

Geographical traceability of West Liguria extravirgin olive oils by the analysis of volatile terpenoid hydrocarbons

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Abstract

This study aimed at evaluating if the volatile terpenoid hydrocarbons of extravirgin olive oils from West Liguria, a North Italy region, could trace their geographical origin. If terpenoid hydrocarbons were individually considered, three compounds, i.e. α -copaene, α -muurolene and α -farnesene, allowed building a simple decision tree and discriminating oils produced in West Liguria from oils produced in other Mediterranean regions. Moreover, the multivariate analysis allowed building West Liguria class-models with high predictive ability, confirming the fundamental role of the volatile terpenoid hydrocarbons for the geographical characterisation of West Liguria oils.

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1. Introduction

Olive oil is a valuable vegetable oil often used without any preliminary refining process. Its extraction is obtained by direct fruit milling and by pressing or centrifugating olive paste, thus preserving the great number of chemical compounds which are characteristic of its aroma and could be damaged by the oil refining process. Extravirgin olive oils are highly appreciated for their excellent and distinctive flavor that results from a great number of chemical compounds of different chemical classes, such as hydrocarbons, aldehydes, alcohols, esters and other compounds [1–4]. These substances originate from metabolic processes that take place inside the fruits and their appearance is dependent on fruit tree species as well as on their cultivars. Most of them significantly suffer from various technological aspects such as olive storage, washing operation, malaxation time and temperature [4–7]. Among olive oil volatiles the terpenoid compounds, also originating from plant metabolism and cultivar related, should mainly suffer from factors clearly dependent on the growing area, such as climatic conditions and parasites devel-

opment. In fact, as reported for other botanical species [8], the constant and continuous presence of parasites promotes the biosynthesis of terpenoids and the amounts of these biochemicals is also influenced by growing temperature and drought, rather typical of the different geographical areas. Thus, manufacturing conditions should affect only marginally their final amounts.

Previous studies proposed the use of the whole hydrocarbons fraction of olive oils for the classification of oil geographical origin and/or cultivar [9–11]. In these studies preliminary and time-consuming extraction steps were required. Nevertheless, volatile hydrocarbons can be detected in olive oil head-space without any purification. Thus, starting from our preliminary observations of the chromatographic plots of olive oils volatiles, this study is focused on the volatile terpenoid hydrocarbons of West Liguria extravirgin olive oils and on their importance for tracing oil geographical origin. The peculiar aroma of these oils, which are particularly valued for their nutritional and sensorial qualities, certainly results both from the olive cultivar (cv. Taggiasca) and from some peculiar features of the growing area. Thus, these niche products deserved the protected designation of origin (PDO) “Riviera Ligure – Riviera dei Fiori” [12] for their high quality. In our previous study [13], the products of lipoxigenase

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(LOX) pathway were used for the distinction between these PDO oils and some extravirgin oils, produced in Spain, having a very similar aroma and sometimes used for their adulteration. Terpenoid hydrocarbons should be less affected than LOX products by technological variables, they can be similarly detected in oil volatile fraction and, in our preparatory studies [14], they appeared quite different in oils from different geographical areas. It was then supposed that they could be, even if individually considered, a straighter and more powerful tool for discriminating the West Liguria oils. Thus, their amounts were determined by direct thermal extraction of oil volatiles, followed by gas chromatography–mass spectrometry (GC–MS). The detected amounts were then compared with those of oils from other Mediterranean areas, whose high production might also induce profitable adulterations.

2. Experimental

2.1. Samples

One-hundred five Mediterranean extravirgin olive oils were analyzed: 30 were oils from West Liguria, 30 from Puglia, 15 from Greece, 15 from Spain and 15 from Tunisia. Oil samples were obtained from the 2003–2004 olive crop by collecting them from farmers in each considered geographical area. Then they were kept in darkness, at room temperature, and analyzed within 3 months from the production date.

2.2. Instruments

GC–MS was performed by an Agilent 6890 GC equipped with an Agilent 5973 mass quadrupole detector (Agilent Technologies, Palo Alto, CA, USA). The injector was a Gertsel CIS4 programmed temperature injector (Gerstel, GmbH, Mülheim an der Ruhr, Germany). It was coupled with an external thermal extraction unit (Gerstel TDS2). In this system, the thermostated block of TDS2, which contained an empty glass tube, formed the extraction unit.

A capillary transfer line connected this block with the CIS4 injector, which can be cooled down to -150°C (with liquid nitrogen) and heated up to 350°C . Helium flowed through the glass tube and the transfer line to the injector. The oil sample was directly introduced in the glass tube and heated. The Helium flow favoured the stripping of volatiles, which were then trapped and focused on the cooled surface of the injector liner.

2.3. Sample analysis

Instrument performances were improved and discussed in details in our previous study [13]. Nevertheless, contrary to what previously reported, the glass tube was left inside the TDS at a controlled 20°C temperature all through the

GC–MS analysis time, thus avoiding the introduction of air and improving repeatability.

In short, volatiles were extracted from $10\ \mu\text{L}$ of oil sample and cryo-focalized in the cooled injection system at -120°C . The starting extraction temperature was 20°C , then it was raised to 40°C at $30^{\circ}\text{C}/\text{min}$ and this temperature was kept for 20 min. At the injection time, the temperature of the injector was raised at a ramp rate of $12^{\circ}\text{C}/\text{s}$ up to 300°C , held for 5 min. In this step the carrier pressure (Helium) was 8.76 psi, the column flow $1.2\ \text{mL}/\text{min}$ and the split vent flow $5\ \text{mL}/\text{min}$.

For the gas chromatographic analysis a $30\ \text{m} \times 0.25\ \text{mm} \times 0.25\ \mu\text{m}$ film thickness DB-5MS fused-silica capillary column (J&W, Folsom, CA, USA) was used at a helium flow rate of $1.2\ \text{mL}/\text{min}$. The oven temperature was kept at 40°C for 5 min and then programmed at $5^{\circ}\text{C}/\text{min}$ to 160°C , kept for 1 min, under flow-controlled conditions (constant flow $1.2\ \text{mL}/\text{min}$). A 10 min post-run at 300°C was then performed. The mass-spectrometer interface temperature was set at 250°C . The temperature of the ion source was 230°C , electron energy 70 eV and quadrupole temperature 150°C .

The chromatographic plots were obtained by total ion current (TIC) mode, with a mass range between 40 and 250 amu (Fig. 1). The relative amount of each compound was calculated by internal normalization and expressed as percentage of the whole volatile fraction. The identification of compounds was obtained by comparison with the spectra reported in Wiley 275 Mass Spectra Library and supported by their Kovats Retention Indexes, since the standards of most terpenoid hydrocarbons were not commercially available.

2.4. Statistical analysis

Statistical data analysis was performed by the packages Systat 5.03 and PARVUS [15].

The univariate statistical parameters of the eight detected terpenoid compounds (original variables) are reported in Table 1. Fisher weights (FW) and correlation coefficients (R) were then computed for the eight original variables and their combinations (sums, differences, products, ratios).

As far as multivariate analysis is concerned, as first step the 105 objects (oil samples) were divided into two categories, the samples coming from West Liguria (L) and all the other samples (Not-Liguria, N), aiming at building a predictive classification rule to discriminate West Liguria samples. A matrix composed by as many rows as samples (105 objects) and by 10 columns ($V + Y$ columns: $V = 8$, i.e. the original variables; $Y =$ the number of their combinations having $\text{FW} > 1.0$ and $R > 0.98$) was built. This data matrix was divided into two subsets by Kennard-Stone algorithm [16]: the former subset was used to build the chemometric rules (*training set*: 84 objects) and the latter was used to validate them (*external test set*: 21 objects, i.e. 20 % of the 105 objects).

Principal component analysis (PCA) was used as unsupervised pattern recognition technique to visualize the data set. Soft independent modeling of class analogy (SIMCA)

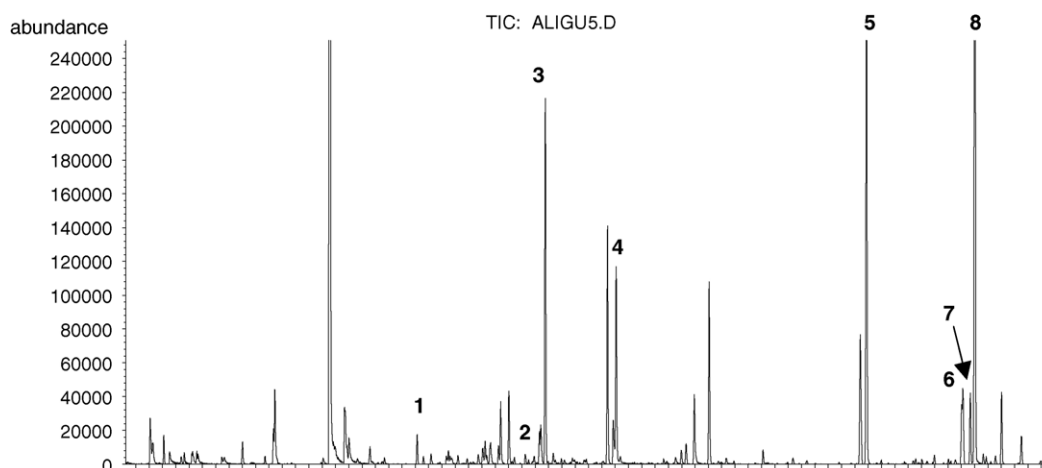


Fig. 1. Chromatographic plot of the volatile fraction of an extravirgin olive oil from West Liguria. (1) α -pinene; (2) limonene; (3) *trans*- β -ocimene; (4) 4,8-dimetil-1,3,7-nonatriene; (5) α -copaene; (6) eremophyllene; (7) α -muurolene and (8), α -farnesene.

Table 1

Univariate statistical parameters of the eight terpenoid hydrocarbons amounts, expressed as percentage of the whole volatile fraction

Variable (% amount)	West Liguria		Puglia		Greece		Spain		Tunisia	
	m ^a	SD ^b	m ^a	SD ^b	m ^a	SD ^b	m ^a	SD ^b	m ^a	SD ^b
(1) α -Pinene	0.89	3.20	0.10	0.18	0.04	0.07	0.04	0.07	0.08	0.06
(2) Limonene	0.88	1.32	0.64	0.81	1.16	0.73	0.87	1.29	0.58	0.25
(3) <i>trans</i> - β -Ocimene	4.69	2.08	0.34	0.37	0.46	0.21	0.89	0.47	2.75	0.91
(4) 4,8-Dimetil-1,3,7-nonatriene	2.17	0.74	0.47	0.55	4.57	2.12	0.66	0.16	0.56	0.22
(5) α -Copaene	5.92	1.13	15.16	6.10	1.13	0.52	1.42	0.64	2.00	1.01
(6) Eremophyllene	1.49	1.69	0.18	0.12	0.13	0.08	0.47	0.24	0.34	0.30
(7) α -Muurolene	0.63	0.17	2.44	0.99	0.09	0.06	0.09	0.08	0.27	0.20
(8) α -Farnesene	10.42	5.96	0.51	0.72	1.57	1.00	10.26	7.20	8.74	4.34

^a m = mean.

^b SD = standard deviation.

was used as class-modeling tool [15]. SIMCA calculates the principal components for each category separately; then, the model functions for each class are calculated using a specified number of principal components. In this study, each class was suitably modelled by using two PCs and a 95% confidence level. The obtained results were validated both internally, using cross-validation with five cross-validation groups, and externally, using the test set selected by Kennard-Stone algorithm. Internal and external prediction rates (the percentages of correctly predicted objects), sensitivity (the non-error rate for each category) and specificity (the percentage of objects of the other class rejected by the class model under study) of the West Liguria model was used to measure its classification and modeling performances (Table 2).

As second step, in order to support the importance of the eight terpenoid hydrocarbons for discriminating West Liguria category against each other category, an analogous chemometric treatment was applied to the 105 objects (oil samples) divided into five categories (West Liguria, L; Puglia, P; Greece, G; Spain, S; Tunisia, T), according to their geographical origin. Thus, four distinct data matrixes were built: each matrix had as many rows as the number of L + X oil samples (X = P or G or S or T) and V + Y columns (Table 2).

Table 2

SIMCA results

Category	%Prediction rate (evaluation set)	%Prediction rate (test set)	Sensitivity (%) ^a	Specificity (%) ^a
L	95.2	100.0	81.0	100.0
N	96.8	100.0	90.5	100.0
L	100.0	100.0	82.6	92.0
P	96.0	100.0	88.0	100.0
L	100.0	100.0	82.6	100.0
G	100.0	100.0	84.6	100.0
L	100.0	100.0	88.0	100.0
S	100.0	100.0	90.9	100.0
L	100.0	100.0	86.4	92.9
T	92.8	100.0	85.7	100.0

^a Sensitivity and specificity are reported at 95% confidence level.

3. Results and discussion

Eight terpenoid hydrocarbons, i.e. α -pinene, limonene, *trans*- β -ocimene, 4,8-dimetil-1,3,7-nonatriene, α -copaene, eremophyllene, α -muurolene, α -farnesene (in order of increasing RRT), were often detected in the analyzed sam-

ples. The relative standard deviation (RSD) was between 3.1 and 7.4 %, with the exception of α -farnesene, which had a 10.1% relative standard deviation.

Just looking at the chromatographic plots, the amounts of some of these compounds immediately appeared quite different in the oils from West Liguria with respect to their amounts in the other four groups of olive oils (Table 1 and Fig. 2).

trans- β -Ocimene content was generally low in the oils from Puglia, Greece and Spain, while in samples from West

Liguria the amounts of this terpenoid hydrocarbon were higher, ranging between 1.7 and 10.0 %, with eight values above 5.0%. Nevertheless, one Puglia and one Spain sample had a *trans*- β -ocimene content next to 1.7%.

The detected amounts of 4,8-dimethyl-1,3,7-nonatriene, a terpenoid C11 unsaturated aliphatic hydrocarbon, were also quite different in the five groups of samples, with values $\leq 1.0\%$ in oils from Spain and Tunisia and $\leq 2.1\%$ in Puglia. In samples from West Liguria and Greece the percent amounts

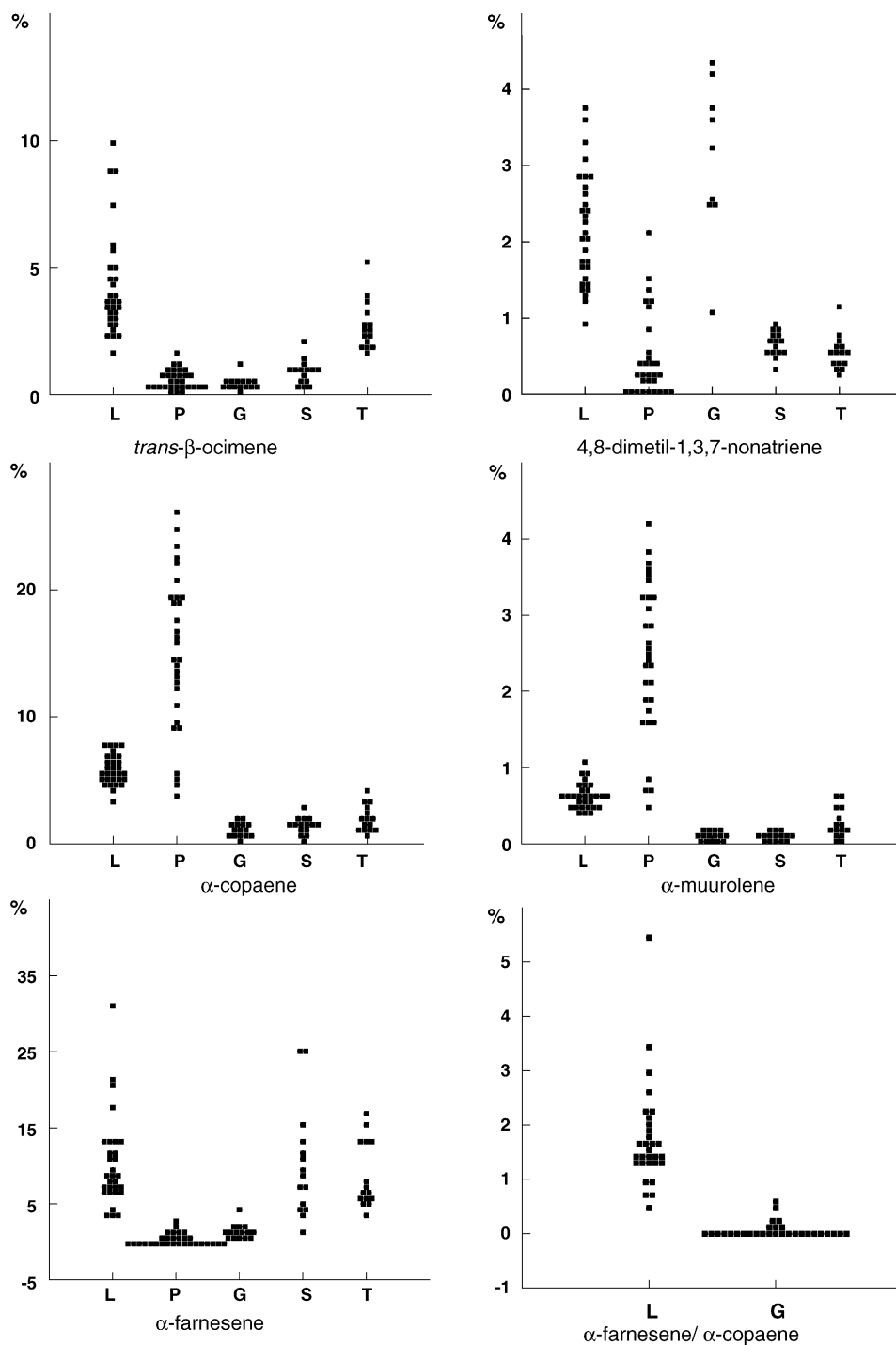


Fig. 2. Contents of *trans*- β -ocimene, 4,8-dimethyl-1,3,7-nonatriene, α -copaene, α -murolene and α -farnesene in the analyzed oil samples.

of this compound were higher, with higher mean and SD values in Greece samples.

α -Copaene appeared as a typical “Italian” compound, since the amounts of this sesquiterpene exceeded 5.0% of the whole volatile fraction in most oils from West Liguria and Puglia and were lower than 2.0% in most analyzed Not-Italian oils. Moreover, this compound might also help to discriminate between the analyzed Italian oils, since in the samples obtained in West Liguria the amounts of α -copaene gathered between 3.5 and 7.9% while in samples from Puglia they were very dispersed in a range between 3.7 and 26.0%.

Although the amounts of α -muurolene were lower than those of α -copaene, their distribution in the five oil groups appeared very similar. Once again in oils from West Liguria the amounts of α -muurolene were rather constant, in a range between 0.4 and 1.0% and appeared quite variable in oils from Puglia, with four samples between 0.5 and 1.0% and twenty-seven samples in a wide range between 1.1 and 4.2%. However, in Spanish and Greek oils the α -muurolene amounts were often lower than 0.2%.

As far as α -farnesene is concerned, the oil samples produced in West Liguria, Spain and Tunisia showed the highest contents of this sesquiterpene, while the samples from Greece and Puglia were poor in this compound, whose amounts were lower than 3% in Puglia oils, with most samples below 1.0%. This time, oils from West Liguria had the most variable content of this compound, which ranged between 3.7 and 31.0%.

As far as the percent amounts of α -pinene, limonene and eremophyllene were concerned, they did not appear very distinctive of oils from West Liguria, if individually considered.

A careful consideration of the obtained numerical results showed that just three of the eight terpenoid compounds allowed the distinction of West Liguria extravirgin olive oils. Fig. 3 displays a simple decision tree for their identification: the percentage of α -copaene and α -muurolene allowed a clear distinction between oils produced in West Liguria or Puglia (α -copaene $\geq 5\%$ and/or α -muurolene $\geq 0.5\%$) and the analyzed Not-Italian oils, while the content of α -farnesene is decisive to distinguish between the West Liguria (α -farnesene $\geq 3\%$) and the Puglia samples. Moreover, the geographical origin of oils from West Liguria could be also traced by some ratios between the amounts of the terpenoid compounds: for example, 4,8-dimethyl-1,3,7-nonatriene/ α -muurolene ratio could distinguish oils from West Liguria and Greece, while α -farnesene/ α -copaene ratio could be useful for the distinction between oils from West Liguria and Puglia (Fig. 2).

Afterwards, the eight detected terpenoid hydrocarbons were used for a multivariate characterization of West Liguria oils by chemometric methods, in order to confirm the importance of these analytical variables as a tool for tracing their geographical origin.

PCA was initially performed on the autoscaled data (105 objects: 84 in the training set and 21 in the test set; 12 variables: $V + Y$) divided in two categories (L and N). The first two PCs ($PC_1 - PC_2$) explained 56.1% of total variance and a very good separation between samples coming from West Liguria (L) and samples belonging to all the other categories (N) was observed (Fig. 4, left side). High values of the products “ 5×8 ”, “ 3×5 ” and “ 4×5 ”, and of the original variables *trans*- β -ocimene (3) and α -farnesene (8) seemed particularly important for characterizing Liguria samples and seemed to

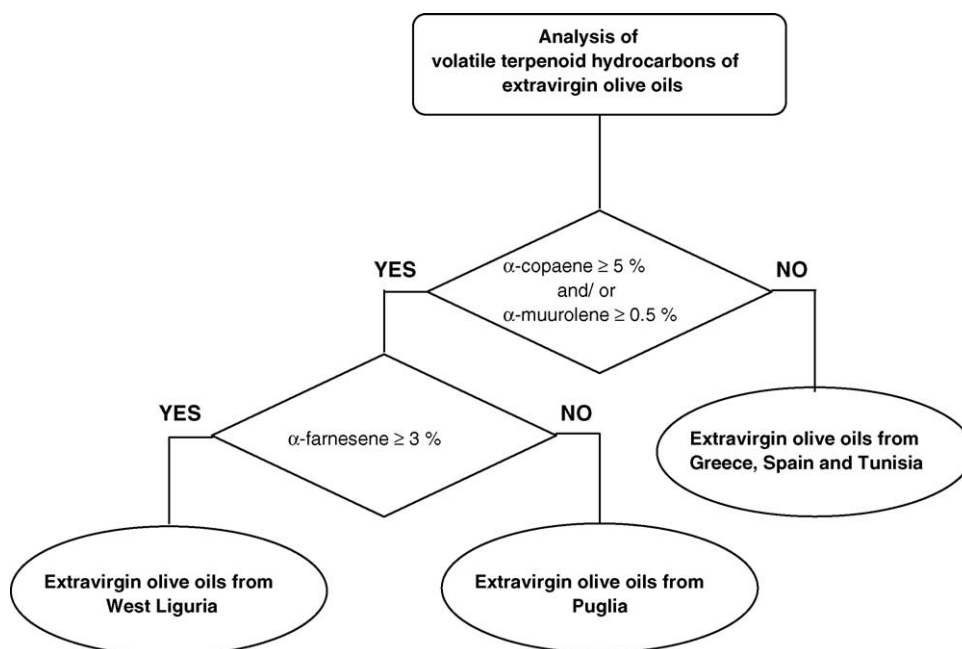


Fig. 3. Decision tree for an easy identification of extravirgin olive oils from West Liguria.

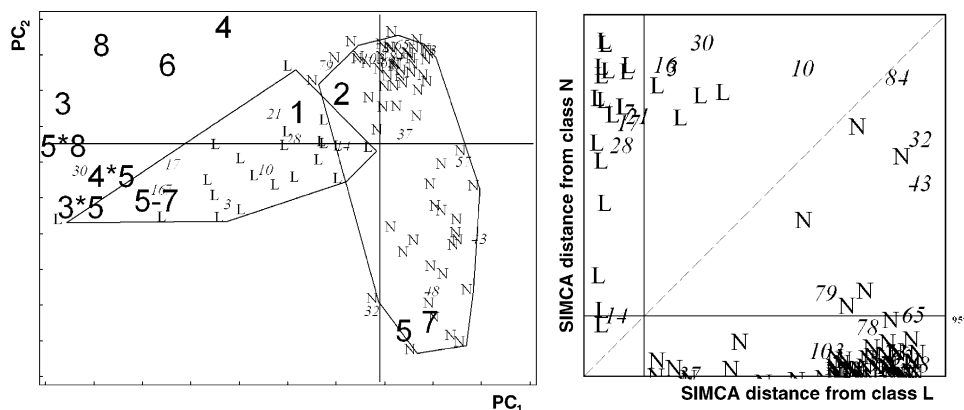


Fig. 4. Loading and score plot on the first two principal components (score axes) and SIMCA Coomans distance plot. Training set objects are represented by their category symbol (L or N). Test set objects are represented by their indexes [1–30: West Liguria (L) category, 31–105: Not-Liguria (N) category]. Loadings are represented by their indexes and reported with a bigger font.

be typical of this geographical area. α -Copaene (5) and α -muurolene (7) were confirmed as typical “Italian” terpenoid hydrocarbons, since all the Puglia and most of the Liguria samples had low scores on PC₂, mainly composed by these two hydrocarbons, while “Not-Italian” oils were characterized by high scores on it.

In order to quantify the class separation, SIMCA was then performed: a total classification rate of 98.8%, a total prediction rate on the evaluation set (five cross-validation groups) of 96.4% and a total prediction rate on the test set of 100.0% were obtained. Sensitivity and specificity of the SIMCA models are reported in Table 2, even if their meaning is limited to Liguria category, since Not-Liguria category is quite heterogeneous. The results are visualized in the Coomans distance plot [15] reported in Fig. 4 (right side), where vertical and horizontal lines represent the boundaries of the class models at 95% of confidence probability.

The results obtained from the comparison of West Liguria category against each other category were also very good. 100.0% correct internal and external predictions were always obtained by SIMCA for L category, whereas the mean sensitivity and specificity were about 85 and 96%, respectively: detailed values are reported in Table 2. The PC₁–PC₂ plots of the couples L and G, L and S, L and T showed a satisfactory separation between these categories, with high α -copaene and α -muurolene loading values, paralleled by high SIMCA discriminant powers of these two hydrocarbons, while α -farnesene similarly confirmed its importance for the couple L and P.

4. Conclusions

The obtained results show that the analysis of volatile terpenoid hydrocarbons is a straight and powerful tool for tracing the geographical origin of extravirgin olive oils from West Liguria. As far as the five considered geographical areas are concerned, just three individual terpenoid hydrocarbons

allow the distinction of West Liguria oils by a simple decision tree. Moreover, the multivariate analysis of the eight detected terpenoids allows building class models with high predictive ability, confirming again that the three terpenoid compounds are fundamental for the characterization of West Liguria oils.

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