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Evaluation of Five Olive Cultivars by Morpho-agronomic, Bio-molecular and Sensory Markers for Nutraceutical Purposes

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Abstract

In the last few years, there has been an increasing interest towards the "nutraceutical" content of typical Mediterranean foods, particularly of extra virgin olive oil, which is rich in monounsaturated fatty acids, antioxidants and polyphenols. In the present work 5 olive cultivars traditionally cultivated in Basilicata region (Southern Italy) have been studied so as to identify the autochthonous cultivars producing oils with the best chemical composition. The analysis was performed on morpho-agronomic, biochemical and sensory properties of oils, followed by genetic screening of 6 SSR locus markers on DNA extracted from leaves. Results showed significant differences within 5 olive cultivars in terms of leaf surface, drupe mean weight and oil yield. Biochemical analyses have shown differences in fatty acid composition of oleic, palmitic, stearic, palmitoleic and linoleic acids. Significant differences have also been found in the content of total phenols, sterols, total fatty acids and in the unsaturated/saturated fatty acid ratios. Sensory profiles have provided valuable information about the organoleptic characteristics of each monovarietal oil. SSR locus markers were able to discriminate among the tested cultivars. The results of this investigation have demonstrated that the cvs *Majatica di Ferrandina* and *Racioppa* have the highest potential for producing high quality extra virgin olive oil. © 2015 Friends Science Publishers

Keywords: Olive cultivars; Extra virgin olive oil; Morphological traits; Bio-molecular markers

Introduction

In recent years poor diet and pollution have led to the dramatic increase in degenerative diseases with metabolic and cardiovascular complications. This has induced to explore the potential benefits of more suitable diets and alternative drug therapies. In this context there has been increasing attention to the "nutraceutical" content of a healthy Mediterranean diet. A fundamental role in the Mediterranean diet is played by olive oil and the health benefits of its free acidity, peroxide value, sterol and phenol content, and fatty acid structure and composition (Medeiros, 2001; Harwood and Yaqoob, 2002; Visioli *et al*., 2002).

The nutraceutical activity of extra virgin olive oil is expressed through the reduction of LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein) factors that leave deposits of "bad cholesterol" on the artery walls. At the same time olive oil enhances HDL (High Density Lipoprotein), or "good cholesterol" that removes deposits from the artery walls. The beneficial action of extra virgin olive oil is due to the high ratio of unsaturated/saturated fatty acids, the high content of oleic acid (monounsaturated) and the significant concentrations of polyunsaturated fatty acids such as linoleic and linolenic acids. Linoleic and linolenic acids, which are important for growth, are essential fatty acids that cannot be synthesized by the human body. It is well known that the saturated fatty acids contained in food, including palmitic (C16:0), myristic (C14:0) and lauric (C12:0) acids increase serum cholesterol. Palmitic acid is the most dangerous: its average concentration in olive oil is around 12% but it may increase up to 19%. Stearic acid (C18:0) does not exceed 7% and, even if saturated, it is less atherogenic because the body desaturates it quickly and turns it into oleic acid (Pannelli, 2006; Alba *et al.,* 2012). On the basis of biochemical and sensory parameters, three categories have been established by the EC for olive oil: extra virgin, virgin and lamp oils (EC, 2003). Extra virgin oil is obtained from olive fruit by mechanical or other physical means in thermal conditions that do not alter oil in any way.

Olive oil, which does not undergo any treatment other than washing, decanting, centrifuging, and filtering, has a free acidity, expressed as oleic acid, of not more than 0.8%. The quality of olive oil can be improved through the identification of superior cultivars within the available national germplasm. In Italy olive cvs are difficult to identify, since they are often wrongly categorised or categorised under similar names (La Mantia *et al*., 2004; Alba *et al*., 2009b). As reported by Council Regulation (EEC) No. 2081/92 (Pasqualone *et al*., 2007), correct

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identification of cvs and derived extra virgin oils with specific organoleptic and sensory characteristics is essential for obtaining the Protected Designation of Origin (PDO). PDO extra virgin oil must be obtained from a specific cultivar or combined with other cultivars in well-defined. To identify the quality of olive oils obtained from different cvs some authors suggest the use of chemical parameters, such as volatile compounds, total phenols, fatty acids and sensory profiles (Allouche *et al*., 2009; Ouni *et al*., 2011).

Traceability of raw and processed materials plays a crucial role in product certification for consumer protection and fraud prevention. The importance of correct identification of cvs can be a useful tool for agriculturalists/ farmers that require product certification. In the last decade different classes of molecular markers (RFLP, RAPDs, AFLPs and ISSRs) have been successfully used to achieve these goals. The aim of this research is to characterize and evaluate monovarietal extra virgin oils obtained from five autochthous cvs cultivated in the Basilicata region (southern Italy) by morpho-agronomic, bio-molecular and sensory markers for nutraceutical value.

Materials and Methods

The survey involved five cultivars traditionally cultivated in Basilicata and particularly widespread in the olive growing areas already identified by Rotundo and Marone (2002): the cvs *Nociara*, *Augellina* and *Maiatica di Ferrandina* in *Colline Materane* and *Medio Agri-Basento* areas; the cvs *Ogliarola del Vulture* and *Racioppa* in *Vulture* area. Five productive and homogeneous samples were labeled at the same stage of growth in each area and for each cultivar. 100 randomly chosen drupes were collected prior to harvest time. 200 leaves from the middle of yielding branches were then measured using a portable area-meter (Lambda Instruments Corporation). For each sample, drupes and their endocarps were weighed before shattering to obtain a homogenous olive paste used for chemical measurement. Lipid content was measured by the standard AOAC (1995) method (Soxhlet extraction by Petroleum Ether 40-60); fatty acids were measured using the methods described in Regulation (EEC) No. 2568/91 (EC, 1991). Total Polyphenols (expressed in mg equivalent of caffeic acid/kg of olive oil) were quantified at 720 nm utilizing the Folin and Ciocalteau colorimetric method. Polyphenolic fractions were separated and analyzed using HPLC according to Montedoro *et al.* (1992); single fractions were eluted from 5 g of olive oil using water/acetonitrile (methyl cyanide) for separation. Following the previous separation method, sterols were measured using HPLC. The mean values of morphological, biochemical and sensory data were computed for the three-year observation period (2008-2011), while molecular analyses were performed only in the third year. Sensory analysis for each oil sample was carried out by a panel test described in Regulation (EEC) No. 2568/91 and backed up by analysis of further attributes available in the form of *Premio Muntiferru* (Oristano 2000). For molecular characterization DNA genome was extracted from fresh leaves using a Gene Elute Plant Kit (Sigma, St. Louis, MO) comprising six pairs of primer microsatellites available from the current literature, and labeled with fluorochromes FAM and HEX (Sefc *et al*., 2000). Amplification reactions were performed in an I-Cycler programmable thermal cycler (Bio-Rad Laboratories, Hercules, CA) using the following composition: 50 ng of DNA, 1 × PCR buffer (10 mM Tris-HCl pH 8.3, 10 mM KCl), 2.4 μM $MgCl_2$, 2 μM dNTP, 2.5 μM primer forward and reverse each, and 1 U of Taq DNA polymerase (Sigma, St. Louis, MO), in a volume of 25 μL. Amplification conditions were as follows: 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at appropriate annealing temperature and 30 s at 72°C; and with final elongation at 72°C for 60 min. Amplification products were first denatured at 94°C for 5 min and then separated by capillary electrophoresis on an ABI PRISM® 3100 Avant Genetic Analyzer (Applied Biosystems). DNA polymorphic alleles were used to create a rectangular binary matrix, where bands were scored as present or absent at a certain molecular weight. A similarity matrix, based on the Dice Coefficient (Dice, 1945) was obtained and subjected to UPGMA clustering analysis to create a dendrogram of genetic similarity, as implemented in NTSYS V2.0 software (Rohlf, 1998).

Results

Mean values and standard deviation of morpho-agronomic and biochemical descriptors are reported in Table 1, where cv *Maiatica* drupes and endocarps were higher in weight than average. The highest oil yield was recorded in cv *Maiatica*, and the lowest in cv *Augellina*. The latter is characterized by greater leaf surface (8.38 cm^2). Oleic acid content varied between 69.4% and 74.5% in cvs *Racioppa* and *Maiatica*, respectively. The highest linoleic acid value (10.6%) was found in cv *Racioppa* and the lowest in cv *Nociara* (6.53%). Sharp differences were also observed for saturated fatty acid content. Cv *Ogliarola del Vulture* showed the highest values for stearic and arachic acids: 2.97% and 0.45%, respectively. Palmitic acid was highest in cv *Augellina* and lowest in cv *Maiatica*, while cv *Racioppa* showed the lowest arachic acid content (0.03%). Cvs *Racioppa* and *Maiatica* showed the lowest (4.70) and the highest (5.60) ratio, respectively. The highest levels in total phenols were found in cvs *Racioppa* and *Nociara*, and the lowest in cv *Maiatica.* Total sterols showed a marked variability, ranging from 7176 mg/kg in cv *Racioppa* to 1537 mg/kg in cv *Ogliarola*.

Sensory profiles for the oils produced from the 5 cultivars are reported in Fig. 1. *Augellina* and *Nociara* cvs showed similar profiles characterized by medium-light fruitiness, a pleasant pungency and a strong and persistent hotness. *Maiatica, Ogliarola* and *Racioppa* cvs showed comparable sensory profiles characterized by slight

Table 1: Mean values and S.D. of quantitative descriptors observed in 5 olive cultivars from Basilicata region: *Augellina*, *Maiatica di Ferrandina*, *Nociara, Ogliarola del Vulture* and *Racioppa*

Cultivar	Augellina	Maiatica di Ferrandina	Nociara	Ogliarola del Vulture	Racioppa
Fatty acids (%)					
Palmitic acid	15.2±0.61	11.9±0.46	14.1±1.12	13.0±1.27	14.7±1.40
Palmitoleic acid	1.25±0.06	0.93±0.17	1.15±0.05	0.76±0.26	1.26±0.05
Stearic acid	1.46±0.04	2.63±0.39	2.10±0.30	2.97±0.65	2.34±0.08
Oleic acid	71.7±0.69	74.5±1.09	74.3±0.07	72.9±3.79	69.4±1.21
Linoleic acid	8.79±0.70	8.30±0.70	6.53±1.01	8.70±2.08	10.6±0.27
Linolenic acid	0.81±0.08	0.66±0.09	0.84±0.13	0.64±0.08	0.73±0.07
Arachic acid	0.24±0.04	0.44±0.10	0.25±0.01	0.45±0.08	0.03±0.03
Fatty acid ratio (n)					
Oleic/Palmitic	4.73±0.21	6.27±0.33	5.28±0.06	5.66±0.79	4.72±0.10
Oleic/Palmitoleic	57.3±2.38	80.3±13.8	64.9±1.37	103.8±36.78	55.2±2.43
Linoleic/Palmitoleic	7.05±0.83	9.11±1.27	5.70±1.13	11.68±6.77	8.40±1.21
Insatured/Satured Fatty ac.	4.88±0.18	5.60±0.30	5.02±0.29	5.06±0.54	4.70±0.85
Phenols (mg/1.000 g oil)					
Hydroxy-Tyrosol	0.15±0.15	0.07±0.02	0.23±0.27	0.08±0.04	0.04±0.01
Tyrosol	1.61±0.24	0.72±0.47	0.94±0.36	1.34±0.32	0.62±0.09
Dialdehydic form of Elenolic ac. linked to Hydroxy-Tyrosol (OHTyEDA)	76.3±9.53	26.1±9.54	99.9±37.2	72.9±43.57	81.0±1.99
Dialdehydic form of Elenolic ac. linked to Tyrosol (TyEDA)	7.66±1.69	2.09±0.50	17.3±9.38	9.19±8.37	8.22±0.88
Oleuropeine-Aglicon Isomer (3,4-DHPEA-EA)	9.9±1.33	10.4±10.8	29.4±7.05	12.9±3.48	14.1±1.99
Total Phenols (mg Caffeic acid/1.000 g oil)	147.9±26.8	121.3±16.4	321.7±108.4	228.8±92.86	326.2±116.0
Sterols (%)					
Cholesterol	0.16±0.23	0.48±0.13	0.48±0.35	0.47±0.35	0.09±0.06
Campesterol	1.91±0.45	1.75±0.46	2.25±0.49	2.34±0.47	1.66±0.38
Stigmasterol	0.29±0.33	0.63±0.31	0.53±0.09	0.78±0.19	0.61±0.25
Clerosterol	0.49±0.29	0.93±0.42	0.78±0.31	0.73±0.23	0.69±0.33
β-Sitosterol	76.7±1.83	57.5±1.68	67.2±2.29	64.9±2.61	73.6±5.12
Δ-5-Avenasterol	15.7±1.23	32.5±0.91	20.8±1.05	25.8±0.85	18.9±2.34
Δ-5,24-Stigmastadienol	0.26±0.12	1.3±0.15	1.01±0.31	0.33±0.06	0.38±0.39
Δ-7-Stigmasterol	0.26±0.11	0.43±0.18	0.40±0.57	0.41±0.36	0.30±0.56
Δ-7-Avenasterol	0.26±0.09	0.95±0.36	0.77±0.38	0.63±0.41	0.20±0.12
Total β-sitosterol	96.7±1.99	94.7±3.11	94.2±3.21	94.7±4.21	96.7±3.94
β-sitosterol/Δ-5-Avenasterol	4.88±0.19	1.77±0.61	3.23±0.51	2.51±0.45	3.88±0.41
Total Sterols (mg/1.000 g oil)	2012±101	2470±257	1898±198	1537±149	7176±242
Morpho-agronomic traits					
Leaf surface (cm²)	8.38±0.47	5.26±0.46	7.82±1.28	5.96±1.39	7.12±1.31
Drupe weight (g)	2.55±1.37	3.84±2.15	3.24±0.78	2.56±0.23	2.28±0.65
Endocarp weight (g)	0.49±0.12	0.55±0.19	0.49±0.21	0.43±0.65	0.41±0.45
Oil yield (% fresh weight)	18.5	22.5	20.5	22.4	21.4

fruitiness, sweet taste, pleasant pungency and bitterness, and a slightly spicy flavor.

Table 2 and Fig. 3 show polymorphic and reproducible electrophoretic profiles for the five olive cvs from 6 SSR locus markers. 3 locus markers (DCA13, DCA17, DCA18) were discriminant for all cvs, while the other 3 locus markers were monomorphic for 2 out of 5 cvs. DCA03 was unable to discriminate between cvs *Nociara* and *Racioppa*; DCA04 was monomorphic between cvs *Racioppa* and *Ogliarola* and DCA07 was monomorphic between cvs *Racioppa* and *Maiatica. Maiatica* was genotypically discriminated from all other cvs by 5/6 SSR locus markers. The most revealing locus markers were DCA13 and DCA17 because both detected as many as 6 different alleles. The lowest mean allelic frequency was given for DCA17, which tends to confirm its genotypic diversity. For DCA17, 4 out of 5 cvs were, as expected in such a predominantly allogamous plant as olive, homozygotes. Other locus markers were mainly heterozygotes with DCA03 heterozygotic in all 5 cvs. Fig. 2 reports a dendrogram of similarity based on Dice Coefficient, in which the huge genetic distance among the olive cvs is evident. In particular, two main clusters formed at 0.25, the first comprising *Nociara*, *Maiatica* and *Racioppa*, while the second was represented by *Ogliarola* and *Augellina*. Moreover, from a genetic point of view based on SSR analysis, the closest cultivars resulted *Maiatica* and *Racioppa* (0.60 Dice Coefficient).

Discussion

As for the morphological parameters, the weight values observed in cv *Maiatica* drupes and endocarps could explain why the oil yield obtained from this cultivar was the highest.

Fatty acid composition of olive oil from the five cultivars showed mean values that meet the requirements of the European Regulation (EC, 1991) for extra virgin olive

Table 2: Simple sequence repeats (SSRs) and size range of 6 SSR markers on the 5 olive cultivars investigated

SSR marker	Cultivars									
	Nociara		Maiatica F.		Racioppa V.		Ogliarola V.		Augellina	
DCA03	239	253	243	253	239	253	232	243	232	239
DCA04	130	152	132	190	130	132	130	132	132	132
DCA07	149	167	151	171	151	171	149	149	129	151
DCA13	120	124	120	122	120	157	120	120	110	113
DCA17	129	179	113	113	115	115	143	143	183	183
DCA18	169	173	173	177	177	177	171	171	171	195

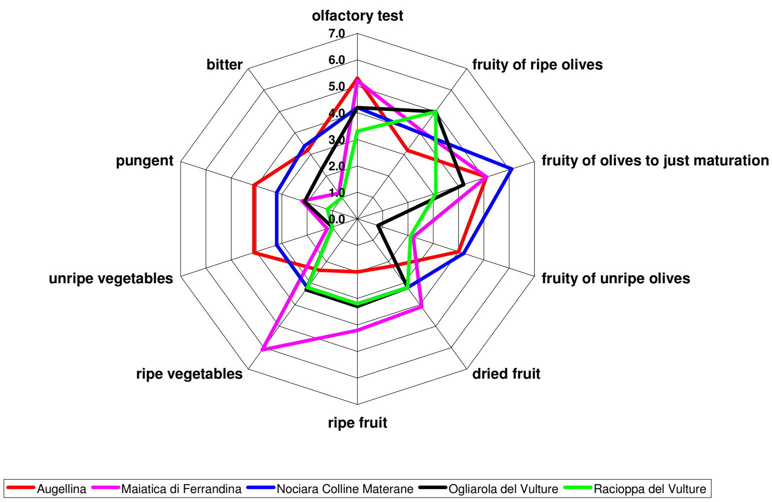

Fig. 1: Sensory profiles of 5 olive cultivars from Basilicata region: *Augellina*, *Maiatica di Ferrandina*, *Nociara*, *Ogliarola del Vulture* and *Racioppa*

oil; significant differences among the five cultivars were observed. The oleic acid content of the *Maiatica di Ferrandina* and *Nociara* oils was similar and higher than other cvs oils. Concerning the composition of saturated fatty acid, it is well known that palmitic acid is the main saturated acid contained in olive oil. The content of this fatty acid in the *Augellina*, *Racioppa* and *Nociara* oils was significantly higher than in the *Maiatica* and *Ogliarola* oils. However, *Racioppa* oil showed the highest linoleic acid content. The unsaturated oleic and linoleic acids are of great importance from a nutritional point of view and essential for oxidative stability (Sánchez-Casas *et al*., 2003). Higher contents of unsaturated fatty acids increase oil quality by modifying the unsaturated/saturated acids ratio, as found in the cv. *Maiatica di Ferrandina*. Similar results on Spanish varieties are reported by Benito *et al*. (2010).

Phenolic composition, which characterizes pungency and bitterness in sensory profiles and is essential for European PDO certification, showed substantial differences between cvs. *Racioppa* and *Nociara* with a very high content, if compared to the other three cultivars; for this reason these oils must be very stable to oxidation. Their antioxidant activity reduces peroxide formation and promotes oil conservation. On the other hand, these chemicals are considered as being powerful antioxidants, they "fight" free radicals in the human body, prevent cancers and other diseases, reduce "bad cholesterol" (LDL) and reduce the risk of heart attack (Visioli *et al.*, 2005).

With regard to the total sterols content the *Racioppa* olive oil had a significantly different value, characterized by a balanced composition. To this end, it is important to underline that sterols, like all phytosterols, tend to limit (30-40%) intestinal absorption of cholesterol. Moreover, β-sitosterol and Δ-5-avenasterol influence drupe ripeness during fruit ripening, while stigmasterol interferes with absorption of carotenoids and other fat-soluble vitamins, such as vitamin E and tocopherols (Ostlund *et al*., 2003).

Negative sensory attributes were not detected for any of the oils analyzed, confirming the category of extra virgin olive oils as stated by Regulation EEC/796/2002 (CE, 2002). The sensory profiles of the oils are quite different; two groups, *Augellina* and *Nociara,* with a similar but less balanced profile were identified; *Racioppa*, *Maiatica* and *Ogliarola* with overlapping oil profiles were characterized by sensory data that are highly desirable for consumption.

SSRs are nowadays preferred for variability studies, germplasm characterization and varietal fingerprinting and identification in olive (Alba *et al*., 2009a). They are transferable, hypervariable, highly polymorphic, multiallelic polymerase chain reaction (PCR)-based co-dominant

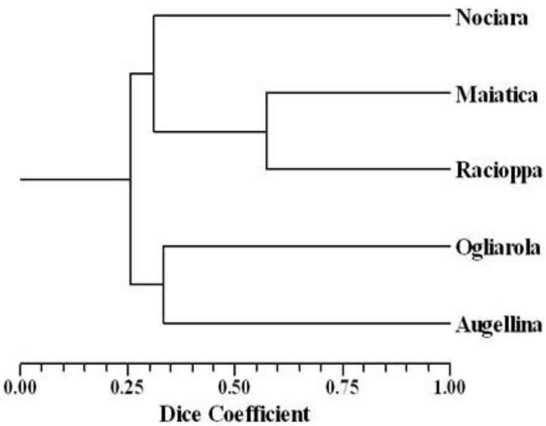

Fig. 2: Dendrogram of genetic similarity based on Dice Coefficient among five olive cultivars from Basilicata region, using the UPGMA clustering obtained with 6 microsatellite loci

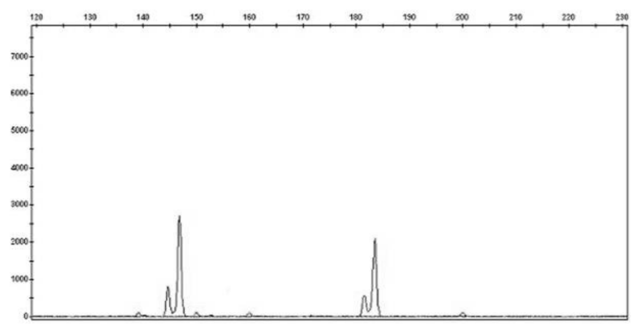

Fig. 3: Electrophoregram showing polymorphic homozygous profiles at locus DCA17 for *Ogliarola* (left peak) and *Augellina* (right peak)

markers, relatively simple to interpret, show a high information content (Ganino *et al*., 2006; 2007). Although for most cvs SSRs assume single locus patterns, in some cases they can amplify non-specific multi-locus patterns. While this might be due to multi loci anchoring of SSR primers along the genome, Cipriani *et al*. (2002) have detected high frequency for two or more amplified loci, which suggests the possibility of a duplicate genome in some olive cvs. Based on current research trends, this could be due to genome fusion or chromosome duplication in species evolution, a phenomenon common to allopoliploide plants (Poljuha *et al*., 2008).

Conclusion

Results suggest that chemical and organoleptic characteristics of cvs *Maiatica* and *Racioppa* are optimal for their production and commercialization as high quality extra virgin monovarietal oils. Apart from the favorable sterol composition and medium-low content in palmitic acid, the high total phenol content in *Racioppa* ensures a good shelf life of oil. A positive sensory profile was detected in *Maiatica* confirming both fruity and slightly spicy oil that makes it a favourite condiment. The SSR markers in association with morpho-agronomic, biochemical and sensory traits were able to discriminate among oil samples and can be considered as a useful tool to characterize monovarietal virgin olive oils.

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