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# The compositional quality and sensory properties of virgin olive oil from a new olive cultivar — I-77

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**Abstract**

Virgin olive oil sampled in different production areas and obtained from a new olive cultivar, I-77, was analysed. It was characterised by: (i) good content of total phenols, *o*-diphenols, tyrosol, hydroxytyrosol, tyrosol-aglycones, hydroxytyrosol-aglycones, tocopherols, *trans*-2-hexenal and total volatiles; (ii) good sensory score, resistance to oxidation, overall quality indices, free acidity, peroxide index, UV (ultraviolet) spectrophotometric indices and carbonyl index; (iii) a balanced fatty acid composition; (iv) a content of triacylglycerols, diacylglycerols, chlorophylls and carotenoids, sterols, triterpene dialcohols, aliphatic and triterpene long-chain alcohols, and waxes comparable to that of several traditional cultivars. 

## 1. Introduction

Virgin olive oil (*Olea europaea* Linn.) is one of the oldest known vegetable oils and the only one that can be consumed in its crude form (unrefined) (Boskou, 1996). It is valued for its fine, balanced, delicious and unique aroma and flavour, and long shelf-life (Kiritsakis, 1998). Today, its biological, nutritional and healthful effects are universally acknowledged (Sciancalepore, 1998; Visioli & Galli, 1998).

Virgin olive oil quality is affected by several factors, such as agronomic techniques, seasonal conditions, sanitary state of drupes, ripening stage, harvesting and carriage systems, method and duration of storage, and processing technology (Sacchi, Mannina, Fiordiponti, Barone, Paolillo & Patumi, 1998). The preservation of quality depends on conditions adopted to store the product and on duration of storage (Ranalli, Sgaramella & Surricchio, 1999). Oils of elevated quality are obtained when olives are fresh and wholesome and harvested at the optimal ripening point (Ranalli, Tombesi, Ferrante & De Mattia, 1998). However, the environmental and genetic (cultivar) factors are those that basically affect quality and typicality of the product (Fontanazza, 1993). Recently, a new, early, productive and cold-resistant olive cultivar, I-77, has been obtained by the Olive Research Institute-NRC, Perugia, Italy, which is being successfully tested according to intensive cultivation methods in different environments of Italy and other olive-growing countries (Fontanazza, 1993). Accordingly, an in-depth survey of the composition and qualitative level of the oil from the new olive variety has been carried out. This study aims to contribute to the optimisation and valorisation of virgin olive oil quality in the world olive-producing areas.

## 2. Materials and methods

### 2.1. Olive sampling and origin areas selected

Three Italian geographical areas, such as Perugia (PG), Campobasso (CB) and Lecce (LE), each including three sub-areas, were selected. In each sub-area, when the ripening index value was ~3, a 15 kg sample of drupes was picked by hand from trees with uniform characteristics. This index (Esti, Cinquanta & La Notte, 1998; Manzi, Panfili, Esti & Pizzoferrato, 1998) was evaluated by a well-established procedure (Uceda & Frías, 1975) consisting of distributing a randomly taken sub-sample of 100 olives in 7 groups according to the skin colours: bright green (group $N=0$), green-yellowish (group $N=1$), green with reddish spots (group $N=2$),

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reddish-brown (group $N=3$), black with white flesh (group $N=4$), black with $<50\%$ purple flesh (group $N=5$), black with $\geqslant 50\%$ purple flesh (group $N=6$), black with 100% purple flesh (group $N=7$). The index is given by $\Sigma(N\mathrm{i}\ n\mathrm{i})/100$, where $N$ is the group number and $n$ is the olive number in that group. Maturation index values range from 0 ($N=0$ bright green olives, $n=100$) to 7 ($N=100$ purple flesh olives, $n=100$). The measurements were run in triplicate, and the mean values had variation coefficients (CVs) all $<7\%$.

### 2.2. Olive sample processing

The collected olive samples were processed in a laboratory oil-mill (Valpesana Company, S. Casciano Val di Pesa, Italy) made up of a hammer crusher, a mixer and a basket centrifuge. The olive paste was kneaded for 5 min at room temperature and centrifuged at $3000\times g$ for 10 min without addition of lukewarm water. Collection, carriage and processing of the olive samples were carried out rapidly and with care. Therefore, there are no drupes damaged by incipient lipase and lipoxidase enzyme actions.

### 2.3. Oil sample analyses

The analytical procedures applied have been outlined in a previous paper (Ranalli et al., 1999); therefore, only some additional details are included in this report. Chemicals, solvents and apparatus used, and their suppliers, have similarly been specified in that paper.

Carotenes and xanthophylls (lutein, violaxanthin and neoxanthin) were quantified colorimetrically after separation by TLC (thin-layer chromatography) (Minguéz-Mosquera, Rejano-Navarro, Gandul-Rojas, Sánchez-Gomez & Garrido-Fernández, 1991), using *N*,*N*-dimethylformamide for the extraction and a mixture of petroleum ether 65–95°C:acetone:diethylamine (10:4:1, v/v) as developer. Chlorophylls *a* and *b* and Mg-free chlorophyll derivatives, such as pheophytins *a* and *b*, were determined by a colorimetric method (Ranalli, Tombesi et al., 1998). Chroma ($\sigma\%$), brightness ($h\%$), hue ($\lambda d$) and integral colour index were determined by transmittance measurements (Ranalli & De Mattia, 1997). Chlorophyll and carotenoid colour indices, and colour ratio ($A^{446}/A^{448}$), were determined spectrophotometrically (Ranalli & Angerosa, 1996).

Oxidative stability was evaluated as induction time (h) of the peroxidising reactions using 5 g oil sample and a "Rancimat" 679 apparatus (Metrohm Co., Basel, Switzerland), which automatically applied the accelerated and repetitive Swift's test (120°C; air flow rate of 20 l $h^{-1}$) (Gordon & Mursi, 1994). With this well-established methodology, the volatile oxidation products were stripped from the oil and dissolved in cold water, whose conductivity thus increased progressively. The time taken to reach a fixed level of conductivity was measured.

Waxes were analysed by HRGC (high-resolution gas chromatography) (E.C. Regulation no. 183, 1993) using a SPB$^{TH}$-5 capillary column (30 m, 0.32 mm i.d., 0.25 mm film thickness, SE-54 stationary phase, from Supelco Inc., Bellofonte, PA, USA). The gas chromatographic conditions were as follows: column temperature program, from 80 to 120°C at 30°C $min^{-1}$, from 120 to 340°C at 5°C $min^{-1}$; flame ionisation detector (FID) temperature 350°C; linear $H_2$ carrier gas speed 30 cm $s^{-1}$; recorder paper speed 30 cm $h^{-1}$; substance amount injected (in the on-column system) 0.8 μl. The internal standard was lauryl arachidate ($C_{32}$) (0.1% w/v, in hexane).

Sterols and triterpene dialcohols were analysed by HRGC (E.C. Regulation no. 2568, 1991) using a 25 m long glass capillary column (0.30 mm i.d., 0.20 μm film thickness, SE-52 stationary phase, from Supelco Inc., Bellofonte, PA, USA). The gas chromatographic conditions were as follows: column temperature 260°C, heater temperature 280°C, FID detector temperature 290°C, linear H2 carrier gas speed 30 cm $s^{-1}$, split ratio 1:50, recorder paper speed 50 cm $h^{-1}$, substance amount injected (in the split injection system) 0.5 μl. The internal standard was 3β-hydroxy-5α-cholestane (0.2% w/v, in chloroform).

Aliphatic and triterpene alcohols were analysed by HRGC (Ranalli, De Mattia & Ferrante, 1997). The analytical procedure was similar to that for sterols with only small differences. The column temperature program was as follows: 8 min at 180°C, from 180 to 260°C at 5°C $min^{-1}$ , 15 min at 260°C. The internal standard was arachidilic alcohol (0.1% w/v, in chloroform).

Triglycerides and partial glycerides were analysed by $^{13}$C-NMR (nuclear magnetic resonance) (Howarth & Vlahov, 1996). The oil sample spectra were run in $CDCl_3$ (deuterochloroform) (250 mg olive oil/0.5 ml $CDCl_3$). Free induction decays (FIDs) were acquired at 25°C using a spectral width of 13.00 Hz, 131 K acquisition points zero filled to 256 K points. A 45° excitation pulse and a 20 s relaxation delay were employed to collect 256 scans. FIDs were processed before Fourier transformation by a Gaussian filter of 0.1 Hz Lorenztian narrowing and 0.15 Gaussian broadening. Chemical shifts were relative to the signal of $Me_4Si$ (tetramethylsilane).

Phenolics were analysed by HRGC (Angerosa, D'Alessandro, Konstantinov & DiGiacinto, 1995), using a 25 m long capillary column (0.32 mm i.d., 0.1 μm film thickness, SE-54 stationary phase, from Lab. Service Analitica Ltd., Anzola Emilia, BO, Italy). The gas chromatographic conditions were as follows: oven temperature program, from 70 to 135°C at 2°C $min^{-1}$, 10 min at 135°C, from 135 to 220°C at 4°C $min^{-1}$, 10 min at 220°C, from 220 to 270°C at 3.5°C $min^{-1}$, 20 min

at 270°C; FID detector temperature 315°C; $H_2$ carrier gas pressure 35 kPa (on the head of the column); recorder paper speed 0.25 cm $s^{-1}$. The internal standard was resorcinol (>99% pure; 0.5% w/v, in methanol).Total polyphenols were determined colorimetrically (Ranalli, Ferrante et al., 1999) with Folin Ciocalteu's reagent ($H_3PW_{12}O_{40} + H_3PMo_{12}O_{40}$ mixture) which is able to oxidise these components affording blue oxides ($W_8O_{23}$ and $Mo_8O_{23}$), whose maximum absorbance is reached at 725 nm wavelength. *Ortho*-diphenols were determined colorimetrically (Ranalli, Tombesi et al., 1998) with Arnow's reagent (equimolecular $NaNO_2 + Na_2Mo_4$ mixture) which is able to develop a pink colour whose maximum absorbance is reached at 450 nm wavelength.

Fatty acids were analysed by HRGC (E.C. Regulation no. 2568, 1991) using a 25 m long capillary column (0.35 mm i.d., 0.25 μm film thickness, polyglycol type stationary phase, from Nordion Ltd., Helsinki, Finland). The gas chromatographic conditions were as follows: oven temperature program, from 120 to 165°C at 30°C $min^{-1}$, 25 min at 165°C, from 165 to 200°C at 5°C $min^{-1}$; 10 min at 200°C, from 200 to 220°C at 5°C $min^{-1}$, 10 min at 200°C, from 200 to 220°C at 5°C $min^{-1}$; FID detector temperature 260°C; $H_2$ carrier gas pressure 50 kPa (on the head of the column); recorder paper speed 0.5 cm $s^{-1}$; substance amount injected (in the on-column system) 0.6 μl. Values of these components were relative to the total fatty acids.

Pleasant and unpleasant volatiles were simultaneously analysed by HRGC (Ranalli & De Mattia, 1997) using a carbowax 20 M capillary column (25 m, 0.32 mm i.d., 0.20 μm film thickness, from Nordion Ltd., Helsinki, Finland). The gas chromatographic conditions were as follows: oven temperature program, 7 min at 28°C, from 28 to 33°C at 0.8°C $min^{-1}$ (no hold), from 33 to 85° at 2.4°C $min^{-1}$ (no hold), from 85 to 180°C at 3.7°C $min^{-1}$, 20 min at 180°C; FID detector temperature 220°C; $H_2$ carrier gas pressure 30 kPa (on the head of the column); recorder paper speed 0.5 cm $s^{-1}$; substance amount injected (in the on-column system) 0.5 μl; a $CO_2$ cryogenic accessory was used to hold the oven at 25°C. The internal standard was nonan-1-ol (>99% pure), that was directly added (7–8 mg) to the oil sample (50 g).

Tocopherols were evaluated by HPLC (high-performance liquid chromatography) (Ranalli et al., 1997) with a direct-phase M-porasil column (3.9×300 mm, 10 μm, from Waters Corporation, Milford, MA, USA), using a hexane-propan-2-ol (98.5:1.5, v/v) eluent and a UV detector at 292 nm wavelength.

The other analytical measurements performed were titrimetric determination of free acidity degree and of peroxide index, determination of spectrophotometric UV indices (absorption coefficients) (E.C. Regulation no. 2568, 1991), spectrophotometric determination of carbonyl index and nephelometric determination of turbidity (Ranalli, Tombesi et al., 1998). By using two algorithms (Ranalli, Ferrante et al., 1999), one involving free acidity extent, peroxide index, $K_{270}$ and sensory score, and another involving also phenol content, the overall quality indices ($OQI_1$ and $OQI_2$) were calculated.

The quantitative descriptive sensory profiling was performed by a fully trained analytical taste panel recognised by International Olive Oil Council (I.O.O.C.), Madrid, Spain, and made up of 12 assessors (E.C. Regulation no. 2568, 1991). Isolated air-conditioned booths within a sensory laboratory were used. Attribute perceptions were flavour (a combination of olfactory-gustatory-tactile-kinesthetic sensations), aroma (sensation perceived indirectly by the olfactory organ when tasting the oil), odour (combination of sensations perceived directly through the nose), taste (sensations perceived when the oil comes into contact with all sensitive areas of the mouth), and after-mouth feel/after-taste (combination of sensations perceived after the stimuli have disappeared from the mouth). Each sensory attribute was evaluated on a six-point intensity scale ranging from 0 (no perception) to 5 (extreme). Next, an overall evaluation of the magnitude of positive and negative attributes (off-flavours) was made, and the sensory score was obtained on a grading-structured nine-point scale from 1 (lowest quality) to 9 (optimal quality). The bitterness index was evaluated according to Gutiérrez, Perdiguero, Gutiérrez and Olías (1992). The overall acceptability was assessed according to McEwan (1994).

The oils were also pyrolysed at Curie-point (Goodacre, Kell & Bianchi, 1993) and, from the pyrolysed materials, by means of a mass-spectrometer, the pyrograms or fragmentograms (fingerprints of the samples) were obtained. A Rapyd-400 instrument (coupled to a mass-spectrometer), which was provided by Horizon instruments Ltd., Sussex, UK, was used.

A set of oil samples was stored at −20°C in darkness until analysed, using green screw-capped glass bottles (250 ml) without head-space which, after filling, were hermetically sealed. Another set of samples was stored at room temperature in darkness and, after 6 and 12 months of storage, only the analytical parameters closely related to quality and shelf-life were determined, to see how they changed with time.

### 2.4. Statistical analyses

A simple experimental design was adopted. Average analytical data were statistically tested with the one-sided variance analysis (ANOVA). If the null hypothesis was rejected, Duncan's multiple range test was applied to separate the means (Martens & Martens, 1986). Probabilities higher than $P = 0.05$ were considered non-significant. Data were also processed by principal component analysis (PCA) (Tabachnick & Fidele, 1983). Barlett's sphericity and Kevin-Meyer-Olkin tests were used to

check that PCA might be applied to the data set. PCA was applied under the following conditions: Kaiser's normalisation, varimax rotation, and tolerance limits for matrix inversion (0.0001). The cross-validation procedure was used to determine the maximum number of significant dimensions to avoid data over-fitting. Hierarchical cluster analysis (HCA) (Goodacre et al., 1993) and partial least squares (PLS1) (Tabachnick & Fidele, 1983) regression were also used. The packages Unscrambler® 6.0 for Windows 95 and Windows NT operating systems (CAMO A/S, Trondheim, Norway) and SPSS Advanced Statistics 6.1 (SPSS Inc., Chicago, IL, USA) were used, together with the softwares Genstat and Excel 5.0 for Windows. A digital 486 computer was used under MS-DOS and Windows 3.10 operating systems.

## 3. Results and discussion

Mean data (and standard deviations) for the major quality, typicality and genuineness parameters are presented in Tables 1 and 2, and refer to the fresh oil samples, while data referring to the oil samples analysed after 6 and 12 months of storage are only discussed in the text.

### 3.1. Fatty acid composition, triacylglycerols, diacylglycerols

Virgin olive oil from the new I-77 cultivar showed a good fatty acid composition (Table 1). In fact, the oleic acid percentage, related to the total fatty acid fraction, was rather high (generally $>83.0\%$ average) compared to several traditional cultivars. The oleic acid + linoleic acid percentage sum was slightly high (85.4%); the linoleic acid percentage was always around a good value (5%); the oleic acid/linoleic acid ratio (16.5) was largely higher than the minimum value of 7 (Kiritsakis, 1998); the saturated fatty acid percentage (10.8%) was good, while the polyunsaturated fatty acid percentage (5.5%) was relatively low; the unsaturated fatty acids/saturated fatty acids ratio (8.4) was good, as well as the unsaturated fatty acids/polyunsaturated fatty acids ratio (16.5). There were, as with the other olive oil varieties, 5 major fatty acids, such as oleic, linoleic, linolenic, palmitic, and stearic acid (Table 1), while the other fatty acids were present in small percentages or were not detected ($<0.005\%$).

The percentage of some individual fatty acids (markers of genuineness), such as myristic, linolenic, arachidonic, eicosenoic, behenic and lignoceric acid were within the

Table 1
Analytical and compositional variables assessed for fresh virgin olive oils from the new I-77 cultivar grown in three different geographical areas (PG = Perugia, CB = Campobasso and LE = Lecce) and selected for multivariate analysis[a]

| Codes | Analytical oil parameters | PG | CB | LE |
|---|---|---|---|---|
| 1 | Acidity (as oleic acid, g $100g^{-1}$) | 0.48 (0.04)a | 0.36 (0.03)a | 0.37 (0.03)a |
| 2 | Peroxide index (meq $O_2$ $kg^{-1}$) | 5.0 (0.3)a | 5.2 (0.4)a | 4.9 (0.3)a |
| 3 | $K_{270}$ | 0.09 (0.02)a | 0.10 (0.03)a | 0.10 (0.03)a |
| 4 | Chlorophylls and pheophytins ($a+b$, mg $kg^{-1}$) | 12.7 (0.9)a | 9.2 (0.4)b | 8.8 (0.6)b |
| 5 | Total carotenoids (mg $1^{-1}$) | 4.92 (0.23)a | 4.36 (0.22)b | 4.01 (0.20)b |
| 6 | Total tocopherols ($a+\gamma$, mg $kg^{-1}$) | 128.4 (3.1)a | 114.5 (1.7)b | 97.3 (4.3)c |
| 7 | Total phenols (as caffeic acid, mg $kg^{-1}$)[b] | 366 (15)a | 299 (13)b | 247 (13)c |
| 8 | Swift's test (h) | 23.5 (1.7)a | 17.8 (1.1)b | 14.4 (0.9)c |
| 9 | Sensory score (panel test) | 8.0 (0.2)a | 7.7 (0.2)ab | 7.5 (0.2)b |
| 10 | Palmitic acid (%)[c] | 8.3 (0.1)a | 8.6 (0.2)a | 9.1 (0.2)b |
| 11 | Stearic acid (%)[c] | 1.4 (0.1)a | 1.5 (0.1)ab | 1.6 (0.1)b |
| 12 | Oleic acid (%)[c] | 83.3 (0.2)ab | 83.5 (0.3)b | 83.2 (0.3)a |
| 13 | Linoleic acid (%)[c] | 5.4 (0.1)a | 5.0 (0.1)b | 4.8 (0.1)b |
| 14 | Linolenic acid (%)[c] | 0.5 (0.1)a | 0.4 (0.1)ab | 0.3 (0.1)b |
| 15 | Oleic acid/linoleic acid ratio | 15.5 (0.2)a | 16.7 (0.4)b | 17.2 (0.2)c |
| 16 | Unsaturated fatty acids/saturated fatty acids ratio | 9.0 (0.2)a | 8.5 (0.2)b | 7.8 (0.1)c |
| 17 | Unsaturated fatty acids/polyunsaturated fatty acids ratio | 15.4 (0.1)a | 16.7 (0.3)b | 17.4 (0.3)c |
| 18 | Pleasant volatiles (as nonan-1-ol, mg $kg^{-1}$) | 499 (21)a | 408 (12)b | 364 (13)c |
| 19 | Total sterols (mg $100g^{-1}$) | 116 (8)a | 123 (9)a | 134 (9)b |
| 20 | Aliphatic alcohols ($C_{22}+C_{24}+C_{26}+C_{28}$, mg $100g^{-1}$) | 6.9 (0.6)a | 7.7 (0.5)b | 8.8 (0.8)c |
| 21 | Geranylgeraniol (mg 100 $g^{-1}$) | 3.5 (0.3)a | 3.8 (0.4)ab | 4.0 (0.4) b |
| 22 | Phytol (mg $100g^{-1}$) | 15.6 (1.2)a | 16.4 (1.5)ab | 17.1 (1.7)b |
| 23 | Triterpene alcohols (mg $100g^{-1}$) | 48.7 (3.7)a | 55.0 (3.9)b | 59.0 (4.0)c |
| 24 | Alcoholic index | 0.06 (0.01)a | 0.08 (0.01)a | 0.08 (0.01)a |
| 25 | Triterpene dialcohols (mg $100g^{-1}$) | 2.0 (0.2)a | 2.4 (0.3)b | 2.2 (0.2)ab |
| 26 | Total waxes (mg $kg^{-1}$) | 156 (8)a | 186 (11)b | 202 (15)a |

[a] Data are means of three independent samples. Significant differences in the same row are showed by different letters ($P<0.05$).
[b] As determined by colorimetric method.
[c] Related to the total area of chromatogram.

Table 2
Other important analytical and compositional characteristics of fresh virgin olive oils from the new I-177 cultivar grown in three different geographical areas (PG = Perugia, CB = Campobasso and LE = Lecce)[a]

| Codes | Analytical oil parameters | PG | CB | LE |
|---|---|---|---|---|
| 27 | Carbonyl index-WMI (E/Y) | 3.0 (0.2)a | 2.3 (0.4)b | 3.0 (0.3)a |
| 28 | $K_{232}$ | 1.22 (0.06)a | 1.30 (0.05)a | 1.35 (0.05)a |
| 29 | Lutein (mg $l^{-1}$) | 3.22 (0.31)a | 2.87 (0.26)b | 2.73 (0.19)b |
| 30 | β-Carotene (mg $l^{-1}$) | 0.80 (0.07)a | 0.72 (0.05)ab | 0.65 (0.06)b |
| 31 | Violaxanthin (mg $l^{-1}$) | 0.51 (0.05)a | 0.44 (0.03)ab | 0.35 (0.04)b |
| 32 | Neoxanthin (mg $l^{-1}$) | 0.39 (0.03)a | 0.33 (0.04)ab | 0.28 (0.03)b |
| 33 | Brightness (%) | 72.9 (1.5)a | 77.6 (2.6)b | 86.5 (0.9)c |
| 34 | Chroma (%) | 68.9 (1.2)a | 76.9 (2.1)b | 81.3 (1.0)c |
| 35 | Integral colour index | 9.5 (0.4)a | 8.5 (0.9)a | 5.9 (0.4)b |
| 36 | *o*-Diphenols (as caffeic acid, mg $kg^{-1}$)[b] | 232 (13)a | 198 (11)b | 163 (22)c |
| 37 | Tyrosol (as resorcinol, mg $kg^{-1}$)[c] | 9.7 (0.8)a | 8.5 (0.7)a | 6.0 (0.6)b |
| 38 | Hydroxytyrosol (as resorcinol, mg $kg^{-1}$)[c] | 11.1 (0.6)a | 9.1 (0.6)b | 8.2 (0.5)b |
| 39 | Tyrosol-aglycones (as resorcinol, mg $kg^{-1}$)[c] | 39.4 (2.9)a | 36.6 (2.6)a | 32.6 (2.7)b |
| 40 | Hydroxytyrosol-aglycones (as resorcinol, mg $kg^{-1}$)[c] | 94.4 (8.2)a | 86.7 (8.0)b | 77.1 (7.1)c |
| 41 | Overall quality index ($OQI_1$) | 8.4 (0.1)a | 7.8 (0.3)b | 7.9 (0.2)b |
| 42 | Turbidity (NTU)[d] | 17 (2)a | 22 (3)b | 37 (2)b |
| 43 | Trans-2-hexenal (mg $kg^{-1}$) | 405 (31)a | 334 (25)b | 300 (19)c |
| 44 | Trans-2-hexenal/hexanal ratio | 78.0 (8.1)a | 44.9 (7.6)b | 34.4 (5.5)c |
| 45 | Trans-2-hexenal/total volatiles ratio (×100) | 74.0 (5.5)a | 72.4 (4.8)a | 68.1 (3.5)b |
| 46 | Unpleasant volatiles (as nonan-1-ol, mg $kg^{-1}$) | 48 (4)a | 53 (7)a | 77 (6)b |
| 47 | Citrostadienol (mg $100g^{-1}$) | 8.5 (0.8)a | 9.4 (0.9)b | 10.2 (1.1)c |
| 48 | Triglycerides (%)[e] | 98.9 (8.2)a | 98.8 (7.9)a | 98.8 (8.0)a |
| 49 | Dyglycerides (%)[e] | 1.1 (0.1)a | 1.2 (0.1)a | 1.2 (0.1)a |
| 50 | 1,2-Diglycerides/1,3-diglycerides ratio | 4.5 (0.4)a | 3.0 (0.2)b | 3.0 (0.2)b |

[a] Data are means of three independent samples. Significant differences in a same row are showed by different letters ($P \leqslant 0.05$).
[b] As determined by colorimetric method.
[c] As determined by HRGC method.
[d] NTU = nephelometric turbidity units.
[e] Related to the total glyceridic classes.

limits set by the official normal standard (E.C. Regulation no. 2568, 1991). However, there were some differences in the fatty acid composition due to the origin area factor.

Fatty acid composition, and noticeably the oleic acid/linoleic acid ratio, affects the taste of virgin olive oil, a condiment which is largely responsible for sapidity and healthful effects of the Mediterranean diet (Boskou, 1996). Oleic acid and linoleic acid are essentially esterified at the position 2 of glycerol (98–99% vs a percentage of esterified saturated fatty acids of $< 2\%$) (Sciancalepore, 1998). Also, the unsaturated fatty acids are originally almost entirely in the *cis* form and do not show a significant degree of conjugated double or triple bonds. However, there are unavoidable changes due to chemical and enzymatic actions, which result in a negative effect on the oil quality (Kiritsakis, 1998). The conjugated diene and triene degrees are proportional to the oxidation extent of the unsaturated fatty acids. The maximum absorbance for dienes and trienes is reached at 232 and 270 nm wavelength, respectively (Sciancalepore).

The triacylglycerol percentage (related to the total glyceridic classes) of the virgin I-77 olive oil was, on average, regardless of origin area, 98.8%, a value which is comparable to that of most olive cultivars. There was no influence of the production area factor. The corresponding percentage of partial glycerides was 1.2%. The 1,2-diacylglycerols/1,3-diacylglycerols ratio was high (3.5), thus suggesting good quality/freshness of the oil (Ranalli, De Nattia et al., 1998). The fatty acid distribution in the triglycerides of olive oil is of 1–3 random type (Kiritsakis, 1998). Seven triacylglycerols are in order more representative, such as OOO, POO, OLO, LOO, PLO, SOO and POP (O = oleic acid, L = linoleic acid, P = palmitic acid and S = stearic acid), which on the whole account for ~90% of total triglycerides (Sciancalepore, 1998). Triacylglycerol composition is responsible for the oil tactile-kinesthetic perceptions (Ranalli, Ferrante et al., 1999).

### 3.2. Phenol, tocopherol and head-space volatile components

The new olive oil variety was rich in total phenols (generally 304 mg $kg^{-1}$ average), which were represented basically by *o*-diphenols (198 mg $kg^{-1}$). Tyrosol (8.1 mg $kg^{-1}$) and hydroxytyrosol (9.5 mg $kg^{-1}$) were major free phenols. The latter component originates

from hydrolysis of oleuropein (bitter glycoside of olive fruit) (Bianco et al., 1998). However, some hydrolysable phenols (Angerosa et al., 1995; Servili, Baldioli, Selvaggini, Mariotti, Federici & Montedoro, 1998) were quantitatively much more important (Table 2). There was a good linear relationship between *o*-diphenol content and hydroxytyrosol content ($r = 0.9307^{+++}$) and between *o*-diphenol content and hydroxytyrosol-aglycone content ($r = 0.9667^{+++}$) (Fig. 1). The resulting adjusted multiple linear correlation coefficient was $0.9708^{+++}$. Fig. 2 shows a typical phenol HRGC chromatogram of the virgin I-77 olive oil.

Phenols and noticeably o-diphenols exert a scavenger effect on peroxyl radicals (Baldioli, Servili, Perretti & Montedoro, 1996; Blekas & Boskou, 1998; Evangelisti, Zumin, Tiscornia, Petacchi, Drava & Lanteri, 1997) and contribute to the flavour and fruity taste of virgin olive oil (Caponio, Alloggio & Gomes, 1999; Hueso-Urenã, Jimenez-Pulido, Moreno-Correntino & Rodriguez-Avi, 1998).

The tocopherol fraction (113 mg $kg^{-1}$) consisted essentially of α-tocopherol (>90%), while only minute amounts of β-, γ-, and δ- tocopherol were detected (data not shown).

These substances exert both vitamin potency and antioxidant action and come essentially from the fruit seed. (Psomiadou & Tsimidou, 1998; Ranalli & Angerosa, 1996). Pleasant volatiles (424 mg $kg^{-1}$) included a major component, *trans*-2-hexenal, which accounted for a high percentage of this fraction, and correlated strongly with total aroma ($r = 0.9705^{+++}$). There were also good correlations of *trans*-2-hexenal with some major phenols (e.g. hydroxytyrosol and hydroxytyrosol-aglycones). Volatiles are greatly responsible for sensory quality and often for typicality of virgin olive oil (Aparicio & Morales, 1998). Both absolute and relative contents of these components (especially aldehydes, alcohols and hydrocarbons) affect the flavour quality (Aparicio, Morales & Alonso, 1997; Morales, Calvente & Aparicio, 1996). A typical aromagram of the virgin I-77 olive oil is given in Fig. 3. Values of the characteristic quality ratios involving

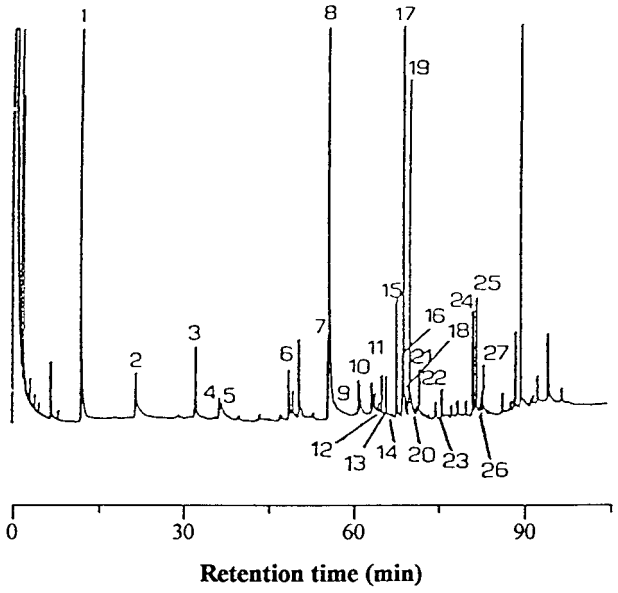


Fig. 2. HRGC profile of the phenolic fraction of a virgin I-77 olive oil. Peaks: (1) resorcinol (internal standard), (2) tyrosol, (3) hydroxytyrosol, (4) unknown, (5) unknown, (6) palmitic acid, (7) linoleic acid, (8) oleic acid, (9) stearic acid, (10) unknown, (11) dialdehydic form of ligstroside-aglycone containing no carbomethoxy group, (12), (13), (17), (19), (20), (21), (22), linked phenols containing tyrosol, (14), (16), (18), (23), (24), (25), (26), (27), linked phenols containing hydroxytyrosol and (15) monoglyceride.

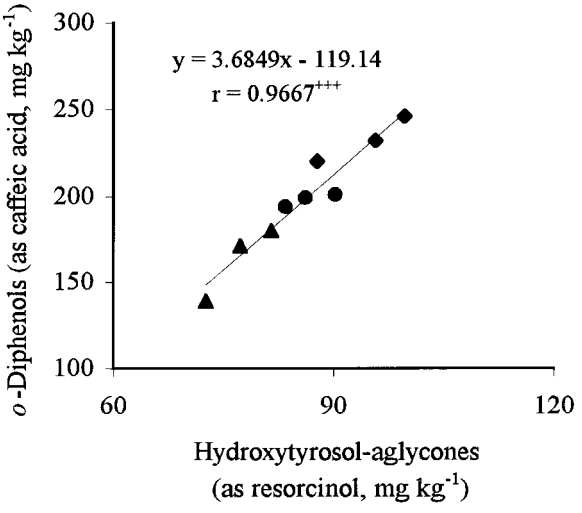


Fig. 1. Positive linear relationship between hydroxytyrosol-aglycones (determined by HRGC) and *o*-diphenols (determined colorimetrically) found for the virgin olive oils from the new I-77 cultivar sampled in the PG (◆), CB (●) and LE (▲) areas. $^{+++}$ $P \leqslant 0.001$.

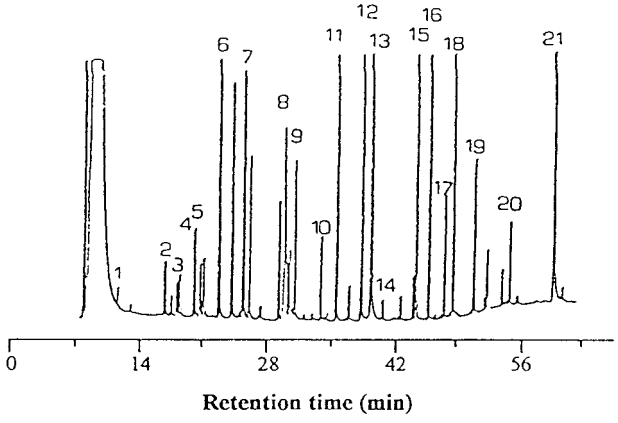


Fig. 3. HRGC profile of the aromatic volatile fraction of a virgin I-77 olive oil. Peaks identified: (1) n-octane, (2) ethyl-acetate, (3) 2-methyl-butyraldehyde, (4) 3-methyl-butyraldehyde, (5) ethanol, (6) 3-pentanone, (7) pent-1-en-3-one, (8) hexanal, (9) isobutyl alcohol, (10) *trans*-2-pentenal, (11) 1-penten-3-ol, (12) isoamyl alcohol, (13) *trans*-2-hexenal, (14) *n*-amyl alcohol, (15) 2-penten-1-ol, (16) hexan-1-ol, (17) *cis*-3-hexen-1-ol, (18) *trans*-2-hexen-1-ol, (19) acetic acid, (20) 1-octanol and (21) nonan-1-ol (internal standard).

*trans*-2-hexenal (Table 2) suggested that this oil could have good aromatic features. Volatile, phenol and tocopherol concentrations decreased by 19–24% after 6 months and by 37–43% after 12 months of storage of the oil samples.

### 3.3. Autoxidation rate, sensory score, overall quality indices

The general mean value of oxidative stability (Swift's test) (Gordon & Mursi, 1994) was rather high (18.6 h) and correlated with the content of *o*-diphenols ($r = 0.9623^{+++}$) (Fig. 4), hydroxytyrosol ($r = 0.9724^{+++}$), hydroxytyrosol-aglycones ($r = 0.9543^{+++}$) and tocopherols ($r = 0.9121^{+++}$). The resulting adjusted multiple linear correlation coefficient was (excluding *o*-diphenols among which are included free and bonded hydroxytyrosol) $0.9867^{+++}$.

The general mean value of sensory score, a basic quality parameter (E.C. Regulation no. 2568, 1991), was 7.7, and correlated with the content of *o*-diphenols ($r = 0.9549^{+++}$), tyrosol ($r = 0.8949^{++}$), hydroxytyrosol ($r = 0.9588^{+++}$), tyrosol-aglycones ($r = 9260^{+++}$) and hydroxytyrosol-aglycones ($r = 0.9773^{+++}$). The resulting adjusted multiple linear correlation coefficient was $0.9936^{+++}$. The correlation of sensory score with total volatile content was less good ($r = 0.7744^{+}$).

The general mean values of the overall quality indices ($OQI_1$ and $OQI_2$) (Ranalli, Ferrante, De Mattia & Costantini, 1999), regardless of origin area, were 8.0 and 39.8, respectively. There was a good linear relationship between the two indices ($r = 0.9265^{+++}$). These and the oxidation rate decreased by 15–19% after 6 months and by 27–34% after 12 months of storage of the oil samples. The sensory score, after 12 months, was still $>6.5$ (minimum limit set by the E.C. Regulation no. 2568, 1991).

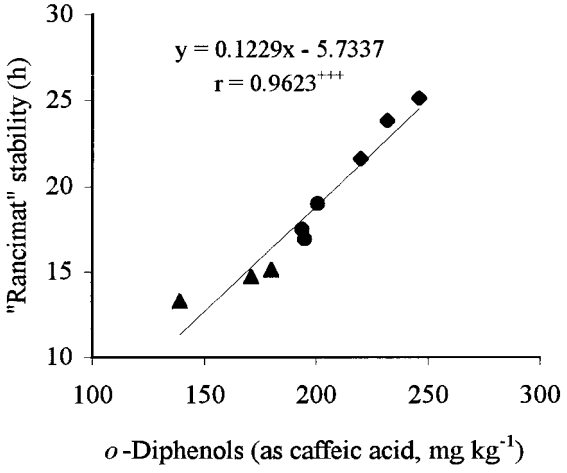


Fig. 4. Positive linear relationship between *o*-diphenol content (determined colorimetrically) and "Rancimat" stability found for the virgin olive oils from the new I-77 cultivar sampled in the PG (◆), CB (●) and LE (▲) areas. $^{+++}$ $P \leqslant 0.001$.

### 3.4. Chlorophylls, carotenoids, chromatic parameters, colour indices, turbidity

The content of chlorophylls $a+b$ (green pigments), including also pheophytins $a+b$ (Mg-free chlorophyll derivatives having a brown-green colour), averaged 10.2 mg $kg^{-1}$ (great mean), which is a medium value (Gandul-Rojas & Mínguez-Mosquera, 1996). The content of carotenoids (yellow pigments), independently of origin area, averaged 4.43 mg $kg^{-1}$. These components were represented most notably by lutein (2.93 mg $kg^{-1}$), β-carotene (0.72 mg $kg^{-1}$), violaxanthin (0.43 mg $kg^{-1}$) and neoxanthin (0.33 mg $kg^{-1}$). Chlorophylls and carotenoids exert an antioxidant action in the dark but an oxidant one in the light (Ranalli & Modesti, 1999). These substances also have biological and health properties (Roca López-Cepero, Gandul-Rojas & Minguez-Mosquea, 1999) and occur in the oil at concentrations which usually correlate with those of phenols and volatiles (whose positive properties have been noted above) (Ranalli, De Mattia & Ferrante, 1998). They undergo, during fruit ripening and oil storage, a natural breakdown which is parallel to that of phenols and volatiles; consequently they could even be considered as a product freshness indicator (Gandul-Rojas, Roca López-Cepero, Carmona-Ramón & Minguez-Mosquera, 1999; Ranalli, De Mattia et al., 1998).

The values of brightness and chroma (Table 1) were consistent with those of the green and yellow lipochromes. Those of hue indicated a predominancy of the yellow colour. Those of carotenoid colour index, chlorophyll colour index and integral colour index agreed with those of lipochromes. Finally, those of turbidity were somewhat low (Table 2).

The pigment content decreased by 18–21% after 6 months and by 33–36% after 12 months of storage of the oil samples. It is noteworthy, that after a storage of 6 months, both chlorophylls *a* and *b* were thoroughly degraded to the corresponding pheophytins *a* and *b*. However, pheophytin *a*, followed by lutein and β-carotene, as with other olive oil varieties, was also the quantitatively most important pigment occurring in the fresh I-77 oil variety. Chlorophyll *a*, which is the major pigment present in the growing fruit, could be thoroughly degraded to pheophytin *a* towards the end of the ripening cycle (Ranalli, Tombesi et al., 1998).

### 3.5. Free acidity, UV spectrophotometric indices, peroxide index, carbonyl index

Cultivar or origin area (Tables 1 and 2) had no significant influence on these analytical parameters, which are basically affected (Kiritsakis, Nauos, Polymenoupoulos, Thomai & Sfakiotakis, 1998) by factors causing damage to the fruits (e.g. olive fly attacks or improper systems of harvesting, carriage and storage of

olives). The injured drupes suffer the actions of endogenous and exogenous lipase and lipoxidase enzymes, which can modify the oxidation state and the acidity of the oil droplets. Lipases are mostly present in the fruit seed (and much less in the mesocarp) and stop their activity at 0°C, whilst lipoxidases are active at more than −20°C (Boskou, 1996).

The above analytical parameters are also hardly influenced by technological treatments (Ranalli & Angerosa, 1996). Toward the end of processing, both lipases and lipoxidases are almost entirely found in the liquid effluent (as they are water-soluble); therefore, all changes involving these parameters during storage of oil are almost exclusively of a chemical nature (Sciancalepore, 1998). Their values, which were all good in the virgin olive oil from the new olive cultivar (Tables 1 and 2), increased by 9–12% after 6 months and by 18–23% after 12 months of storage of the oil samples.

### 3.6. Sterols and triterpene dialcohols

The oil sterol composition is helpful when biochemically characterising a vegetable species (Kiritsakis, 1998). In almost all animal and vegetable fats, sterols are the most quantitatively important non-saponifiable components (Boskou, 1996). In olive oil they are less important than hydrocarbons, which are essentially represented by squalene (Sciancalepore, 1998).

In the virgin I-77 olive oil, total sterols, regardless of origin area, averaged 124 mg 100 $g^{-1}$, a value which is significantly higher than the minimum limit set by the E.C. Regulation no. 2568 (1991). The most representative sterols, similar to other olive oil varieties, were β-sitosterol, $\Delta^5$-avenasterol, campesterol and stigmasterol (data not shown). These (excluding $\Delta^5$-avenasterol), together with brassicasterol, cholesterol and $\Delta^7$-stigmastenol, are considered authenticity markers. Their individual percentage was, for the above oil, lower than the maximum official limit (E.C. Regulation no. 2568, 1991).

The HRGC chromatogram of the sterol fraction included two peaks corresponding to erythrodiol and uvaol (Table 1). These are triterpene dialcohols whose overall content averaged 2.2 mg 100 $g^{-1}$. Their percentage (1.5%) was far lower than the maximum official limit. The fruit epicarp is a major source of them (Ranalli et al., 1997). They are extracted in large amounts by organic solvents (hexane) and, therefore, are present in high percentage in the olive-pomace oil (Ranalli, De Mattia et al., 1998).

### 3.7. Waxes and aliphatic and triterpene long-chain alcohols

These substances (firstly waxes) are mostly present in the external fruit wax cuticle (Bianchi, Murelli & Vlahov, 1992). The waxes ($C_{40}+C_{42}+C_{44}+C_{46}$) are useful in detecting the presence of olive-pomace oil in pressed oil (E.C. Regulation no. 2568, 1991). The general average content of waxes (181 mg $kg^{-1}$) and that of superior alcohols (77 mg 100 $g^{-1}$) of the virgin I-77 olive oil paralleled those of several traditional virgin olive oil varieties. The alcoholic index, whose value is inversely related to quality (Ranalli & De Mattia, 1997), was very low (0.07). Phytol, a diterpene alcohol, corresponding to the first peak of the HRGC chromatogram of the alcohol fraction, and geranylgeraniol, corresponding to a newly identified peak, were included in the set of variables selected for multivariate analysis (Table 1).

### 3.8. Multivariate analysis results

A summary of total variation of the 26 selected analytical oil parameters (Table 1) is presented by their factor loadings from the first two factors of the PCA (Fig. 5). However, three dimensions of the PCA model were found to be significant and explained 80% of total variance.

PC1, accounting for 68% of the total variance, was dominated by the variables, triterpene alcohols, unsaturated fatty acids/polyunsaturated fatty acids ratio, total waxes, total sterols, aliphatic alcohols, geranylgeraniol, phytol, palmitic acid and stearic acid (negative values), and the variables, total phenols, total tocopherols, oxidative stability, linoleic acid, pleasant volatiles, unsaturated fatty acids/saturated fatty acids ratio, oleic acid/linoleic acid ratio, chlorophylls + pheophytins, total carotenoids, linolenic acid, sensory score and free acidity (positive values).

PC2, accounting for 7% of the total variance, was dominated by the variables, peroxide index, oleic acid and alcoholic index (negative values), and the variables, palmitic acid, chlorophylls + pheophytins and geranylgeraniol (positive values).

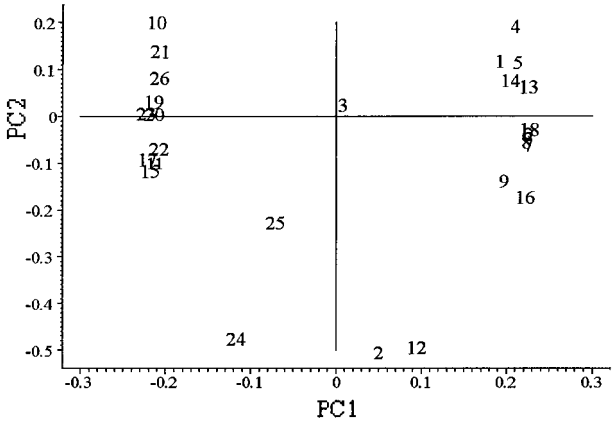


Fig. 5. PCA loadings of the selected analytical oil variables (Table 1) for dimensions 1 and 2.

Finally, PC3, accounting for 5% of total variance, was dominated by the variables, $k_{270}$, alcoholic index, peroxide index, geranylgeraniol and linoleic acid (positive values), and the variable triterpene dialcohols (negative value).

The PCA scores of samples on the 1 and 2 eigenvectors are given in Fig. 6. Two PCA factors were effective in discriminating between origin areas. These were the CB area, discriminated along the second component and the LE and PG areas along the first component (negative and positive side, respectively). This was confirmed by Hierarchical Cluster Analysis (Goodacre et al., 1993). In fact, the dendrogram (Fig. 7) showed three distinct blocks, each representing an origin area, with a similarity percentage >70% (PG area), >80% (CB area) and >85% (LE area).

The comparison between score plot and loading plot indicated that the variables, oleic acid/linoleic acid ratio, stearic acid, unsaturated fatty acids/polyunsaturated fatty acids ratio, geranylgeraniol, phytol, palmitic acid, triterpene alcohols, aliphatic alcohols, total sterols and total waxes, were mainly responsible for discrimination of the LE area, while the CB area was mainly differentiated by the variables, peroxide index, oleic acid, alcoholic index and triterpene dialcohols, and the PG area by the variables, unsaturated fatty acids/saturated fatty acids ratio, sensory score, total phenols, oxidative stability, pleasant volatiles, linoleic acid, linolenic acid, free acidity degree, total carotenoids and chlorophylls + pheophytins.

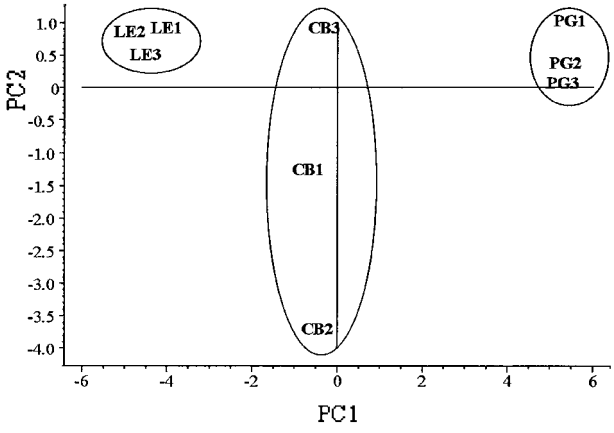


Fig. 6. Score plot by the dimensions 1 and 2 from PCA of the data set (Table 1) referring to virgin I-77 olive oils sampled in three different geographical areas (PG = Perugia, CB = Campobasso and LE = Lecce).

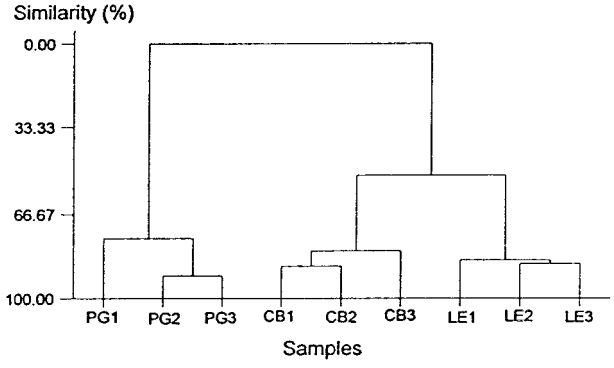


Fig. 7. Dendrogram showing the clustering of the production areas (PG = Perugia, CB = Campobasso and LE = Lecce) of virgin I-77 olive oil using the analytical variable data set (Table 1).

## 4. Conclusion

The new olive cultivar, I-77, gave an oil kind showing good chemical, sensorial and nutritional properties. The good quality and stability of its aroma and flavour (which was ascribable to the composition of its volatile and phenol fraction) along with its marked fruity notes, medium bitterness index and good overall acceptability, suggested that it could even have some typicality characters. Also, its shelf-life was of concern as suggested by the non-marked variation during storage (one year), of some analytical parameters (autoxidation rate, peroxide index, free acidity extent, carbonyl index, spectrophotometric UV indices, pigment content and others). Finally, its composition suggested that the characteristic ratios between fatty acids were good. However, its overall analytical picture indicated that several quality descriptors displayed significantly different values due to the origin area. This was confirmed by examination of the pyrograms.

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