ELSEVIER

Food Chemistry 80 (2003) 331–336

Food Chemistry

www.elsevier.com/locate/foodchem

Minor polar compound and fatty acid analyses in monocultivar virgin olive oils from Tuscany

P. Pinelli[a,*], C. Galardi[a], N. Mulinacci[a], F.F. Vincieri[a], A. Cimato[b], A. Romani[a]

[a]*Dipartimento di Scienze Farmaceutiche, via G. Capponi 9, 50121 Firenze, Italy*
[b]*Istituto sulla Propagazione delle Specie Legnose, CNR, Scandicci, Firenze, Italy*

Received 22 March 2002; received in revised form 11 June 2002; accepted 11 June 2002

Abstract

Virgin olive oil is a typical component of the Mediterranean diet, consumed unrefined and rich in important molecules, such as minor polar compounds (hydroxytyrosol, tyrosol, secoiridoids and flavonoids) and fatty acids. These molecules not only influence the sensorial properties of both olives and virgin oil but they are also important markers for typicity, biodiversity and quality determination of this product. The aim of this study was to evaluate the minor polar compound and fatty acid contents of 10 monocultivar virgin olive oils, typical of Tuscany, in order to have better knowledge about the quali-quantitative profiles of these compounds in samples obtained from both the same collecting season and same processing technique. Quali-quantitative analysis (performed by HPLC/DAD, HPLC/MS and GC) could be a useful tool to better correlate the typicity of the virgin olive oil with its minor polar compound and fatty acid pattern. Further studies are in progress to isolate the unknown compounds and to further investigate the quality index of this food product.

Keywords: Fatty acids; HPLC/DAD; Minor polar compounds; Typicity; Tuscany olive oils; Quality

1. Introduction

Consumption of antioxidant-rich foods, such as olive oil, in the Mediterranean diet, is now expanding in non-traditional producer countries (i.e. the United States, Australia and Japan) due to the large body of epidemiological studies that show a decreased incidence of cardiovascular disease (Keys, 1995; Navarro, Periago, Pita, & Hortelano, 1994).

From a health perspective, the high content of oleic acid in olive oil serves to slow down penetration of fatty acids into arterial walls (Charbonnier, 1982). Oils which are much higher in monounsaturated fatty acids (MUFAs) and lower in saturated fatty acids (SFAs) are preferred because of the proven beneficial effect of MUFAs on serum cholesterol levels (Mensink and Katan, 1992). In southern European populations there is strong evidence that MUFAs may influence breast cancer risk, as pointed out by studies where the intake of oleic acid, particularly from olive oil, appears protective (Simonsen et al., 1998). Recent data have suggested that polyunsaturated fatty acids (PUFAs) could be deeply involved in nutritional regulation of cellular fatty acid levels by inhibiting lipogenesis, and also show a modulator effect on vascular function (Abeywardena & Head, 2001). The varying contents of these molecules affect the stability of the oil: higher polyunsaturation tends to yield a more unstable oil, as the un-conjugated double bonds in these oils are particularly sensitive to thermal- and photo-oxidation. This leads to an important benefit of olive oil, namely its superior high temperature behaviour with respect to highly polyunsaturated seed oils.

Moreover, the biological properties of olive oil are also related to its antioxidant composition, namely, tocopherols and minor polar compounds, in particular phenols and secoiridoids. The antioxidant activity of some polyphenols of *Olea europaea* L. has been compared with synthetic derivatives by in vitro tests: caffeic acid and hydroxytyrosol showed a stronger activity than BHT (Perrin, 1992). Studies in vivo have also been performed (Ciappellano, Simonetti, Brighenti, Bermano, & Testolin, 1994) to compare the effects of minor compounds present in virgin olive oil on oxidative stress indices in rats. This

* Corresponding author. Tel.: +39-55-2757288; fax: 39-55-240776.
E-mail address: patrizia.pinelli@unifi.it (P. Pinelli).

0308-8146/03/$ - see front matter
PII: S0308-8146(02)00268-6

type of oil is effective in reducing some metabolic effects of oxidative stress. Investigations have also been carried out in human cells of intestinal epithelium to study the injurious effects of reactive oxygen metabolites and the possible protective role played by some olive oil phenolic compounds (Manna et al., 1997). This latter study suggests that dietary intake of olive oil phenols may lower the risk of oxygen metabolite-mediated diseases such as gastrointestinal diseases and arteriosclerosis.

Visioli's group (Visioli & Galli, 1998; Visioli et al., 1999; 2001) has evidenced the antiatherogenic effect of a polyphenolic extract of olive and olive oil waste waters by protecting the LDL against oxidative modifications. Incubation of platelets with dihydroxyphenylethanol causes a significant decrease in platelet aggregation, induced by collagen, under conditions of oxidative stress (Driss, Duranthon, & Viard, 1996). Moreover, some studies (Ficarra, Ficarra, De Pasquale, Monforte, & Calabrò, 1991; Le Tutor & Guedon, 1992) have shown a hypocholesterolemic and hypoglycemic activity of the oleuropein, the main secoiridoid present in the olive fruit, as well as in the olive oil, as an aglycone.

It appears that the minor polar compounds of virgin olive oil play an important role in human nutrition as preventive agents against several diseases.

The phenol content of olive oil can vary depending on several factors: e.g. technology used in the processing of olives, type of cultivar, growing conditions and time of ripening. Knowledge of the composition in phenolic substances makes it possible to hypothesize a relationship among the quality and typicity of this alimentary product as well as its pattern in natural antioxidants. The qualitative potential of virgin olive oil is an undeniable fact, but objectively to define the exact components of that quality is extremely difficult. However, quality can be evaluated with regard to four aspects: commodity (genuineness, adulteration, alteration, according to provisions of the law); health (absence of foreign and toxic residues); nutrition and dietetics (chemical composition which fulfils the physiological needs of the consumer); organoleptic characteristics (chemical composition which makes it pleasant and appetizing). Typicity is peculiar to those foods whose features depend on the geographic environment of production. Geographic environment means not only natural factors (climate, soil, soil exposure), but also human factors (historical, cultural, traditional), special processing techniques and finally the organoleptic properties of the end-product resulting from them.

The virgin olive oil samples considered in this study were obtained from plants growing in a specific Tuscan area. These cultivars belong to a collection of the autochthonous species created in order to preserve and improve their biodiversity. A previous quali-quantitative analysis allowed separation and identification of many compounds from the polar fraction of olives and the relative monocultivar virgin olive oil (Romani, Mulinacci, Pinelli, Vincieri, & Cimato, 1999; Romani et al., 2001).

The aim of the present investigation was to evaluate the minor polar compound and fatty acid contents of 10 Tuscan monocultivar virgin olive oils in order to gain knowledge about quali-quantitative profiles of these compounds in samples obtained from the same collecting season and processed using the same mill.

2. Materials and methods

2.1. Olive sampling and origin areas selected

Eight years ago all the mother plants, representing most of Tuscan olive varieties, were cloned and used to establish a germplasm nursery in Follonica (Tuscan coastal area), maintained in collection by the National Research Council of Italy. These genotypes are mostly "landraces", in some cases well known by their names since ancient times, and described at the phenotypic level. DNA fingerprinting was by microsatellite markers, confirming their specific and different profiles. These cultivars were not registered since they have been widespread among farmers for years. The olive fruits were harvested near technological ripening (1–10 November 1999) and the oils were immediately obtained by crushing the olives (25 kg) at room temperature (25 °C) in a benchtop biphasic mill (Oliomio, Ditta Mori, Grosseto, Italy) without water addition, and 30 min kneading time. The oil yield was in the range 16–18%, depending on the cultivar. The following monocultivar olive oils were considered: Tondino (T), Mignolo Cerretano (MC), Rossellino (R), Leccione (L), Olivastra Seggianese (OS), Morchiaio (M), Leccio Maremmano (LM), Emilia (E), Ginestrino (G) and two Frantoio samples (F1 and F2).

2.2. Sample preparation

A volume of 50 ml of each oil sample was extracted in triplicate with 150 ml of the following solution: EtOH/H_2O 70:30 v/v; the water was acidified by formic acid (pH = 2.5). Defatting with *n*-hexane was performed to completely remove the lipid fraction. The raw alcoholic extract of each sample was concentrated under reduced pressure to aqueous phase and rinsed with 2 ml of extraction solvent and then analysed by HPLC/DAD and HPLC/MS for the minor polar compounds' determination.

2.3. Fatty acid analysis

The preparation of methyl esters of the fatty acids was carried out according to the EC Official Gazette. A gas chromatograph (Perkin Elmer, mod. 8420) was employed, with a FID detector (Flame Ionization Detector), a split-splitness injector, capillary column SP-2380 (0.35 mm i. d.,

Table 1
Polar minor compound concentrations (mg l^{-1}) evaluated by HPLC-DAD in ten Tuscany olive oil cultivars[a]

	T	F1	F2	MC	R	L	OS	M	LM	E	G
OH-Tyr	6.0±0.14	7.6±0.12	12.9±0.23	12.8±0.28	0.6±0.01	17.9±0.38	5.2±0.14	27.9±0.64	2.9±0.07	13.4±0.25	55.2±0.83
Tyr	4.5±0.12	32.4±0.67	45.5±0.82	134±0.24	3.7±0.09	11.4±0.21	5.9±0.14	3.7±0.05	2.1±0.05	6.9±0.07	17.1±0.18
EA	32.1±0.54	118±0.22	135±2.02	183.9±3.11	65.2±0.98	166±2.94	39.0±0.74	218±3.28	46.2±0.91	86.7±1.02	178±3.03
Eag	9.5±0.19	198±3.02	10.9±0.25	308±4.32	109±1.20	1.8±0.04	3.1±0.09	12.4±0.27	10.6±0.21	3.6±0.07	1.9±0.05
EA der.	0.4±0.01	1.4±0.03	2.0±0.05	7.7±0.15	n. d.[b]	n. d.	n. d.	3.5±0.10	1.5±0.04	1.1±0.03	1.7±0.04
DAcOlagl	273±4.16	1.8±0.05	n. d.	15.4±0.25	traces	42.9±1.03	183±3.09	176±3.01	64.8±1.27	115.5±2.98	n. d.
Oleur. agl.	44.2±0.79	15.6±0.37	25.9±0.45	55.8±0.84	7.9±0.21	266±3.98	48.9±1.03	143±2.44	15.5±0.28	146. 6±2.71	91.5±1.73
Secoir. der.	279±3.31	156±2.80	147±2.64	45.2±0.95	179±2.97	92.3±1.58	155±2.68	93.7±1.67	86.7±1.65	141±2.38	96.0±1.71
Lut	2.8±0.07	1.5±0.03	4.7±0.09	n. d.	3.5±0.08	n. d.	7.9±0.22	4.8±0.11	1.7±0.04	3.1±0.05	1.7±0.03
Apig	0.9±0.02	0.2±0.01	1.1±0.03	n. d.	2.7±0.07	n. d.	1.1±0.04	traces	traces	0.5±0.01	0.3±0.01

[a] Average of three analyses±S.D. Tondino (T), Mignolo Cerretano (MC), Rossellino (R), Leccione (L), Olivastra Seggianese (OS), Morchiaio (M), Leccio Maremmano (LM), Emilia (E), Ginestrino (G) and two Frantoio samples (F1 and F2). Compounds: OH-Tyr = hydroxytyrosol; Tyr = tyrosol; EA = elenolic acid; EAg = elenolic acid glucoside; EA der. = sum of two elenolic acid derivatives; DAcOlagl = deacetoxyoleuropein aglycone; Oleur. agl. = oleuropein aglycone; Secoir. der. = sum of four secoiridoid derivatives; Lut = luteolin; Apig = apigenin.
[b] n. d. = not detected

0.25 m film thickness, silica phase, from Supelco, Italy). The gas chromatographic conditions were as follows: oven temperature programme: from 160 to 174 °C at 2 °C min^{-1}, from 174 to 190 °C in 15 min at 4 °C min^{-1}. FID detector temperature 250° C. H_2 carrier gas pressure 18 psi.

2.4. Minor polar compounds analyses

The analysis of the minor polar compounds was performed on an HP 1100 liquid chromatograph equipped with HP DAD and 1100 MS detectors. The interface was an HP 1100 MSD API-electrospray. The experimental conditions were similar to those previously reported (Romani, Pinelli, Mulinacci, Vincieri and Tattini, 1999). In detail, the used column was a 250×4.6 mm (5 μm) LiChrosorb RP18 (Merck) maintained at 26 °C, equipped with a 10×4 mm LiChrosorb RP18 precolumn. The eluent was H_2O (pH 3.2 by H_3PO_4)/CH_3CN. A four-step linear solvent gradient was used, starting from 100% H_2O up to 100% CH_3CN, during a 106 min period, at a flow rate of 1 ml min^{-1}. Tyrosol and derivatives, elenolic acid and derivatives, oleuropein aglycone, deacetoxyoleuropein aglycone, other secoiridoid derivatives and flavone aglycones were analysed according to a previous work (Romani et al., 2001).

2.5. Quantitative evaluation of minor polar compounds

The quantitative evaluation of individual phenols was performed using four-point regression curves ($r^2 \geqslant 0.998$) operating in the range 0–30 μg, through the use of authentic standards purchased from Extrasynthese (Lyon, France).

The tyrosol (Tyr) and hydroxytyrosol (OH-Tyr) amounts were calculated at 280 nm using tyrosol as reference; the oleuropein aglycone, deacetoxyoleuropein aglycone (DAcOLagl) and the other secoiridoids were calculated at 280 nm, using oleuropein as standard; elenolic acid (EA) and elenolic acid glucoside (oleoside) were evaluated at 240 nm, using oleuropein as standard. For oleuropein aglycone, deacetoxyoleuropein aglycone, elenolic acid and its glucoside, correction of the molecular weight was applied. The flavonoid aglycones, luteolin (Lut) and apigenin (Apig), were evaluated by measuring the absorbance at 350 nm of the corresponding pure standards.

3. Results and discussion

The technological process applied, using a benchtop bi-phasic mill, made it possible to obtain a controlled olive oil of high quality without waste water production, and thus to collect information of typical Tuscan monocultivar olive oil. The acidity of the all analysed samples was below 0.3%.

The performed extraction method was carried out to obtain a total recovery of the minor polar compounds; therefore to preserve the quality and typicity of this food product.

The content of each phenol present in the polar fraction of the oil samples is expressed in mg l^{-1} of oil, as reported in Table 1. The derivatives of elenolic acid (EA) and secoiridoid (Secoir. der.) are the main minor polar compounds in the different analysed samples. The results shown in Table 1 suggest the use of some compounds, particularly EA and DAcOLagl, as specific markers. The flavones, luteolin and apigenin, are at low concentrations with respect to the total (less than 2%) in all the tested samples.

The total amount of minor polar compounds varies from 232 to 652 mg l^{-1} among the considered cultivars. In particular, M, MC and T show the highest values, while LC represents the monocultivar olive oil with the lowest content.

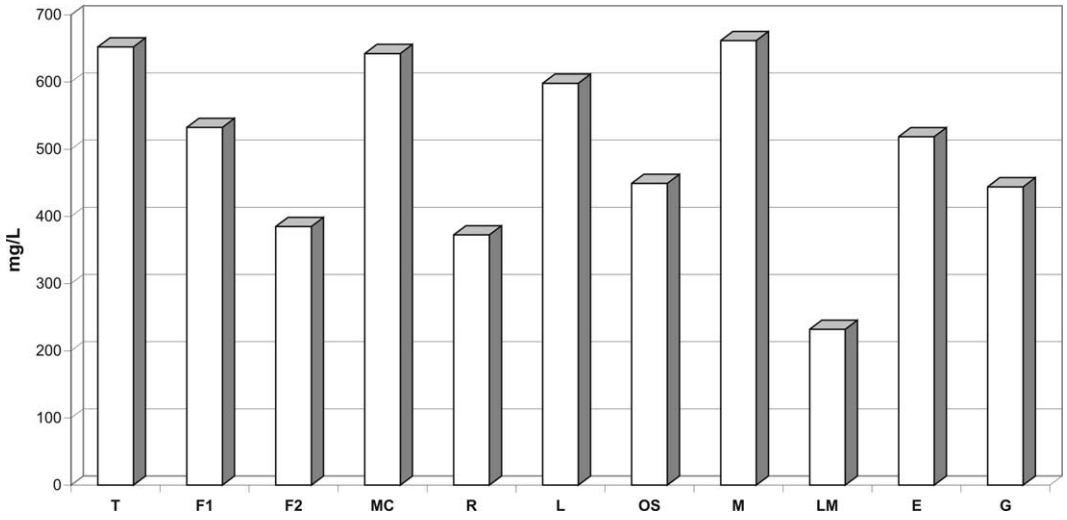

Fig. 1. Total minor polar compound contents in monocultivar virgin olive oils from Tuscany.

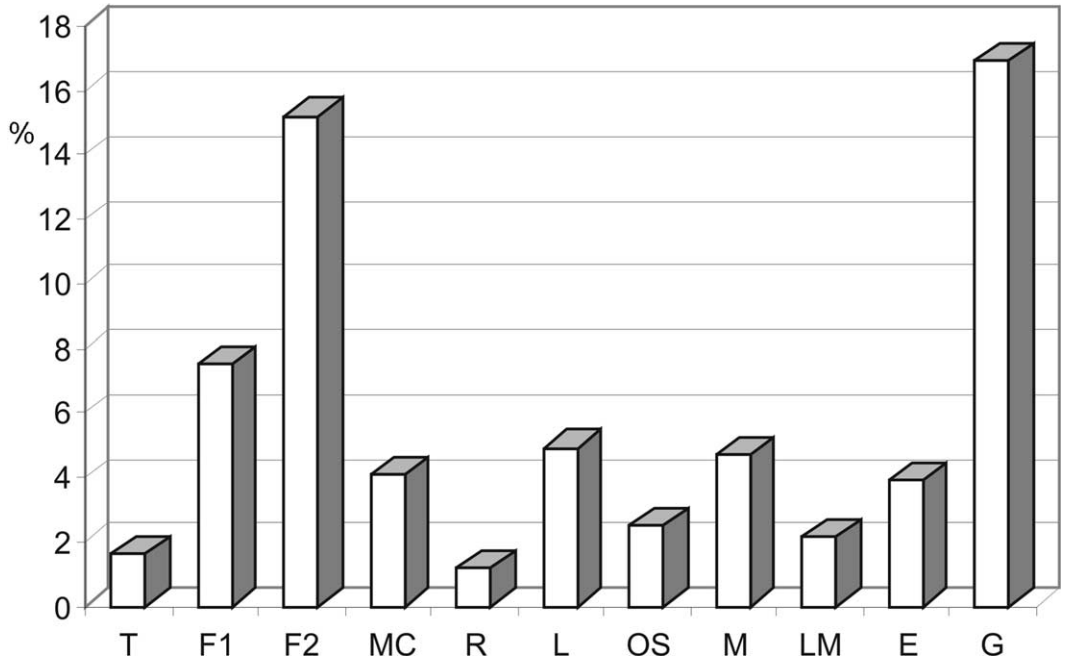

Fig. 2. Hydrolysis percentage [(OH-Tyrosol + Tyrosol)/Total Phenols] in the analysed olive oil samples.

With regard to the total phenol content, a previous investigation (Romani et al., 2001) reports virgin olive oil (from Abruzzo, Italy) in a medium range (200–500 ppm). As shown in Fig. 1, among the analysed cultivars, F2, R, OS, LM and G, effectively belong to the medium range with a phenolic content ranging from 232 (LM) to 449 (OS) mg l^{-1} of olive oil. The other samples, such as E, T, MC, L M, F1, show a higher content (500–650 ppm) and this evidence is not in complete agreement with previous findings reported for Tuscan olive oils (Montedoro, Servili, Baldioli, & Miniati, 1992). A high content of minor polar compounds could protect against auto-oxidation during the shelf life and add functional value to this traditional product of Mediterranean food culture.

The percentage of hydrolysis of each oil sample (percentage of tyrosol and hydroxytyrosol on total polyphenols) is reported in Fig. 2. Hydroxytyrosol content is in the range 0.6–55.2 mg/l of olive oil. This

Table 2
Ratios of secoiridoid derivatives/elenolic acid derivatives in the analysed Tuscan monocultivar virgin olive oils

	T	F1	F2	MC	R	L	OS	M	LM	E	G
Secoir. der./EA der.	14.2	0.5	1.2	0.2	1.1	2.4	9.2	1.7	2.8	4.4	1.0

Table 3
Fatty acids detected in olive oil samples (data are expressed in percentage values)

	T	F1	F2	MC	R	L	OS	M	LM	E	G
Palmitic ac.	14.6	13.1	12.6	14.3	13.7	9.76	14.34	13.05	16.4	14.0	13.2
Palmitoleic ac.	1.93	0.81	0.65	0.8	1.50	0.35	1.43	1.00	1.06	1.76	1.26
Margaric ac.	0.02	0.04	0.05	0.04	0.04	0.04	0.04	0.03	0.04	0.03	0.03
Margaroleic ac.	0.08	0.09	0.10	0.06	0.10	0.06	0.07	0.07	0.07	0.06	0.08
Stearic ac.	1.81	1.91	2.04	2.06	1.75	2.65	2.08	2.07	2.17	1.72	1.98
Oleic ac.	75.2	75.7	76.1	72.5	74.6	77.2	72.4	76.1	59.0	72.2	73.6
Linoleic ac.	5.40	7.1	7.19	8.91	6.95	8.63	8.57	6.46	19.8	9.19	8.62
Linolenic ac.	0.52	0.63	0.66	0.73	0.69	0.63	0.55	0.57	0.67	0.57	0.73
Arachid ac.	0.27	0.34	0.32	0.33	0.36	0.41	0.34	0.35	0.37	0.31	0.27
Eicosenoic ac.	0.17	0.28	0.33	0.23	0.33	0.28	0.22	0.34	0.43	0.25	0.20
Σ SFAs	16.7	15.4	15.0	16.7	15.9	12.9	16.8	15.5	19.0	16.0	15.5
Σ PUFAs	5.92	7.73	7.85	9.64	7.64	9.26	9.12	7.03	20.5	9.76	9.35
Oleic/linoleic	13.9	10.7	10.58	8.14	10.7	8.94	8.44	11.8	2.98	7.85	8.54
MUFAs/SFAs	5.0	5.5	5.7	5.0	5.3	6.8	4.9	5.4	4.3	5.2	5.4
MUFAs/PUFAs	14.1	10.9	10.8	8.6	11.0	9.4	9.1	12.0	3.95	8.60	9.00

SFAs = total content of saturated fatty acids; PUFAs = total content of polyunsaturated fatty acids; MUFAs = total content of monounsaturated fatty acid.

important antioxidant compound, originating from oleuropein degradation during fruit ripening, processing and storage of the oil, could be useful for evaluating stability. In fact, a high percentage of hydrolysis is correlatable to an aged oil, which is in an advanced oxidized condition. G and F2 samples show the highest values (16.9 and 15.2%), whereas T and R oils, which have low contents of tyrosol and hydroxytyrosol with respect to the total polyphenols, show a hydrolysis percentage near 1%.

In addition, a high ratio of secoiridoid derivatives/elenolic acid derivatives (Secoir. der./EA der.) is presumably correlated to high stability of the olive oil. In fact, Fig. 2 and Table 2 show that high ratios correspond to low percentages of hydrolisis. This characteristic is particularly evident in samples T, OS, G, F1 and F2.

Fatty acid evaluation was also performed on the olive oils, following the usual product analyses. Table 3 reports the identified fatty acids: palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), margaric ($C_{17:0}$), margaroleic ($C_{17:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), arachidic ($C_{20:0}$) and eicosenoic acid ($C_{20:1}$). In all the samples, the oleic acid is always the most abundant compound, never less than 72% of the total fatty acid with the exception of the sample LM (59%). Curiously, this sample is less rich in minor polar compounds (232 mg/l). Moreover, LM is characterized by a high content of linoleic acid (19.8%) in contrast to all other samples which vary from 5.4% (T) to 9.19% (E). The sum percentages of oleic and linoleic acid were 78.8 (M) and 85.8 (L); the oleic acid/linoleic acid ratio was always over 7.85 with the exception of sample LM which had an unusual value (2.98) with respect to previous data (Ranalli, Modesti, Patumi, & Fontanazza, 2000). The SFA percentage (12.9–18.9) was good; the PUFAs percentage was relatively similar for all the samples, except for T (5.9) and LM (20.5). The MUFA/SFA ratio (5.3 average value) and the MUFA/PUFA ratio (9.8 average value) were relatively low; however, the high phenol content could indicate that oil quality was maintained without lipid deterioration.

These oils, with the exception of the LM sample, have similar fatty acid compositions but they differ in their unsaponified fraction.

4. Conclusion

The work attempts to develop an initial database on Tuscan germplasm collection in order to establish the criteria and indicators useful to correlate the agronomic characteristics with quality and typicity of the olive oil. To this end the in situ conservation of each country's biological diversity can be focused on conserving genes, species, and ecosystems in their natural surroundings, for example by establishing protected areas, such as in the Tuscan germplasm collection. Since the minor polar compounds are significantly related to the quality and typicity of virgin olive oil (contribution to the oxidative oil stability and nutritional role), future investigations will aimed at the isolation of the unknown phenolic compounds.

These results can be essential tools for a registered trademark, such as the Denomination of Protected Origin (DOP) and Indication of Protected Geographical Area (IGP).

Acknowledgements

The authors gratefully acknowledge the skilled technical assistance of Mr. G. Sani. This work was supported in part by research grants from MIUR.

References

Abeywardena, M. Y., & Head, R. J. (2001). Dietary polyunsaturated fatty acid and antooxidant modulation of vascular dysfunction in the spontaneously hypertensive rat. *Prostaglandins Leukotrines and Essential Fatty Acids*, *65*(2), 91–97.

Charbonnier, A. (1982). *Main conclusion dawn from the International Symposium on the recent medical researches on the value of the olive oil to health* (pp. 1–4) Paris, 17 November.

Ciappellano, S., Simonetti, P., Brighenti, F., Bermano, G., & Testolin, G. (1994). Some nutritional benefits of extra virgin olive oil. *Grasas y Aceites*, *45*(1–2), 48–52.

Driss, F., Duranthon, V., & Viard, V. (1996). Effects biologiques des composés polyphénoliques de l'olivier. *Corps Gras*, *3*(6), 448–451.

Ficarra, P., Ficarra, R., De Pasquale, A., Monforte, M. T., & Calabrò, M. L. (1991). HPLC analysis of oleuropein and some flavonoids in leaf and bud of Olea europaea L. *Il Farmaco*, *46*(6), 803–809.

Keys, A. (1995). Mediterranean diet and public health: personal reflections. *American Journal of Clinical Nutrition*, *61*, 1321–1323.

Le Tutor, B., & Guedon, D. (1992). Antioxidative activities of Olea europaea L. leaves and related phenolic compounds. *Phytochemistry*, *31*(4), 1173–1176.

Manna, C., Galletti, P., Cucciolla, V., Moltedo, O., Leone, A., & Zappia, V. (1997). The protective effect of the olive oil polyphenol (3,4-dihydroxyphenyl)-ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *Journal of Nutrition*, *127*(2), 286–292.

Mensink, R. P., & Katan, M. B. (1992). Effect of dietary fatty acids on serum lipids and lipoproteins. *Arteriosclerosis Thrombosis, and Vascular Biology*, *12*, 911–919.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992). *Journal of Agricultural and Food Chemistry*, *40*(9), 1577–1580.

Navarro, M. D., Periago, J. L., Pita, M. L., & Hortelano, P. (1994). *Lipids*, *29*, 845–848.

Perrin, J. T. (1992). Les composés mineurs et les antioxigènes naturels de l'olive et de son huile. *Corps Gras*, *39*(1/2), 25–32.

Ranalli, A., Modesti, G., Patumi, M., & Fontanazza, G. (2000). The compositional quality and sensory properties of virgin olive oil from a new olive cultivar—I77. *Food Chemistry*, *69*(1), 37–46.

Romani, A., Pinelli, P., Mulinacci, N., Galardi, C., Vincieri, F. F., Liberatore, L., & Cichelli, A. (2001). HPLC and HRGC analyses of polyphenols and secoiridoid in olive oil. *Chromatographia*, *53*(5/6), 279–284.

Romani, A., Pinelli, Mulinacci, Vincieri, F. F., & Tattini, M. (1999). Identification and quantitation of polyphenols in *Myrtus communis* L. Leaves. *Chromatographia*, *49*, 17–20.

Simonsen, N. R., Fernandez-Crehuet Navajas, J., Martin-Moreno, J. M., Strain, J. J., uttunen, J. K., Martin, B. C., Thamm, M., Kardinaal, A. F., van't Veer, P., Kok, F. J., & Kohlmeier, L. (1998). Tissue stores of individual monounsaturated fatty acids and breast cancer: the EURAMIC study. European community multicenter study on antioxidants, myocardial infarction, and breast cancer. *American Journal of Clinical Nutrition*, *68*(1), 134–141.

Visioli, F., Caruso, D., Plasmati, E., Patelli, R., Mulinacci, N., Romani, A., Galli, G., & Galli, C. (2001). Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Research*, *34*, 301–305.

Visioli, F., & Galli, C. (1998). The effect of minor constituents of olive oil on cardiovascular disease: new findings. *Nutrion Reviews*, *56*(5), 142–147.

Visioli, F., Romani, A., Mulinacci, N., Zarini, S., Conte, D., Vincieri, F. F., & Galli, C. (1999). Antioxidant and other biological activities of olive mill waste waters. *Journal of Agricultural and Food Chemistry*, *47*(8), 3397–3401.