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Statistical analysis on Sicilian olive oils

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Abstract

A robust multivariate statistical procedure applied to an accurate gas chromatography (GC) analysis was used to analyze 1004 monovarietal and multivarietal Sicilian extra virgin olive oils coming from 22 cultivars of different geographical areas of Sicily and collected in nine years (1993, 1995, 1996, 1997, 1998, 1999, 2000, 2001 and 2002). The effect of the cultivar, of pedoclimatic conditions as well as of the year of harvesting on the olive oil fatty acid composition and therefore on their classification was investigated. Oleic, linoleic and palmitic fatty acids, important for the nutritional properties of an olive oil, showed a crucial rule in the characterization of olive oils. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Olive oil; Cultivar; Gas chromatography; Statistical analysis

1. Introduction

The characterization of olive oils in terms of cultivar and geographical origin is a current debated problem: the quality and the peculiarity of an olive oil are, in fact, the result of several factors such as the cultivar, the pedoclimatic condition and the production practices. In Italy, the olive oil germoplasm is estimated to include about 500 cultivars (Cimato & Sani, 1999): however, many studies have been performed and are currently in progress on the morphological evaluation of olive germoplasm present in different Italian regions (Cimato, 2002; Gemas, Almadanim, Tenreiro, Martins, & Fevereiro, 2004) to answer to the controversial issue concerning the actual number of varieties.

The fatty acid composition of olive oils has been frequently used to group olive oils according to the cultivar of origin (Bianchia, Giansantea, Shawb, & Kellb, 2001; Bucci, Magrì, Magrì, Marini, & Marini, 2002; Lanteri, Armanino, Perri, & Palopoli, 2002; Paz et al., 2005). Specifically, in a previous paper, (Mannina et al., 2003) we have reported a Gas Chromatographic and ¹³C NMR study on the cultivar-composition relationship in Sicilian olive oils coming from four monovarietal cultivars grown within a well-limited geographical area with a very homogeneous microclimatic condition: the results proved the usefulness of fatty acids to group monovarietal olive oils belonging to the same cultivar and suggest that the fatty acid content and the distribution of the fatty chains on the glycerol moiety can be used to the fingerprint each specific cultivar. The punctual description of a specific cultivar on the base of the fatty acid content is important since the fatty acid content is a fundamental parameter to determine nutritional properties of olive oils (Grigg, 2001; Owen et al., 2000). A healthy diet must contain a limited amount of saturated fatty acids to reduce the total cholesterol content and a high amount of monounsaturated fatty acids which prevent the risk of cardiovascular diseases, reduce the insulin body-requirement and decrease the plasma concentration of glucose. Therefore, an accurate fatty acid determination can be extremely important when specific dietetic regimes are required. Moreover, the relationship between the intake of olive oil, the richest dietary of the

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monounsaturated fatty acid oleic acid and breast cancer risk and progression is a current discussed issue (Perez-Jimenez, Lopez-Miranda, & Mata, 2002; Servili et al., 2004; Tuck & Hayball, 2002).

Another important aspect concerns the geographical characterization of olive oils. An import 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. The PDO certification improves the commercial value of the product and it is particularly important in Italy where many different "typical" olive oils are present. Many papers are reported on the classification of olive oils according to their geographical origin (Alessandri et al., 1997; Aparicio, Albi, Cert, & Lanzon, 1988; Lanteri et al., 2002) by means of multivariate statistical analysis applied to fatty acids (Forina & Tiscornia, 1982), triacylglycerols (Tsimidou, Macrae, & Wilson, 1987), sensory attributes, chemical components (Alessandri et al., 1997; Boggia, Zunin, Lanteri, Rossi, & Evangelisti, 2002; Bucci et al., 2002) and also to minor compounds determined using ¹H NMR technique (García-González, Mannina, D'Imperio, Segre, & Aparicio, 2004; Mannina et al., 1999, 2000; Mannina, D'Imperio, Lava, Schievano, & Mammi, 2005; Mannina, Luchinat, Emanuele, & Segre, 1999; Mannina, Patumi, Proietti, Bassi, & Segre, 2001; Mannina, Patumi, Proietti, & Segre, 2001; Sacchi et al., 1996, 1998; Segre & Mannina, 1997).

Generally, the results obtained using different methodologies refer to specific conditions of cultivar, geographical area or year of harvesting and therefore the results requires further validations using, for instance, more cultivars, more geographical areas, more samples and more years.

In this paper, the previously reported statistical approach was definitely tested and improved extending the analysis to a large fatty acids library obtained using accurate Gas Chromatographic data. This library contains the composition of fatty acids of 1004 monovarietal and multivarietal olive oils samples coming from 22 cultivars grown in different pedoclimatic areas of Sicily and collected in nine years. This exhaustive library allowed us to answer questions on the effects of the cultivar, of the geographical area as well as of the year of harvesting. The discriminant power of the different fatty acids is discussed.

2. Materials and methods

2.1. Sampling

Samples (1004) of monovarietal (918 samples), multivarietal (65 samples) extra virgin olive oils, together with some olive oils samples coming from unknown cultivars (21 samples) were analyzed. These olive oils, obtained within the project POM B02 Misura 2 ("reduction of production costs, improving of the quality and protection of environment in the olive oil processing chain"), were collected in nine years (1993, 1995, 1996, 1997, 1998, 1999, 2000, 2001 and 2002) and came from 22 cultivars grown in different geographical areas of Sicily (see Fig. 1). An explorative statistical analysis allowed us to remove anomalous samples and therefore to reduce the number of samples to 998. The geographical origin, the geographical area (East Coast, North Coast, South Coast and Inland Area of Sicily), the cultivar and the year of harvesting of the 998 extra virgin olive oils are reported in Table 1.

2.2. 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 Carbowax fused silica capillary column, $30 \text{ m} \times 0.25 \text{ mm}$ ID, $0.20 \mu \text{m}$ film thickness (Supelco, Inc. Bellefonte, PA) was employed.

The following chromatographic conditions were used: column temperature from 160 °C (kept for 10 min) to 190 °C at 4 °C/min (maintained for 13 min); injector and detector temperatures 220 °C; hydrogen linear velocity 50 cm/sec.

Peak areas (data not shown) of 13 fatty acids (C14, C16, C'16, C17, C'17, C18, C'18, C''18, C'''18, C20, C'20, C22, C24) and C'18/C''18, C16/C''18 and unsaturated/saturated ratios were calculated using Shimadzu CLASS-VP 5 software. The 16 GC normalized variables were submitted to the statistical procedure.

2.3. Statistical method

GC data were submitted to Statistica software package for Windows (1997 edition by Statsoft, Inc.). The statistical procedure, reported in previous papers (Dugo, La Pera, Pellicanò, Di Bella, & D'Imperio, 2005; Mannina et al., 2003; Mannina, Fontanazza, Patumi, Ansanelli, & Segre, 2001), was completed by an initial *explorative analysis* performed to remove anomalous samples and therefore to reduce the initial data matrix.

The *explorative analysis* uses a box plot based on the median and the quartiles; it allows the anomalous samples which are in extreme and outlier areas of the box plot to be removed.

Therefore, the statistical procedure was based on *explorative analysis, principal component analysis (PCA), analysis of variance (ANOVA)* and *linear discriminant analysis (LDA)*. The variables with the highest index of variability were selected according to their *p*-level and *F* values. The *F* value is the ratio between within-group variability and between groups variability: larger is this ratio, larger is the discrimination power of the corresponding variable.

In the case of LDA the Mahalanobis distances, i.e. the distances between the centroids of each groups were also reported.



Fig. 1. Map of Sicily: the geographical origin of the analyzed olive oils and the four different geographical areas corresponding to the North Coast, the East Coast, the South Coast and the Inland Area are reported.

3. Results and discussion

GC applied to 1004 olive oil samples allowed a complete fatty acid profile (data not reported) to be obtained: peak areas of 13 fatty acids (C14, C16, C'16, C17, C'17, C18, C'18, C"18, C"'18, C20, C'20, C22, C24) and C'18/C"18, C16/C"18 and unsaturated/saturated ratios were measured. The 16 GC values corresponding to 1004 olive oil samples were submitted to a multivariate statistical procedure. An explorative statistical analysis added to the previously reported statistical methodology allowed us to remove anomalous samples and to reduce the number of samples to 998. These 998 olive oil samples came from 22 cultivars (Baratta & Campisi, 2001, chap. 3) widespread in Sicily (see Fig. 1) and collected in nine years (see Table 1).

The different effects observed on the fatty acid composition will be discussed separately.

3.1. Effect of the cultivar on the fatty acid content

The 998 analyzed olive oils came from 22 cultivars: some more common cultivars were more represented, with a number of samples larger than 80, than the other ones. However, some of the less-common less-represented cultivars are important for the local olive growing areas (Baratta et al., 2001). Therefore, in a first explorative statistical approach we chose not to discard either samples or cultivars. The 16 GC variables of 998 olive oils samples of nine years were submitted to the PCA to individuate the eventual presence of some principal groupings. The result of the PCA is reported in Fig. 2. A careful analysis of the graph allowed three principal groupings to be identified: two groups, A and B, correspond mainly to olive oil samples of two cultivars, namely, *Ogliarola messinese* and *Cerasuola*, whereas the last large group C contains olive oil samples from many different cultivars. However, within this large group it is possible to identify four statistically representative sub-groups corresponding to olive oils from *Minuta, Biancolilla, Tonda Iblea* and *Santagatese* cultivars.

Samples relative to the other 16 cultivars (22 cultivar minus the above mentioned six cultivars) give values scattered all over in the plot areas previously identified. Therefore, we decided to simplify the analysis reducing the number of the cultivars from 22 to 6. The mean values of the 16 GC variables corresponding to the six more representative Sicilian cultivars are reported in Table 2.

A qualitative "similarity" relationship (see Table 3) among the olive oil samples of these six cultivars and the other olive oils can be obtained by the PCA plot: in fact, olive oils with similar fatty acids composition are grouped in the same area of the plot. Olive oils from *Passalunara* cultivar are similar to olive oils from *Ogliarola messinese*; *Cerasuola* olive oils are well grouped together and are not similar to other olive oil samples. The gas chromatographic

Table 1

Geographical origin, geographical area, cultivar and year of harvesting of 998 Sicilian olive oils

$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Geographical origin ^a	Geographical area	Origin CODE ^b	Cultivar (Cv)	Cultivar code ^c
Angeon (AG)SCAra (2)Bela i spagnaBSS (1)Brigh (ME)ECBri (1)Bianco(DillaBia (9)Brole (MF)NCBro (5)CalabreacCal (8)Bronte (CT)IABro (5)CarabacaCar (7)Busto Palizable (TF)NCBuPa (9)CerasubaCer (97)Calubelletta (AG)SCCal (2)CreaturCar (10)Casal Vacchin (ME)ECCaVe (1)CuercianaCue (1)Casal Urberto (ME)NCCalo (6)MantaMan (1)Casal Vactano (TP)SCCas (16)MantaMan (1)Casal Vactano (ME)NCCol (19)Moreca Mor (74)Colesno (PA)NCCol (19)Moreca Mor (74)Cortone (PA)IACor (12)Nocellara mestineseNoMe (4)Ferra D'Agri (ME)ECGal (1)OutabraticaMaf (5)Gampilieri (ME)ECGal (1)OutabraticaMaf (5)Gampilieri (ME)ECGal (1)PartiginaPar (1)Calouforo (ME)ECGal (1)PartiginaPar (1)Leanta (FC)IALeFr (1)StrausanaSir (13)Leanta (Fed)IALeFr (1)StrausanaSir (13)Leanta (Fed)SCMac (2)FordesVer (37)Maria (TP)SCMat (1)Sir (13)Sit (13)Leanta (Fed)SCMac (2)FordesVer (37)Maria (FG)SCMac (2)FordesVer (37)	Antillo (ME)	EC	Ant (4)	Ascolana tenera	AsTe (1)
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Casel Vertano (TP)SCCas (16) <i>Hindta</i> Min (100)Collesno (PA)NCCol (19) <i>Moresca</i> Mor (74)Corleone (PA)IACor (12) <i>Nocellara at helice</i> NoBe (32)Forza D'Agro (ME)ECFoAg (1) <i>Nocellara at thelice</i> NoBe (32)Francolone (SR)IAFra (31) <i>Nocellara at thelice</i> NoBe (4)Furci (ME)ECGal (1) <i>Ottobratica</i> OttobGalledoro (ME)ECGal (1) <i>Ottobratica</i> Pas (6)Lentini (SR)ECLon (44) <i>Parsiginan</i> Par (1)Learan Fredid (PA)IALoo (43) <i>Satusgatese</i> Sa (82)Learan Fredid (PA)IALof (7) <i>Tonda thea</i> Tol (120)Mazara (TP)SCMar (2) <i>Veralese</i> Ver (37)Meta (AG)SCMer (10)Unknown CvCv(G (21)Meta (RG)SCMon (10)Mixtor of CvCv(H (65)Moracelo (PA)NCMon (10)MoracelosVer (37)Monracole (PA)NCMon (10)MoracelosVer (37)Monracole (PA)NCMon (10)MoracelosVer (37)Monracole (PA)NCMon (10)MoracelosVer (37)Moracelos (PA)NCMon (10)MoracelosVer (37)Moracelos (PA)NCMon (10)1499415Moracelos (PA)NCMon (10)153SoMoracelos (PA)NCMos (10)199-199526Palaz	Castell'Umberto (ME)	NC	CaUm (66)	Mantonica	Man (1)
Collesano (PA)NCCol (19)MorecaMor (74)Corlence (PA)IACor (12)Nacellara del holieNoble (32)Forza D'Agro (ME)ECFoAg (1)Nacellara etneaNoE (13)Francofonte (SR)IAFra (31)Nacellara messineseOgMe (160)Galladoro (ME)ECGal (1)OttobattacaOttobattacaGalanpilieri (ME)ECGai (1)OttobattacaPar (1)Lentini (SR)ECLen (44)PastulanaraPas (6)Leonforte (EN)IALeo (43)SantagateseSan (82)Lecata (AG)SCMaz (2)VerdeseVer (37)Mazara (TP)SCMaz (2)VerdeseVer (37)Meazin (AG)SCMar (2)Variation988Modica (RG)SCMod (84)YaarNo. of samplesModica (RG)SCMod (80)YaarNo. of samplesMoreaCole (PA)NCMod (10)No. of samplesNo. of samplesNoto (SR)ECNot (5)1995-199646Pacco (TP)NCNCPact (10)1992-199646Pacco (TP)NCPact (49)1997-199865Partana (TP)SCPar (9)1994-199135Noto (SR)IAPacc (49)1997-199865Partana (TP)SCPar (10)1992-10936Patrano (CL)SCPar (10)1992-10936Scate (RG)IAPacc (49)1997-1998	Castel Vetrano (TP)	SC	Cas (16)	Minuta	Min (100)
Corlcone (PA)IACor (12)Nocellara atheaNobel (32)Foraz D'Agro (ME)ECFoAg (1)Nocellara atheaNobel (4)Francofonte (SR)IAFra (31)Nocellara atheaNobel (4)Francofonte (SR)IAFra (31)Nocellara atheaOttobGalledoro (ME)ECGal (1)OttobraticaOttobGianopilieri (ME)ECGal (1)OttobraticaOttobLentini (SR)ECLen (4)PassuluaraPas (6)Lentini (SR)ECLen (4)PassuluaraSa (6)Lerara Freddi (PA)IALeo (43)SatagateseSa (82)Lerara Freddi (PA)IALeft (1)SiracusanaSir (13)Licata (AG)SCMac (2)FerdeceVer (37)Maziar (IP)SCMac (10)Unknown CvCVG (21)Maziar (IP)NCMas (10)Unknown CvCVG (21)Meziojuso (PA)IAMez (19)Mixter of CvCVMI (65)Misilmeri (PA)NCMon (10)YearNo. of samplesMoneaCole (PA)NCNas (1)YearNo. of samplesMotica (RG)SCNis (4)1993-1994135Noto (SR)ECNis (4)1993-1994135Noto (SR)ECNis (4)1993-1994135Partinico (PA)NCPac (49)199-1994153Partinico (PA)NCPac (49)199-1994153Partinico (PA)NCPac (10) <td< td=""><td>Collesano (PA)</td><td>NC</td><td>Col (19)</td><td>Moresca</td><td>Mor (74)</td></td<>	Collesano (PA)	NC	Col (19)	Moresca	Mor (74)
Form Francofont (SR)ECFoAg (1)Nocellara messineseNoEt (59)Francofont (SR)IAFra (31)Nocellara messineseOgM (160)Gallodoro (ME)ECGal (1)OttobraticaOtt (5)Gianpilieri (ME)ECGal (1)OttobraticaOtt (5)Lentini (SR)ECLen (44)ParstigianaPart (1)Lentini (SR)ECLen (44)ParstigianaSin (82)Leonfort (EN)IALeo (43)SantagateseSan (82)Letrata Freddi (PA)IALeo (43)SantagateseSin (82)Leata (AG)SCLic (7)Tonda libadTolb (120)Mazar (TP)SCMax (2)VerdeseVer (37)Menfi (AG)SCMax (2)Inknown CvCvIG (21)Mezcojuso (PA)IAMcc (19)Mixture of CvCvMI (65)Misimeri (PA)NCMas (1)YearNo. of samplesModica (RG)SCMod (84)YearNo. of samplesMonrea Cole (PA)NCMot135ScNoto (SR)ECNot (5)1995-199646Paracolo Acreide (SR)IAPack (49)1997-199865Partania (TP)SCPart (10)1999-1996153Partania (PA)NCPart (10)1999-2000280Partania (RG)SCRag (30)2001-2002129Partania (RG)SCRag (30)2001-2002129Ragusa (RG)SCRag (30)	Corleone (PA)	IA	Cor (12)	Nocellara del belice	NoBe (32)
Francionte (SR)IAFra (31)Nocle (and maximize) Optimular messimeseNoMe (4)Furci (ME)ECFur (1)Optimular messimeseOpt (50)Galadoro (ME)ECGal (1)OttobraticaOtt (5)Giandificri (ME)ECLen (44)PartiginanPart (1)Lentini (SR)ECLen (44)PassidumaraPas (6)Leontore (EN)IALeF (1)SincusanaSir (13)Licata (AG)SCLic (7)Tonda liblaTolls (120)Mazara (TP)SCMaz (2)VerdeseVer (37)Mear (AG)SCMac (10)Unknown CvCvIG (21)Misimer (PA)NCMor (10)Unknown CvCvIG (21)Misimer (PA)NCMod (84)YearNo. of samplesMoneaCole (PA)NCMon (10)YearNo. of samplesNoso (ME)SCNot (5)195-199646Noso (SR)ECNot (5)195-199646Partana (TP)SCPart (10)199-199865Partana (TP)SCPart (10)199-199865Partana (TP)SCPart (10)199-199865Partana (TP)SCPart (10)199-199865Partana (TP)SCPart (10)199-199865Partana (TP)SCPart (10)199-2000280Parterio (CT)ECPart (10)199-200136Ragusa (RG)SCRag (30)201-2002129<	Forza D'Agro (ME)	EC	FoAg (1)	Nocellara etnea	NoEt (59)
Furci (ME)ECFur (1)Opticula messineseOptic (160)Galladoro (ME)ECGia (14)PartiginaPart (1)Giampilieri (ME)ECGia (14)PartiginaPart (1)Lentini (SR)ECLen (44)PassuhunaraPas (6)Leonforte (EN)IALeo (43)SontagateseSan (82)Lercara Freddi (PA)IALeFr (1)StracusanaSir (13)Licata (AG)SCLic (7)Tonda iblaTolb (120)Mazara (TP)SCMax (2)VerdeseVer (37)Menfi (AG)SCMen (10)Unknown CvCvIG (21)Mezzojus (PA)IAMez (19)Mixture of CvCvMIG (5)Misilmeri (PA)NCMon (10)Mixture of CvCvMIG (5)Noncaclole (PA)NCMon (10)Nas (1)Not (5)135Noto (SR)ECNot (5)1995-1994135Noto (SR)ECNot (5)1995-199646Palazolo Acreide (SR)IAPac (49)1997-1994135Patana (TP)SCPar (9)1998-1999153Partana (TP)SCPar (9)1998-1999153Patrinico (PA)NCPar (12)2000-200136Patazolo Acreide (SR)IAPac (49)1997-1994135Patinico (PA)NCPart (12)2000-2003128Patazolo Acreide (SR)IAPac (49)1997-1994135Patinico (PA)NCPart (12)200	Francofonte (SR)	IA	Fra (31)	Nocellara messinese	NoMe (4)
Gallodoro (ME)ECGal (1)OrtobraticaOtt (5)Giamplieri (ME)ECGia (14)PartiginaPar (1)Lentini (SR)ECLen (44)PassidinaraPar (3)Leotar Fredi (PA)IALeo (43)SantagateseSan (82)Leotar Fredi (PA)IALeo (43)SantagateseSan (82)Licata (AG)SCMax (2)VerdeseVer (3)Mazara (TP)SCMax (2)VerdeseVer (3)Menf (AG)SCMen (10)Mixture of CvCVG (21)Mezzojuso (PA)IAMez (19)Mixture of CvCVMI (65)Misilmeri (PA)NCMis (52)Total998Modica (RG)SCMod (84)YearNo. of samplesMonracOle (PA)NCNas (1)NoSantagateseSantagateseNaso (ME)NCNas (1)SantagateseSantagateseSantagateseParceco (TP)NCNas (1)SantagateseSantagateseSantagatesePartana (TP)SCPar (9)1993-1994135SantagatesePartana (TP)SCPar (9)1994-199726SantagatesePartana (TP)SCPar (9)1998-199726Partana (TP)SCPar (9)1998-1992153Partana (AG)SCRag (30)2001-2002129Rosolini (RR)ECRag (30)2001-2002129Rosolini (RR)ECRag (30)2001-2002129S. G	Furci (ME)	EC	Fur (1)	Ogliarola messinese	OgMe (160)
Giamplieri (ME)ECGia (14)PariginaPar (1)Lentini (SR)ECLen (44)PassulmaraPas (6)Lenoforte (EN)IALeo (43)SantagateseSan (82)Lercara Freddi (PA)IALefr (1)StratesamaSir (13)Licata (AG)SCLic (7)Tonda libeaTolb (120)Mazara (TP)SCMaz (2)VerdeseVer (37)Menfi (AG)SCMar (10)Unknown CvCVG (21)Mezojuso (PA)IAMez (19)Mixture of CvCvMI (65)Modica (RG)SCMon (10)NoScModMonreaCole (PA)NCMon (10)Misture of CvCvMI (65)Moscen (CL)SCNo (5)1993–1994135Noto (SR)ECNo (5)1995–199646Palazzola Acreide (SR)IAPac (49)1997–199865Partanna (TP)SCPar (1)1998–1999153Partinico (PA)NCPar (1)1998–1999153Partinico (PA)NCPar (10)1998–1999153Partinico (PA)SCRag (30)2001–2002129Partonico (CT)ECPar (1)Eac CoastEC (162)Rosolini (SR)ECRos (6)Total998S. Agata di Miltello (ME)NCAgMi (42)Geographical areaS. Agata di Miltello (ME)NCAgMi (42)Geographical areaS. Caterina (CL)IACat (17)Eac CoastEC (162) <td>Gallodoro (ME)</td> <td>EC</td> <td>Gal (1)</td> <td>Ottobratica</td> <td>Ott (5)</td>	Gallodoro (ME)	EC	Gal (1)	Ottobratica	Ott (5)
Lenthin (SR)ECLen (44)PasuharaPas (6)Leonforte (EN)IALeo (43)SantagateseSan (82)Lecarar Freddi (PA)IALeFr (1)SiracusanaSir (13)Licata (AG)SCLic (7)Tonda ibleaTolb (120)Mazara (TP)SCMax (2)VerdeseVer (37)Menfi (AG)SCMax (2)VerdeseVer (37)Menfi (AG)SCMax (2)Total98Modica (RG)SCMon (10)Wixture of CvCvNI (65)Misilmeri (PA)NCMoo (10)YearNo. of samplesMonaco (kPA)NCNo. (10)No. SCNis (4)1993-1994135Noto (SR)ECNot (5)1995-199646Paeceo (TP)NCPac (50)1996-199726Palazolo Acreide (SR)IAPaAc (49)1997-199865Partaina (TP)SCPart (10)1999-2000280Paterink (CT)ECPat (12)2002-2003128Patrinko (CT)ECRag (30)2001-2002129Ragusa (RG)SCRag (30)2001-2002129S. Agata di Militelo (ME)NCPat (12)2002-2003128S. Agata di Militelo (ME)NCPat (12)2002-2003128S. Giovanni Gemini (AG)SCGiGe (3)North CoastSC (15)S. Gierna (CL)IACat (17)Eac CoastEC (162)S. Agata di Militelo (ME)NCPiNi (5)<	Giampilieri (ME)	EC	Gia (14)	Partigiana	Par (1)
Leon(orbre (EN)IALeo (43)SantagateseSan (82)Lercara Fredii (PA)IALeFr (1)SiracusanaSir (13)Licata (AG)SCLic (7)Tonda ibleaTolb (120)Mazara (TP)SCMaz (2)VerleseVer (37)Menfi (AG)SCMaz (2)VerleseVer (37)Medi (AG)SCMen (10)Unknown CvCvIG (21)Mezojuso (PA)IAMez (19)Mixture of CvCvMI (65)Misimer (PA)NCMod (84)YearNo. of samplesMonreaCole (PA)NCMas (1)NoNas (1)Naso (ME)NCNas (1)NoNas (1)Niseeni (CL)SCNo (5)1995-199646Paceco (TP)NCPac (50)1995-199646Paceco (TP)NCPac (50)1995-199665Partana (TP)SCPar (9)1998-1999153Partana (TP)SCPart (10)1999-2000280Partenio (PA)NCPart (10)1999-200136Ragusa (RG)SCRa (30)2001-2002129Ribera (AG)SCRa (30)2001-2002129Sciorani (SR)ECRos (6)Total998S. Caterina (CL)IACat (17)East CoastEC (162)S. Giovanni Gemini (AG)SCGiGe (3)North CoastSC (192)S. Tress Riva (ME)ECTeRi (11)Inland AreaIA (192)S. Vio Lo Capo (TP) <td>Lentini (SR)</td> <td>EC</td> <td>Len (44)</td> <td>Passulunara</td> <td>Pas (6)</td>	Lentini (SR)	EC	Len (44)	Passulunara	Pas (6)
Lercara Freddi (PA)IALeFr (1)SiracusanaSir (13)Licata (AG)SCLic (7)Tonda tibleaTolb (120)Mazara (TP)SCMaz (2)VerdeseVer (37)Menfi (AG)SCMen (10)Unknown CvCVIG (21)Mezzijuso (PA)IAMez (19)Mixture of CvCVMI (65)Misilmeri (PA)NCMos (52)Total998Modica (RG)SCMod (84)YearNo. of samplesMonracCole (PA)NCMon (10)NcNas (1)Naso (ME)NCNas (1)135Noto (SR)ECNot (5)1996-199726Palaczolo Acreide (SR)IAPaAc (49)1997-199865Partanna (TP)SCPard (4)1997-199865Partanna (TG)SCRag (30)2001-2002129Paterinic (PA)NCParti (10)1999-2000280Paterinic (CA)SCRag (30)2002-2003128Ragusa (RG)SCRag (30)2002-2003128Rosolini (SR)ECRos (6)Total998S. Agata di Miltello (ME)NCAgMi (42)Geographical areaS. Sagata di Miltello (ME)NCPiNi (5)South CoastSC (192)S. Fress Riva (ME)ECTeki (1)Inland AreaIA (192)S. Vito Lo Capo (TP)NCPiNi (5)South CoastSC (192)S. Troina (EN)IASaf (12)Total998Sileenci (Leonforte (EN)	IA	Leo (43)	Santagatese	San (82)
Licata (AG)SCLic (7)Tonda ibleaTolb (120)Mazara (TP)SCMaz (2)VerdeseVer (37)Menfi (AG)SCMen (10)Unknown CvCVIG (21)Mezonjuso (PA)IAMez (19)Mixture of CvCvVII (65)Misilmeri (PA)NCMot (84)YearNo of samplesModica (RG)SCMod (84)YearNo of samplesMoracole (PA)NCMon (10)NCNot (5)1993-1994135Niscemi (CL)SCNot (5)1995-199646Noto (SR)ECNot (5)1995-199646Paceco (TP)NCPac (49)1997-199865Partanic (TP)SCPar (9)1998-199726Patzalo Acreide (SR)IAPac (49)1999-1996153Partinic (PA)NCPart (10)1999-2000280Patarana (TP)SCPart (12)2000-200136Ragusa (RG)SCRag (30)201-2002129Rosolini (SR)ECRos (6)Total998S. Agata di Militello (ME)NCAgMi (42)Geographical areaGeographical area code ⁴⁴ S. Caterina (CL)IACat (17)East CoastEC (162)S. Giovanni Gemini (AG)SCGiGe (3)North CoastNC (135)S. Pier Niceto (ME)NCPiNi (5)South CoastSC (192)S. Teresa Riva (ME)ECFeki (1)Inland AreaIA (192)S. Vito Lo Capo (TP)	Lercara Freddi (PA)	IA	LeFr (1)	Siracusana	Sir (13)
Mazara (TP)SCMaz (2)VerdeseVer (37)Menfi (AG)SCMen (10)Unknown CvCVIG (21)Mezzojuso (PA)IAMez (19)Mixture of CvCVMI (65)Misilmeri (PA)NCMol (84)YearNo. of samplesModreaCole (PA)NCMon (10)Naso (ME)No. of samplesMoareaCole (PA)NCMon (10)Naso (ME)IstaNaso (ME)NCNo. (5)1995-1994135Noto (SR)ECNot (5)1995-199646Paceco (TP)NCPac (50)1996-199726Palazzolo Acreide (SR)IAPaAc (49)1997-199865Partana (TP)SCPart (9)1998-1999153Partinico (PA)NCParti (10)1999-2000280Paternò (CT)ECPat (12)2000-200136Ragus (RG)SCRag (30)201-2002129Rosolini (SR)ECRos (6)Total998S. Agata di Militello (ME)NCAgMi (42)Geographical area code ^d S. Caterina (CL)IACat (17)East CoastEC (162)S. Giovanni Gemini (AG)SCTeRi (1)Inland AreaIA (192)S. Jere Nicet (ME)NCPiNi (5)South CoastSC (192)S. Teresa Riva (ME)ECTeRi (1)Inland AreaIA (192)S. Vie Lo Capo (TP)NCYie (1)Yie (1)Yie (1)S. Vie Lo Capo (TP)NCSci (11)Sira	Licata (AG)	SC	Lic (7)	Tonda iblea	ToIb (120)
Menf (AG)SCMen (10)Unknown CvCVG (21)Mezzojuso (PA)IAMez (19)Mixture of CvCVMI (65)Misilmeri (PA)NCMod (84)YearNo. of samplesModica (RG)SCMod (84)YearNo. of samplesMonreaCole (PA)NCMon (10)NcNcNsistemi (CL)Niscemi (CL)SCNis (4)1993–1994135Noto (SR)ECNot (5)1995–199646Paceco (TP)NCPac (49)1997–19865Patazolo Acreide (SR)IAPaAc (49)1997–199865Patrana (TP)SCPar (9)1998–1999153Patrinic (PA)NCPat (12)2000–200136Paterno (CT)ECPat (12)2002–2003128Rosolin (SR)ECRos (6)Total998S. Agata di Militello (ME)NCAgMi (42)Geographical areaGeographical area code ^d S. Caterina (CL)IACat (17)East CoastSC (162)S. Giovanni Gemini (AG)SCGiGe (3)North CoastNC (192)S. Fier Nice (ME)NCFiNi (5)South CoastSC (192)S. Vic Lo Capo (TP)NCVica (1)UnknownUN (137)Salemi (TP)IASal (12)Total998S. Giovanni Gemini (AG)SCSci (11)Sracus (SR)Sci (11)Siracusa (SR)ECSci (11)Total998Siracusa (SR)ECSci	Mazara (TP)	SC	Maz (2)	Verdese	Ver (37)
Mezzojuso (PA)IAMez (19)Mixture of CvCvMI (65) TotalMisimeri (PA)NCMis (52)Total998Modica (RG)SCMod (84)YearNo. of samplesMonreaCole (PA)NCMon (10)NcNaso (ME)NCNaso (ME)NCMon (10)Nc135Noto (SR)ECNot (5)1995–199646Paceco (TP)NCPac (50)1996–199726Palazolo Acreide (SR)IAPAAc (49)1997–199865Partanan (TP)SCPart (10)1999–2000280Patrinic (PA)NCPart (10)1999–2000280Patrino (CTA)ECPat (12)2002–2003129Ragusa (RG)SCRag (30)2001–2002129Rosolini (SR)ECRos (6)Total998S. Agata di Militello (ME)NCAgMi (42)Geographical areaGeographical area codedS. Caterina (CL)IACat (17)East CoastEC (162)S. Giovanni Gemini (AG)SCGiGe (3)North CoastNC (315)S. Pier Niceto (ME)NCPiNi (5)South CoastSC (192)S. Vito Lo Capo (TP)IASal (12)Total998S. Sicovanni Gemini (AG)SCSci (11)Inland AreaIA (192)S. Vito Lo Capo (TP)IASal (12)Total998S. Vito Lo Capo (TP)IASal (12)Total998Sicacca (AG)SCSci (Menfi (AG)	SC	Men (10)	Unknown Cv	CvIG (21)
Misilmeri (PA)NCMis (52)Total998Modica (RG)SCMod (84)YearNo. of samplesMonreaCole (PA)NCMon (10)Naso (ME)NCNas (1)Niscemi (CL)SCNis (4)193–1994135Noto (SR)ECNot (5)1995–199646Pacco (TP)NCPac (50)1996–199726Palazzolo Acreide (SR)IAPaAc (49)1997–199865Partana (TP)SCPar (10)1999–2000280Patrinico (PA)NCPatri (10)1999–2000280Patrinico (PA)NCPatri (10)2000–200136Ragusa (RG)SCRag (30)2001–2002129Ribera (AG)SCRag (30)2001–2002129S. Agata di Militello (ME)NCAgMi (42)Geographical area codedS. Caterina (CL)IACat (17)East CoastSC (192)S. Givanni Gemini (AG)SCGiGe (3)North CoastNC (315)S. Pier Niceto (ME)NCPiNi (5)South CoastSC (192)S. Teresa Riva (ME)ECTeix (11)Inland AreaIA (192)S. Vito Lo Capo (TP)NCSci (11)Sci (11)Sci (11)S. Vito Lo Capo (TP)NCSci (11)Total998Sciacca (AG)SCSci (11)Total998Sciacca (AG)SCSci (11)Total998Sciacca (AG)SCSci (11)Total998	Mezzojuso (PA)	IA	Mez (19)	Mixture of Cv	CvMI (65)
Modica (RG)SCMod (84)YearNo. of samplesMonreaCole (PA)NCMon (10)Naso (ME)NCNas (1)Niscemi (CL)SCNis (4)1993-1994135Noto (SR)ECNot (5)1995-199646Paceco (TP)NCPac (50)1996-199726Palazzolo Acreide (SR)IAPaAc (49)1997-199865Partana (TP)SCPar (10)1999-2000280Paterno (CT)ECPat (12)2000-200136Ragusa (RG)SCRag (30)2001-2002129Ribera (AG)SCRagu (30)2001-2002129Ribera (AG)SCRagu (42)Geographical areaGeographical area codedS. Agata di Militello (ME)NCAgMi (42)Geographical areaCeographical area codedS. Caterina (CL)IACat (17)East CoastNC (315)S. Pier Niceto (ME)NCPiNi (5)South CoastSC (192)S. Teresa Riva (ME)ECTeRi (1)Inland AreaIA (192)S. Vito Lo Capo (TP)NCViCa (1)UnknownUN (137)Salemi (TP)IASal (12)Total998Scacca (AG)SCSir (11)Total998Vizzini (CT)IATro (2)Vizzini (CT)Viz (1)UnknownUN (137)Total998998	Misilmeri (PA)	NC	Mis (52)	Total	998
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Unknown UN un (137) Total 998	Vizzini (CT)	IA	Viz (1)		
Total 998	Unknown	UN	un (137)		
		Total	998		

^a ME: Messina; AG: Agrigento; CT: Catania; TP: Trapani; SR: Siracusa, EN: Enna; PA: Palermo; CL: Caltanissetta; RG: Ragusa.

^b The number of samples for each geographical origin is reported in brackets.

^c The number of samples for each cultivar is reported in brackets.

^d The number of samples for each geographical area is reported in brackets.

results show that *Cerasuola* olive oils have the highest content of oleic fatty acids and the lowest content of palmitic fatty chains. Therefore, *Cerasuola* olive oils, well distinguishable from all the other olive oils, have a favorable impact in specific dietetic regimes. Moreover, in a previous paper (Mannina et al., 2001) it was shown that this cultivar has a good pedoclimatic adaptability giving acceptable olive oils even in extreme pedoclimatic conditions.

The similarity relationship of fatty acid composition among olive oils coming from different cultivars would be



Fig. 2. PCA on 16 GC variables of 998 Sicilian olive oils collected in nine years and coming from 22 cultivars. Samples are labeled according to Table 1. Ellipses group the three principal groups (A, B and C) described in the text.

Table 2

Mean values and standard deviations of the 16 GC variable areas relative to olive oil samples from six Sicilian cultivars; the number of samples for each cultivar is reported in brackets

GC variables	Biancolilla (93)	Cerasuola (97)	Minuta (100)	Ogliarola messinese (160)	Santagatese (82)	Tonda iblea (120)
C14 Tetradecanoic (myristic)	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
C16 hexadecanoic (palmitic)	14.01 ± 1.78	9.97 ± 1.17	15.78 ± 1.48	14.76 ± 2.23	13.39 ± 1.33	14.86 ± 1.70
C'16 Z-9-hexandecenoic(palmitoleic)	0.89 ± 0.20	0.34 ± 0.19	1.54 ± 0.40	0.87 ± 0.34	0.79 ± 0.33	1.16 ± 0.37
C17 heptadecanoic (margaric)	0.19 ± 0.06	0.06 ± 0.05	0.15 ± 0.06	0.20 ± 0.05	0.14 ± 0.05	0.07 ± 0.06
C'17 Heptadecenoic	0.33 ± 0.08	0.08 ± 0.07	0.26 ± 0.08	0.28 ± 0.06	0.22 ± 0.06	0.11 ± 0.10
C18 Octadecanoic (stearic)	2.31 ± 0.49	2.64 ± 0.45	2.05 ± 0.52	2.71 ± 0.55	2.31 ± 0.39	2.08 ± 0.31
C'18 Z-9- octadecenoic (olieic)	71.71 ± 3.72	76.35 ± 2.32	65.40 ± 3.13	70.73 ± 6.89	70.24 ± 2.51	69.48 ± 3.46
C"18 Z, Z -9-12- octadienoic (linoleic)	9.17 ± 2.01	9.17 ± 1.64	12.99 ± 1.93	8.80 ± 5.06	11.87 ± 1.62	10.21 ± 1.64
C ^{'''} 18 Z, Z, Z -9-12-15- octadecatrienoic (linolenic)	0.49 ± 0.19	0.52 ± 0.11	0.60 ± 0.16	0.62 ± 0.19	0.49 ± 0.15	0.60 ± 0.23
C20, Icosanoic (arachidic)	0.36 ± 0.09	0.40 ± 0.07	0.34 ± 0.10	0.41 ± 0.09	0.35 ± 0.10	0.36 ± 0.06
C'20, Z -11- eicosenoic	0.15 ± 0.11	0.25 ± 0.14	0.15 ± 0.10	0.19 ± 0.11	0.11 ± 0.08	0.19 ± 0.09
C22 Docosanoic (behenic)	0.05 ± 0.04	0.06 ± 0.04	0.06 ± 0.04	0.08 ± 0.05	0.05 ± 0.04	0.06 ± 0.04
C24, Tetracosanoic (lignoceric)	0.04 ± 0.04	0.05 ± 0.05	0.04 ± 0.03	0.05 ± 0.04	0.02 ± 0.02	0.05 ± 0.03
C'18/C''18	8.33 ± 2.48	8.62 ± 1.70	5.18 ± 1.08	11.63 ± 6.59	6.08 ± 1.21	7.02 ± 1.40
C16/C"18	1.59 ± 0.38	1.12 ± 0.21	1.24 ± 0.18	2.21 ± 1.00	1.15 ± 0.19	1.48 ± 0.24
Unsaturated/saturated	4.95 ± 0.67	6.63 ± 0.67	4.43 ± 0.47	4.54 ± 0.60	5.18 ± 0.49	4.73 ± 0.68

better razionalized by a careful morphological and biogenetic characterization. It is important to note that cultivars known with different names not necessarily have different genotype. It is worth noticing that, from the PCA analysis, it was also possible to gain information in terms of similarity of fatty acid composition, on the olive oil samples coming from unknown cultivars (see Table 3).

Table 3

Groupings identified by PCA analysis applied on 998 samples coming from 22 cultivars; the six principal cultivars together with the corresponding "similar" ones are reported

Group	Principal cultivar	Similar cultivars
A	Ogliarola messinese	Passalunara, Unknown cultivar (1 sample)
В	Cerasuola	Unknown cultivar (4 samples)
С	Minuta, Biancolilla,	Moresca, Siracusana, Partigiana, Ottobratica, Nocellara messinese, Nocellara etnea,
	Santagatese, Tonda iblea	Mantonica, Bella di spagna, Ascolana tenera, Carolea, Verdese, Crastu, Calabrese, Cuccitana,
		Nocellara del belice, Unknown cultivar (16 samples)

The analysis of variance, (ANOVA) applied to the 16 GC variables relative to the six main cultivars allowed the variables with the highest discriminant power to be determined. The more discriminant variables are C'16 (F = 89,2697), C'17 (F = 84,1076), C16 (F = 49,4382), C18' (F = 36,3523) and C"18 (F = 22,5147).

3.2. Effect of the year of harvesting

In order to investigate the effect of the year of harvesting on the fatty acid composition and therefore on the olive oil classification, the PCA was performed separately on olive oil samples of the each cultivar collected in nine years. In this way, it is possible to understand whether the fatty acid composition of a specific cultivar can be considered conservative in different years or undergoes significant modifications. It was previously reported (Boggia et al., 2002) that good climatic conditions such as high summer temperatures and poor autumn rainfalls improve olive oil quality limiting *Dacus oleae* infestation; on the other hand bad climatic conditions with low summer temperatures and strong autumn winds and rainstorms worse the olive oil quality favoring the spreading of infestation. The PCA results, reported in Fig. 3, show that almost all olive oil samples are completely spread not showing grouping according to the specific year; only olive oils samples of 1999–2000 year



Fig. 3. PCA of olive oils collected in nine years coming from each of the 6 selected principal cultivars. Ellipses represent the 95% confidence regions and group olive oils of the "anomalous" year (1999–2000).

are always grouped together and slightly separated from the others. In Italy, this year was characterized by bad climatic conditions and a widespread infestation (Boggia et al., 2002). These observations allow us to conclude that the year does not influence significantly the olive oil fatty acid composition unless extremely bad climatic conditions occur.

3.3. Pedoclimatic effect

The obtained results suggested us to perform the PCA only on olive oils from the six principal cultivars in all

the years with the exclusion of 1999–2000. The results are reported in Fig. 4A. Olive oil samples from *Ogliarola* and *Cerasuola* are well separated from all the other samples. The olive oils of the other four cultivars are grouped together showing a clear overlapping of data. In order to analyze the PCA plot in details, the PCA results corresponding to each cultivar are reported separately (see Fig. 4B). Four different geographical areas can be identified namely, the East Coast, the North Coast, the South Coast and the Inland Area of the Sicily. Following this geographical distinction, olive samples of each cultivar can be further grouped: in fact, *Ogliarola messinese* olive oils can



Fig. 4. (A) PCA of olive oils, collected in eight years coming from the selected 6 principal Sicilian cultivars: Bia: *Biancolilla*; Cer: *Cerasuola*; Min: *Minuta*; OgMe: *Ogliarola messinese*; San. *Santagatese*; ToIb: *Tonda iblea*. (B) Details of the same PCA: each cultivar is reported separately. Groupings corresponding to olive oil samples of different geographical areas are indicated with a dashed line.

be grouped in two classes corresponding to olive oils from the North Coast and the East Coast, respectively whereas Biancolilla olive oils can be grouped in two classes corresponding to olive oils from the South Coast and the North Coast; finally Tonda Iblea olive oils can be grouped in two classes corresponding to olive oils from the South Coast and Inland Area. Minuta Santagatese and Cerasuola olive oils are well grouped together coming from the same geographical area. Note that in the case of *Cerasuola* olive oils only one sample coming from the South Coast is well separated from the group corresponding to olive oil samples from the North Coast. These results suggest that although the effect of the cultivar is predominant in the olive oil classification based on the fatty acid composition, a minor but well defined geographic effect is also present. The analysis of the factor loadings allows us to select C"18, C'18, C'18/C''18, C16/C''18 as the variables with the highest discriminant power for geographic effect; these variables are all included in Factor 1.

Therefore, oleic, linoleic and palmic fatty acids and the corresponding ratios play a fundamental rule not only in the cultivar characterization but also in the pedoclimatic characterization.

All these observations suggested us to perform the statistical analysis on olive oils including also other typical cultivars according to the four geographical areas. In this way it is possible to have an idea of the difference and the similarity among cultivars. The LDA plot and the corresponding Mahalanobis distances are reported in Fig. 5 and in Table 4, respectively. The similarity among cultivars, in fact, can be useful and can suggest appropriate mixtures of olive oils. The LDA plot of the olive oils of the North Coast show Ogliarola messinese olive oils well separated from *Cerasuola*; the other olive oil samples are grouped together according to their cultivar with a small overlapping which suggests a similarity among these cultivars. In the case of East Coast, Moresca olive oils are well separated from all the others which, in turns, are similar. Again, in the case of the South Coast, Moresca olive oils well separated from the other olive oils coming from Biancolilla, Nocellara del Belice, Tonda Iblea and Verdese. Finally, the olive oils of the inland area coming from Biancolilla, Cerasuola, Moresca and Tonda Iblea are well separated according to their cultivar. The Squared Mahalonobis distances i.e. the distances between the centroids of each group allow the similarity between different



Fig. 5. LDA analysis performed on Sicilian olive oils according to their geographical areas (North Coast, east Coast, South Coast and Inland Area): Bia, *Biancolilla*; Cer, *Cerasuola*; Min, *Minuta*; OgMe, *Ogliarola messinese*; San; *Santagatese*; Mor, *Moresca*; NoEt, *Nocellara etnea*; ToIb, *Tonda iblea*; Ver, *Verdese*; Sir, *Siracusana*. Ellipses represent the 95% confidence regions for each group.

Table 4	
Squared Mahalanobis distances between the centroids of each group identified by olive oils of	the specific cultivar

North Coast				East Coast						
	Bia ^a	Cer ^a	Min ^a	OgMe ^a	San ^a		Mor ^a	NoEt ^a	OgMe	Sir ^a
Bia	0.0					Mor	0.0			
Cer	38.6	0.0				NoEt	123.2	0.0		
Min	22.7	51.3	0.0			OgMe	123.2	9.4	0.0	
OgMe	51.7	68.4	76.6	0.0		Sir	123.2	36.9	23.1	0.0
San	11.0	19.6	26.7	55.1	0.0					
South Coa	ıst					Inland Are	ea			
	Bia	Mor	NoBe ^a	ToIb ^a	Ver ^a		Bia	Cer	Mor	ToIb
Bia	0.0					Bia	0.0			
Mor	71.4	0.0				Cer	394.3	0.0		
NoBe	35.2	98.8	0.0			Mor	72.6	597.5	0.0	
ToIb	20.2	77.4	15.0	0.0		ToIb	104.0	336.1	161.1	0.0
Ver	13.0	77.9	56.4	29.1	0.0					

^a Bia, Biancolilla; Cer, Cerasuola; Min, Minuta; OgMe, Ogliarola messinese; San; Santagatese; Mor, Moresca; NoEt, Nocellara etnea; ToIb, Tonda iblea; Ver, Verdese; Sir, Siracusana.

cultivars to be valuated. For instance, the *Nocellara del Belice* cultivar, a very important Sicilian cultivar, known both for olive oils and for the excellent table olives, produce olive oils well comparable to olive oils produced by Tonda Iblea.

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