JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Traceability of Italian Protected Designation of Origin (PDO) Table Olives by Means of Microsatellite Molecular Markers

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**ABSTRACT:** The aim of this work was to develop a DNA microsatellite-based method of analysis to allow traceability of the three Italian Protected Designation of Origin (PDO) table olives in comparison with fruits of another seven highly diffused table olive cultivars. The analyses were carried out by using 16 primer pairs, with a mean of five different alleles detected per primer set, and power of discrimination from 0.56 to 0.90. Allelic error rates in the range of 0−3.8% were observed. By combining data from the most reliable and highly informative microsatellites (DCA3, DCA16, DCA17, DCA18, UDO-043, and GAPU101), it was possible to identify the PDO fruits over the panel of 10 cultivars, with the probability of a chance match between different cultivars as low as $10^{-9}$ and with 0.5% error rate. The amplification profile is independent of environmental and processing conditions and is helpful to verify the authenticity of PDO samples.

**KEYWORDS:** *table olives, PDO and traditional food, traceability, DNA microsatellites*

## ■ INTRODUCTION

Besides being used for oil extraction, olive (*Olea europaea* L.) is cultivated to obtain a wide variety of table olives, which are one of the main preserved vegetable products prepared throughout the world.[1] The mean yearly world production of table olives is close to 2 500 000 tons (in the 2010/2011 season), and the main producing area is Europe, accounting for about 32% of the world production. Italy, with about 60 200 tons (in 2010/2011 season), is the third European producer, after Spain and Greece.[2]

Depending on their main characteristics, olive cultivars are classified for oil, table, and dual purpose. Table olives are generally characterized by bigger size, higher flesh-to-pit ratio, and lower oil content than oil cultivars.[3] The use of dual purpose cultivars for table purposes depends on market requests and season performances with regard to the biggest fruits harvested.[4]

The Italian olive cultivar panorama is very rich, with 395 entries registered in the national list approved by Decree of the Italian Agricultural Ministry no. 573/93. Many cultivars have been grown since ancient times in restricted areas of origin, which is a prerequisite to be awarded the Protected Designation of Origin (PDO) mark, according to the EC Regulation no. 510/2006.

Up to now, three Italian vegetable preserves of the marketing category "table olives" have been included in the list of PDO products: Nocellara del Belice in 1998 (EC Commission Regulation no. 134/98), Bella della Daunia in 2000 (EC Commission Regulation no. 1904/2000), and Ascolana del Piceno in 2005 (EC Commission Regulation no. 1855/2005). In particular, the protected designation Nocellara del Belice refers to fruits picked at green or black stage (more frequently green) from cv. Nocellara del Belice grown in the area of Castelvetrano (Sicily), treated with dry salt or brine to ensure adequate shelf life. Bella della Daunia PDO refers to fruits of cv. Bella di Cerignola harvested in the area of Foggia (Apulia), picked green or black and treated with brine. Ascolana del Piceno PDO are the fruits, generally green, from cv. Ascolana tenera grown in the area of Ascoli Piceno and Teramo (central Italy). They can be treated with brine or, thanks to the easy detachment of the stone from the flesh, they are typically stuffed with a mixture of meat, eggs, and cheese and consumed after frying.

Moreover, the olives of Termite di Bitetto cultivar, long appreciated and locally known as "olive dolci" or "olive mele" (olives sweet as apples, in Italian), have been recently included in the list of the Italian traditional agri-food products (approved by Decree of the Italian Agricultural Ministry of June 7, 2012, 12th edition, sublist Apulian products, entry no. 108), and its producers are going to request a PDO recognition.

In the presence of seals of quality, such as PDO, there is a special need for effective checking systems to avoid food adulteration. Food traceability systems represent a very helpful tool for guaranteeing levels of quality and increasing transparency throughout the entire food chain. Tracing and tracking systems are essentially based on documental procedures, but analytical methods can support them, allowing regular controls. Most of these methods are based on DNA analysis and are used to check label claims regarding both the species and the varietal composition.[5−8]

Microsatellites are highly polymorphic, species-specific, reproducible PCR-based markers consisting of repetitive small-sized DNA sequences, abundant throughout the genome.[9] A previous study has demonstrated the feasibility of the assessment of authenticity of PDO extra virgin olive oil by targeting selected DNA microsatellites.[10]

The aim of this work was to develop a microsatellite-based method of analysis able to allow traceability of fruits of the Italian

Received: July 31, 2012
Revised: March 4, 2013
Accepted: March 5, 2013
Published: March 5, 2013

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PDOs Nocellara del Belice, Bella della Daunia, and Ascolana del Piceno in comparison with fruits of other very diffused table cultivars including Termite di Bitetto.

## MATERIALS AND METHODS

**Samples.** Fruits and leaves of the Italian cultivars Ascolana tenera, Bella di Cerignola, Dolce di Cassano, Leccino, Peranzana, Nocellara del Belice, Nolca, Pasola di Andria, Sant'Agostino, and Termite di Bitetto were collected at the experimental field of the Olive Premultiplication Centre Concadoro at Palagiano (Italy). The field was isolated with respect to other olive cultivations. All of the plant materials were certified as qualifying for Conformitas Agraria Comunitatis (CAC), with special reference to their identity and plant health, according to the requirements of the Directive 2008/90/EEC. Fruits of the PDO cultivars (Ascolana tenera, Bella di Cerignola, and Nocellara del Belice), of the traditional cultivar Termite di Bitetto, and of Peranzana were sampled also after processing. In particular, fruits of Bella di Cerignola, Termite di Bitetto, and Peranzana were processed with 8% (w/w) NaCl brine, according to the so-called "natural-style" method, at a local olive-processing factory (Puglia Conserve, Modugno, Italy). Fruits of Nocellara del Belice were treated in the laboratory with dry salt for 3 weeks, daily pouring off the water released. Commercial samples of in-brine Ascolana del Piceno PDO table olives were purchased from local retailers.

**DNA Extraction.** Fresh (all cultivars), dry-salted (cv. Nocellara del Belice), and in-brine fruits (cv. Ascolana tenera, Bella di Cerignola, Peranzana, and Termite di Bitetto) were subjected to DNA extraction. The dry-salted and in-brine samples were previously accurately rinsed with distilled water. An amount of 200 mg of olive flesh was used to extract DNA using NucleoSpin Food (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions, except that the elution volume was decreased to 50 $\mu$L. DNA was extracted also from fresh leaves, after grinding them with liquid nitrogen, according to the method of Doyle and Doyle.[11] After extraction, DNA was checked for degradation level and concentration by 0.8% agarose gel electrophoresis and spectrophotometric measure (Nanodrop 2000, Thermo, Wilmington, MA, USA) at 260 nm. In particular, DNA extracted from leaves showed no degradation and had a concentration of 100−300 ng/$\mu$L. DNA from fresh olives was intact but less concentrated (10−60 ng/$\mu$L), whereas DNA extracted from dry-salted and in-brine processed fruits showed similar concentrations but were partially degraded. Two replicates were performed per each extraction.

**Amplification and Detection of Microsatellite Markers.** Sixteen primer pairs were considered; sequences and annealing temperatures are reported in the corresponding references: DCA3, DCA4, DCA5, DCA9, DCA13, DCA14, DCA15, DCA16, DCA17, DCA18;[12] EMO-L and EMO-90;[13] GAPU45, GAPU71B, and GAPU101;[14] and UDO-043.[15] The majority of the microsatellites used (namely, DCA3, DCA5, DCA9, DCA14, DCA16, DCA18, UDO-043, GAPU71B, GAPU101, and EMO-90) were previously selected in a collaborative study among four independent laboratories, for high power of discrimination and reproducibility due to low peak stuttering, strong peak signal, and absence of null alleles.[16] Other markers were chosen for good quality of amplification observed in previous works of the same authors.[10,17−20] The amplification reactions were carried out with 50 ng of genomic DNA, 1× buffer (10 mM Tris-HCl, pH 8.3, 10 mM KCl), 250 $\mu$M each dNTPs, 2.0 mM $MgCl_2$, 0.5 $\mu$M each microsatellite primer, and 0.06 U Taq polymerase (Euroclone, Milano, Italy) in a final volume of 25 $\mu$L. The forward primer of each microsatellite was fluorescently labeled with 6-FAM or 8-HEX Sigma Genosys (St. Louis, MO, USA). The amplification program was performed in a MyCycler programmable thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) as follows: 5 min at 95 °C; 35 cycles at 95 °C for 30 s, 30 s at the primer-specific annealing temperature, 30 s at 72 °C; final extension at 72 °C for 60 min. Preliminary electrophoresis of PCR products was performed on 2.5% SeaKem LE Agarose gel (Lonza, Switzerland), whereas the labeled amplicons were separated by capillary electrophoresis, as described in Pasqualone et al.,[21] using an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The molecular weight internal standard was 500-ROX (Applied Biosystems). Allele sizing was carried out by means of GeneMapper genotyping software (3.7 v., Applied Biosystems).

**Allele Cloning and Sequencing.** The pGEM-T Vector System I (Promega, Madison, WI, USA) was used to clone the fragments obtained by amplifying EMO-L with DNA from Leccino and Nolca and amplifying GAPU45 with Bella di Cerignola. The recombinant vectors were used to transform heat shock sensitive *Escherichia coli* competent cells following the manufacturer's protocol. After the ampicillin/IPTG/X-gal screening, the recombinant cells were isolated, and plasmid DNA was extracted after an overnight growth at 37 °C using the mini-prep protocol. PCR was performed on 10 plasmid DNA for each amplicon with the universal primers T7/SP6 at the annealing temperature of 50 °C. Cloned PCR products were purified with DNA purification kit NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany) quantified by Nanodrop 2000 (Thermo, Wilmington, MA, USA) and sequenced by Macrogen Europe (Amsterdam, The Netherlands).

**Data Analysis.** For each microsatellite, the frequency of alleles detected over the cultivars was computed by POPGENE software (v. 1.32)[22] and used to calculate some indices as a measure of microsatellite informativeness. In particular, the band informativeness ($I_b$) and resolving power (RP) were calculated as in Prevost et al.,[23] and the power of discrimination (PD)[24] (sometimes referred to as polymorphism information content,[25] or diversity index[26]) was calculated as in Kloosterman et al.[24] The probability of identity (PI) was calculated as in Paetkau et al.[27] Error rates were quantified on each microsatellite by replicating the whole procedure of sampling, DNA extraction, amplification, and electrophoresis seven times for Leccino and three times for the other cultivars. True alleles were considered to be those having intensity well above the background signal present at least twice over the replicates. The mean allelic error rate was calculated as the ratio between the number of allelic mismatches and the number of replicated alleles, whereas the mean error rate per multilocus genotype over a combination of the best microsatellites was calculated as the ratio between the number of multilocus genotypes including at least one allelic mismatch and the number of replicated multilocus genotypes.[28]

## RESULTS AND DISCUSSION

Fruits of Nocellara del Belice PDO, Bella della Daunia PDO, Ascolana del Piceno PDO, and Termite di Bitetto traditional olive cultivar were compared, by means of DNA microsatellite analysis, with fruits of the main Italian table olive cultivars. The study also included Leccino (mainly used for oil extraction, but also destined to table use, according to market requests) because it is the reference cultivar in a ring test involving nine laboratories within the framework of the Italian research project OLVIVA.[16,29] In fact, the adoption of different fragment analysis procedures in different laboratories may cause variations by a few base pairs (bp) in the size assessment of the amplified fragments. Hence, correct allele recognitions require the inclusion of reference cultivars, which amplify representative alleles. They can be used as "allelic ladders" for direct comparisons at each locus.[29]

A number of alleles between three and eight was scored per microsatellite, sized in the range of 107−266 bp (Table 1), with a mean frequency of 0.4. The $I_b$ value[23] varied from 0.4 to 0.7, with the highest results in UDO-043 and GAPU71B. Various indices of microsatellite informativeness were determined (Table 2). These indices provide an estimate of the discrimination ability of each microsatellite by taking into account not only the number of revealed alleles/patterns but also their relative frequencies. The RP value,[23] referred to allele frequency, varied from 1.2, in DCA15, to 4.0, in UDO-043. Different values of RP for microsatellites detecting the same number of alleles were due to their different allelic distributions (for example, UDO-043 and

**Table 1. Band Informativeness ($I_b$) and Frequency of Alleles Amplified by 16 Microsatellites in 10 Olive Cultivars**

| microsatellite | detected alleles (bp) | $I_b$ | allele frequency | microsatellite | detected alleles (bp) | $I_b$ | allele frequency |
|---|---|---|---|---|---|---|---|
| DCA3 | 232 | 0.6 | 0.3 | DCA17 | 107 | 0.2 | 0.1 |
| | 237 | 0.2 | 0.1 | | 113 | 0.8 | 0.4 |
| | 243 | 0.8 | 0.6 | | 115 | 0.8 | 0.6 |
| | 245 | 0.2 | 0.1 | | 117 | 0.4 | 0.2 |
| | 249 | 0.8 | 0.4 | | 143 | 1.0 | 0.5 |
| | 253 | 0.8 | 0.4 | | 161 | 0.4 | 0.2 |
| mean | | 0.6 | 0.3 | mean | | 0.6 | 0.3 |
| DCA4 | 130 | 1.0 | 0.5 | DCA18 | 165 | 0.2 | 0.1 |
| | 132 | 0.6 | 0.7 | | 171 | 0.6 | 0.3 |
| | 140 | 0.2 | 0.1 | | 173 | 0.4 | 0.2 |
| | 162 | 0.2 | 0.1 | | 175 | 0.2 | 0.1 |
| | 165 | 1.0 | 0.5 | | 177 | 0.8 | 0.6 |
| | 190 | 0.6 | 0.3 | | 179 | 0.4 | 0.2 |
| | 193 | 0.2 | 0.1 | | 185 | 0.6 | 0.3 |
| mean | | 0.5 | 0.3 | | 187 | 0.2 | 0.1 |
| DCA5 | 198 | 0.6 | 0.3 | mean | | 0.4 | 0.2 |
| | 202 | 0.2 | 0.1 | UDO-043 | 172 | 0.6 | 0.3 |
| | 206 | 0.2 | 0.9 | | 174 | 0.6 | 0.3 |
| | 208 | 0.6 | 0.3 | | 176 | 0.8 | 0.4 |
| mean | | 0.4 | 0.4 | | 188 | 0.2 | 0.1 |
| DCA9 | 162 | 0.6 | 0.7 | | 210 | 0.8 | 0.6 |
| | 172 | 0.6 | 0.3 | | 216 | 1.0 | 0.5 |
| | 182 | 0.4 | 0.2 | mean | | 0.7 | 0.4 |
| | 186 | 0.2 | 0.1 | GAPU45 | 180[a] | 0.4 | 0.2 |
| | 194 | 0.4 | 0.2 | | 183 | 0.6 | 0.7 |
| | 208 | 0.2 | 0.1 | | 185 | 0.8 | 0.4 |
| mean | | 0.4 | 0.3 | | 195 | 0.2 | 0.1 |
| DCA13 | 118 | 0.2 | 0.1 | mean | | 0.5 | 0.4 |
| | 120 | 0.4 | 0.8 | GAPU71B | 121 | 0.6 | 0.3 |
| | 122 | 0.6 | 0.3 | | 124 | 0.8 | 0.6 |
| | 140 | 0.8 | 0.4 | | 127 | 0.6 | 0.3 |
| mean | | 0.5 | 0.4 | | 130 | 0.6 | 0.3 |
| DCA14 | 153 | 0.4 | 0.2 | | 144 | 0.8 | 0.4 |
| | 173 | 0.2 | 0.1 | mean | | 0.7 | 0.4 |
| | 179 | 0.4 | 0.2 | GAPU101 | 183 | 0.2 | 0.1 |
| | 181 | 0.6 | 0.7 | | 192 | 0.2 | 0.1 |
| | 191 | 0.8 | 0.6 | | 198 | 0.8 | 0.6 |
| | 193 | 0.2 | 0.1 | | 199 | 0.2 | 0.1 |
| mean | | 0.4 | 0.3 | | 200 | 1.0 | 0.5 |
| DCA15 | 246 | 0.4 | 0.8 | | 206 | 0.6 | 0.3 |
| | 257 | 0.4 | 0.2 | mean | | 0.5 | 0.3 |
| | 266 | 0.4 | 0.8 | EMO-L | 192[a] | 0.8 | 0.6 |
| mean | | 0.4 | 0.6 | | 198[a] | 0.4 | 0.8 |
| DCA16 | 120 | 0.4 | 0.2 | | 200 | 0.2 | 0.1 |
| | 122 | 0.4 | 0.2 | mean | | 0.5 | 0.5 |
| | 150 | 1.0 | 0.5 | EMO-90 | 188 | 0.6 | 0.7 |
| | 153 | 0.6 | 0.3 | | 190 | 0.6 | 0.3 |
| | 155 | 0.2 | 0.1 | | 194 | 0.6 | 0.7 |
| | 167 | 0.2 | 0.1 | mean | | 0.6 | 0.6 |
| | 174 | 0.2 | 0.1 | | | | |
| | 176 | 0.8 | 0.4 | general mean | | 0.5 | 0.4 |
| mean | | 0.5 | 0.2 | | | | |

[a]Allele size confirmed by sequencing (for EMO-L the allele of 192 bp was amplified in Nolca and that of 198 bp in Leccino; for GAPU45 the allele of 180 bp was amplified in Bella di Cerignola).

DCA9, both detecting six alleles, had RP values of 4.0 and 2.4, respectively), with the most discriminative loci showing many alleles in similar frequencies. As observed in previous works,[31] high values of RP do not always correspond to high efficiency in discriminating among cultivars, due to a lower correlation of RP than other indices, such as PD, to the number of distinguishable cultivars. RP and PD are two indices commonly used to evaluate informativeness degree of microsatellite markers, but RP is based on allele frequencies[23] whereas PD is based on banding pattern or genotype frequencies at a given locus.[24] Hence, high RP values

**Table 2. Informativeness of Microsatellite Markers Amplified in 10 Olive Cultivars[a]**

| microsatellite | NA | NADSC | NDC | PD | RP | PI | AE |
|---|---|---|---|---|---|---|---|
| DCA3 | 6 | 1 | 9 | 0.88 | 3.4 | 0.026 | 0.000 |
| DCA4 | 7 | 2 | 9 | 0.90 | 3.8 | 0.026 | 0.035 |
| DCA5 | 4 | 1 | 4 | 0.70 | 1.6 | 0.153 | 0.032 |
| DCA9 | 6 | 1 | 7 | 0.83 | 2.4 | 0.055 | 0.014 |
| DCA13 | 4 | 1 | 5 | 0.68 | 2.0 | 0.166 | 0.038 |
| DCA14 | 6 | 1 | 6 | 0.76 | 2.6 | 0.088 | 0.014 |
| DCA15 | 3 | 1 | 3 | 0.56 | 1.2 | 0.263 | 0.023 |
| DCA16 | 8 | 1 | 8 | 0.86 | 3.8 | 0.042 | 0.007 |
| DCA17 | 6 | 1 | 8 | 0.87 | 3.6 | 0.035 | 0.007 |
| DCA18 | 8 | 1 | 9 | 0.88 | 3.2 | 0.026 | 0.000 |
| UDO-043 | 6 | 2 | 8 | 0.85 | 4.0 | 0.042 | 0.000 |
| GAPU45 | 4 | 1 | 5 | 0.72 | 2.0 | 0.131 | 0.000 |
| GAPU71B | 5 | 1 | 7 | 0.82 | 3.4 | 0.055 | 0.033 |
| GAPU101 | 6 | 1 | 7 | 0.82 | 3.0 | 0.055 | 0.000 |
| EMO-L | 3 | 1 | 4 | 0.64 | 1.4 | 0.216 | 0.000 |
| EMO-90 | 3 | 1 | 5 | 0.74 | 1.8 | 0.106 | 0.023 |
| mean value | 5 | 1 | 6 | 0.78 | 2.7 | 0.093 | 0.014 |

[a]NA, number of alleles detected; NADSC, number of allelic differences between the two more similar cultivars apart the nondistinguishable; NDC, number of distinguishable cultivars; PD, power of discrimination; RP, resolving power; PI, probability of identity; AE, allelic error.

**Table 3. Amplification Data of Olive Cultivars with 16 Microsatellite Markers**

| cultivar | no. of alleles | allele size range (bp) | distinctive allele combinations (bp) |
|---|---|---|---|
| Ascolana tenera | 27 | 115–266 | 122–153 (DCA16) |
| | 29 | 113–266 | 167–167 (DCA16) |
| Bella di Cerignola | | | 180–180 (GAPU45) |
| | | | 183–200 (GAPU101) |
| Dolce di Cassano | 27 | 120–266 | 179–191 (DCA14) |
| Leccino | 27 | 107–266 | 243–253 (DCA3) |
| Nocellara del Belice | 31 | 115–249 | 153–179–191 (DCA14) |
| Nolca | 29 | 115–246 | 192–192 (EMO-L) |
| Pasola di Andria | 26 | 115–266 | 122–155 (DCA16) |
| Peranzana | 32 | 113–266 | 120–153 (DCA16) |
| Sant'Agostino | 32 | 113–266 | 120–176 (DCA16) |
| Termite di Bitetto | 30 | 113–266 | 181–191–193 (DCA14) |

did not necessarily correspond high PD in all cases. The PD, which can range between 0 (monomorphism) and 1 (highly informative microsatellite), varied from 0.56 to 0.90. The high mean PD value observed (0.78) confirmed the efficacy of preliminary selection for highly polymorphic primers. The highest value of PD was recorded for DCA4, followed by DCA3 and DCA18. DCA4, in particular, was already found to be the most polymorphic microsatellite in several different panels of olive cultivars.[18,30] Each of these three markers was potentially able to distinguish 9 cultivars over 10 examined (Table 2), confirming the significant correlation between PD and number of distinguishable cultivars observed in previous works.[31] However, some microsatellites showed a non-negligible allelic error rates, exceeding the range of 0.5−1% that is usual in many laboratories.[28] Hence, to obtain reliable genotyping, microsatellites with higher error rates were discarded (i.e., DCA4, DCA5, DCA13, DCA15, GAPU71B, and EMO-90), irrespective of high RP or PD value.

Some alleles or allele combinations were of particular interest because they appeared to be "new" with respect to a wide allele database recorded over the main olive cultivars (Table 3).[16] They were detected uniquely in specific cultivars and, hence, could be defined as "distinctive alleles", that is, markers of reference for the detection of the fruits of a certain cultivar in industrial checks. Some of these distinctive alleles were cloned and sequenced to assess precisely their length and sequence, so as to validate the method. In particular, this procedure was applied to the PCR products of GAPU45 with Bella di Cerignola and of EMO-L with Nolca and Leccino. Direct sequence analysis at loci GAPU45 and EMO-L confirmed for all cultivars the allele length assessed by capillary electrophoresis (Table 1). Size assessment of Leccino allele agreed with results from other laboratories involved in the ring test of the OLVIVA project (data not shown). Besides, the obtained sequences showed the expected tandem repeat unit motif. In particular, GAPU45 was based on AG tandem repeat, namely $(AG)_7$, whereas EMO-L was a GA-based microsatellite, $(GA)_{12}$, in both cases according to data reported in the literature.[13,14]

However, only one microsatellite locus, even distinctive, is not sufficient to identify a cultivar, because genotyping errors could occur (due to mis-scoring of bands, amplification faults, electrophoretic artifacts, etc.). Hence, a combination of microsatellites is always needed. Apart from the discarded microsatellites, the probability of a chance match between any two different cultivars at a given locus, measured through the PI value (Table 2), ranged from 2.6% (for hyperpolymorphic markers such as DCA3 and DCA18) to 21.6% (for EMO-L). In particular, both DCA3 and DCA18 were able to distinguish nine cultivars each, and these two microsatellites together were able to identify the full set of cultivars considered. The PI over a combination of microsatellites can be calculated as the product of individual PI values. The PI of the combination of DCA3 and DCA18 accounted for $6.76 \times 10^{-4}$. Moreover, the overall PI at the six best loci, characterized by highest polymorphism and lowest allelic error (namely, DCA3, DCA16, DCA17, DCA18, UDO-043, and GAPU101), was as low as $2.29 \times 10^{-9}$. The error rate of this multilocus genotype, reflecting its reliability,[28] was 0.5%.

Wiht regard to processed fruits, they were treated with either dry salt or brine and compared with untreated fruits of the same cultivar. Figure 1 shows the electropherogram of microsatellite GAPU71B amplified with DNA extracted from Nocellara del Belice fruits at harvest and dry-salted fruits of the same cultivar. The profiles perfectly overlapped, indicating that dry-salt processing did not affect DNA analysis. A perfect match was observed also by comparing the amplification profiles obtained from fruits at harvest and in-brine, indicating that also in-brine processing did not affect DNA quality, and confirming the correctness of PDO labeling in the examined samples, collected at a local olive-processing factory and destined to market.

In conclusion, by combining data of six highly informative and reliable microsatellites, it was possible to distinguish 3 Italian PDO table olive cultivars over a panel of 10 cultivars, with a theoretical probability for nondistinguishable profiles from different cultivars as low as $10^{-9}$. This DNA profiling method is independent from environmental and processing conditions and is helpful to verify the authenticity of PDO samples. The method might be transferred to a company or PDO consortium, and the obtained information could be used to make decisions about bottling and to verify labeling correctness.

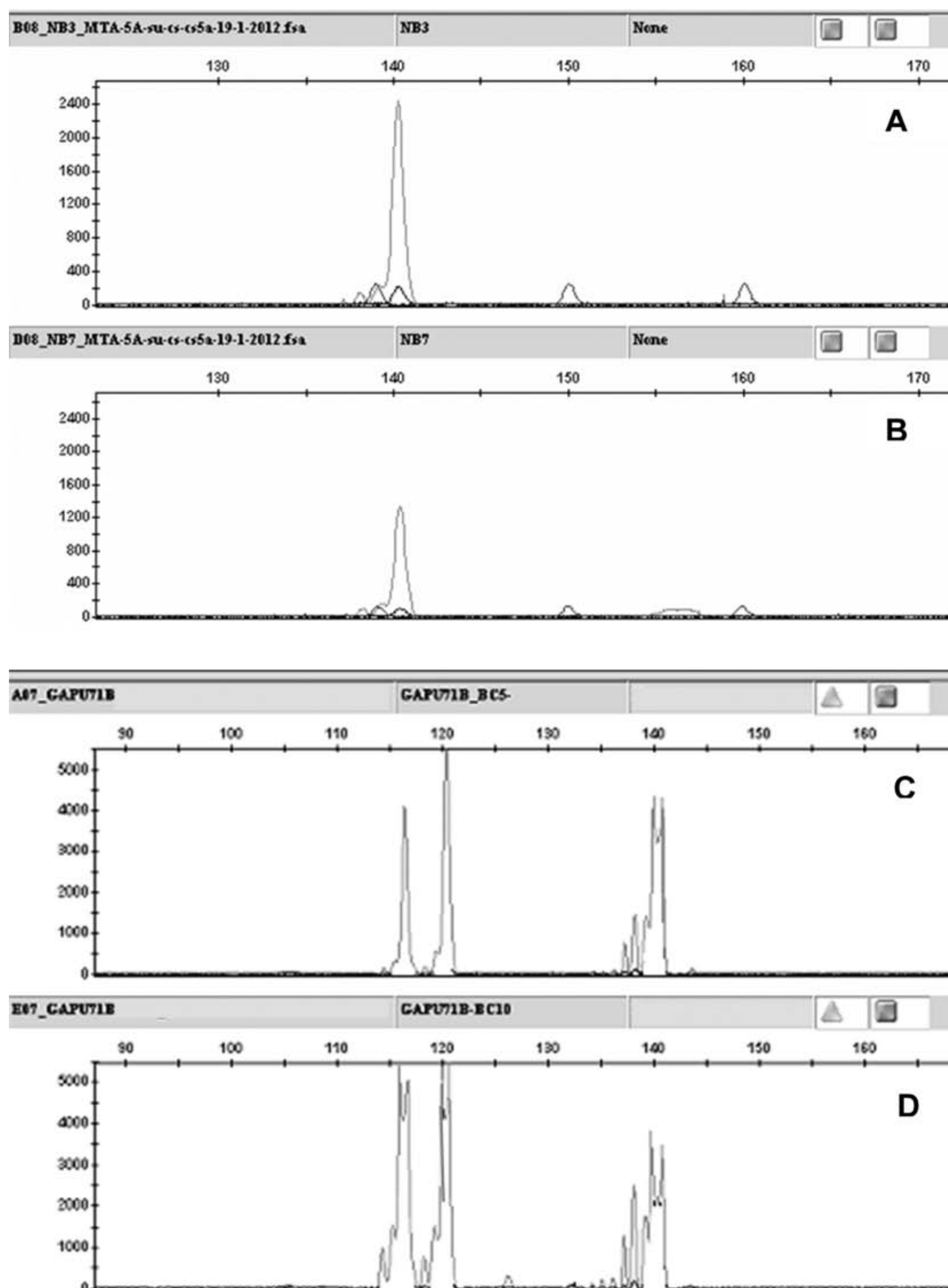


**Figure 1.** Capillary electropherogram of the amplified fragments obtained by microsatellite GAPU71B with DNA extracted from fruits of Nocellara del Belice cultivar at harvest (A) and dry-salted (B) and from fruits of Bella di Cerignola cultivar at harvest (C) and in-brine (D).

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### Funding

The research was carried out within the framework of Project OLVIVA, funded by "Programma Interregionale Sviluppo Rurale, sottoprogramma Innovazione e Ricerca", and Project "Sviluppo ed applicazione di protocolli per la certificazione dell'autenticità e tracciabilità di prodotti tipici pugliesi", funded by Fondazione CRP.


### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

We are grateful to Dr. Chiara Consonni for linguistic revision.

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