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Genetic and geochemical signatures to prevent frauds and counterfeit of high-quality asparagus and pistachio

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ABSTRACT

A fingerprinting strategy based on genetic (simple sequence repeat) and geochemical (multielement and $^{87}Sr/^{86}Sr$ ratio) analysis was tested to prove the geographical origin of high-quality Italian products "*White Asparagus from Bassano del Grappa*" and "*Green Pistachio from Bronte*". Genetic analysis generated many polymorphic alleles and different specific amplified fragments in both agriproducts. In addition, a core set of markers was defined. According to variability within production soils and products, potential candidate elements linking asparagus (Zn, P, Cr, Mg, B, K) and pistachio (Mn, P, Cr, Mg, Ti, B, K, Sc, S) to the production areas were identified. The Sr isotopic signature was an excellent marker when Italian asparagus was compared with literature data for Hungarian and Peruvian asparagus. This work reinforces the use of Sr isotope composition in the soil bioavailable fraction, as assessed by 1 mol/L NH_4NO_3, to distinguish white asparagus and pistachio originating from different geographical areas.

1. Introduction

Nowadays, food quality and safety are topics of great attention for consumers and any case of food adulteration has a strong impact on public opinion. The traceability of food products is becoming increasingly important for the global economy as a consequence of the pressure that consumers exert on knowing the nutritional value of food as well as its geographical origin and authenticity. Consumers in the European Union require guarantees on the origin of food products, which they take as a pledge for safety and quality (regulations EEC 2081/92 and EC 1898/06). Finding the appropriate tools to provide a fingerprint for geographic origin determination of food products and to establish their 'traceability' may be challenging (Adamo et al., 2012). The traceability system is an important tool for tracking, monitoring and managing product flows through food supply chains, potentially verifying the presence of credence attributes in consumer food purchases (Myae & Goddard, 2012). The Council Regulation (EC) N. 510/2006 established some brands with legal protection, such as Protected Designation of Origin (PDO) "that covers agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognized know-how". Among Italian PDO brands, *White Asparagus from Bassano del Grappa*", produced in province of Vicenza (north of Italy) and "*Green Pistachio from Bronte*", produced in province of Catania, Sicily (south of Italy), represent important examples of food products with an urgent need for analytical approaches that guarantee authentication.

In recent years, there has been an increasing interest in defining analytical techniques to trace food products. Thanks to the new advances in the field of molecular biology, molecular markers have become rapid, sensitive and efficient tools to identify the origin of commercialized products (Martin-Lopes, Gomes, Pereira, & Guedes-Pinto, 2013). Of particular interest are microsatellites (simple sequence repeat (SSR) markers) because, in comparison to other DNA markers, they are faster to use and the results are more clear-cut. One additional advantage of SSR markers is that the small dimension of their target sequences may allow the amplification of DNA degraded or extracted from processed food (Pasqualone, Alba, Mangini, Blanco, & Montemurro, 2010). Molecular markers alone cannot ascertain the geographical origin of the products, thus the use of complementary techniques have been proposed (Drivelos & Georgiou, 2012). In this context, the investigation of stable isotopes has gained increasing importance. In

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particular, the isotopes of H, C, O, N and S have been widely used. Over the last years, geogenic isotopes, all above Sr isotopes, have become increasingly popular as they provide a unique link from soil to primary agricultural products (Brunner, Katona, Stefánka, & Prohaska, 2010; Swoboda et al., 2008). Sr is generally present in food at trace levels (<0.1% (w/w)) and shows a distinct variation in its isotopic composition due to the geochemical differences of soils. In addition, the Sr system does not show a significant isotopic fractionation during plant uptake. As a consequence, the bioavailable fraction in soils provides a unique system to direct link the soil to the plant. Moreover, it has been shown that the seasonal and annual variation of the $^{87}Sr/^{86}Sr$ isotope ratio is not significant, thus it represents a reliable tool that lasts over time (Swoboda et al., 2008). Examples on the use of the isotope ratio measurement to authenticate and trace the geographical origin of agricultural products are reported in cider (Garcìa-Ruiz, Moldovan, Fortunato, Wunderli, & Garcìa Alonso, 2007), grape or wine (Marchionni et al., 2013) and tomato (Trincherini, Baffi, Barbero, Pizzoglio, & Spalla, 2014).

The main objective of this study was to establish a combined analytical tool based on molecular and geochemical markers to identify the geographical provenance of high-quality protected white asparagus and pistachio downstream of the production chain to prevent fraud and counterfeiting. The genetic approach was based on the use of SSR markers. The geochemical fingerprint was built on multi-element data and the isotope ratio of $^{87}Sr/^{86}Sr$ assessed both in soil and plant materials.

2. Materials and methods

2.1. Geo-pedological properties of the cultivation areas, soil and plant sampling

2.1.1. White Asparagus from Bassano del Grappa

The geographical area devoted to the production of PDO *White Asparagus from Bassano del Grappa* (*Asparagus officinalis* var. "*Comune*", hereafter coded BSN) is the plain surrounding the town of Bassano del Grappa (in the province of Vicenza, north Italy), 129 m a.s.l. at the foothills of the Venetian Prealps, where the Brenta river flows. The soil parent material consists of river sediments and gravelly sandy deposits of alluvial fan. Soils have been classified as Cutanic Luvisols (Hypereutric, Endoskeletic, Endoarenic) (WRB, 2006). The land use is dominated by cultivation of corn, whereas autumn-winter cereals (wheat, barley, oats) have a secondary importance. Turions and related cultivation (0–30 cm) soils were sampled from five different farms situated in the Brenta river plain. The altitude of the sampling sites ranged between 112 and 155 m a.s.l. At harvest, turions were in the full-ripe stage. A sampling strategy with three replications per farm was applied, with ten turions collected for each replication. A total of 15 soil and turion samples were collected. More details of samples and sampling sites are given in Table 1. Cultivation soils were characterized by acidic pH ranging between 5.3 and 5.6. Accordingly, soils were poor of carbonates (always below 7 g/kg), sandy-clay and exhibited a moderately high organic carbon content (values ranging from 17.3 to 25.0 g/kg) and a low cation exchange capacity as measured by $BaCl_2$ pH 8,1 (between 8.2 and 10.7 $cmol_+$/kg) (ISO 13536, 1995). In all farms, the asparagus cultivation was managed in full compliance with the PDO *White Asparagus from Bassano del Grappa* production guidelines (European Community Council Regulation EC 1050/2007; GUCE 240/2007; Dossier number IT/PDO/0005/0338). In order to maintain soil fertility, mature cow manure is applied in pre-plantation (60 tons per hectare) followed by yearly NPK fertilization, with nitrogen supply for at least 50% of organic nature. Phosphate and part of potassium fertilization takes place in the fall or at the end of winter, while nitrogen and the rest of potassium in the post-harvest period (no later than July). The annual supply of N, P and K never exceeds the maximum amounts of 150, 80 and 180 kg per hectare, respectively.

2.1.2. Green pistachio from Bronte

The production area of PDO *Green Pistachio from Bronte (Pistachia vera* cv. "*Bianca* or *Napoletana*", hereafter coded BRNT) is located on the north-western foot slope of the Mount Etna, an active stratovolcano on the east coast of Sicily. The altitude of the sampling sites ranges between 460 and 672 m a.s.l. The soil parent material is made of lava flows, scoria cones, spatter ramparts and pyroclastic fall deposits related to flank and summit eruptions. Lava types range from basalt to benmoreite, aphyric to highly porphyritic in texture, with phenocrysts of plagioclase, pyroxene, olivine, variable in quantity and size. Seeds of BRNT and corresponding cultivation soil (0–30 cm) were sampled from five different farms situated in the province of Catania. At the collection time seeds were at the full-ripe stage. A sampling strategy with three replication per farm was applied, with about 500 g of seeds collected for each replication. A total of 15 soil and seed

Table 1
Code and geographical location of the studied samples.

PDO	Region/Municipality	Sample code	Sample	Locality	Latitude N	Longitude E
White Asparagus from Bassano del Grappa	Veneto/Bassano del Grappa	Farm I	Soil Turions	ROSA'	45° 43′ 41,734″	11° 46′ 3907″
		Farm II	Soil Turions	ROSA'	45° 43′ 32,879″	11° 47′ 10,518″
		Farm III	Soil Turions	CASSOLA	45° 44′ 28,416″	11° 45′ 26,903″
		Farm IV	Soil Turions	BASSANO DEL GRAPPA	45° 44′ 16,319″	11° 44′ 52,996″
		Farm V	Soil Turions	CARTIGLIANO	45° 42′ 40,740″	11° 42′ 18,785″
Green Pistachio from Bronte	Sicily/Bronte	Farm I	Soil Nuts	ROCCARELLA	37° 44′ 46,708″	14° 47′ 48,463″
		Farm II	Soil Nuts	GINESTROLA	37° 42′ 45,305″	14° 40′ 1695″
		Farm III	Soil Nuts	MOSCARELLO	37° 43′ 11,602″	14° 48′ 41,357″
		Farm IV	Soil Nuts	ROCCARELLO	37° 44′ 40,903″	14° 48′ 29,742″
		Farm V	Soil Nuts	DAGADI	37° 45′ 19,992″	14° 49′ 6372″