), C18:0–C20:0 ($r = 0.811$), and C18:2–C18:3 ($r = 0.734$). Conversely, strong negative correlation coefficients were found between C18:1–C18:2 ($r = -0.841$), C18:1–C18:3 ($r = -0.741$), and C18:1–C20:0 ($r = -0.761$).

The scatter plot of olive oil samples in two axes, regression factor 1 (axis x) and regression factor 2 (axis y) as extracted by principal component analysis, is presented in Figure 1. The two cultivars constitute two distinctive groups above and below a line on the plane of the two regression factors. The classification of olive oils according to the location of origin was efficiently achieved by canonical discriminant analysis in Figure 2. The oils from low-altitude locations (Chrysopigi and Falasarna) can be discriminated from the oils of high-altitude locations. Furthermore, within the low-altitude group, the oils from Chrysopigi and Falasarna constitute two distinctive groups. This can be explained since although both are low-altitude areas, other environmental factors such as relative humidity and rainfall significantly differ. The samples of oils from the high-altitude locations constitute one group. This happens because both sampling sites belong to the same central mountainous area with similar environmental conditions.

Results of this study prove that fatty acid compositional data have a significant discriminating power even within close geographical areas.

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