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# Effectiveness of Microsatellite DNA Markers in Checking the Identity of Protected Designation of Origin Extra Virgin Olive Oil

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Collina di Brindisi is an Italian extra virgin olive oil that obtained the mark of protected designation of origin (PDO) according to EC Regulation 2081/92. The varietal requirements of the official production protocol of this oil foresee that this oil is prepared from cultivar Ogliarola (minimum 70%) and other *Olea europaea* L. cultivars that are diffused in the production area, accounting for a maximum of 30%. The aim of this work was to verify the effectiveness of microsatellite analysis in verifying the identity of Collina di Brindisi PDO olive oil. A preliminary assessment of product's quality by means of chemical analyses was also carried out. Microsatellite analysis clarified that the generic name Ogliarola, indicated in the technical sheet of this PDO oil, actually corresponded to the Ogliarola salentina cultivar. Furthermore, the obtained results showed that the examination of a limited number of DNA microsatellites enables the identification of the Ogliarola salentina cultivar in this PDO oil.

**KEYWORDS: Extra virgin olive oil; microsatellites; PDO**

## INTRODUCTION

Apulia is the Italian region with the highest olive oil production (*1*). Part of this production is represented by oils that, because of their typicality, have obtained marks of protected designation of origin (PDO) at a European level according to EC Regulation 2081/92. To gain the PDO quality mark, food products should derive from few local cultivars (*2*). The concept of "typicality", in fact, implies excellent and peculiar sensory features related to environmental factors present in a well-defined and restricted geographic area where the production takes place. Therefore, the PDO products possess a higher economic value as compared to non-PDO foodstuffs. Consequently, to guarantee the consumers about a product's authenticity, it is important to avoid possible mixtures or substitutions of raw materials and to set up effective methods to enable cultivar checks during processing.

Molecular markers allow the detection of DNA polymorphisms, and they make it possible to effectively distinguish different cultivars in an effective way, without any environmental influence. Microsatellites, or simple sequence repeats (SSR), are characterized by a high polymorphism level, due to variations of the number of repeats. They are easily obtained by amplification and subsequent electrophoresis on agarose or polyacrylamide gels. Their high polymorphism level has been exploited to differentiate cultivars and to analyze genetic diversity in various edible species, such as wheat (*3*−*5*), rice (*6*), grape (*7*), tomato (*8*, *9*), oilseed rape (*10*, *11*), and olive (*Olea europaea* L.) (*12*−*16*), but mainly as an aim to identify plant material.

Up to now, some applications of molecular markers have been developed for extra virgin olive oil traceability by direct analysis of the end product, mainly by using amplified fragment length polymorphism markers (AFLP) (*17*, *18*) or random amplified polymorphic DNA (RAPD) (*17*, *19*). As compared to AFLP and RAPD markers, microsatellites usually have a better specificity and reliability (*20*) and are very useful for possible forensic applications. Thus, these markers have been used successfully to detect alien alleles in olive oils from single cultivars (*21*−*23*). The combined analysis of various microsatellite electrophoretic profiles and multiplex polymerase chain reaction (PCR) enabled the elaboration of an identification key of the monovarietal oils from the most diffused Italian cultivars (*24*, *25*). No studies have been carried out, however, to evaluate the efficiency of microsatellites in the case of composite oils derived from various cultivars.

Collina di Brindisi PDO olive oil, one of the Apulian typical oils, is prepared from Ogliarola (minimum 70%), Cellina di Nardò, Coratina, Frantoio, Leccino, Picholine, and other unspecified *O. europaea* cultivars that are diffused in the production area (such as Cima di Melfi and Nociara), alone or together, accounting for a maximum of 30%, with no other indications of their single ratios (*26*, *27*). Among these cultivars, Ogliarola

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**Table 1.** Characteristics of the Microsatellites Analyzed in the Investigation and Electrophoretic Data of the Patterns Detected in the Collina di Brindisi PDO Oils and in Ogliarola Salentina Monovarietal Oils

| microsatellite | primer sequence (forward and reverse, 5′ to 3′) | annealing temp (°C) | band size (base pairs) |
|---|---|---|---|
| UDO99-009 | TTGATTTCACATTGCTGACCA<br>CATAGGGAAGAGCTGCAAGG | 45 | 90, 110 |
| UDO99-019 | TCCCTTGTAGCCTCGTCTTG<br>GGCCTGATCATCGATACCTC | 55 | 80, 180 |
| UDO99-025 | AACATGCCGTTGCATTTTTA<br>GGCATCAATCTACTTCCCACA | 55 | 158 |
| UDO99-035 | AATTTAATGGTCACACACAC<br>ATTGCGAAATAGATCTACGA | 50 | 80, 180 |
| UDO99-044 | AATTCCGACAAGTTGTGTGTG<br>CACAGCACCCAACCAGATTT | 50 | 90 |
| GAPU89 | GATCATTCCACACACGAGAG<br>AACACATGCCCACAAACTGA | 55 | 180 |
| GAPU101 | CATGAAAGGAGGGGGACATA<br>GGCACTTGTTGTGCAGATTG | 55 | 80, 205 |

is especially appreciated by growers for its good productivity; in fact, Ogliarola means "giving good oil yields".

The aim of this work was to verify the effectiveness of the microsatellite analysis for the purpose of setting up a method to check the identity of Collina di Brindisi PDO olive oil. A preliminary assessment of product quality by means of chemical analyses was also carried out.

## MATERIALS AND METHODS

**Samples.** Six samples of Collina di Brindisi PDO oil (four unfiltered and two filtered oils) were collected from different oil mills, together with six samples of oil from the following cultivars: Cima di Melfi, Leccino, Nociara, Ogliarola, and Picholine, used for the preparation of the PDO mix according to the official production protocol (*26*, *27*). The total number of samples was 12.

**DNA Extraction.** A volume of 50 mL of unfiltered oil was centrifuged at 3000 rpm for 10 min in a J2-21 centrifuge (Beckman Instruments, Palo Alto, CA), while for filtered oils, 200 mL was centrifuged at 13000 rpm for 10 min in a Biofuge Pico centrifuge (Heraeus, Hanau, Germany). Then, a volume of centrifuged pellet of 50 $\mu$L was used to extract DNA using a Gene Elute Plant kit (Sigma, St. Louis, MO), with the following modifications to the manufacturer's instructions: In the first step of the protocol, 500 $\mu$L instead of 350 $\mu$L of lysis solution A and 60 $\mu$L instead of 50 $\mu$L of solution B were added; in the second step, 170 $\mu$L instead of 130 $\mu$L of precipitation solution was used; in the third step, 900 $\mu$L instead of 700 $\mu$L of binding solution was added, with a final elution of the extracted DNA in 100 $\mu$L of 1× TE buffer [10 mM Tris-HCl, pH 8.0, and 1 mM ethylenediaminetetraacetic acid (EDTA)]. The concentration of the extracted DNA was determined on 0.8% agarose gel by comparison with $\lambda$DNA solutions at known concentration.

**Microsatellite Primer Sequences.** A total number of seven primer pairs were used as follows: UDO99-009, UDO99-019, UDO99-025, UDO99-035, UDO99-044 (*15*), GAPU89, and GAPU101 (*14*). Sequences and annealing temperatures are reported in **Table 1**. Primers were synthesized by Sigma Genosys (St. Louis, MO).

**Amplification and Detection of Microsatellite Markers.** Amplification reactions were performed in a I-Cycler programmable thermal cycler (Bio-Rad Laboratories, Hercules, CA) in a reaction mix with the following composition: 30 ng of di DNA, 1× PCR buffer, 0.25 mM dNTP, 2.5 $\mu$M primer forward and reverse each, and 1 U of REDTaq DNA polymerase (Sigma), in a volume of 25 $\mu$L. The amplification conditions were as follows: 5 min at 95 °C; 35 cycles composed of 1 min at 95 °C, 1 min at the appropriate annealing temperature (*14*, *15*), and 2 min at 72 °C; final elongation at 72 °C for 10 min. The amplification products were separated by electrophoresis on 2.5% agarose gels in 1× TBE buffer (0.045 M Tris-borate and 0.001 M EDTA), stained by ethidium bromide, and visualized under UV light. Fragment sizes were quantified by comparison to 100 base pair molecular size markers (Bio-Rad Laboratories, Hercules, CA).

**Chemical Determinations.** Analyses of the free fatty acids, the peroxide value, and determination of the UV spectrophotometrical indices were carried out for each sample according to the official methods of analyses of the European Communities (*28*). The phenolic compounds were extracted, purified, and determined according to the method described in a previous paper (*29*), using Folin−Ciocalteau as a reagent and measuring the absorption at 765 nm. The results obtained were expressed as mg of gallic acid per kg of oil. The oxidative stability index was evaluated by Rancimat apparatus (Methrohm Co., Basel, Switzerland) at 120 °C, with an air flow of 20 L/h. The results were expressed as induction time (h). The polar compounds of the oil were separated by silica gel column chromatography according to the AOAC method (*30*). After elution of the nonpolar components with 150 mL of petroleum ether−diethyl ether (87:13, v/v), the polar compounds were recovered with 150 mL of diethyl ether. The efficacy of separation was checked by thin-layer chromatography as recommended by the same method. Then, the polar compounds recovered in $CH_2Cl_2$ were analyzed by means of high-performance size-exclusion chromatography (HPSEC) using $CH_2Cl_2$ as the eluant, at flow rate of 1 mL/min. The HPSEC system consisted of a series 200 pump (Perkin-Elmer, Norwalk, CT) with a Rheodyne injector, a 50 $\mu$L loop, a PL-gel guard column (Perkin-Elmer, Beaconsfield, United Kingdom) of 5 cm length × 7.5 mm i.d., and a series of three PL-gel columns (Perkin-Elmer) of 30 cm length × 7.5 mm i.d. each. The columns were packed with highly cross-linked styrene−divinylbenzene copolymer with a particle diameter of 5 $\mu$m and a pore diameter of 500, 500, and 100 Å, respectively. The refractive index detector was a series 200 (Perkin-Elmer) connected to an integrator. Peaks on the chromatograms were identified and quantified as reported in previous papers (*31*, *32*). The fatty acid composition was determined by gas chromatographic analysis of fatty acids methyl esters (*33*), using a gas chromatographic system composed of a Fisons (Milan, Italy) HRGC mega 2 series, equipped with a WCOT fused silica capillary column (Chrompack, Middleburg, The Netherlands), FFAP-CB coating, film thickness of 0.30 $\mu$m, 25 m length × 0.32 mm i.d., with a flame ionization detector and a split-splitless injector. Hydrogen was used as the carrier gas. The operative conditions were the same as in Caponio et al. (*31*). The identification and quantification of fatty acids were carried out by using a lipid standard purchased from Sigma. Several analyses carried out on the same sample produced a repeatability coefficient of percent variation (CV%) of about 5%.

## RESULTS AND DISCUSSION

The flow sheet of the processing technology of Collina di Brindisi PDO oil is shown in **Figure 1**. It can be noticed that, after the production of monovarietal oils (or binary mixtures not containing Ogliarola), mixing operations take place to fulfill the varietal requirements, preparing the blend where the Ogliarola cultivar is the limiting factor (*26*, *27*).

Regarding its quality requisites, Collina di Brindisi PDO oil should present the following parameters (maximum values): 0.8% free fatty acids (expressed as oleic acid), a peroxide value of 14 meq $O_2$/kg oil, and $K_{232}$ and $K_{270}$ values of 2.40 and 0.16, respectively (*26*, *27*). To examine the quality level of the samples, chemical analyses were carried out, including both the above cited parameters and other indices such as induction time, total phenol content, and amount of polar compounds.

The results of these analyses (**Table 2**) indicated that all of the samples belonged to the extra virgin olive oil category, fulfilling the quality requirements of the current rules. In addition, the data obtained by analyzing the samples collected from mills 3 and 4 indicated that the filtration carried out before bottling caused a slight increase of the oxidative degradation of the oil but without exceeding the limits provided. Regarding the total phenolic compounds, the values observed were quite low and corresponded to those usually found in sweet-tasting

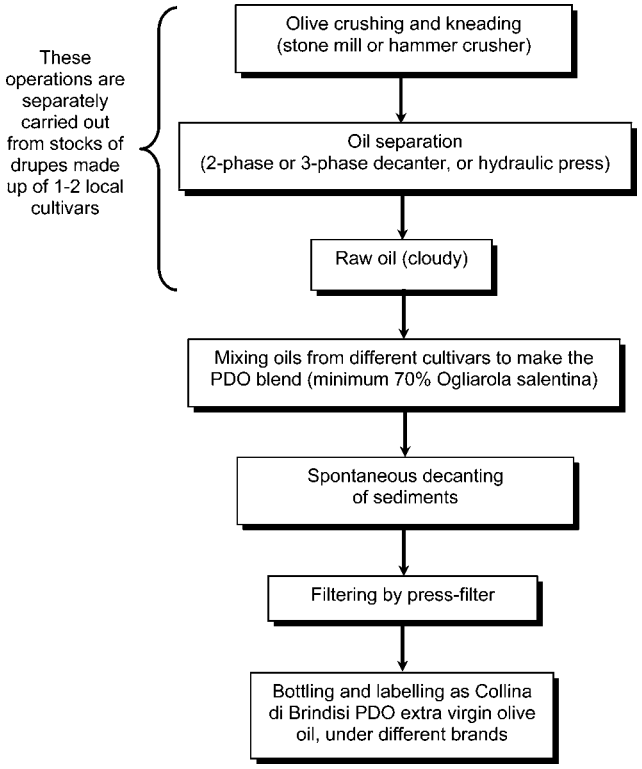


**Figure 1.** Flow sheet of the processing technology of Collina di Brindisi PDO extra virgin olive oil based on its official production protocol.

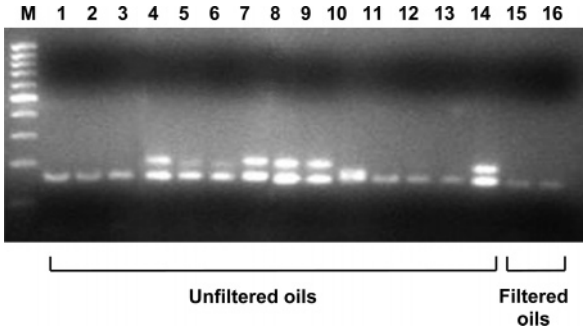


**Figure 2.** Electrophoretic patterns of the amplification products obtained by microsatellite GAPU89 with the samples: 1 and 2, two samples of Collina di Brindisi oil from different mills; 3, monovarietal oil of Leccino (wrong cultivar attribution made by the miller); 4, Leccino from DNA bank; 5, composite oil of Cima di Melfi and Nociara from the miller; 6, Cima di Melfi from DNA bank; 7, Nociara from DNA bank; 8, composite oil of Leccino and Picholine from the miller; 9, Leccino from DNA bank; 10, Picholine from DNA bank; 11 and 12, two samples of monovarietal oils of Ogliarola from the miller; 13, Ogliarola salentina from DNA bank; 14, Ogliarola barese from DNA bank; and 15 and 16, two samples of Collina di Brindisi oil from different mills. On the left was loaded the 100 bp marker.

oils (*29*, *34*). Furthermore, the Rancimat test was used to assess the antioxidant capacity of the examined oils. This method, which is specific for oils and fats, evaluates the ability of the antioxidants to inhibit the lipid oxidation under accelerated conditions (at high temperature, such as 120 °C, and under an air flow of 20 L/h) and allows one to measure the induction time. The low content of phenolic compounds in the examined oils justified the observed limited length of induction times, due to the significant positive correlation among these two parameters evinced in previous papers (*29*, *35*). Induction times were found to be shorter in filtered than in unfiltered oils.

Furthermore, the single classes of substances constituting the oil polar compounds were determined (**Table 2**). Polar compounds are substances with a higher polarity than unaltered triacylglycerols, such as triacylglycerol oligopolymers, oxidized triacylglycerols, and diacylglycerols. Interest in the determination of triacylglycerol oligopolymers and oxidized triacylglycerols was spurred by the observation that they may be potentially toxic when ingested in high amounts (*36*, *37*), and they are also thought to have a pro-oxidant activity (*38*, *39*). Moreover, these substances indicate the oxidative degradation of oils. The triacylglycerol oligopolymers, stable substances due to the secondary oxidation of oils and fats (*40*, *41*), were present in very low levels, while the oxidized triacylglycerols, indices of the primary oxidation of oils, were determined in mean amounts of about 0.50%. The amounts of these substances increased after oil filtering, confirming the results already provided by the other analyses. Among the polar compounds, the diacylglycerols were found to be the most abundant but with low values, slightly higher than 1%, denoting a very limited hydrolytic degradation of the analyzed oils. These results agreed with those found in other extra virgin olive oils (*42*).

**Table 3** reports the fatty acid composition of the examined oils. The obtained data indicated that all of the oils had the common fatty acid composition of the extra virgin olive oil (*43*, *44*), with oleic acid as the most abundant. Furthermore, these data did not provide evidence to indicate any relevant difference between Collina di Brindisi PDO oil and monovarietal oils. Besides being a varietal character, the fatty acid composition is influenced by agronomic and environmental factors (*45*). Thus, the small differences observed among these oils could not be effectively used to assess any possible inconsistency in the varietal profile of Collina di Brindisi PDO oil, due also to the limited number of samples.

Regarding the molecular analyses, **Figure 2** shows the amplification patterns of microsatellite GAPU89 with different samples of Collina di Brindisi PDO oil, collected from distinct oil mills, as well as of oils used by millers for the preparation of the PDO mix.

It can be noticed that good amplification levels were obtained both from cloudy unfiltered and from clear filtered oils, although oil filtration is known to dramatically decrease the amount of cell residues in the oil, thus having a detrimental effect on the results of DNA extraction (*24*). In particular, a sufficient cellular pellet for extraction of nucleic acids was obtained from filtered oils by centrifuging greater oil volumes, at higher speeds and for longer times, than required for unfiltered oils. Besides, for both kinds of oils, an improvement in the extractive capability of the commercial kit used for DNA extraction was attained by using higher amounts of the extracting reagents.

A preliminary phase of cultivar check was carried out on the raw materials (monovarietal oils and binary blends from two cultivars), taking advantage of an olive DNA bank composed of 60 olive cultivars native to Italy, Spain, France, and Greece that has been previously set up (*16*). Each oil, according to the cultivar attribution stated by the millers, was compared to the corresponding cultivar, or couple of cultivars, from the DNA bank. The obtained electrophoretic profiles (**Figure 2**) indicated that in one case the putative cultivar identification effected in the olive mill solely based on morphologic characters was wrong (a sample supposed to be Leccino was actually an Ogliarola oil), thus marking the need for more reliable and sophisticated means of cultivar checking.

**Table 2.** Chemical Characteristics of the Examined Oils (FFA, Free Fatty Acids; PV, Peroxide Value; TGP, Triacylglycerol Oligopolymers; ox-TG, Oxidized Triacylglycerols; DG, Diacylglycerols; and PC, Polar Compounds)

| type of oil | FFA (% oleic acid) | PV (mequiv $O_2$/kg) | $K_{232}$ | $K_{270}$ | $\Delta K$ | total phenols (mg/kg) | induction time (h at 120 °C) | TGP (%) | ox-TG (%) | DG (%) | PC (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| monovarietal and composite oils prior to PDO mixing | | | | | | | | | | | |
| Ogliarola | 0.31 | 8.9 | 1.95 | 0.16 | 0.00 | 125 | 10.8 | 0.02 | 0.30 | 0.98 | 1.82 |
| Ogliarola | 0.35 | 13.0 | 2.39 | 0.16 | 0.00 | 107 | 7.7 | 0.03 | 0.58 | 1.15 | 2.37 |
| Ogliarola | 0.42 | 9.9 | 2.12 | 0.16 | 0.00 | 98 | 8.6 | 0.02 | 0.37 | 1.29 | 2.34 |
| Leccino | 0.25 | 6.0 | 1.55 | 0.10 | 0.00 | 65 | 8.7 | 0.02 | 0.21 | 1.00 | 1.69 |
| mix of Nociara and Cima di Melfi[a] | 0.24 | 10.0 | 1.79 | 0.13 | 0.00 | 121 | 8.8 | 0.02 | 0.39 | 1.17 | 2.02 |
| mix of Leccino and Picholine[b] | 0.31 | 5.9 | 1.73 | 0.15 | 0.00 | 100 | 9.8 | 0.02 | 0.56 | 0.94 | 2.01 |
| Collina di Brindisi PDO oils | | | | | | | | | | | |
| sample from mill 1 (unfiltered) | 0.28 | 13.9 | 2.31 | 0.15 | 0.00 | 79 | 6.7 | 0.03 | 0.65 | 1.32 | 2.41 |
| sample from mill 2 (unfiltered) | 0.46 | 12.5 | 2.37 | 0.16 | 0.00 | 123 | 7.7 | 0.03 | 0.55 | 1.27 | 2.47 |
| sample from mill 3 (unfiltered) | 0.71 | 10.9 | 2.08 | 0.09 | 0.00 | 99 | 7.0 | 0.02 | 0.47 | 1.31 | 2.78 |
| sample from mill 3 (filtered) | 0.70 | 12.7 | 2.34 | 0.13 | 0.00 | 99 | 6.8 | 0.03 | 0.57 | 1.36 | 2.90 |
| sample from mill 4 (unfiltered) | 0.27 | 7.9 | 1.87 | 0.10 | 0.00 | 106 | 13.5 | 0.02 | 0.35 | 1.06 | 1.92 |
| sample from mill 4 (filtered) | 0.28 | 9.9 | 2.16 | 0.13 | 0.00 | 104 | 13.1 | 0.02 | 0.41 | 1.08 | 2.03 |

[a] Composed of 90% Cima di Melfi and 10% Nociara. [b] Composed of 70% Leccino and 30% Picholine.

**Table 3.** Percent Fatty Acid Composition of the Examined Oils

| type of oil | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{17:0}$ | $C_{17:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ | $C_{20:0}$ | $C_{20:1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| monovarietal and composite oils prior to PDO mixing | | | | | | | | | | | |
| Ogliarola | 0.03 | 12.42 | 2.03 | 0.06 | 0.11 | 1.81 | 73.74 | 8.90 | 0.53 | 0.20 | 0.18 |
| Ogliarola | 0.02 | 12.22 | 2.08 | 0.06 | 0.11 | 1.88 | 73.33 | 9.39 | 0.51 | 0.24 | 0.16 |
| Ogliarola | 0.01 | 12.15 | 1.87 | 0.05 | 0.08 | 2.00 | 72.98 | 9.83 | 0.57 | 0.27 | 0.19 |
| Leccino | 0.05 | 13.84 | 1.66 | 0.05 | 0.09 | 2.45 | 74.03 | 6.61 | 0.62 | 0.34 | 0.26 |
| mix of Nociara and Cima di Melfi[a] | 0.04 | 13.50 | 1.08 | 0.04 | 0.07 | 2.28 | 73.57 | 8.02 | 0.70 | 0.36 | 0.33 |
| mix of Leccino and Picholine[b] | 0.04 | 13.23 | 2.04 | 0.06 | 0.10 | 2.02 | 74.45 | 7.01 | 0.54 | 0.29 | 0.23 |
| Collina di Brindisi PDO oils | | | | | | | | | | | |
| sample from mill 1 (unfiltered) | 0.01 | 12.76 | 1.91 | 0.04 | 0.12 | 2.10 | 72.42 | 9.61 | 0.58 | 0.23 | 0.21 |
| sample from mill 2 (unfiltered) | 0.03 | 12.93 | 1.97 | 0.05 | 0.09 | 1.97 | 72.53 | 9.42 | 0.61 | 0.22 | 0.18 |
| sample from mill 3 (unfiltered) | 0.01 | 11.97 | 1.73 | 0.04 | 0.10 | 2.01 | 74.42 | 8.72 | 0.53 | 0.30 | 0.17 |
| sample from mill 3 (filtered) | 0.01 | 12.08 | 1.79 | 0.03 | 0.08 | 2.25 | 74.00 | 8.74 | 0.56 | 0.26 | 0.20 |
| sample from mill 4 (unfiltered) | 0.02 | 12.42 | 1.85 | 0.03 | 0.09 | 2.05 | 73.53 | 8.99 | 0.58 | 0.24 | 0.20 |
| sample from mill 4 (filtered) | 0.02 | 12.57 | 1.91 | 0.04 | 0.07 | 1.99 | 73.19 | 9.16 | 0.60 | 0.27 | 0.18 |

[a] Composed of 90% Cima di Melfi and 10% Nociara. [b] Composed of 70% Leccino and 30% Picholine.

At the times of the Collina di Brindisi PDO mark approval, lack of both accurate means of cultivar identification and systematic and organized procedures of cultivar cataloguing led to the indication of the general name "Ogliarola" in the list of cultivars foreseen by the official production protocol of this oil (*26*, *27*). Ogliarola, indeed, was also the registered name present in the national list of *O. europaea* Italian cultivars (*46*). More recently, however, the presence of three different types of Ogliarola in the Apulia region has been assessed; the cultivars Ogliarola barese, Ogliarola salentina, and Ogliarola garganica (*47*−*49*) derive their qualifications from the three different districts where they are mainly diffused, the areas of Bari (central Apulia), Salento (the Southern subregion of Apulia), and Gargano (the Northern part of Apulia).

Considering that the production area of Collina di Brindisi PDO oil is the area of main diffusion of the Ogliarola salentina cultivar, while Ogliarola barese and Ogliarola garganica are almost absent, the identity of the generic Ogliarola with Ogliarola salentina was strongly presumed. This assumption was confirmed by the microsatellite analysis of Ogliarola samples originating from oil mills in comparison to Ogliarola salentina, Ogliarola barese, and Ogliarola garganica derived from the DNA bank. In fact, considering the electrophoretic profiles obtained with microsatellites GAPU89 (**Figure 2**) and GAPU101 (**Figure 3**), it was unambiguously assessed that Ogliarola was genetically different from the "barese" and the "garganica" types.

Furthermore, the effectiveness of microsatellites in checking the identity of Collina di Brindisi PDO olive oil was evaluated by analyzing seven microsatellites selected based on both the degree of polymorphism and the simplicity of their patterns, as assessed in previous studies (*16*, *25*). These DNA markers were chosen since the amplification patterns of microsatellites are usually composed by one or two fragments (**Figure 4**), while other markers, such as AFLPs, RAPDs, or inter-SSRs, show numerous bands and are not suitable in cases of composite oils from assorted cultivars, as for PDO oils (*50*, *51*). In fact, the overlapping of complex profiles, each corresponding to one of the cultivars present in the mix, could prevent a reliable interpretation.

In spite of the intrinsic simplicity of the electrophoretic pattern of microsatellites, we expected a complex profile due to the sum of the bands imputable to each of the single cultivars present in the oil. The obtained results showed, on the contrary, that the electrophoretic pattern of PDO oil was very uncomplicated because, in all of the examined samples, it was mainly consistent with that of Ogliarola (more precisely, Ogliarola salentina) monovarietal oil (**Figure 4**). In fact, this cultivar prevails in Collina di Brindisi PDO oil and it is present in amounts higher than 70%, levels that can hide the electrophoretic patterns of other cultivars. Very often, this cultivar was the only present. Thus, the absence of the Ogliarola salentina pattern in the case of oils sold with the Collina di Brindisi PDO mark can be revealed to be a fraud.

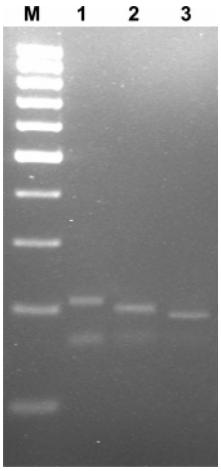


**Figure 3.** Agarose gel (2.5%) loaded with the amplification products of DNA extracted from (1) Ogliarola salentina, (2) Ogliarola barese, and (3) Ogliarola garganica, with the microsatellite GAPU101. On the left was loaded the 100 bp marker.

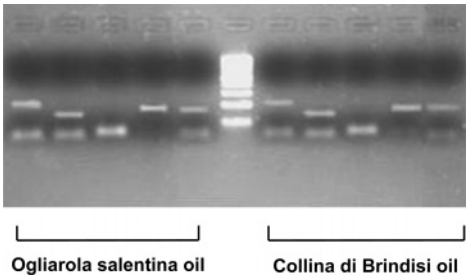


**Figure 4.** Agarose gel (2.5%) loaded with the amplification products of DNA extracted from Ogliarola salentina monovarietal oil (first five lanes, from left to right) and Collina di Brindisi PDO oil (last five lanes) with five different microsatellites: 1, GAPU101; 2, UDO99-019; 3, UDO99-044; 4, GAPU89; and 5, UDO99-035. The central lane was loaded with the 100 bp marker.

On the whole, the seven microsatellites examined in Collina di Brindisi oil led to amplicons ranging from 80 to 205 base pairs, and the same amplicons were obtained from monovarietal oil of Ogliarola salentina (**Table 1**). The small dimensions of the target sequences facilitated the amplification in the presence of degraded DNA, such as that extracted from the analyzed oils.

In conclusion, the results obtained by microsatellite analysis clarified that the generic name Ogliarola, indicated in the technical sheet of the PDO oil Collina di Brindisi, actually corresponded to the Ogliarola salentina cultivar. In addition, our data demonstrate that based on the examination of a limited number of DNA microsatellites, it is possible to check the presence of the Ogliarola salentina cultivar in Collina di Brindisi PDO oil, thus verifying the identity of the product. This is of major importance since some problems still occur, at the mill level, in guaranteeing the varietal composition foreseen by the official regulations. Regarding the chemical features of this PDO oil, they were found to completely fulfill the current law's requirements.

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