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Fatty acids, Sterols and Antioxidant compounds of minor and neglected cultivar of Sicilian virgin olive oils

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Titolo
Acidi grassi, Steroli e composti Antiossidanti in oli di oliva vergini siciliani da varietà minori e neglette

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Acidic composition, olive oil, polar phenols, sterols, tocopherols, total phenols

Parole chiave
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Summary
Acidic and sterol composition, pigment fraction and antioxidant compounds (tocopherols, total phenols and polar phenols) from eight monovarietal Sicilian olive oil samples, analyzed during the crop seasons from 2003/2004 to 2005/2006, are reported. Cultivars were divided, according to their diffusion, in main, minor and neglected cultivars. Encouraging data were observed for minor and neglected cultivars, for some revaluation in regard to their relevance, because they showed the highest content in tocopherols, in total phenols and polar phenols compared with main cultivars.

Riassunto
La presente ricerca è stata volta a determinare la composizione acidica e sterolica, la frazione dei pigmenti ed i composti antiossidanti, presenti in otto campioni di olio di oliva monovarietali provenienti dalla Sicilia. Sono state prese in considerazione le campagne olearie che andavano dal 2003/2004 al 2005/2006. Le cultivar sono state divise in base alla loro diffusione in: principali, minori e neglette. Dati incoraggianti sono emersi riguardo alle cultivar minori e neglette, poiché queste ultime hanno mostrato il contenuto più elevato in tocoferoli, fenoli totali e fenoli polari rispetto alle cultivar principali, fattore di notevole importanza per un eventuale recupero di cultivar potenzialmente in via di estinzione.

Introduction

It is well recognised that the diet plays a very important role in maintaining the human wellness. Many studies emphasise that the "Mediterranean diet", based on cereals, fruits, vegetables, olive oil, etc., could lower the risk for cardiovascular disease and for some kinds of cancer. The beneficial effects of extra virgin olive oils (EVOOs) are linked not only to their fatty acid composition but also to the presence of minor compounds, i.e. polyphenolic substances, which have antioxidant activity.
The demand of virgin olive oil (VOO) is increased thanks to modern dietary guide lines, it helps to assimilate vitamins A, D and K and contains essential fatty acids and antioxidant compounds that slows down the aging process (1-4). Moreover recent studies show that phytosterols apparently help to reduce the total plasma and LDL-cholesterol,

and as a result these compounds are being considered as ingredients of functional foods (5-7).

This paper refers on the quality characteristics of monovarietal VOO from eight cultivars collected in Sicily and grown in the experimental orchard Carboj, in Agrigento province. This study is inserted in a project that aims at the safeguard of the biodiversity of Sicilian patrimony. The diffusion of olive in this area has origin in ancient time and Sicilian native germplasm is notable. Over the centuries many of the varieties became extinct and currently it is tried to recover the remaining autochthonous patrimony. This work wants to contribute to the recovery and characterization of minor and neglected varieties in order to preverse them from the risk of extinction. The olives were produced during the crop season from 2003/04 to 2005/06, and were processed with a identical three phases centrifugal decanter. For the characterization of the oils both the conventional and health parameters were taken into account. Particularly, for this last aspect, percentage acidic composition, total and hydrophilic phenols, tocopherols, phytosterols and pigments were determined and compared between them.

Material and methods

Eight monovarietal Sicilian olive oil samples, were analyzed during the crop seasons from 2003/2004 to 2005/2006. Samples were collected at Campo Carboj, Experimental Camp of Agricoltural and Forest Assessorship of Sicilian Region, situated in Sciacca (Agrigento).

Samples were divided in three different categories: main varieties (Cerasuola, Nocellara del Belice, Tonda Iblea), minor varieties (Calatina, Crastu, Minuta), neglected varieties (Bottone di Gallo, Vaddarica). According to methods of CEE n. 2568/91, fatty acid methyl esters (FAME), were determined. The qualitative and quantitative Sterol contents of the samples were determined according to the European Official Methods Analysis described in Annexes V and VI of Regulation EEC/2568/91 of the European Union Commission (8). Separation and quantification of the silanized sterol fraction was carried out by capillary column gas chromatography, on a CE-Instruments GC-8000 Top chromatograph equipped with a SAT-5, Supelco, column, 30 m lenght. The working conditions of the chromatograph were: injector 300°C, isothermal column 266°C, and detector 300°C. The injected quantity was 1 μL at a flow rate 1 mL/min, using helium as carrier gas. Sterols peak identification was carried out according to the reference method. Quantification was achieved by addition of an internal standard (α-colestanol). The chlorophyll fraction at 670 nm and the carotenoid fraction at 476 nm were

Table 1 - Tocopherols, total phenols and pigment content

CULTIVAR	*Tocopherols (α-Tocoferol mg/kg)*		*Total phenols (GAE mg/L)*		*Clorophyll (Clorophyll "a" mg/kg)*		*Carotenoids (β-carotene mg/kg)*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Bottone di Gallo	503	175	528	90	5,53	4,94	5,72	4,28
Calatina	778	185	434	122	14,15	11,19	15,79	10,72
Cerasuola	275	69	268	160	7,3	6,41	5,17	1,67
Crastu	358	91	206	120	4,35	3,64	3,24	0,76
Minuta	276	54	352	26	8,66	8,7	10,56	9,81
Nocellara del Belice	269	69	253	153	7,17	7,82	4,97	1,82
Tonda Iblea	240	23	145	79	3,89	2,57	5,66	4,19
Vaddarica	499	131	653	108	5,3	0,57	3,64	1,15

evaluated from the absorption spectrum of each virgin olive oil sample (5 g) dissolved in hexane (25 mL). The chlorophyll and carotenoid contents are expressed as mg of chlorophyll "a" and β-carotene for kg of oil, respectively. Whereas Total phenols, was determined according to Solinas et al. (9), and Polar Phenols according to Fogliano et al. (10). Analysis conditions for the latter were as follows: Varian column S5 ODS-3 (250x4.6 mm); T 25°C; λ 279 nm; flow rate 1 mL/min; volume injected 20 μL. Tyrosol (Fluka), was used as external standards for quantitative analysis. Finally Tocopherols content was determined according to Rovellini et al. (11). Analysis conditions were as follows: Merck column ODS-2 (100x4.6 mm); T 25°C; λ 292 nm; flow rate 1 mL/min; volume injected 20 μL. α-Tocopherol (Fluka), was used as external standards for quantitative analysis. Instruments used were: spectrophotomer Beckman DU 620 for total phenols, clorophyll and carotenoid fraction, gas-chromatograph Perkin-Elmer AutoSystem XL equipped with FID detector for FAME, HPLC Perkin-Elmer Series 200 equipped with diode array detector for Polar phenols and HPLC Thermo-Electron Finnigan Surveyor equipped whit PDA for Tocopherols.

Results and discussion

For acidic composition was verified a wide variability for oleic acid, ranging from 64.17% for cv Tonda Iblea to 79.53% for cv Calatina. About Lignoceric acid (C 24:0) was verified amount not measurable, moreover evaluating oleic/linoleic ratio were come out remarkable differences among samples, ranging from 5.75 for cv Minuta to 23.13 for cv. Calatina.

About linolenic acid four samples have exceeded the legal limit of 0.9%. This is unfortunately an ascertained problem for Sicilian cultivars. Acidic composition's data were reported in table 3.

For sterol composition, among varieties considered, Calatina and Vaddarica varieties showed the highest content in β-sitosterol, keeping costantly mean value above 91%.

Some samples of minor cultivars didn't reach the minimum limit of 1000 mg/kg for total sterols content estabilished by current EU legislation (Regulation 2568/91/EEC and later amendments); Crastu in 2003 & 2005 and Minuta in 2004 & 2005. The lowest total sterol content in these varieties, probably is a characteristic of the variety that makes it no marketable

Table 2 - Polar phenols distribution.

	OHTy		*Ty*		*OHTy-EDA*		*Ty-EDA*		*AP*		*OHTy-EA*		*Ty-EA*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Bottone di gallo	9,05	4,41	17,92	11,67	64,70	30,04	194,46	101,41	17,18	0,50	27,74	17,18	28,43	28,99
Calatina	7,36	8,35	3,63	2,88	91,59	51,81	46,27	18,08	2,78	1,50	34,10	23,69	10,18	5,01
Cerasuola	16,25	18,84	20,33	26,36	96,32	74,98	121,69	78,91	27,97	7,54	26,55	24,79	12,58	14,26
Crastu	5,33	6,49	7,17	6,81	21,00	23,93	48,38	44,87	15,10	7,27	10,33	12,54	6,75	3,18
Minuta	4,71	2,53	4,34	1,09	63,03	2,97	71,43	31,13	28,16	5,98	40,11	22,31	18,21	13,17
Nocellara del Belice	7,07	7,01	7,96	7,11	50,07	66,62	37,14	22,52	14,79	3,33	5,01	2,05	2,21	0,53
Tonda Iblea	2,66	3,36	11,50	14,00	2,67	1,29	23,45	11,23	2,69	1,61	3,49	1,22	3,78	1,05
Vaddarica	24,94	23,04	33,55	32,23	107,43	47,29	198,54	42,95	27,78	13,44	84,75	44,67	55,55	20,79

Legend

(OHTy = idroxityrosol; Ty = tyrosol; OHTy-EDA = dialdeidic form of elenolic acid linked to OHTy; Ty-EDA = dialdeidic form of elenolic acid linked to Ty; AP = (+)-1-Acetoxypinoresinol; OHTy-EA = Oleuropein aglycon; Ty-EA = Ligustroside aglycon.)

Table 3 - Fatty acid composition, percentage distribution.

	C 16:0		*C 16:1*		*C 18:0*		*C 18:1*		*C 18:2*		*C 20:0*		*C 20:1*		*C 18:3*		*C 22:0*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Bottone di gallo	17,26	2,92	2,46	0,89	1,62	0,29	68,91	2,67	7,07	1,20	0,28	0,15	0,23	0,08	1,02	0,14	0,10	0,03
Calatina	10,45	0,92	0,47	0,14	2,78	0,66	79,53	1,51	4,62	1,00	0,39	0,12	0,27	0,01	1,00	0,11	0,10	0,01
Cerasuola	9,97	1,02	0,32	0,10	2,33	0,48	75,73	1,83	9,82	0,80	0,38	0,13	0,40	0,02	0,64	0,04	0,10	0,03
Crastu	13,49	1,31	1,48	0,43	1,99	0,49	75,16	1,96	5,71	0,96	0,36	0,15	0,28	0,06	0,83	0,16	0,14	0,04
Minuta	15,59	1,29	1,62	0,60	2,01	0,27	66,36	2,90	12,30	0,82	0,36	0,20	0,24	0,05	0,85	0,31	0,13	0,04
Nocellara del Belice	13,27	1,99	0,98	0,11	2,42	0,44	71,32	5,47	9,88	4,45	0,34	0,21	0,20	0,14	0,81	0,19	0,11	0,03
Tonda Iblea	16,10	0,91	1,59	0,80	1,86	0,10	64,17	5,14	13,81	3,72	0,50	0,12	0,16	0,14	1,10	0,09	0,12	0,01
Vaddarica	15,77	0,99	2,14	0,17	2,03	0,15	65,76	2,20	12,11	1,69	0,31	0,11	0,24	0,01	0,72	0,07	0,11	0,01

Table 4 - Phytosterols composition, percentage distribution.

Variety →	*Bottone di gallo*		*Calatina*		*Cerasuola*		*Crastu*		*Minuta*		*Nocellara del Belice*		*Tonda iblea*		*Vaddarica*	
Sterols ↓	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Cholesterol	0,11	0,06	0,09	0,08	0,19	0,01	0,28	0,2	0,17	0,09	0,15	0,06	0,08	0,04	0,12	0,07
Brassicasterol	0,08	0,02	0,03	0,05	0,08	0,03	0,15	0,07	0,12	0,03	0,15	0,1	0,05	0,01	0,07	0,02
24 Methylencolesterol	0,02	0,02	0,02	0,02	0,12	0,04	0,1	0,09	0	0	0,18	0,06	0,05	0,02	0,01	0,01
Campesterol	2,72	0,33	2,75	0,04	2,84	0,03	3,27	0,55	3,1	0,04	3,11	0,12	3,38	0,45	2,07	0,07
Campestanol	0,49	0,06	0,47	0,07	0,56	0,05	0,84	0,37	0,79	0,23	0,71	0,19	0,3	0,07	0,61	0,12
Stigmasterol	0,72	0,13	0,57	0,16	0,57	0,13	1,07	0,34	1,06	0,25	1,06	0,33	1,07	0,41	0,89	0,26
Clerosterol	0,68	0,03	0,64	0,16	0,75	0,11	0,68	0,19	0,68	0,01	0,74	0,04	0,48	0,21	0,72	0,14
β-Sitosterol	89,39	2,76	91,97	1,07	85,78	0,64	86,42	4,69	88,81	1,47	85,55	1,52	89,3	1,05	91,86	0,32
Sitostanolo	0,48	0,08	0,33	0,29	0,32	0,33	0,46	0,41	0,46	0,11	0,47	0,21	0	0	0,42	0,11
D5 Avenasterol	4,11	2,84	2,1	0,71	7,31	0,88	5,3	2,24	3,03	0,93	6,65	1,21	4,49	0,7	2,46	0,19
D5,24 Stigmastadienol	0,63	0,16	0,46	0,04	0,72	0,12	0,66	0,22	0,94	0,28	0,71	0,15	0,5	0,12	0,34	0,01
D7 Stigmastenol	0,24	0,13	0,27	0,12	0,2	0,01	0,25	0,14	0,4	0,11	0,15	0,04	0,13	0,02	0,14	0,11
D7 Avenasterol	0,35	0,05	0,3	0,05	0,57	0,11	0,53	0,2	0,45	0,08	0,38	0,1	0,43	0,1	0,31	0,03
Uvaol + Erythrodiol	0,92	0,54	1,12	0,47	0,74	0,3	2,31	1,24	1,08	0,72	1,5	0,24	0,86	0,29	0,79	0,33
Total Sterols mg/kg	1359	201	1355	292	1086	66	892	518	882	287	912	232	2060	503	1079	209

like monovarietal oil. Sterol composition are reported in table 4.
Data showed a large variability in samples for total phenols content, ranging from 145 mg/kg for cv Tonda iblea to 653 mg/kg for cv Vaddarica. Same state showed tocopherols content, ranging from 240 mg/kg for cv Tonda Iblea to 778 mg/kg for cv Calatina.
Moreover for all period considered, minor and neglected cultivars showed the highest content in tocopherols (considerable the content of cv Calatina, 965 mg/kg in 2003) and total phenols (777 mg/kg for cv Vaddarica in 2005 and 598 for cv Bottone di gallo in 2005) compared with main cultivars. For pigment's fraction cvs Calatina and Minuta, minor varieties, showed the highest content in clorophylls and carotenoids. In table 1 are reported mean values of tocopherols and total phenols content of single samples.
For polar phenols, neglected varieties (Bottone di gallo and Vaddarica) showed, for all period considered, the highest content. In particular these cultivars have shown the highest content in 2003, with Ty-EDA (dialdeidic form of elenolic acid linked to Tyrosol) content for Bottone di gallo of 292 mg/kg and for Vaddarica of 202 mg/kg. Among main cultivars, only Cerasuola variety showed similar content whit Ty-EDA in 2003 of 187 mg/kg.
Ty-EDA is ligstroside derivate and has healt properties because its anti-infiammatory activity is notable (Oleocanthal), similar to that of ibuprofen (12).
Is important yet underline that hydrophilic phenols reported in table 2 showed high variableness as appeared from high value of standard deviation. That due to, different climatic conditions occurred during 3 year of research, forced harvesting in different ripening stages.
In conclusion, minor and neglected cultivars are little known and threatened with extinction, but this research showed interesting data, for some revaluation in regard to their relevance because they showed highest content in "nutraceutical" compounds compared with main cultivars. Infact tocopherols, phenols and sterols are natural antioxidant that prevent the formation of free radicals and decrease the concentration of total and LDL cholesterol in human body.

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