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Statistical Characterization of Sicilian Olive Oils from the Peloritana and Maghrebian Zones According to the Fatty Acid Profile

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This paper deals with the characterization of 475 Sicilian virgin olive oils (VOO) produced in 10 different crop years (from 1993 to 2004), according to the cultivar and the geographical origin by means of multivariate statistical analysis applied to fatty acids. In particular, the studied VOOs came from the Peloritana and Maghrebian geological zones. The fatty acid composition was determined by using the official gas chromatographic method. The results suggest that although the effect of the cultivar is significant in the olive oil classification based on the fatty acid composition, a predominant and well-defined geographic effect is also present. This study demonstrated that it is possible to employ an official and inexpensive analytical method coupled with the statistical analysis to ascertain the geographical origin and the cultivar of an extra virgin olive oil.

KEYWORDS: Cultivar; fatty acid; geographical origin; olive oil; statistical analysis

INTRODUCTION

The quality of an olive oil is the result of several factors such as the cultivar, the pedoclimatic condition, and the production practices. Since the 1950s medical and nutritional investigations have confirmed the importance of olive oil, with a high content of monounsaturated fatty acids, in reducing mortality from cardiovascular disease. It has also been suggested that a high intake of olive oil may offer protection against a number of cancers (*1*). Olive oil is promoted as part of the "Mediterranean diet", which is currently viewed as making a favorable dietary contribution and has a positive image in terms of consumer appeal. Sicily is the largest island in the Mediterranean Sea; the mild climate and fertile soil of the seaside plains create conditions for one of the most successful agricultural economies in Italy. Olive oil is of the utmost importance in Sicily, both economically and nutritionally. The production and consumption of olive oil mostly concern the Mediterranean countries. Spain is the major producer (620000 T per year), followed by Italy (500000 T per year), Greece, Portugal, Tunisia, and Turkey. The amount of olive oil consumed in Greece is 18 kg per year per capita, that in Italy is 13 kg per year per capita, and that in Spain is 11 kg per year per capita (*2*); due to the increasing popularity of the Mediterranean diet, in which olive oil is the major fat component, its consumption is expanding to nonproducing countries such as the United States, Canada, and Japan (*3*). The quality and peculiarity of the olive oil depend on several factors including the cultivar and zone of origin; therefore, it is of great importance to find parameters that allow olive oil classification according to these variables (*4*). Furthermore, olive oil classification according to its chemical parameters has been considered to be an important tool for the monitoring of adulteration (*5*) and mislabeling (*6*), which often consists in false labeling concerning the geographic origin or the olive variety. Many researchers have reported the classification of olive oils according to their cultivar or geographical origin by means of statistical analysis applied to fatty acids and triacylglycerols (*7*−*12*), sensory attributes (*13*), and also minor compounds (*14*−*16*).

During recent years there have been several research studies aimed at the development of analytical methods to authenticate the origin of extra virgin olive oils, but most of these methods are time- and money-consuming and involve instruments that are not often present in quality control laboratories (e.g., ^{1}H and ^{13}C NMR spectroscopy and isotope ratio mass spectrometry) (*14*−*20*). This study describes the use of an official and inexpensive analytical method based on gas chromatographic determination of fatty acid coupled with multivariate statistical analysis to ascertain the geographical origin and cultivar of an extra virgin olive oil. The first studies on the multivariate analysis applied for the geographic classification of virgin olive oil (VOO) based on gas chromatographic data were carried out by Forina et al. in 1983 (*21*). Oleic, linoleic, and palmitic fatty acids are important for the nutritional properties of an olive oil and showed a crucial role in the characterization of olive oils.

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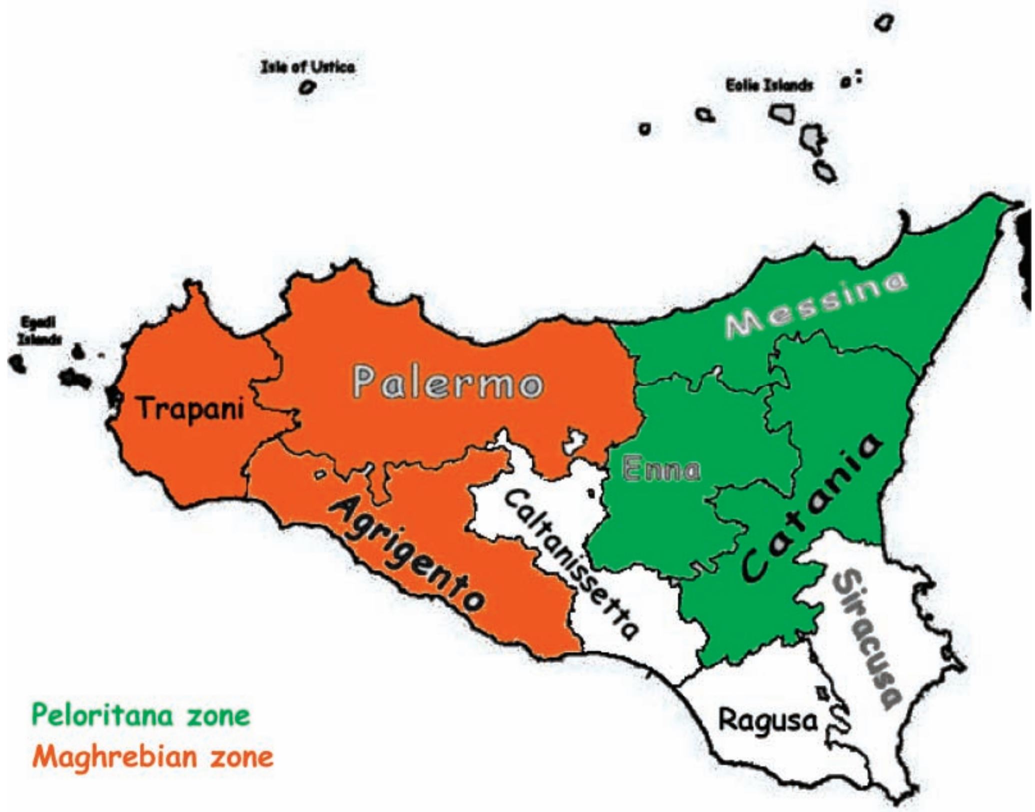

Figure 1. Sicilian provinces belonging to the Peloritana and Magrhebian areas.

Sicily is perhaps the region with the greatest number of olive varieties and cultures in the whole of the Mediterranean basin. Studies conducted on the olive culture in the region have indicated more than 60 different olive types with myriad synonyms and homonyms; in fact, the different methods of identifying and cataloging the different plant types have in the past led to a certain amount of confusion. The entire island is involved in the culture of olives, but certain regions distinguish themselves by the characteristics of the nature and peculiarity of its oils. An important aspect concerns the geographical characterization of olive oils. An act of legislation, the ''protected designation of origin" PDO (1992; 1997; 2001), allows some European extra virgin olive oils to be labeled with the names of the production areas. In Sicily there are six PDO olive-growing zones: "Monti Iblei" (Reg. CEE 2523/97), "Valli Trapanesi" (Reg. CEE 2523/97), "Val di Mazara" (Reg. CEE 138/01), "Monte Etna" (Reg. CEE 1491/03), "Valle del Belice" (Reg. CEE 1486/04), and "Valdemone" (Reg. CEE 205/05). The most widely grown olive varieties in western Sicily, that is, the Maghrebian area, which comprises the Valli Trapanesi, Valle del Belice, and Val di Mazara PDO zones, are Cerasuola and Nocellara del Belice. Minuta and Ogliarola Messinese are the most important varieties in eastern Sicily, that is, the Peloritana area, which comprises the Valdemone and Monte Etna PDO zones (*22*). In this paper data expressing fatty acids concentration of 475 extra virgin Sicilian olive oils produced in 10 different crop years (from 1993 to 2004) in two different geological areas—Maghreb and Peloritana—were elaborated by multivariate statistical analysis to classify olive oils according to their zone of origin. At first, the cultivars were studied separately, then variables such as the province of provenience and the geological area were included in the statistical analysis.

MATERIALS AND METHODS

Sampling. Four hundred and seventy-five extra virgin olive oils were analyzed. These olive oils were collected in 10 years (1993–2004) and came from four cultivars grown in two different geological areas of Sicily: Maghrebian and Peloritana. Cerasuola and Nocellara del Belice varieties came from the Maghrebian area (western Sicily), whereas Minuta and Ogliarola Messinese varieties came from the Peloritana area (Eeastern Sicily); only eight samples of the Ogliarola Messinese variety came from the Maghrebian area. As **Figure 1** shows, each of the studied geological areas comprised three different Sicilian provinces. Further information concerning the studied olive oil samples, such as year of harvesting, the geological area, the cultivar, and the country of origin, are reported in **Tables 1 −3**.

Gas Chromatographic Analysis. The fatty acid composition was released as methyl ester by the official GU of the CEE methylation procedure (European Community Regulation, 1991) and analyzed by gas chromatography (GLC). A Shimadzu GC 17A (Milano, Italy) instrument, equipped with a split/splitless injector (split ratio 70:1) and flame ionization detector, was used.

A Mega 10 fused silica capillary column, 50 m $\times$ 0.32 mm i.d., 0.25 μm film thickness (Legnano, Milano, Italy), was employed.

The following chromatographic conditions were used: column temperature, 170 °C; injector and detector temperatures, 250 and 280 °C, respectively; carrier gas, hydrogen, linear velocity = 50 cm/sec.

Peak areas of 13 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) were calculated using a HP 3394A integrator, the obtained results are expressed as mean $\pm$ standard deviation (SD) of three replicates (**Table 4**). The 13 normalized variables were submitted to the statistical procedure.

Statistical Method. Chemical data were processed using the software packages SPSS 12.0 and Statistica 6.0 for Windows. An initial explorative analysis was performed, using box-plots based on the median and the quartiles, to allow the anomalous samples to be removed. Then, to group variables expressing significant differences

Table 1. Olive Oil Samples Classified According to Cultivar

cultivar	years	samples
Cerasuola	1995/1996	6
	1996/1997	6
	1997/1998	9
	1998/1999	11
	1999/2000	39
	2000/2001	8
	2001/2002	11
	2002/2003	15
	2003/2004	7
	total	112
Minuta	1993/1994	46
	1995/1996	1
	1996/1997	1
	1997/1998	1
	1998/1999	9
	1999/2000	9
	2001/2002	18
	2002/2003	14
	total	99
Nocellara del Belice	1995/1996	20
	1996/1997	22
	1997/1998	31
	1998/1999	18
	1999/2000	41
	2000/2001	28
	2001/2002	12
	total	172
Ogliarola Messinese	1993/1994	28
	1995/1996	9
	1996/1997	1
	1997/1998	6
	1998/1999	15
	1999/2000	13
	2000/2001	2
	2001/2002	12
	2002/2003	6
	total	92
total	1993/1994	74
	1995/1996	36
	1996/1997	30
	1997/1998	47
	1998/1999	53
	1999/2000	102
	2000/2001	38
	2001/2002	53
	2002/2003	35
	2003/2004	7
	total	475

Table 2. Olive Oil Samples Classified According to the Zone of Provenience

zone	cultivar	no. of samples
Maghreb	Cerasuola	112
	Nocellara del Belice	172
	Ogliarola Messinese	8
	total	292
Peloritana	Minuta	99
	Ogliarola Messinese	84
	total	183
total	Cerasuola	112
	Minuta	99
	Nocellara del Belice	172
	Ogliarola Messinese	92
	total	475

Table 3. Olive Oil Samples Classified According to Provenience and Cultivar

cultivar	country of provenience	no. of samples
Cerasuola	Buseto Palizzolo (TP)	9
	Monreale (PA)	8
	Paceco (TP)	68
	Partinico (PA)	9
	San Vito Lo Capo (TP)	1
	Salemi (TP)	12
	Sciacca (AG)	5
	total	112
Minuta	Castell'Umberto (ME)	52
	Naso, Sinagra (ME)	1
	Capo d'Orlando(ME)	46
	total	99
Nocellara del Belice	Aragona (AG)	1
	Campobello di Mazara (TP)	10
	Castelvetrano (TP)	140
	Mazara del Vallo (TP)	1
	Partanna (TP)	20
	total	172
Ogliarola Messinese	Altavilla Milicia (PA)	1
	Brolo (ME)	41
	Bronte (CT)	1
	Castell'Umberto (ME)	1
	Forza d'Agrò (ME)	1
	Gallodoro (ME)	1
	Misilmeri (PA)	7
	Patti, Barcellona, Milazzo (ME)	28
	Sant'Agata di Militello (ME)	6
	San Pier Niceto (ME)	5
	total	92

between the two olive-growing zones, a descriptive statistical analysis was applied. Subsequently, factorial analysis by principal component analysis (PCA) and linear discriminant analysis (LDA) were performed for different categorized groups to establish in which way fatty acids contribute to discriminate samples.

RESULTS AND DISCUSSION

The gas chromatographic analysis of 475 olive oil samples gave the complete fatty acid profile: 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.

The normalized variables corresponding to 475 samples of olive oils were submitted to a multivariate statistical procedure. The initial explorative analysis confirmed that all of the samples contributed to the statistical model. Olive oils from the Maghrebian area include samples from the Cerasuola and Nocellara del Belice varieties, whereas olive oils from the Peloritana area include samples from the Minuta and Ogliarola Messinese varieties. Only 8 samples from the Ogliarola Messinese variety came from the Maghrebian area (**Table 2**).

The preliminary descriptive analysis gave evidence that samples from the Peloritana area had higher concentrations of C16:0, C17:0, C17:1, C18:2, and C16:1 fatty acids than samples from the Maghrebian area. On the other hand, samples from the Maghrebian area showed higher amounts of C18:0, C18:1,

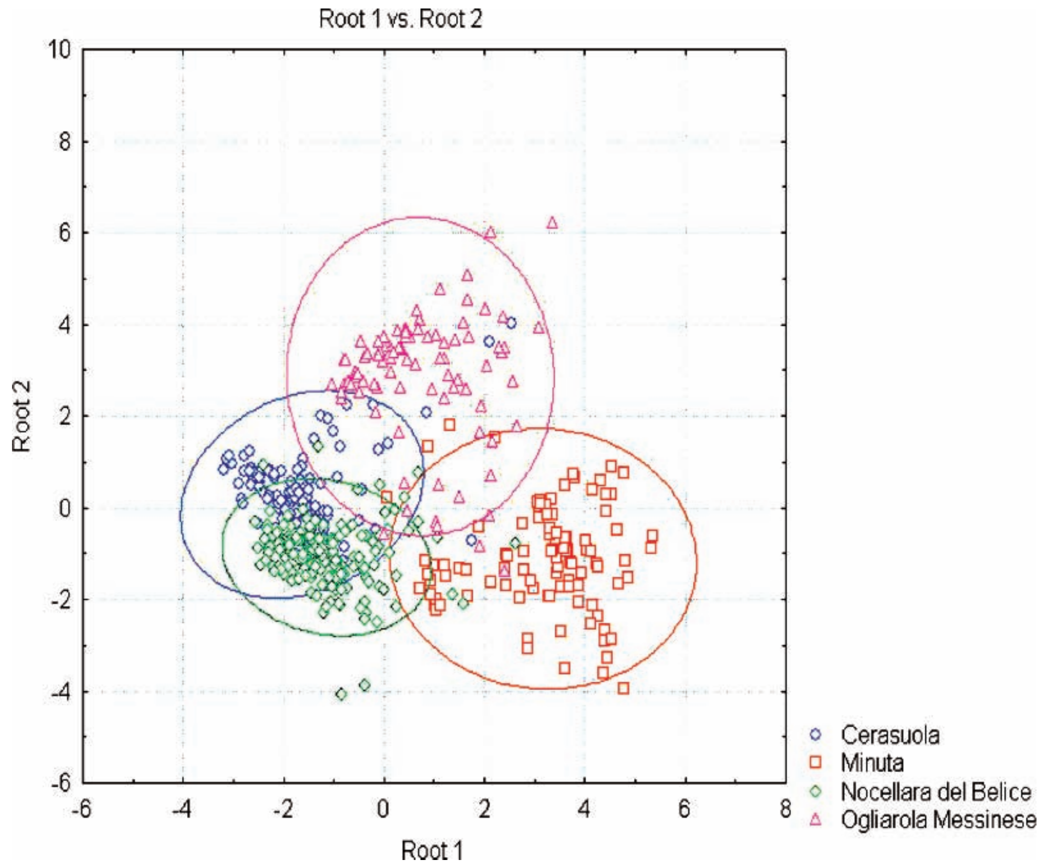

Figure 2. Linear discriminant analysis resulting from applying the discriminant functions to the data expressing fatty acid levels (independent variables) of olive oils from different cultivars (grouping variables).

Table 4. Fatty Acid Concentrations in the Studied Olive Oil Samples (Mean ± SD, $n = 3$)

fatty acid	Cerasuola	Minuta	Nocellara del Belice	Ogliarola Messinese
C14:0	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
C16:0	10.00 ± 1.26	15.8 ± 1.46	13.42 ± 1.90	13.4 ± 1.60
C16:1	0.34 ± 0.17	1.54 ± 0.40	0.98 ± 0.27	0.75 ± 0.28
C17:0	0.06 ± 0.04	0.15 ± 0.06	0.05 ± 0.03	0.21 ± 0.06
C17:1	0.08 ± 0.06	0.26 ± 0.08	0.08 ± 0.03	0.27 ± 0.06
C18:0	2.55 ± 0.32	2.02 ± 0.44	2.70 ± 0.48	2.93 ± 0.52
C18:1	76.30 ± 2.27	66.05 ± 3.11	73.05 ± 2.26	75.80 ± 2.9
C18:2	9.32 ± 1.66	13.00 ± 1.92	8.35 ± 1.08	5.37 ± 2.53
C18:3	0.53 ± 0.11	0.60 ± 0.17	0.66 ± 0.19	0.56 ± 0.15
C20:0	0.40 ± 0.06	0.34 ± 0.10	0.39 ± 0.09	0.41 ± 0.10
C20:1	0.26 ± 0.13	0.15 ± 0.10	0.21 ± 0.12	0.16 ± 0.11
C22:0	0.07 ± 0.05	0.06 ± 0.04	0.05 ± 0.03	0.07 ± 0.04
C24:0	0.05 ± 0.05	0.04 ± 0.03	0.05 ± 0.03	0.05 ± 0.03

and C20:1. The effect of the cultivar and of the area of provenience on olive oil fatty acid composition is discussed separately.

Influence of Cultivar on Fatty Acid Content. The statistical importance of the influence of the cultivar on the fatty acid content was investigated using LDA. First, the discrimination between samples belonging to different cultivars, without considering the geological area of origin, was studied. LDA (**Figure 2**) shows that four groups of olive varieties can be identified. The fairly good discrimination shows a light overlap between Cerasuola and Nocellara del Belice samples, both belonging to the Maghrebian area, whereas a higher degree of discrimination was noted for Ogliarola Messinese from both the Maghrebian and Peloritana zones and for Minuta from the Peloritana zone. To discriminate among several type of cultivars, two discriminant functions have been considered, explaining on average about 81% of the total variance over the samples and both including variables with the highest index of variability. The analysis of correlations between variables and discriminant functions shows a positive correlation between C17:1, C16:1 and function 1 and a negative correlation between this function and C18:1.

Effect of Cultivar on Fatty Acid Content with Respect to Geological Area of Origin. To investigate the effect of cultivar on the fatty acid composition with the two geological areas of interest also considered, samples from the Maghrebian and Peloritana areas have been selected separately. LDA applied on samples from the Maghrebian area gives the results shown in **Figure 3**: two discriminant functions explaining 73% of the total variance allowed a good discrimination among the three cultivars (Cerasuola, Nocellara del Belice, and Ogliarola Messinese). In particular, the discriminant power of the model allowed eight samples belonging to the Ogliarola Messinese cultivar to be separated, which establishes their own position in the graph.

PCA was chosen as a statistical tool to study the Peloritana area because it gave a more significant model than LDA. The scatterplot shown in **Figure 4** points out a discrete separation between the two cultivars Minuta and Ogliarola Messinese, even if the first two principal components account for only almost 60% of the total variance.

These observations allow us to conclude that the cultivar significantly influences the olive oil fatty acid composition unless samples come from different areas of harvest.

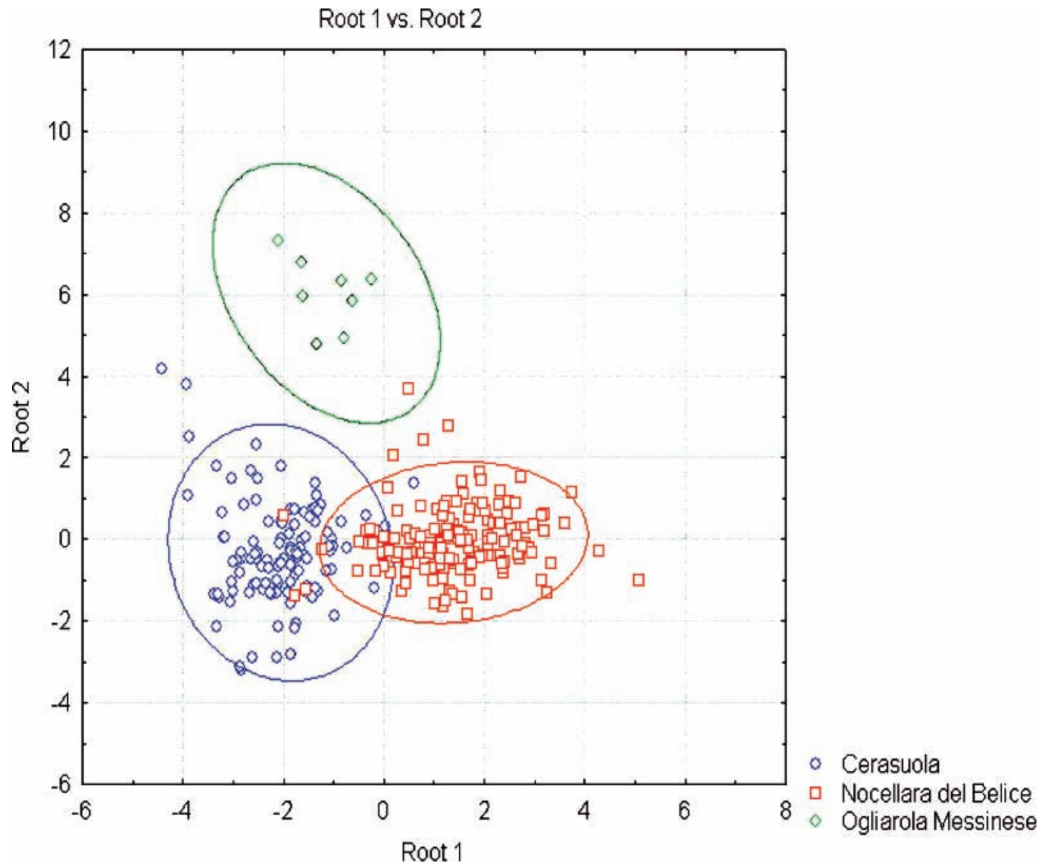

Figure 3. Linear discriminant analysis resulting from applying the discriminant functions to the data expressing fatty acid levels of olive oils from the Maghrebian zone.

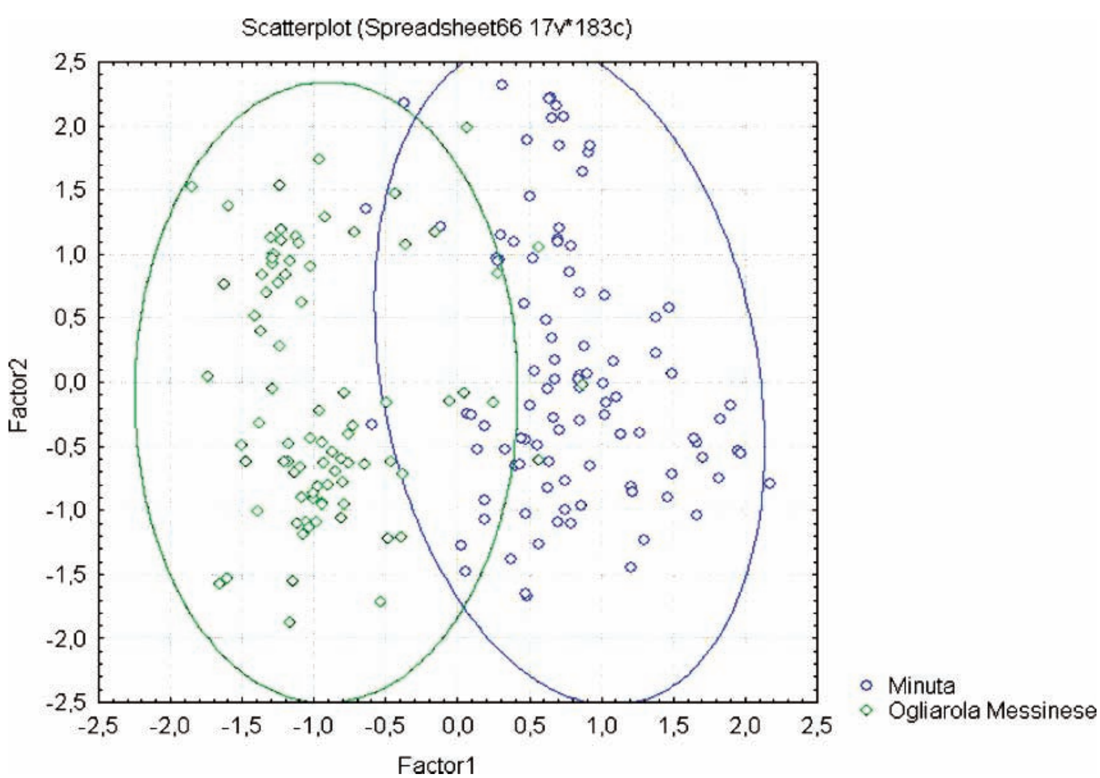

Figure 4. Principal component analysis applied to the data expressing fatty acid levels of olive oils from the Peloritana zone.

Effect of Area of Origin on Fatty Acid Content. The obtained results suggested that a further LDA be performed on olive oils from all of the cultivars to investigate a possible discrimination between the geological areas of origin.

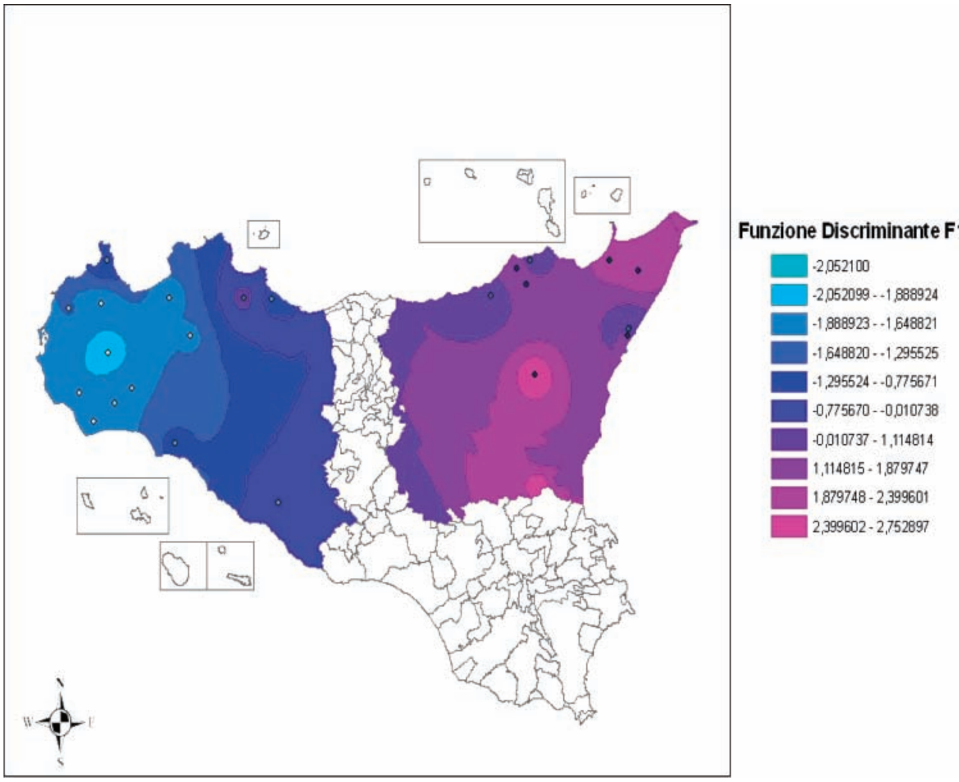

Figure 5. Map of the studied geological area created by representing the F1 value with an inverse distance weighted interpolation.

This analysis has been applied to obtain a geological characterization among the olive oil samples. In this case, only a discriminant function (F1) has been obtained as a linear combination of 10 variables:

$$F1 = 0.62 \times C17{:}1 - 0.44 \times C18{:}0 + 0.44 \times C17{:}0 - 0.59 \times C18{:}1 + 0.28 \times C22{:}0 - 0.21 \times C20{:}1 - 0.11 \times C18{:}3 - 0.08 \times C14{:}0 - 0.23 \times C16{:}1 - 0.23 \times C18{:}2$$

This function is positively correlated with C17:0 and C17:1 variables; moreover, the classification matrix shows that almost 94% of the total samples are correctly classified in relation to the geological area of origin. In particular, only 13 of 292 samlpes from the Maghrebian area and 15 of 183 samples from the Peloritana area were incorrectly classified.

Finally, to obtain a comprehensive and more incisive visualization of the discrimination of the olive oil samples with respect to the obtained function, a mapping has been constructed by a geographic information system (GIS). The map (**Figure 5**) has been obtained by creating a raster surface representing the F1 value with an inverse distance weighted (IDW) interpolation to cover the area without observation samples.

As we can see, the two geological Sicilian areas are well distinguished by the value of the function, represented in a different color with a continued gradation.

These results suggest that although the effect of the cultivar is significant in the olive oil classification based on the fatty acid composition, a predominant and well-defined geographic effect is also present. The variables that contributed the most to this effect were C17:0 and C17:1, whereas the other 10 variables gave a lower contribution to the geological separation.

This work represents the results of a 10 year study during which the importance of olive oil production for the economy of Sicily has increased and, as a result, much more attention has been given to the monitoring of its quality parameters. Furthermore, olive oil has become one of the most regulated alimentary products in the European Union, and some rigid guidelines for its production, marketing, and labeling are applied. In this context, this study is of great importance because the Sicilian virgin olive oils can be classified according to cultivar and geographical origin by means of multivariate statistical analysis applied to fatty acids. Moreover, Sicilian VOO characterization is of great concern because the island has six PDO olive-growing zones; therefore, the method described provides a useful tool for the monitoring of adulteration and mislabeling.

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