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Direct thermal extraction and gas chromatographic–mass spectrometric determination of volatile compounds of extra-virgin olive oils

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Abstract

The instrumental performances of a Thermo Desorption-Cooled Injection System coupled with a gas chromatography–mass spectrometer (GC–MS) were improved by a Plackett-Burman experimental design for the direct thermal extraction of volatile compounds from extra-virgin olive oils. The obtained experimental conditions were applied to the analysis of samples from West Liguria (cv. Taggiasca ≥90%) and Spain (cv. Arbequina), which shared such similar sensorial features that Taste Panel did not distinguish them. Principal component analysis (PCA) was then applied to the experimental data. Three linear combinations of the amounts of the lipoxygenase oxidation products proved to be decisive and sufficient in the differentiation of the two groups of samples.

Keywords: Olive oil; Direct thermal extraction; Principal component analysis; Plackett–Burman design; Lipoxygenase oxidation products; Volatile organic compounds

1. Introduction

Extra-virgin olive oils are extracted from the fruit of olive tree, *Olea europea*, and are consumed without any refining process. Thus, they retain their volatile compounds, which are often present at extremely low concentrations but are responsible for their fragrant flavor and aroma [1].

Gas chromatography–mass spectrometry (GC–MS) has been widely used for the instrumental analysis of olive oil flavor. The preliminary extraction of volatiles from oils has been frequently obtained by dynamic headspace techniques, generally employing Tenax® or Charcoal as adsorbents [2–4]. Some authors have also proposed an extraction by Solid Phase Micro Extraction (SPME) with polydimethylsiloxane fibers, in order to detect polyunsaturated aldehydes that might be underestimated by Tenax® [4], or by Supercritical Fluid Extraction (SFE) followed by Tenax® trapping [5].

In the late 90's, Gerstel proposed the Thermo Desorption-Cooled Injection System TDS2-CIS4 for the extraction of volatiles or semi-volatiles from stir bars coated with the sorbent polydimethylsiloxane (PDMS). Nevertheless, the manufacturer itself proposed also the use of TDS2-CIS4 for the direct flavor extraction of olive oils and rancidity monitoring [6]: the employed analytical method avoided the use of stir bars and of any adsorbent, that might modify the ratios among the volatiles content. This approach has been considered particularly interesting and potentially useful for discrimination among olive oils showing similar organoleptic features. Thus, in this study the TDS2-CIS4 system coupled with GC–MS was used for the direct extraction and analysis of the volatiles of two groups of extra-virgin olive oils: these oils had been obtained from different olive cultivars in different geographical areas but had such similar sensorial features that the Taste Panel did not distinguish them. Since these oils had a delicate and light flavor, experimental conditions had to be improved and an experimental design was carried out in order to enhance method sensitivity. The resulting settings were then applied to the analysis of Italian "Riviera Ligure-Riviera dei Fiori" Protected Designation of Origin (PDO) [7] oils and of Spanish extra-virgin olive oils of the same year crop (2001–2002) obtained from cv. Arbequina. The chemical composition of the volatile fraction of the samples was examined and the obtained data were

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elaborated by multivariate statistical methods in order to verify their ability to distinguish the two groups of samples.

2. Experimental

2.1. 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 (Gerstel, GmbH, Mülheim an der Ruhr, Germany) programmed temperature injector and was coupled with an external Gerstel TDS2 thermal extraction unit (Gerstel, GmbH, Mülheim an der Ruhr, Germany). In this system, the thermostatated block of TDS2, which contains 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. For the direct sample extraction, the oil sample was directly introduced in the glass tube and heated. The carrier gas flow favored the stripping of volatiles, which were then trapped and focused on the cooled surface of the injector liner. Volatile compounds were transferred to the cooled injector in splitless mode. The control of the numerous operating parameters of the extraction unit was obtained by an external automatic controller.

2.2. Instruments performance improvement

From preliminary investigation it was found that 13 experimental variables could affect the instrumental response (Table 1). The 13 parameters taken into account could be classified as follows: 11 were instrumental parameters of the TDS2-CIS4 system (six related to the extraction step and five to the injection step), and two were GC–MS instrumental parameters. The values proposed for these variables by the manufacturer are reported in Table 1 (V_m).

As far as "TDS2 final temperature" is concerned (the second parameter reported in Table 1) a 40 °C temperature was preliminary set (V_1, V_2 and V_f), obviously causing a drop of the chromatographic signal, but high extraction temperatures might promote oxidation and degradation reactions, whose results are extremely difficult to keep under control. Moreover, this choice allowed also an easier comparison of the obtained results with literature data, which have been almost always obtained after extraction of oil flavor at 38–40 °C, at a temperature close to human body's temperature. Thus, in order to improve the instrumental performance the effect of the remaining 12 parameters was studied by means of a Plackett–Burman design (two level design) requiring 16 experiments [9]. In Table 1 are reported the two tested levels (V_1 and V_2) and the final setting (V_f) of each analytical variable.

The Plackett–Burman design showed that "CIS4 split flow" (i.e. the flow at split vent of CIS4 during injection) and "CIS4 helium vent flow" (i.e. helium flow out of CIS4 during sample extraction and transfer) had a significant effect on the method sensitivity. The other experimental parameters were found not to have significant influence on the instrumental performance and so they were set at the most convenient values on the basis of analysis time and cost. The final conditions of all the screened instrumental variables are summarized in Table 1 (V_f). It is interesting to note that the two significant factors are involved in the

Table 1
The screened analytical variables

	V_m[a]	V_1[b]	V_2[b]	V_f[c]
TDS2-CIS4-extraction step				
TDS2 temperature ramp rate (°C/min)	60	30	60	30
TDS2 final temperature (°C)	80	40	40	40
TDS2 final time (min)	20	20	30	20
TDS2 transfer line temperature (°C)	280	250	300	300
CIS4 cooling temperature (°C)	−150	−75	−120	−120
CIS4 helium vent flow (ml/min)	50	50	100	100
TDS2-CIS4-injection step				
CIS4 temperature ramp rate (°C/s)	12	5	12	12
CIS4 final temperature (°C)	280	250	300	300
CIS4 final time (min)	5	3	7	3
CIS4 splitless time (min)	0.02	0.02	1.01	0.02
CIS4 split flow (ml/min)	10	5	15	5
GC–MS				
Helium flow (ml/min)	0.7[d]	1.0	1.2	1.2
GC-MS transfer line temperature (°C)	280	250	300	250

[a] V_m: proposed by manufacturer.
[b] V_1 and V_2: levels used for the Plackett–Burman design.
[c] V_f: setting for the analysis of oil volatiles.
[d] The column employed by the manufacturer was a 50 m × 0.2 mm × 0.5 μm HP1.

two different TDS-CIS4 steps, while the parameters related to GC–MS resulted not significant.

The performance and repeatability (relative standard deviation of each component between 3.7 and 15.6%) obtained under these conditions were considered to be satisfactory for the analysis of the flavor of the analyzed oils. A further and finer optimization of the analytical variables will be the goal of a following study.

2.3. Samples

21 extra-virgin olive oils were analysed: 14 of them were PDO oils "Riviera Ligure–Riviera dei Fiori" (cv. Taggiasca ≥90%), while seven oils were Spanish extra-virgin olive oils from cv. Arbequina.

2.4. Sample analysis

2.4.1. Volatiles extraction

Ten microlitres of sample were directly introduced as a thin layer into a glass thermal extraction tube. The starting extraction temperature was 20 °C, then it was raised to 40 °C at 30 °C/min, and this temperature was kept for 20 min. The splitless transfer of volatiles was performed at a 300 °C transfer line temperature. During the extraction procedure the CIS4 temperature was kept at −120 °C and helium was vented out of the injector at a 100 ml/min flow. At the end of extraction and before chromatographic analysis, the extraction tube was removed and substituted by a clean one.

2.4.2. GC–MS analysis

After the insertion of the clean extraction tube, the injector temperature was re-equilibrated at −120 °C. Then, at the injection time, this temperature was kept for 1 min more, then raised at a ramp rate of 12 °C/s up to 300 °C, held for 5 min. In this step the helium pressure was 8.76 psi, the column flow 1.2 ml/min and the split vent flow 5 ml/min.

For the gas chromatographic analysis of the volatile compounds a 30 m × 0.25 mm × 0.25 μm film thickness DB-5MS fused-silica capillary column (J&W, Folsom, CA, USA) was used at a helium flow rate of 1.2 ml/min. The oven temperature was kept at 40 °C for 5 min and then programmed at 5 °C/min to 160 °C, kept for 1 min, under flow-controlled conditions (constant flow 1.2 ml/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 plot (Fig. 1) was obtained by total ion current (TIC) mode: the acquired mass ranges were 40–250 amu up to a 20 min retention time, and 50–350 amu for higher retention times. In order to avoid the MS detection of CO_2, a solvent delay of 2 min was used. The identification of compounds was obtained by comparison with Wiley 275 Mass Spectra Library.

The relative amounts of each compound were calculated by internal normalization, since the absolute amounts of compounds were not necessary for the aim of this study.

2.4.3. Statistical analysis

A multivariate statistical analysis of the data set obtained from the chromatographic plots was performed using the principal component analysis (PCA), as implemented in Q-PARVUS 3.0 [10].

3. Results and discussion

The products of lipoxygenase cascade (LOX) (Fig. 2) were generally the major components of the volatile fraction

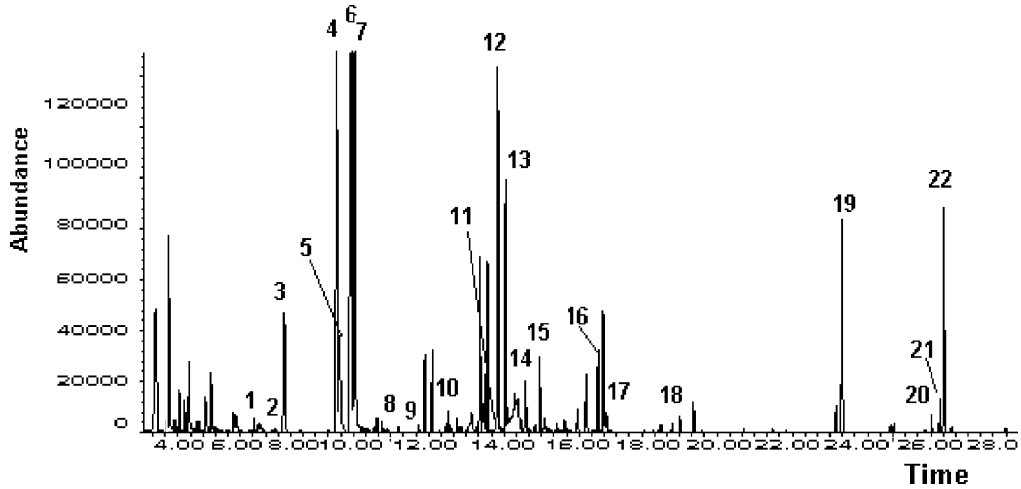

Fig. 1. Chromatographic plot of the volatile fraction of a PDO oil "Riviera Ligure-Riviera dei fiori" obtained by direct thermal extraction coupled with GC-MS. (1) Toluene; (2) *n*-octane; (3) hexanal; (4) *trans*-2-hexenal; (5) *cis*-3-hexen-1-ol; (6) *trans*-2-hexen-1-ol; (7) 1-hexanol; (8) heptanal, (9) α-pinene; (10) *trans*-2-heptenal; (11) *n*-octanal; (12) *cis*-3-hexenyl acetate; (13) hexyl acetate; (14) limonene; (15) *trans*-β-ocimene; (16) *n*-nonanal; (17) 4,8-dimethyl-1,3,7-nonatriene; (18) naphthalene; (19) α-copaene; (20) aromadendrene; (21) α-muurolene; (22) α-farnesene.

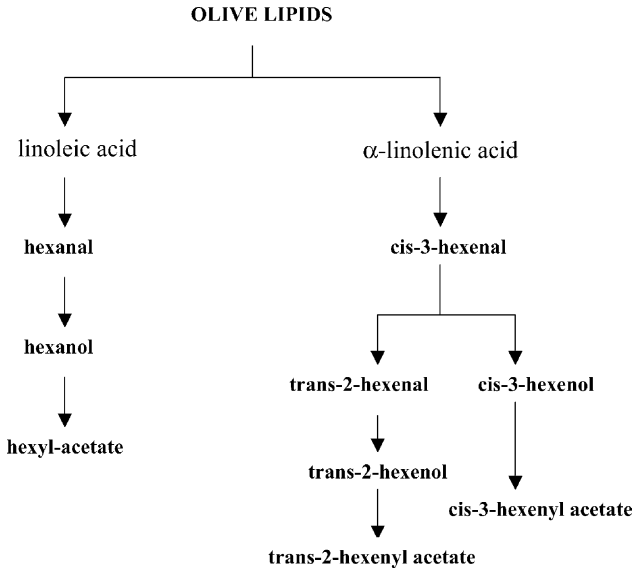

Fig. 2. The development of lipoxygenase attack to linoleic and linolenic acids in olive oils.

of the analyzed oils and the sum of the areas of their peaks ranged between 48 and 82% of the total area. The percentage of C_6 aldehydes ranged between 33 and 94% of total LOX products, with the exception of one sample with a 22% value. Although these values were often lower than previously reported for other oils [11] it must be pointed out that the different extraction and analysis methods might have affected both the number and the amounts of the detected compounds. The ratios between C_6 alcohols and their acetate appeared quite variable. However, linolenic acid (LnA) was confirmed as the favorite target of LOX enzymes of olive fruits: in fact, unsaturated metabolites were generally between 71 and 95% of total LOX products and *trans*-2-hexenal was generally present in the largest amounts. Nevertheless, in the sample having few aldeydes even the amounts of LOX products originating from La and LnA were unexpectedly similar and 1-hexanol accounted for 42% of the products of LOX cascade. Among LnA oxidation products, *trans*-2 compounds were predominant and ranged between 80 and 97% in PDO samples and 62–74% in Spanish oils. Although it was not possible to know the method employed for oil extraction for all the analysed samples, the information available for most samples allowed to reject the hypothesis that these differences were consequence of different extraction methods (pressing/percolation against centrifugation). The smaller amounts of *trans*-2-hexenal with respect to other oils [11] could explain the typical low astringent and not bitter flavor of PDO oils from West Liguria and of the similar Spanish oils.

As far as hydrocarbons are concerned, recently several authors have also extensively studied the hydrocarbon fraction of virgin oils and its possible usefulness for their characterization [12]. Thus, the simultaneous determination in oil flavor of the amount of the major LOX products and of the most representative hydrocarbons might be of great potential interest for the identification of oil origins. In the

Table 2
The variables employed for the multivariate analysis

Variable	Volatile compounds	Mol. ion	Main ions
1	Toluene	92	65, 91
2	*n*-Octane	114	43, 57, 71, 85
3	Hexanal	100	43, 44, 56, 57, 72, 82
4	*trans*-2-Hexenal	98	41, 55, 57, 69, 83
5	*cis*-3-hexen-1-ol	100	41, 55, 67, 82
6	*trans*-2-Hexen-1-ol	100	41, 43, 57, 82
7	1-Hexanol	102	43, 56, 69, 84
8	Heptanal	114	41, 43, 44, 55, 57, 70, 81, 86, 96
9	α-Pinene	136	53, 67, 77, 91, 92, 93, 105, 121
10	*trans*-2-Heptenal	112	41, 55, 69, 83
11	*n*-Octanal	128	43, 55, 56, 57, 67, 68, 69, 84
12	*cis*-3-Hexenyl acetate	n.d.[a]	43, 67, 82, 94, 97, 112
13	Hexyl acetate	n.d.[a]	43, 56, 61, 69, 84, 111, 116
14	Limonene	136	53, 68, 79, 93, 107, 121
15	*trans*-β-ocimene	136	41, 53, 79, 93, 105, 121
16	*n*-Nonanal	142	43, 57, 70, 82, 98, 114, 124
17	4,8-Dimethyl-1,3,7-nonatriene	150	41, 69, 79, 81, 107, 135
18	Naphthalene	128	51, 63, 102, 127, 129
19	α-Copaene	204	55, 69, 81, 93, 105, 119, 161, 175, 189
20	Aromadendrene	204	55, 67, 79, 91, 105, 119, 133, 147, 161, 189
21	α-Muurolene	204	55, 69, 77, 81, 93, 105, 119, 133, 147, 161, 189
22	α-Farnesene	204	55, 69, 79, 93, 107, 119, 123, 135, 147, 161, 189
Sum 1	Var. 4 +Var. 6		
Sum 2	Var. 5 + Var. 12		
Sum 3	Var. 3 + Var. 7 + Var. 13		

[a] n.d.: not deleted.

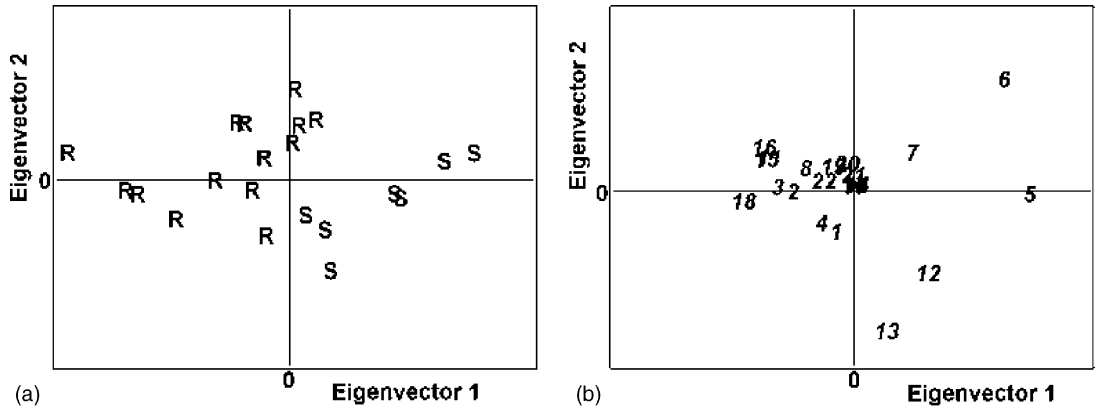

Fig. 3. Results of PCA applied to the data set of the 21 samples (14 "Riviera Ligure-Riviera dei Fiori" DOP olive oil, labelled as R, and 7 Spanish olive oils, labelled as S) described by 22 volatiles (numbered as reported in Table 2). (a) Score and (b) loading plot. Eigenvector 1: 45.5% explained variance; eigenvector 2: 18.2% explained variance.

analyzed oils small amounts of several alifatic and aromatic hydrocarbons were detected. Moreover, several terpenic hydrocarbons, such as limonene, α-pinene, α-copaene and α-farnesene, were often detected and the sum of their areas accounted for 5–33% of the detected volatiles. α-Farnesene and α-copaene were detected in all the analyzed samples.

Chemometric treatment was then applied in order to evaluate if the measured data allowed to distinguish the two groups of samples using only a data visualization technique (PCA). The classification-modeling methods were not applied due to the low number of samples.

For each sample, the 22 peaks (among more than 100 detected compounds) attributable to the major LOX products and to the most representative aldehydes and hydrocarbons (Table 2), were considered. A data matrix having as many rows as the oil samples (21) and 22 columns, the per cent area of each peak, was built.

The data set was initially autoscaled in order to give the same importance to all the peak areas. Then each variable was weighted by multiplying it by a factor inversely proportional to its relative experimental error (the inverse of the coefficient of variation previously measured). Thus, a visualisation of the data set was performed using the Principal component analysis to extract all the significant peak areas information. The first two eigenvectors concentrated about the 67% of the total variance and the corresponding loading and score plots are shown in Fig. 3a and b.

The analysis of the score plot emphasised a separation between the "Riviera dei Fiori" PDO samples (R) and the Spanish ones (S) on the first principal component, which is mainly built by *cis*-3-hexen-1-ol, *trans*-2-hexenol, 1-hexanol, *cis*-3-hexenyl acetate, naphthalene, *n*-nonanal, *n*-octanal and *trans*-β-ocimene. Compared to the Spanish samples, "Riviera dei Fiori" PDO oils were characterised by a relatively high content of naphthalene, *n*-nonanal, *n*-octanal and *trans*-β-ocimene and by a relatively low level of *cis*-3-hexen-1-ol, *trans*-2-hexenol, 1-hexanol, *cis*-3-hexenyl acetate. The amounts of C_6 aldeydes, alcohols and their acetate (LOX products) are mainly related to the different olive cultivar [3,8,11], while other aldeydes such as *trans*-2-heptenal and *n*-nonanal are known to be produced during oil oxidation [13]. In order to eliminate

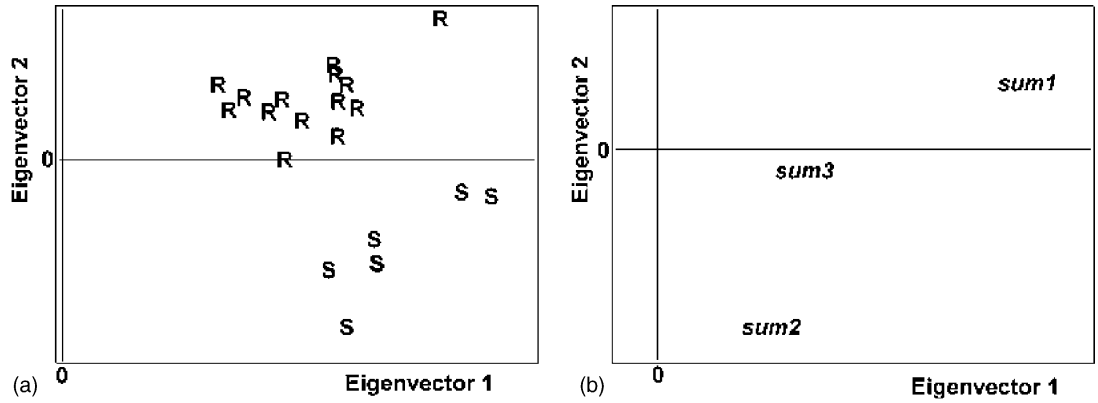

Fig. 4. Results of PCA applied to the data set of the 21 samples (14 "Riviera Ligure-Riviera dei Fiori" DOP olive oils, labelled as R, and 7 Spanish olive oils, labelled as S) described by the three sums of volatiles (labelled as reported in Table 2). (a) Score and (b) loading plot. Eigenvector 1: 95.5% explained variance; eigenvector 2: 3.1% explained variance.

the possibility of a chance separation among samples due to a different oil oxidation level rather than to their different origin, all the variables strongly related to the oil oxidation off-flavors as heptanal, *trans*-2-heptenal, *n*-octanal and *n*-nonanal, were deleted from the data set. Thus, PCA was repeated and an analogous differentiation among samples was obtained.

Then in order to rationalize the relationship between some LOX products and the oil origin, three linear combinations of them were considered as new variables for each sample. LOX products derived from linolenic acid were separated into two groups (sum 1 = Var. 4 + Var. 6 and sum 2 = Var. 5 + Var. 12 of Table 2), which corresponded to the two branches of the development of LOX cascade (Fig. 2). The products derived from linoleic acid were summed together (sum 3 = Var. 3 + Var. 7 + Var. 13 of Table 2). These sums were calculated on the basis of the original variables weighted for the inverse of their variance coefficients. The loading and score plots of the first two eigenvectors, obtained from the PCA on the new data matrix of 21 rows and 3 columns, is shown in Fig. 4a and b. The first two eigenvectors contain 99% of the total variance. The reported plots show that all the three sums contribute to separate samples of different geographical origin probably due to their different cultivar, and that the sum of *cis*-3-hexen-1-ol and *cis*-3-hexenyl acetate is the variable that gives the highest contribution to discriminate the samples.

4. Conclusions

The application of the new analytical method to "Riviera Ligure–Riviera dei Fiori" PDO oils has allowed to highlight for the first time some peculiar features of their aroma and could be extremely useful for their better characterisation. In fact, the comparison of the results obtained for PDO oils and for some extra-virgin olive oils having very similar organoleptic features but originated from different olive cultivars has shown a clear distinction between the two groups of samples. Thus, the obtained results have stressed that the new instrumental analysis of oils volatile fraction allows a strict connection between the fine composition of oils aroma and olive cultivars.

Since no reference method is still available for the analysis of volatile compounds of extra-virgin olive oils, the proposed method is particularly interesting. In fact the direct analysis of oil volatiles by TDS2-CIS4 system coupled with GC–MS gives a complete picture of oil flavor composition avoiding sorbent use and any kind of sample preparation.

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