

Discriminating Power of the Hydrocarbon Content from Virgin Olive Oil of Extremadura Cultivars

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ABSTRACT: Samples of virgin olive oils (105) from seven Extremaduran olive varieties (Cacereña, Carrasqueña, Cornezuelo, Corniche, Morisca, Picual, and Verdial de Badajoz) in three stages of maturity (green, semi-ripe, and ripe) were collected and the alkane, alkene, and sesquiterpene contents determined. There were significant differences at the 0.01 probability level in most of the hydrocarbons, both by variety and by state of maturity. Discriminant analysis applied to 70 samples explained 72.3% of the variance between the different groups of varieties and allowed 90% of the samples to be classified according to their variety. The acceptability of the model was verified against the remaining 35 samples, giving a mean level of correct classification of 94%.

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KEY WORDS: Discriminant analysis, Extremadura, hydrocarbons, single variety, virgin olive oil.

As part of the nonsaponifiable content of vegetable oils, the hydrocarbon fraction has for some time been the subject of studies aimed at the authentication of virgin olive oils and the recognition of fraudulent adulteration with cheaper oils (1).

Squalene is the major constituent of the hydrocarbon fraction of the nonsaponifiable content of olive oils, accounting for 85–90% of the total (2). The remaining 10–15% can provide information that is useful for determining not only the absence of adulterant vegetable oils (1–3) or prior industrial processing (4), but also the variety (5) and even the geographical origin at a national (1,6) or latitudinal (7) level. It has recently been shown that the presence of other compounds, such as alkenes (8) and sesquiterpenes (6), is a major differentiating factor in a hydrocarbon analysis, especially for single-variety virgin olive oils. The composition of the alkane fraction is essentially unique, particularly in virgin oils, and has even been described as the fingerprint of each crop. It consists principally of *n*-alkanes in the range C8–C35, the most abundant being between C21 and C35 (8). The *n*-alkanes with chain lengths C27, C29, and C31 predominate in all vegetable oils except olive oil, in which C23, C25, and C27 are the most important, although there are varieties in which longer chains—C29 and C31—predominate (1,5–8). These differences have been explained as reflecting different environmental conditions (7), different varieties such as Arbequina, Cornicabra, Empeltre, Hojiblanca, or

Picual (5), and even different geographical origins (1,6), with there being a notably lower alkane content in oils from Italy and Spain than in those from Greece and Tunisia. Although fewer in number, there have also been studies showing that the state of maturity of the fruit affects these differences (9), but not the zone or the system of production.

Apart from such facts as the major reductions in hydrocarbon content that result from the different stages of the refining process that has to be applied to some virgin olive oils that need rectification, and that allow the detection of mixtures of these two types of oil (1,10), the group of hydrocarbons under study—despite the pathway of their biosynthesis still being unclear—are a natural part of a virgin olive oil and are therefore linked to its most genuine characteristics. Given this context, the objective of the present work was to quantify the alkanes, alkenes, and sesquiterpenes in virgin oils from the main cultivars grown in Extremadura and to analyze statistically the discriminating capacity of these levels, thereby contributing to a fuller characterization of the oils both by variety and by the state of maturity of the milled olives.

MATERIALS AND METHODS

Samples. Samples of virgin olive oil were collected from different olive varieties considered as most representative of the Extremadura region of Spain, and for each case selected at three states of maturity—green (M.I. < 2.0), semi-ripe (M.I. = 2.0–3.5), and ripe (M.I. > 3.5)—as classified according to the Maturity Index they present (11). In Table 1 are presented descriptions of the oil samples, specifying the variety, the state of maturity, the number of samples, and the location of the groves. The oil was obtained in a uniform fashion from the olive samples using the “Abencor” olive-paste centrifugation system (12). The oil samples were cold-stored in opaque containers until assay.

Chromatographic analysis. (i) *Sample preparation.* Aliquots of 20.00 g of oil were saponified with a 10% solution of KOH in ethanol. The nonsaponifiable fraction was assayed in a chromatography column (50 cm length, 1.5 cm i.d.) filled with silica gel prepared with hexane. Hexane was used because of the low levels of hydrocarbons involved: Alkanes, alkenes, and sesquiterpenes are in the first fraction to be eluted with hexane.

(ii) *Chromatography.* The separation and detection of the hydrocarbons present in this first fraction were performed by injecting 1 μ L of the sample into a Hewlett-Packard model

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TABLE 1
Variety, State of Maturity, Number of Samples, and Origin of the Samples of Extra Virgin Olive Oil Analyzed

Variety	No. of samples ^a	Origin
Cacereña	15	Cáceres. Gata-Hurdes, Ambroz-Jerte-La Vera
Carrasqueña	15	Badajoz. Tierra de Barros
Cornezuelo	15	Badajoz. La Serena
Corniche	15	Badajoz. Vega del Guadiana. Cáceres. Villuerca-Ibores
Morisca	15	Badajoz. Tierra de Barros
Picual	15	Badajoz. La Serena
Verdial de Badajoz	15	Badajoz. Vega del Guadiana. Cáceres. Montanchez

^aFive samples for each state of maturity.

6890 gas chromatograph equipped with an FID and a TRB-5 (30 m) capillary column (Tracer; Sant Cugat del Vallès, Barcelona, Spain). The carrier gas was helium. The starting temperature of the column was 110°C (6 min), with a programmed double ramp up to 200°C (6°C/min) and 300°C (4°C/min). The injector and detector temperatures were 300 and 320°C, respectively. The internal standard used was *n*-icosene. Figure 1 shows a chromatogram in which the different hydrocarbons present in an oil sample are identified.

Statistical analysis. Data for the different hydrocarbons that were detected were subjected to a multivariate ANOVA, taking the variety and state of maturity as fixed factors. A discriminant analysis was then performed on two-thirds of the data, using as variables all the hydrocarbons that were uncorrelated with each other and as grouping variable the olive variety. The remaining data were used to validate the model. All calculations were carried out with the statistical program package SPSS.Base 10 (Chicago, IL).

RESULTS AND DISCUSSION

A total of 105 oil samples, corresponding to the seven varieties and three states of maturity, were assayed for their content of

alkanes, alkenes, and sesquiterpenes having chain lengths between C12 and C35. Table 2 lists the mean values of these determinations.

A study of these data allows us to highlight the fact that alkanes (especially C23 to C33) do not generally exceed a level of 5 ppm in virgin olive oils of Spanish origin, as also indicated by other authors (1), although the results for Cacerañian Manzanilla and Corniche varieties are near this limit. The total content of alkanes, between 18 and 32 ppm, concurs with the mean value of 25.7 ppm established by Webster *et al.* (1).

Other authors (6) indicate slightly higher ranges, 30 to 65 ppm, for the total alkane content in Spanish varieties, including the Picual variety. As regards the C13:1 alkene content, the level of 3.99 ppm found concurs with the level higher than 1 ppm established by Bortolomeazzo *et al.* (6). This minimum level is clearly exceeded in the Corniche and Carrasqueña varieties, 6.56 and 12.12 ppm, respectively. On the other hand, in none of the samples was C17:1 above the 1 ppm established by Bortolomeazzo *et al.* (6). Only the Badajoz Verdial variety is slightly above these limits.

The data were analyzed with a multivariate general factorial GLM (general linear model) using the SPSS.Base 10 program package, taking into account the variety and state of maturity

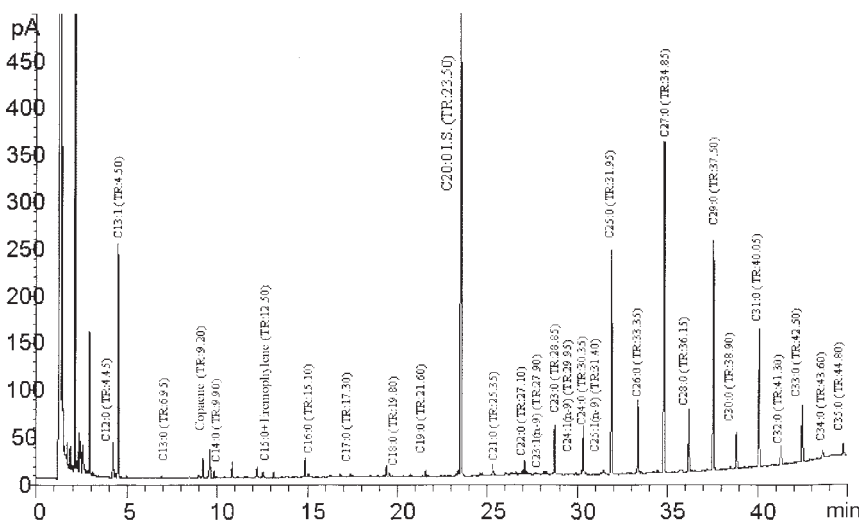


FIG. 1. Chromatogram of alkanes, alkenes, and sesquiterpenes of a sample of olive oil, Picual variety. TR, retention time; I.S., internal standard

TABLE 2
Mean Hydrocarbon Content^a (ppm) (C12:0 to C23:0 and total) of Oils from the Different Varieties of Olive Studied

Variety	<i>n</i>	C12:0	C13:1	C13:0	Copaene	C14:0	Er+ C15:0 ^b	C16:0	C17:1n-8	C17:0	C18:0	C19:0	C21:0	C22:0	C23:1n-9	C23:0
Cacerena	15	0.02a	0.16a	0.03a	1.24b,c	0.04a	0.23a	0.07a	0.15a	0.07a	0.04a	0.08a	0.31a	0.26b	0.06b	1.24b
Carrasqueña	15	0.05a	12.12d	0.03a	0.79a,b	0.05a,b	0.12a	0.06a	0.11a	0.06a	0.06a	0.08a	0.25a	0.21a,b	0.06b	1.06a,b
Comezuelo	15	0.04a	0.42a	0.03a	2.82d	0.06a,b	1.01c	0.06a	0.16a	0.07a	0.06a	0.10a	0.36a	0.22a,b	0.05a,b	1.00a,b
Corniche	15	0.09b	6.56c	0.04a	0.48a	0.05a,b	0.04a	0.06a	0.16a	0.08a	0.09b	0.11a	0.30a	0.27b,c	0.04a,b	1.62c
Morisca	15	0.04a	1.00a	0.03a	1.59c	0.06b	0.68b	0.05a	0.46a	0.10a	0.05a	0.12a	0.40a	0.18a	0.02a	1.12b
Picual	15	0.04a	3.99b	0.03a	0.13a	0.05a,b	0.06a	0.04a	0.13a	0.05a	0.06a	0.07a	0.19a	0.18a	0.02a	0.71a
Verdial Badajoz	15	0.04a	0.22a	0.03a	1.47b,c	0.07b	0.21a	0.06a	1.11b	0.25b	0.05a	0.28b	1.66b	0.32c	0.11c	2.24b
Green	35	0.05a	2.44a	0.03a	1.31a	0.05a	0.32a	0.05a	0.07a	0.06a	0.05a	0.08a	0.25a	0.23a	0.05a	1.24a
Semi-ripe	35	0.05a	3.90b	0.03a	1.12a	0.05a	0.31a	0.06a	0.41b	0.10b	0.06a	0.13b	0.58b	0.23a	0.05a	1.26a
Ripe	35	0.04a	4.15b	0.03a	1.22a	0.06a	0.39a	0.06a	0.49b	0.13b	0.06a	0.15b	0.65b	0.24a	0.05a	1.35a
Variety	<i>n</i>	C24:1n-9	C24:0	C25:1n-9	C25:0	C26:0	C27:0	C28:0	C29:0	C30:0	C31:0	C32:0	C33:0	C34:0	C35:0	Total
Cacerena	15	0.08b	0.71a	0.08b	4.00b	0.86b,c	5.30c	1.21d	7.02d	1.10b	5.20c	0.57c	2.05c	0.23c	0.54c	32.92c
Carrasqueña	15	0.05a,b	0.69a	0.15c	2.98a	0.72a,b	2.39a	0.48a	2.17a	0.35a	1.46a	0.21a	0.76a,b	0.13a,b	0.21a,b	27.87b
Comezuelo	15	0.3a	0.59a	0.03a	2.34a	0.65a	2.56a	0.59a,b	2.70a,b	0.44a	1.65a	0.24a,b	0.82a,b	0.15a,b	0.24a,b	19.49a
Corniche	15	0.04a,b	1.62b	0.03a	5.87c	1.75d	5.53c	1.06d	5.52c	0.89b	3.15b	0.33b	1.07b	0.18b	0.28b	37.32d
Morisca	15	0.02a	0.59a	0.02a	2.34a	0.65a	2.24a	0.53a	2.34a	0.39a	1.52a	0.21a	0.86a,b	0.14a,b	0.27b	18.69a
Picual	15	0.02a	0.53a	0.02a	2.61a	0.95c	3.78b	0.80c	2.74a,b	0.49a	1.50a	0.22a	0.62a	0.11a	0.19a,b	20.30a
Verdial Badajoz	15	0.03a,b	0.66a	0.10b	2.89a	0.73a,b	4.02b	0.71b,c	3.29b	0.45a	1.52a	0.22a	0.56a	0.11a	0.15a	23.54a,b
Green	35	0.04a	1.10c	0.07a	3.96b	1.06a	3.58a	0.89b	4.27b	0.68b	2.67b	0.32b	1.03a	0.15a	0.27a	26.38a
Semi-ripe	35	0.04a	0.67b	0.05a	3.13a	0.86a	3.60a	0.71a	3.31a	0.52a	1.94a	0.25a	0.83a	0.14a	0.24a	24.88a
Ripe	35	0.04a	0.55a	0.06a	2.78a	0.78a	3.90a	0.71a	3.46a	0.57a,b	2.24a	0.29a,b	1.02a	0.16a	0.29a	25.93a

^aMeans in a column followed by different letters are significantly different ($P < 0.01$).

^bEr = eremophylene.

TABLE 3
Standardized Coefficients of the Canonical Discriminant Functions

	Function					
	1	2	3	4	5	6
C12:0	0.081	0.1	0.299	0.426	-0.143	-0.468
C13:1	1.515	0.985	0.541	0.212	0.095	0.689
C13:0	-0.179	0.298	0.213	-0.402	0.159	0.456
Copaene	0.273	0.518	-0.246	0.317	0.926	0.602
C14:0	-0.164	0.241	-0.322	0.601	-0.186	0.342
Erem ^a + C15	0.035	-0.136	0.003	-0.143	0.176	0.18
C16:0	0.212	-0.381	0.244	-0.36	0.699	-0.633
C17:1n-8	-0.211	0.027	-0.455	0.296	-0.441	0.94
C17:0	0.098	-0.129	-0.341	0.539	-0.173	0.029
C18:0	-0.075	-0.065	-0.342	0.056	-0.319	0.49
C19:0	0.424	-0.008	0.722	-1.001	1.271	-0.296
C21:0	0.298	-0.142	-0.01	1.519	-0.508	0.551
C22:0	-1.571	0.627	1.085	0.85	-0.361	0.833
C23:1n-9	-0.172	0.27	0.377	0.521	-0.071	0.696
C23:0	1.394	0.033	-1.087	-1.734	1.076	-3.107
C24:1n-9	0.056	0.091	-0.02	0.616	-0.427	0.362
C24:0	-0.416	0.733	-0.992	1.675	-1.72	1.122
C25:1n-9	0.54	0.874	0.032	0.453	0.253	-0.371
C25:0	-0.914	1.383	2.234	-0.328	0.734	2.38
C26:0	3.31	-1.894	-1.069	0.253	1.172	-1.003
C27:0	-0.41	0.542	-0.357	1.642	-0.279	0.947
C28:0	-1.701	0.976	-1.821	0.656	-2.693	-0.18
C29:0	0.647	-1.169	3.552	-0.668	2.214	0.499
C30:0	0.833	0.022	0.032	0.427	0.765	0
C32:0	-0.86	0.317	-0.027	0.393	-0.487	0.665
C33:0	0.457	0.685	-1.784	0.013	-1.205	-0.916
C34:0	0.15	-0.654	0.691	-1.022	1.083	0.54
Total	-0.985	-1.78	-0.548	-2.009	-0.879	-2.201

Function	Eigenvalues			
	Eigenvalue ^b	% of variance	% accumulated	Canonical correlation
1	31.962a	51	51	0.985
2	13.338a	21.3	72.3	0.964
3	8.336a	13.3	85.6	0.945
4	4.670a	7.5	93.1	0.908
5	3.591a	5.7	98.8	0.884
6	.745a	1.2	100	0.653

^aErem, eremophylene.

^bThe first 6 canonical discriminant functions were used in the analysis.

as factors of variation. The resulting model was significant at the 0.01 level of probability. The variety was the factor having the greater weight in the explanation of the total variance, and the variables C13:1, C26:0, C29:0, and C31:0 were of notable importance ($R^2 = -0.920, -0.880, -0.831, \text{ and } -0.802$, respectively). With respect to this factor, all the hydrocarbons except C13:0 and C16:0 were found to be significant. With respect to the maturity factor, at this same significance level only the hydrocarbons C13:1, C17:1n-8, C17:0, C19:0, C21:0, C24:0, C25:0, C28:0, C29:0, C30:0, C31:0, and C32:0 presented differences between states of maturity.

The comparison of means obtained in this analysis showed that there was always some hydrocarbon that was capable of differentiating one of the varieties from the rest. Indeed, in the cases of Cacereña and Corniche there were numerous distinguishing hydrocarbons, with the long-chain hydrocarbons (C29:0 to C34:0) being the most appropriate for the former, and

the medium-chain hydrocarbons (C24:0 to C29:0) for the latter. There was a notably high C13:1 content in the varieties Carrasqueña, Corniche, and Picual, with the Carrasqueña level being twice that of Corniche and four times that of Picual. With respect to the maturity factor, the comparison of means showed that only the hydrocarbon C24:0 allowed the three states of maturity to be differentiated, although there were others that differentiated the green state of maturity from semi-ripe and ripe.

In view of these results, we looked for functions that would relate the different variables and allow oils to be classified by variety according to the levels of hydrocarbons that they present. To this end, we used the SPSS.Base10 package to perform a discriminant analysis with the results of 70 of the samples, using the variety as the grouping variable. A prior correlation analysis between the variables showed some of them to be highly correlated with each other, and hence one of each correlated pair had to be excluded from the discriminant analysis. In

TABLE 4
Results of the Classification^{a,b}

	Variety	Predicted membership group							Total	
		Cacereña	Carrasqueña	Cornezuelo	Corniche	Morisca	Picual	Verdial de Badajoz		
Cases selected	Count	Cacereña	10	0	0	0	0	0	0	10
		Carrasqueña	0	10	0	0	0	0	0	10
		Cornezuelo	0	0	10	0	0	0	0	10
		Corniche	0	0	0	10	0	0	0	10
		Morisca	0	0	1	0	9	0	0	10
		Picual	0	0	0	0	0	10	0	10
		Verdial B.	0	0	0	0	0	0	10	10
	%	Cacereña	100	0	0	0	0	0	0	100
		Carrasqueña	0	100	0	0	0	0	0	100
		Cornezuelo	0	0	100	0	0	0	0	100
		Corniche	0	0	0	100	0	0	0	100
		Morisca	0	0	10	0	90	0	0	100
		Picual	0	0	0	0	0	100	0	100
		Verdial B.	0	0	0	0	0	0	100	100
Cases not selected	Count	Cacereña	5	0	0	0	0	0	0	5
		Carrasqueña	0	5	0	0	0	0	0	5
		Cornezuelo	0	0	5	0	0	0	0	5
		Corniche	0	1	0	4	0	0	0	5
		Morisca	0	0	1	0	4	0	0	5
		Picual	0	0	0	0	0	5	0	5
		Verdial B.	0	0	0	0	0	0	5	5
	%	Cacereña	100	0	0	0	0	0	0	100
		Carrasqueña	0	100	0	0	0	0	0	100
		Cornezuelo	0	0	100	0	0	0	0	100
		Corniche	0	20	0	80	0	0	0	100
		Morisca	0	0	20	0	80	0	0	100
		Picual	0	0	0	0	0	100	0	100
		Verdial B.	0	0	0	0	0	0	100	100

^aCorrectly classified 98.6% of the selected original grouped cases.

^bCorrectly classified 94.3% of the not-selected original grouped cases.

particular, C31:0 was excluded because of its correlation with C29:0 (0.956) and C33:0 (0.941), and C33:0 because of its correlation with C35:0 (0.964).

In Table 3, the standardized coefficients of the estimated dis-

criminant functions are given. One observes the great weight of the hydrocarbons C26:0, C28:0, C22:0, C13:1, and C23:0 in the first function, and of C26:0, total hydrocarbons, C25:0, and C29:0 in the second. From the table of eigenvalues, one sees that these two functions explain 72.3% of the variance between the different groups of varieties.

Table 4 (top half) gives the classification given by the model. One observes that only the variety Morisca presents any uncertainty in its classification, with only 90% of the oils correctly classified. The bottom half of Table 4 gives the results of the validation of the model with the remaining 35 samples. One here observes that there was some problem with the classification of the varieties Morisca and Corniche. Even so, the mean level of correct classification was greater than 94 %, indicative that the model is quite acceptable.

Figure 2 is a graphical representation of the differentiated groups of varieties. One observes a notable clustering of the varieties Morisca, Cornezuelo, and Verdial de Badajoz.

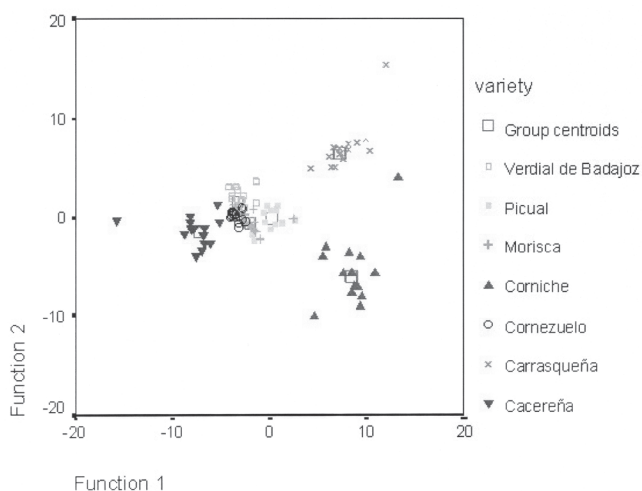


FIG. 2. Canonical discriminant functions.

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