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ORIGINAL PAPER

Characteristics of Virgin Olive Oils from the Olive Zone of Extremadura (Spain), and an Approximation to their Varietal Origin

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Abstract We studied the differences in the characteristics that typify virgin olive oils produced in three representative zones of Extremadura: Sierra Norte of Cáceres, Serena–Siberia, and Tierra de Barros. A total of 156 samples from those three zones were assayed for fatty acids, triglycerides, sterols, and erythrodiol + uvaol. Statistically significant differences ($p < 0.05$) were found between the mean values of the parameters corresponding to the three zones. These profiles were then used to classify the varietal origin of the oils using the discriminant functions that had previously been defined by the authors for these parameters. This classification, together with the production data, confirmed the use of the different varieties of olives in the study zones. The results showed Manzanilla Cacereña to be the most used variety in the Sierra Norte of Cáceres zone, Cornezuelo and Verdial de Badajoz with a slight presence of Picual in the Serena–Siberia zone, and Carrasqueña and Morisca in Tierra de Barros.

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Keywords Virgin olive oil · Discriminant function · Triglycerides · Fatty acids · Sterols · Erythrodiol and uvaol

Introduction

Extremadura is the third-ranked Spanish Region in terms of area of olive groves, representing 11% of the nation's total. The European Commission's Regulation EEC/2138/97 [1] delimits the zones of homogeneous production of olive oil, establishing 12 such zones for Extremadura—6 each in the provinces of Badajoz and Cáceres. The zones which comprise the greatest areas devoted to this crop in Extremadura are Serena–Siberia, Tierra de Barros, and Vegas del Guadiana in Badajoz, and the Sierra Norte of Cáceres consisting of the zones of Gata–Hurdes, La Vera, Jerte, and Ambroz.

The main varieties of olives grown and distributed in these zones are Carrasqueña, Manzanilla Cacereña, Cornezuelo, Corniche, Morisca, and Picual Verdial de Badajoz. The composition and quality parameters of the oils obtained from each of these varieties have been studied in earlier work [2, 3]. It is to be expected that the composition and characteristics of the oils produced by the industries of each zone respond to those of the varieties grown there, although this is not always the case given the possibility that some mills produce oil from olives grown in other zones due to commercial interests.

Numerous studies have indicated the usefulness of determining the quality parameters of olive oils, in particular their fatty acid and triglyceride profiles, to distinguish different types of oil [4], and even to reveal the varietal origin [5–9] or area of production [10, 11]. Recently too, their sterol profiles have begun to be widely used to differentiate oils with specific characteristics [12], for fraud detection [13], and to study their varietal origin [14].

It would be very useful, drawing on the studies which have linked the varietal origin of oils with the characteristics of their composition, to be able to identify the varieties of olives used industrially in each production zone, and to detect any possible deviations that occur with respect to the use of the varieties grown there, taking into account that the parameters such as profiles of fatty acids, triglycerides and sterols are characteristic of each variety of olive and are not affected by the agricultural growing conditions [3].

The objective of the present work is to present the fatty acid, triglyceride, and sterol and erythrodiol + uvaol profiles of oils produced industrially in the zones of Sierra Norte of Cáceres, La Serena–Siberia, and Tierra de Barros. It is also to use the discriminant functions that were defined from the profiles of many oils produced from strictly controlled olives [15–17] to classify these industrial oils by defining the variety or varieties from which they were produced. If, moreover, one takes into account the production data for these varieties grown in the aforementioned zones, one could expect to attain an improved definition of the characteristics of the oil produced in each zone, independently of the variety of olives grown there.

Materials and Methods

Oil Samples

A total of 156 samples of virgin olive oil were studied. Of these, 46 were collected from 10 olive oil mills located in the olive-oil producing zones of Gata–Hurdes, Jerte, La Vera, and Ambroz (Cáceres, Extremadura), grouped together as from the "Sierra Norte of Cáceres", during the 2000–2001 and 2001–2002 campaigns; 49 were collected from 9 mills in the production zone of Serena–Siberia (Badajoz, Extremadura) during the 2003–2004 and 2004–2005 campaigns; and 61 were from 10 mills in the Tierra de Barros zone (Badajoz, Extremadura) during the 2005–2006 and 2006–2007 campaigns.

The samples were taken directly from the vertical centrifuge, filtered, and then stored until assay at 4 °C in topaz-colored glass bottles of 500 ml capacity, leaving the least possible head-space, in order to protect the oil from the oxidizing action of air and light. Assays were performed during the corresponding sampling campaign.

Analytical Methods

Fatty Acid Assays

The analytical method used was that laid out in the European Commission's Regulation EEC/2568/91 [18] and subsequent amendments, based on gas–liquid chromatography of the methyl esters of the fatty acids extracted with hexane.

Triglyceride Assays

Triglycerides were assayed by high performance liquid chromatography (HPLC), using the official EU method (Regulation EEC/2472/97) [19] established to determine the composition in olive oils of triglycerides identified by their equivalent carbon number (ECN).

Sterol and Erythrodiol + Uvaol Assays

Sterols and erythrodiol + uvaol were determined following the procedures set out in Annexes V and VI of Regulation EEC/2568/91 [18] and subsequent amendments.

Statistical Analysis

The data (fatty acid, triglyceride, and sterol plus erythrodiol + uvaol concentrations for the three different zones) were analyzed by ANOVA (Analysis of Variance), using the SPSS 13.0 software package. Differences were considered statistically significant at a 95% confidence level ($p < 0.05$).

The oils of each zone were classified by varietal origin on the basis of their assay profiles (fatty acids, triglycerides, and sterols plus erythrodiol + uvaol) using the discriminant functions that had already been calculated for the varieties Carrasqueña, Manzanilla Cacereña, Corniche, Cornezuelo, Morisca, Picual, and Verdial de Badajoz, which presented an average percentage of success in their validation of 87.5% for the corresponding fatty acids, 83.5% for the function obtained from the triglycerides and 100% for the resulting profile Sterols and Erythrodiol–Uvaol. For this, we used the discriminant analysis procedures of the same SPSS 13.0 statistical package, with the parameters of the oils of each zone as validation samples for those functions. Figures 1, 2 and 3 plot the first two functions, showing the respective coefficients.

Results and Discussion

To characterize the oils produced in the three study zones, their fatty acid, triglyceride, and sterol plus erythrodiol + uvaol profiles were analyzed.

Fatty Acids

Table 1 lists, for the three zones, the mean values of the different fatty acids quantified. These values lie within the

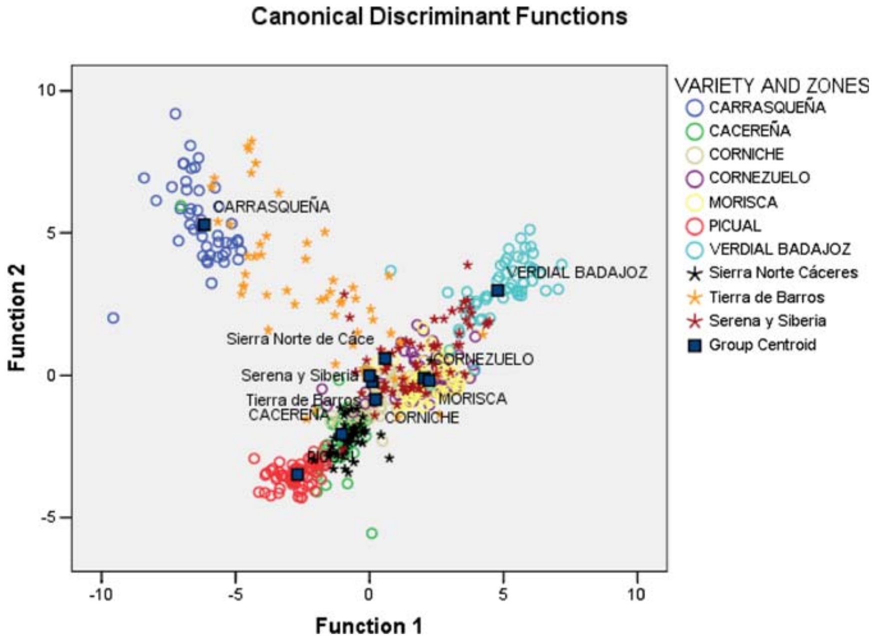


Fig. 1 Discriminant functions obtained from the fatty acid profiles

Function		Palmitic	Palmitoleic	Margaric	Stearic	Linoleic	Linolenic	Gadoleic	Behenic	Lignoceric
	1	0,565	-0,498	-0,641	0,353	0,852	-0,104	0,525	0,633	-0,373
	2	0,273	0,077	0,737	0,299	0,671	-0,659	0,496	0,184	0,240

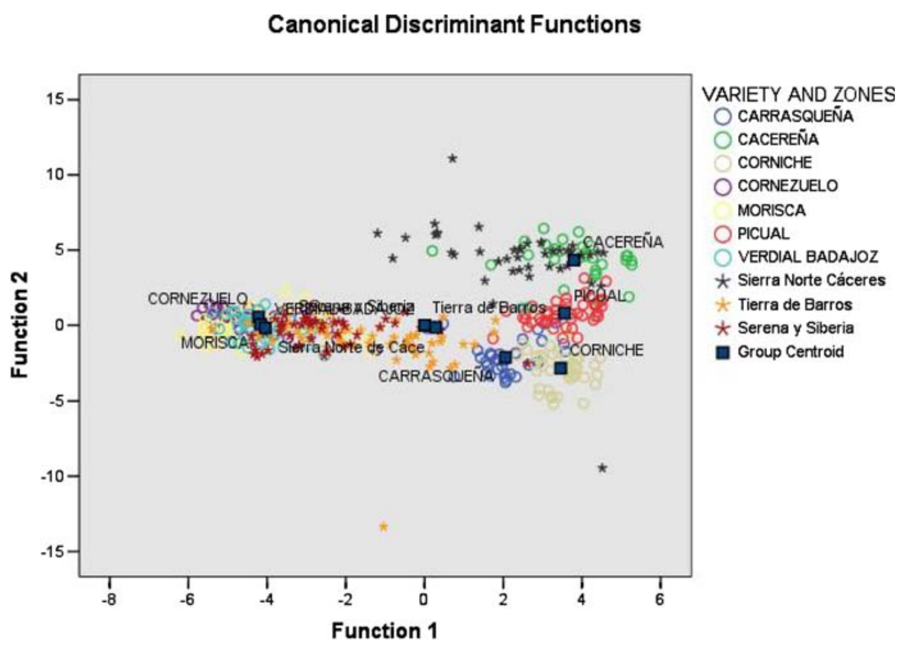
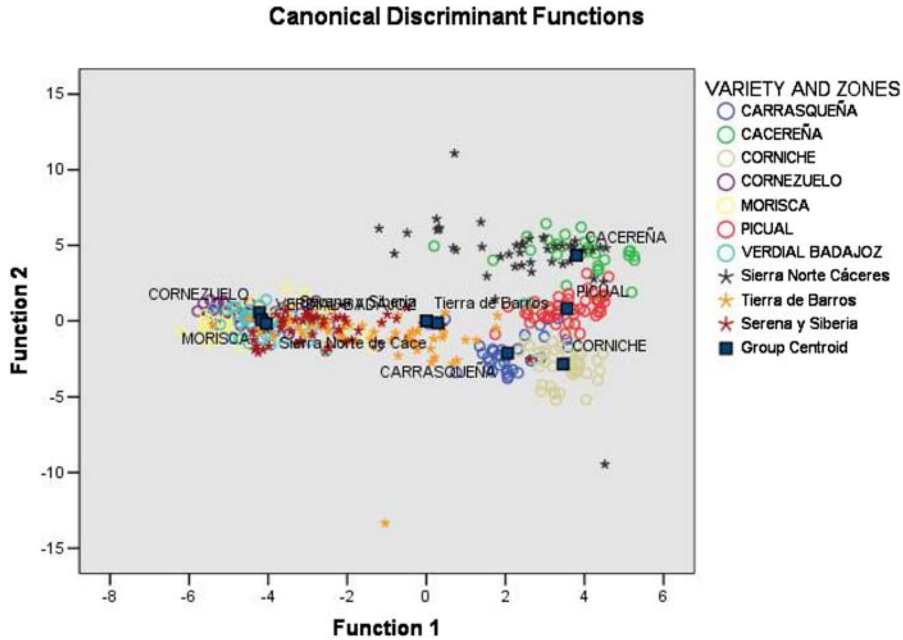


Fig. 2 Discriminant functions obtained from the triglyceride profiles

Function		LLL	OLLn	PLLn	OLnO	OLO	PLO+ SLL	OOO	POO	PPO	PPP	SOO	SLS+ POS
	1	0,696	0,074	-0,302	0,279	-0,497	-0,734	0,35	0,348	0,107	0,476	0,184	-0,155
	2	0,34	-0,161	-0,064	-0,178	0,555	1,082	1,643	1,592	-1,236	0,634	-1,409	0,644

range considered normal for virgin olive oils, with high levels of oleic acid and low levels of linoleic acid. Nonetheless, there were marked differences between the groups, with, as one observes in the table, the ANOVA showing the differences to be significant for most of the acids in the profile. One clearly appreciates that the oils produced in

Fig. 3 Discriminant functions obtained from the sterol and erythrodiol + uvaol profiles



Function		Cholesterol	24-Metilencholesterol	Campesterol	Campestanol	Estigmasterol	Δ7-Campesterol	Clerosterol
	1	0,241	0,608	0,015	0,728	-0,412	0,548	0,059
	2	0,396	0,118	-1,254	0,147	-0,059	0,092	0,308

Sitostanol	5,24-Estigmastadienol	Δ5-Avenasterol	Δ7-Estigmastenol	Δ7-Avenasterol	Total sterols	Erytrodiol +Uvaol
-0,222	0,174	-0,193	0,642	-0,329	1,540	0,106
-0,166	-0,148	0,219	0,857	-0,029	0,133	0,122

Table 1 Mean fatty acid content of the olive oils and standard deviation of the three zones

	Sierra Norte de Cáceres	Serena–Siberia	Tierra de Barros
No. of samples	46	49	61
Palmitic (%)	11.93 ± 0.95 †	11.81 ± 0.52 †	12.96 ± 0.55 ‡
Palmitoleic (%)	0.91 ± 0.09 †	0.95 ± 0.14 †	1.16 ± 0.16 ‡
Margaric (%)	0.04 ± 0.01 †	0.06 ± 0.01 ‡	0.10 ± 0.04 §
Stearic (%)	1.95 ± 0.27 †	3.50 ± 0.37 ‡	3.33 ± 0.20 §
Oleic (%)	77.57 ± 1.57 †	71.08 ± 2.43 ‡	70.27 ± 3.20 ‡
Linoleic (%)	4.45 ± 1.39 †	10.77 ± 2.00 ‡	10.52 ± 2.83 ‡
Linolenic (%)	0.69 ± 0.05 †	0.81 ± 0.04 ‡	0.69 ± 0.08 †
Arachidic (%)	0.36 ± 0.03 †	0.45 ± 0.08 ‡	0.40 ± 0.06 §
Gadoleic (%)	1.89 ± 1.19 †	0.27 ± 0.02 ‡	0.20 ± 0.03 ‡
Behenic (%)	0.12 ± 0.01 †	0.14 ± 0.01 ‡	0.13 ± 0.01 §
Lignoceric (%)	0.06 ± 0.02 †	0.06 ± 0.01 †	0.06 ± 0.01 †

†, ‡, § Different symbols for the same parameter mean significant differences between zones

the Sierra Norte of Cáceres present significantly higher oleic and gadoleic acid levels than those produced in the other two zones, as well as significantly lower levels of margaric, stearic, linoleic, arachidic, and behenic acids. The lignoceric acid content showed no significant differences between the three zones.

These data, differentiated by production zone, were used as the validation set to test the discriminant functions that had been established in previous work [15] for classification according to varietal origin. The results for each zone are presented in Table 2.

It is noteworthy that of the oil samples from the Sierra Norte of Cáceres zone, 91.3% were classified as deriving from the Manzanilla Cacereña variety. Of this group, some oils were classified as Carrasqueña, Cornezuelo, and Picual, but in no case did they surpass 5%.

The oils collected in the Serena–Siberia zone were mostly classified as originating from the variety Cornezuelo (65.3%). Also, however, 22.4% of the samples

Table 2 Classification obtained for the oils according to their fatty acid profiles

Origin of sample	Sample	Carrasqueña		Cacereña		Corniche		Cornezuelo		Morisca		Picual		Verdial Badajoz	
	Totals	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%
Sierra Norte de Cáceres	46	1	2.2	42	91.3			2	4.3			1	2.2		
La Serena–Siberia	49					5	10.2	32	65.3			1	2.0	11	22.4
Tierra de Barros	61	26	42.6			3	4.9	18	29.5	11	18.0	3	4.9		

corresponded to the Verdial de Badajoz variety, and 10.2% to Corniche.

The samples from Tierra de Barros corresponded to the varieties Carrasqueña (42.6%), Cornezuelo (29.5%), and Morisca (18.0%). These levels are indicative of very diverse origins.

Triglycerides

Table 3 lists the mean triglyceride values for the oils from the three zones under study. The levels are consistent with the literature values for genuine virgin olive oils, with the most representative triglycerides being OOO, POO, OLO, PLO + SLL, and SOO.

In general, all the oils had very low levels of LLL, in no case exceeding 0.5%. There were significant differences (ANOVA, $p < 0.05$) between the three production zones, particularly in that the Sierra Norte of Cáceres oils had higher OOO and POO contents, and lower OLO, PLO + SLL, SOO, OLL, and SLS + POS contents than the other two zones.

Table 4 presents the varietal origin classification obtained for the samples from the different zones. The oils from the Sierra Norte of Cáceres zone were mostly classified (80.4%) as of the Manzanilla Cacereña variety origin, with 13% classified as Picual. Most of the oils from the Serena–Siberia zone were classified as Cornezuelo (57.1%) and Morisca (32.7%). Most of the Tierra de Barros oils were classified as Carrasqueña (39.3%), Morisca (34.4%), and Cornezuelo (18.0%). The classification in both of these zones is thus very diverse.

Sterols

The sterol composition of an olive oil together with its triterpene alcohol (erythrodiol + uvaol) content are widely used parameters for the detection of adulteration, and are considered to be criteria of purity. Table 5 presents the profiles found for all the samples. One observes that the most representative sterols are β-sitosterol and Δ-5-avenasterol.

Table 3 Mean triglyceride content of the olive oils and standard deviation of the three zones

	Sierra Norte de Cáceres	Serena–Siberia	Tierra de Barros
No. of samples	46	49	61
LLLn (%)	0.09 ± 0.06 †	0.00 ± 0.00 ‡	0.00 ± 0.02 ‡
LLL (%)	0.01 ± 0.05 †	0.16 ± 0.04 ‡	0.17 ± 0.10 ‡
OLLn (%)	1.00 ± 1.18 †	0.33 ± 0.05 †	0.29 ± 0.10 ‡
PLLn (%)	0.00 ± 0.02 †	0.11 ± 0.02 ‡	0.09 ± 0.04 §
OLL (%)	0.59 ± 0.76 †	2.91 ± 0.72 ‡	2.52 ± 1.00 §
OLnO (%)	1.08 ± 0.79 †	2.14 ± 0.18 ‡	1.96 ± 0.51 ‡
PLL (%)	0.95 ± 2.37 †	0.55 ± 0.04 †	0.80 ± 1.22 †
OLO (%)	8.78 ± 1.61 †	14.79 ± 1.91 ‡	13.08 ± 2.21 §
PLO + SLL (%)	3.93 ± 0.88 †	7.85 ± 1.19 ‡	7.60 ± 1.96 ‡
PPL (%)	0.06 ± 0.18 †	0.80 ± 0.47 †‡	1.39 ± 3.14 ‡
OOO (%)	48.79 ± 3.63 †	36.15 ± 3.65 ‡	34.81 ± 4.90 ‡
POO (%)	25.80 ± 1.96 †	22.52 ± 0.83 ‡	24.66 ± 2.34 §
PPO (%)	3.26 ± 0.30 †	3.30 ± 0.22 †	3.96 ± 1.39 ‡
PPP (%)	0.75 ± 0.19 †	0.47 ± 0.06 ‡	0.56 ± 0.51 ‡
SOO (%)	3.83 ± 0.45 †	5.69 ± 0.49 ‡	5.62 ± 0.87 ‡
SLS + POS (%)	0.87 ± 0.19 †	1.49 ± 0.12 ‡	1.62 ± 0.31 §

†, ‡, § Different symbols for the same parameter mean significant differences between zones

The ANOVA showed clearly significant differences ($p < 0.05$) for β-sitosterol, Δ-5-avenasterol, campesterol, and stigmasterol in the Sierra Norte of Cáceres oils compared to the other two zones. No statistically significant differences were found for the cholesterol, sitostanol, Δ-5,4-stigmastadienol, and Δ-7-stigmastenol contents.

The campesterol and stigmasterol levels comply with the legislation established for virgin olive oils, with less than 4% for the former, and a lower content for the latter.

The sum of the triterpene diols (erythrodiol + uvaol) also differentiated ($p < 0.05$) the oils from the Sierra Norte of Cáceres zone, although none of the three groups had levels above the established limit (4.5%) for extra virgin olive oils.

Table 4 Classification obtained for the oils according to their triglyceride profiles

Origin of sample	Sample	Carrasqueña		Cacereña		Corniche		Cornezuelo		Morisca		Picual		Verdial de Badajoz	
	Totals	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%
Sierra Norte de Cáceres	46	1	2.2	37	80.4	1	2.2					6	13.0	1	2.2
La Serena–Siberia	49					1	2.0	28	57.1	16	32.7	4	8.2		
Tierra de Barros	61	24	39.3			1	1.6	11	18.0	21	34.4	3	4.9	1	1.6

Table 5 Mean sterol and Erythrodiol + Uvaol content of the olive oils and standard deviation of the three zones

	Sierra Norte de Cáceres	Serena–Siberia	Tierra de Barros
No. of samples	46	49	61
Cholesterol (%)	0.14 ± 0.10 †	0.81 ± 4.28 †	0.12 ± 0.03 †
24-Methylenecholesterol (%)	0.19 ± 0.04 †	0.26 ± 0.08 ‡	0.20 ± 0.05 †
Campesterol (%)	2.50 ± 0.13 †	2.80 ± 0.28 ‡	2.40 ± 0.20 §
Campestanol (%)	0.09 ± 0.01 †	0.20 ± 0.17 ‡	0.10 ± 0.02 †
Stigmasterol (%)	1.54 ± 0.76 †	1.05 ± 0.85 ‡	0.80 ± 0.15 ‡
Δ-7-Campesterol (%)	0.01 ± 0.01 †	0.05 ± 0.10 †	0.33 ± 0.50 ‡
Clerosterol (%)	0.96 ± 0.14 †	1.14 ± 0.34 ‡	1.02 ± 0.08 †
b-Sitosterol (%)	81.18 ± 1.70 †	83.55 ± 1.76 ‡	82.82 ± 2.15 ‡
Sitostanol (%)	0.43 ± 0.08 †	0.53 ± 0.42 †	0.53 ± 0.09 †
Δ-5-Avenasterol (%)	11.82 ± 1.84 †	10.05 ± 4.39 ‡	10.19 ± 2.15 ‡
Δ-5,24-Stigmastadienol (%)	0.55 ± 0.06 †	0.50 ± 0.76 †	0.66 ± 0.08 †
Δ-7-Stigmastenol (%)	0.16 ± 0.05 †	0.33 ± 1.04 †	0.23 ± 0.08 †
Δ-7-Avenasterol (%)	0.42 ± 0.07 †	0.45 ± 0.53 †‡	0.58 ± 0.12 ‡
Erythrodiol + Uvaol (%)	2.12 ± 0.56 †	2.86 ± 0.68 ‡	2.70 ± 0.60 ‡

†, ‡, § Different symbols for the same parameter mean significant differences between zones

Table 6 Classification obtained for the oils according to their sterol and erythrodiol + uvaol profiles

Origin of samples	Sample	Carrasqueña		Cacereña		Corniche		Cornezuelo		Morisca		Picual		Verdial de Badajoz	
	Totals	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%	Classified	%
Sierra Norte de Cáceres	46	8	17.4	21	45.7							1	2.2	16	34.8
La Serena–Siberia	49	6	12.2	1	2.0	1	2.0			11	22.4	8	16.3	21	42.9
Tierra de Barros	61	5	8.2	2	3.3			1	1.6	38	62.3	2	3.3	13	21.3

As was done with the fatty acid and triglyceride data, we classified the oils produced in the three zones according to varietal origin, based on the discriminant functions that had already been defined [17] from the sterol and erythrodiol + uvaol profiles.

The results of the classification are presented in Table 6. One observes that the greatest percentage of the classification of the Sierra Norte of Cáceres samples was as of Manzanilla Cacereña origin (45.7%), followed by Verdial de Badajoz (34.8%) and Carrasqueña (17.4%). Most of the oils from the Serena–Siberia zone were classified as Verdial de Badajoz (42.9%), Morisca (22.4%), Picual (16.3%), and Carrasqueña (12.2%). For the Tierra de Barros oils, the greatest percentage was Morisca (62.3%), followed by Verdial de Badajoz (21.3%) and Carrasqueña (8.2%).

Table 7 Distribution of the olive varieties grown in the three zones

Variety	Sierra Norte of Cáceres		La Serena + Siberia		Tierra de Barros	
	ha cultivated	% ha	ha cultivated	% ha	ha cultivated	% ha
Carrasqueña	10.3	0.0	63.9	0.2	24163.0	42.7
Cacereña	35267.8	98.8	37.9	0.1	31.3	0.1
Corniche	11.6	0.0	192.5	0.6	5.9	0.0
Cornezuelo	12.5	0.0	14942.7	44.6	18.1	0.0
Morisca	9.5	0.0	204.3	0.6	20812.5	36.8
Picual	4.8	0.0	2180.5	6.5	216.5	0.4
Verdial	68.4	0.2	2317.4	6.9	627.9	1.1
Other varieties	321.7	0.9	13590.5	40.5	10655.7	18.8
Total	35706.5	100.0	33529.7	100.0	56530.9	100.0

Olive-Oil Producing Zones

The distribution of the olive varieties grown in Extremadura is available in zoning reports [20], in which field data are contrasted with data already tabulated in the Agricultural Registry of the Extremadura Regional Government. Table 7 presents a summary of the area in production by variety for the three study zones.

Sierra Norte of Cáceres

In the Sierra Norte of Cáceres zone (Gata–Hurdes, Jerte, Vera, Ambroz), there is a clear predominance of the variety Manzanilla Cacereña, which accounts for 98.8% of the area of olive groves. It is thus to be expected that this variety will also be that which is most used for the production of oil. This was confirmed by the classifications (Tables 2, 4) corresponding to the fatty acid and triglyceride profiles, which explained (91.3%) and (80.4%) of the samples as having this origin, respectively. The graphical representation of the samples (Figs. 1, 2) shows that the group of samples originating from the Manzanilla Cacereña variety is well differentiated in a cluster. It also confirms the use of the Picual variety with 13.0% categorized. Since the cultivation of hectares of this variety in this area is negligible, we can conclude that there is an error in the classification or origin in olive oil for milling in other olive-growing regions.

The corresponding classification obtained from the sterol plus erythrodiol + uvaol content also confirmed the use of the variety Manzanilla Cacereña given that the level reached was 45.7%, and of Verdial de Badajoz which is second in production in this zone (68.4 ha), followed by Carrasqueña which is much less representative (10.3 ha). In these last two cases, it must also be taken into account that the differences between the groups of the Manzanilla Cacereña, Morisca, and Picual varieties (Fig. 3) are not very pronounced, and could lead to misclassification increasing the levels of both Morisca and Picual.

Serena–Siberia

In the Serena–Siberia zone, the variety of olive grown the most is Cornezuelo (44.6%), followed by Verdial de Badajoz (6.9%) and Picual (6.5%). The mills in this zone thus have ready access to these three varieties of olives. The classification of the oils produced in this zone made on the basis of their fatty acid profiles confirms the use of the Cornezuelo variety (65.3%), and to a lesser extent of Verdial de Badajoz (22.4%). The percentage of oils classified as of Picual origin was, however, minimal. This may be because of possible confusion in classifying some oils as Corniche instead of Picual given the minimal differences between the two groups as shown in Fig. 1.

The classification on the basis of these oils' triglyceride profiles also confirms the use of the varieties Cornezuelo (57.1%) and Picual (8.2%). However, a large percentage (32.7%) is classified as Morisca, even though there are relatively few hectares producing that variety (204.3 ha) making it unrepresentative of the zone. This could be justified because there is an error in the classification from the discriminant function or the oil industry is obtaining these olives from other areas.

On the basis of the sterol and erythrodiol + uvaol profiles, most of the oil samples are classified as coming from the Verdial de Badajoz variety (42.9%), followed by the varieties Morisca (22.4%) and Picual (16.3%), coherent with the industry's use of these varieties in the zone. It is noteworthy, however, that certain oils are classified as corresponding to the variety Carrasqueña whose production is of minimal importance, and none of them are classified as Cornezuelo. The proximity of the two groups as shown in Fig. 3 could well be the cause of this erroneous classification.

Tierra de Barros

The main varieties of olives grown in the Tierra de Barros zone are Carrasqueña and Morisca (Table 7), and these are confirmed as the varieties most used by the industry in this zone by the classification based on the fatty acid profiles with 60.6% of the samples corresponding to these two varieties. These profiles also indicate use of the Cornezuelo variety (29.5%), even though this variety is not representative of production in this zone. This result was also confirmed by the triglyceride profile classification with a large number of oils (18.0%) being categorized as Cornezuelo. Both the triglyceride and sterol plus erythrodiol + uvaol profiles confirmed the majority use of the varieties Carrasqueña and Morisca by the industries in this zone, with levels of classification of the two varieties taken together representing 73.7 and 70.5%, respectively.

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