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Multi-element profiles and Sr isotopic signatures to authenticate the geographical origin of high-quality food.

Ph. D. dissertation by
Carmela Zannella

Tutor: Prof. Paola Adamo **Coordinator: Prof. Guido D'Urso**

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ABSTRACT

Nowadays the authentication of food products remains a major issue, especially for those products that receive excellent labels from European Union (i.e. PDO, PGI, TSG) due to their higher price compared to all other non-branded products. The defense of these products helps to prove product authenticity, to combat fraudulent practices and to control adulteration, which are important issues for economic and cultural reasons, as well as contribute to valorize the cultivation territories.

The focal point of this thesis was to study the potential of the elemental composition and $^{87}Sr/^{86}Sr$ isotopic ratio as promising tools for the authentication of agricultural products in relation to their geographic area of cultivation. In this view, four Italian agro-products covered by a Geographic Indication mark were chosen and investigated: "PDO White Asparagus from Bassano del Grappa", "PDO Green Pistachio from Bronte", durum wheat flour to make the "PDO Bread from Altamura" and "PGI Red Onion from Tropea". In addition, the identification of geochemical markers for fish meat directly linked to the ambient water was investigated at the Viris Laboratory at BOKU University (Vienna, Austria) to determine the geographical origin of Austrian fish food.

Both soil and plant materials (from 5 farms per agro-product for three replications per farm) were analysed. The soils were characterized for their physical and chemical properties (pH, organic carbon, cationic exchange capacity, total carbonate content, particle-size distribution), content of 41 macro- and micro-elements (included rare earths) and Sr isotopic ratio ($^{87}Sr/^{86}Sr$) of total and 1M NH_4NO_3-extractable (i.e. bioavailable) Sr. The plant material was analysed for the total content of 33 elements and the total Sr isotopic ratio.

The Sr isotopic ratio was highly promising for agro-product authentication. In particular, the isotopic ratio of Sr found in agro-foods (i.e. Sr uptaken by the plants) was very similar to the isotopic ratio of bioavailable Sr measured in the soils. In contrast, the isotopic ratio of Sr measured in the plants was significantly lower than the isotopic ratio of total Sr measured in the soils. This important outcome has been confirmed in all the analyzed products, except for *Tropea Red Onion* for which the investigation of isotopic ratio of Sr is still ongoing. The isotopic ratio of Sr found in 'White asparagus from Bassano del Grappa' was distinctively different from the Literature values for Sr isotopic ratio of Hungarian and Peruvian asparagus. In contrast, a certain overlap was observed with white asparagus from Austria.

A coefficient of variation (CV) was calculated to estimate the degree of similarity among geochemical profiles of soils and plants collected in the same production areas (i.e. variability within production areas). For 'White asparagus from Bassano del Grappa', twenty elements were characterized by a CV < 10%. Among these elements Ca, Fe, K, Mg, Mn, Na and P were able to

discriminate between asparagus from Bassano del Grappa and asparagus from USA and Spain. For "Green Pistachio from Bronte", P, Cr, Mg, K, Sc and S were the main discriminating elements (CV < 10%). For "durum wheat flour from Altamura", Co, Fe, P, Cr, Mg, Ti and K were the elements with a CV < 10%. From a Literature comparison, the Sr content and the Ca/Sr ratio were able to well discriminate "Green Pistachio from Bronte" from pistachios from Iran, Turkey and USA, the main pistachio world producers. The ratio between $^{87}Sr/^{86}Sr$ vs δ ‰ well discriminate Altamura from China wheat flour.

In the case of "Red Onion from Tropea", the chemical composition of both soils and onions was found highly variable within the production area. None element was characterized by a CV < 10%. Hence, it was not possible to identify a unique geochemical signature for the *Tropea Red Onion*. This was likely caused by the large geographical scale (both in latitude and altitude) of the production area.

The Sr/Ca ratio along with the $^{87}Sr/^{86}Sr$ isotopic ratio was also applied to determine the geographical provenance of fish meat from different Austrian regions. The development of an analytical approach (including sampling, digestion and analysis) was assessed for the usage of Sr isotopes and multi-element fingerprints as tracer of origin in fish meat. In the next step, the acquainted information will to be linked to the water bodies, which has been tested on a limited number of samples as preliminary study. Further studies based on a larger number of samples of both products covered by Geographical Indication label and similar products with no labels (e.g. agro-products from PDO or PGI areas and similar products outside PDO or PGI areas) might improve the robustness of identified 'geochemical signatures' and hence the potential of soil-based indicators for authenticity and geographical provenience traceability.

INTRODUCTION

Food frauds are a submerged business difficult to identify. Over the last few years, this phenomenon is becoming more and more common and requires efficient and rapid tools as well as a robust control system.

Consumers are increasingly aware to the choice of brands and ingredients selection, with greater predisposition to spend more for typical and quality products with quality certifications.

Hygiene, safety and food quality problems are multiple, and are even more amplified when considering the global market context

Ensuring complete food security is a difficult and complex task that requires penetrating control systems, not just "upstream" and "downstream" of production processes, which may have different phases and involve the participation of various raw materials imported from different countries. It is therefore intuitive that the protection of consumer health cannot and should not be confined to national laws.

In this context European Union set out several regulations regarding food authenticity, food quality and geographical origin (Council Regulation no. 510/2006), such as Protected Designation of Origin (PDO), Protected Geographic Indication (PGI) and Traditional Speciality Guaranteed (TSG). These products represent the excellence of European agro-food production and are the result of a unique combination between human and environmental factors linked to their territory. For this reason, the European Union sets out precise rules for their safeguarding with the aim to provide producers with concrete tools to identify and promote their products to protect them from unfair practices.

For health and food safety issues it is important to specify differences between traceability and authentication (although authentication is part of traceability) . In the first case there is the necessity to date back to the origin of the products along the entire supply chain, from production, processing and retailing from the point of origin to the point of sale (Furness et al., 2013). The authentication, indeed, also refers to the possibility to identify food components and its characteristics (Gall et al., 2003).

Since the 1980’s analytical methods are reported to try to determine the geographical provenance of agrifood; in this context it is possible to order the analytical indicators into two main groups: i) direct indicators which include variables that directly relate some chemical characteristics of agrifood with the same characteristics measured in the field area (i.e., metals and isotopes); ii) indirect indicators which are related to the compositional characteristics of the agri-food and

processing processes (with different analytical techniques). In this case, the chemiometric processing of analytical data, are indispensable tools for the these purposes.

Various techniques have been proposed to discriminate agri-food products on the basis of their production area (Luykx and van Ruth, 2008). The main techniques are: mass Spectrometry (isotope ratio mass spectrometry (IRMS), inductively coupled plasma mass spectrometry (ICP-MS), proton transfer reaction mass spectrometry (PTR-MS), gas chromatography mass spectrometry (GC-MS)); spectroscopy (nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), fluorescence spectroscopy, atomic spectroscopy); separation (high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE)); others (sensor technology, DNA technology, sensory analysis).

The geochemical analytical methods for traceability and food safety include also the stable isotope ratio and the multielement analysis.

Most of traceability and authentication studies focus on the employment of stable isotope ratio (both light and heavy) as potential geochemical tracers. Although isotopes have the same number of electrons (and protons), they have different atomic masses; therefore two isotopes of the same element have similar chemical properties, because of the same number of protons, but different physical properties, due to the different mass.

During natural or artificial biogeochemical phenomena, isotopic fractionation occurs, and the degree of fractionation is generally dependent on the relative difference in mass between the stable isotopes of a given element and the temperature at which the fractionation process occurs (Nakano, 2016). This is at the basis of the variability of the isotope ratios in the environment that can be used as potential tracers to assess, for example, if equal agro-products, which are chemically similar, have different provenance.

Among the most studied stable isotopes, there are H, C, N, O, and S, which are also the main elemental constituents of bio-organic materials and their isotopes, classified as light stable isotopes, can be used as geochemical tracers (^{2}H, ^{1}H; ^{13}C,^{12}C; ^{15}N,^{14}N; ^{18}O,^{17}O,^{16}O; ^{36}S, ^{34}S, ^{33}S, and ^{32}S) (Camin et al., 2016). They have a large isotopic fractionation that reflect the large relative difference in mass between their various isotopes, due to different biological processes.

As reported by Kelly et al. (2005), the fractionation phenomena is strictly related to each type of isotope: for the D/H and $^{18}O/^{16}O$ the evaporation, condensation and precipitation; for $^{13}C/^{12}C$ ratio the carbon fixation by plants, for the $^{15}N/^{14}N$ ratio the agricultural practices and for $^{34}S/^{32}S$ the sulfate-reduction bacteria.

Another interesting alternative to the light stable isotope analysis is the investigation with heavy elements, especially those that are classified as petrogenetic elements (i.e. Sr, Nd, Pb).

In last few years many works focused on the employment of the Sr isotopic ratio as a possible geochemical marker to determine the geographical origin of many agricultural products, such as rice (Lagad et al., 2017), olive oil (Medini et al., 2015), wine (Mercurio et al., 2014) and many others.

Strontium is an alkaline earth metal, and natural strontium is a mixture of four stable isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr. Only the isotope ^{87}Sr is radiodiogenic, indeed it derives from the natural radioactive β^- decay of ^{87}Rb. Rb has been widely used to date rocks, since it occurs naturally in the minerals leucite, pollucite, carnallite, and zinnwaldite. The $^{87}Sr/^{86}Sr$ ratio is strongly influenced by the the initial Rb and Sr content in rocks or minerals and the time elapsed since its formation. The Sr isotope ratio is characteristic of different rock types, indeed $^{87}Sr/^{86}Sr$ ratios are high in old continental rocks, like granites, and low $^{87}Sr/^{86}Sr$ ratios are found in the mantle and rocks (basalts).

This is one of the most interesting techniques used for food traceability and authentication, based also on the assumption that during nutrient uptake by plants, the transfer of Sr from soil to plant occurs with a minimal isotopic fractionation.

The use of multielement analysis employed for food provenance studies is another interesting approach to authenticate the products in relation to the geographical area of cultivation. Environmental factors are characteristic of the quality of agro products. Climate factors, agronomic management and soil quality, for example, play an important role in obtaining a quality product that is typically linked to their territory of origin. The multi-element approach is based on the fact that the element composition can be transferred from soil to agricultural products and therefore any differences in the multi-element distribution between the different geographic areas can be reflected in agro products. Sometimes this approach is based only on the analysis of selected elements, as some kind of metals: alkaline metals (e.g. Li, Na, Rb), alkaline earth metal (e.g. Ca, Mg, Sr, Ba), transition element (e.g. Mn, Ni), lanthanides (e.g. La, Ce, Nd).

One interesting approach to authenticate agri-food provenance is the use of the Sr/Ca ratio. Indeed Sr and Ca are divalent elements with similar ionic radii, so they are considered geochemically similar (Faure & Mensing, 2004); it has the similar Ca-behavior in many geo- and bio-geochemical processes, hence it should represents a tracer for the nutrients flow into the soil-plant system (Nakano et al., 2016). It is biologically considered to be a nonessential element and since the Sr cycle is dominated by carbonate, attention has often focused on the Sr/Ca ratio as a potential tool to reconstruct and authenticate the environment in which agro-products have grown (Phillis et al., 2011).

Different works have focused on food traceability and authentication by the employment of multi element approaches both for agri-food in relation to the cultivation soil in which they were grown, such as for olive oil (Benincasa et al., 2007), tomatoes (Spalla et al., 2009; Lo Feudo et al., 2010), rice (Cheajesadagul et al., 2013; Gonzálvez et al., 2011), wheat grain (Zhao et al., 2013).

However, identifying the right variables in this context is very difficult, due to the great complexity and heterogeneity of the soils as a chemical matrix that can reflect the characteristics of the products.

OBJECTIVES

The Geographical Indication labels are designed to protect the European most precious food and drinks, with production processes and geographical areas qualifying the foodstuff. Italy is the European top seller of protected foods, but is also one of the top countries to have an high number of cases of food frauds. Consumers are willing to pay more for protected foods and that is why there is interest in defrauding products with Geographical Indication labels. Unfortunately, currently it does not exist a real formal system to examine the counterfeits and hence protecting the uniqueness of the foodstuff.

In this context, this thesis aims to explore the potential of soil-related indicators (i.e. multi-element composition and $^{87}Sr/^{86}Sr$ isotopic ratio) as options for the authentication of some agri-food covered by Geographical Indication label, to enhance their traceability and geographical provenance.

In **Chapter 1**, a first approach is tested based on a genetic (simple sequence repeat) and geochemical (multi-element and $^{87}Sr/^{86}Sr$ ratio) analysis to prove the geographical origin of two high-quality PDO (Protected Designation of origin) Italian products "*White Asparagus from Bassano del Grappa"* (Veneto region, north Italy) and "*Green Pistachio from Bronte"* (Sicily region, south Italy).

In **Chapter 2,** a geochemical fingerprint of Altamura wheat flour is proposed, by the use of elemental and Sr isotopic ratio to authenticate the first bread in Europe which has gained the PDO certificate in its category *"Bread from Altamura PDO"*, made from durum flour of Apulia region (south Italy).

In **Chapter 3,** a multi-element approach is used to determine the geographic origin of *PGI Tropea Red Onion* grown in Calabria region (south Italy).

In **Chapter 4**, the investigation focused on the development of an analytical approach (including sampling, digestion and analysis) to assess the usage of Sr isotopes and multi-element fingerprints as tracer of origin in fish meat. In the next step, the acquainted information will to be linked to the water bodies, which has been tested on a limited number of samples as preliminary study. This part of the thesis was carried out at the Viris Laboratory of BOKU University (Vienna, Austria) where I spent 6 months (from 17/10/2016 to 30/04/2017) of my PhD course.

In Chapters 1, 2 and 3 all the analysis were carried out both on soil and plant materials, along with a geo-pedological account of the production areas. Wherever possible, a validation of the geochemical fingerprints of the investigated food products was done through a comparison of our data with data from Literature for similar products cultivated in other geographic areas.

Chapter 1

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

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Carmela Zannella [a,1], Francesca Carucci [a,1], Riccardo Aversano[a], Thomas Prohaska[b], Simona Vingiani[a], Domenico Carputo[a], Paola Adamo[a]*

[1]Co-first authors.

*Corresponding author at Department of Agricultural Sciences, University of Naples Federico II, 80055, Via Università 100, Portici, Italy.

E-mail address: paola.adamo@unina.it, Tel.: +39 0812539172, fax: +39 0812539186

[a] Department of Agricultural Sciences - University of Naples Federico II, 80055, Via Università 100, Portici, Italy

[b] Department of Chemistry - VIRIS Laboratory, University of Natural Resources and Life Sciences, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria

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.

Keywords: *Fingerprinting, Protected Designation of Origin mark, traceability, molecular markers, strontium isotopic signature, multielement analysis, geographic origin*

Highlights

- DNA fingerprint, $^{87}Sr/^{86}Sr$ ratio and geochemical profiling were applied for PDO signature;
- Microsatellites were useful in varietal identification and discrimination;
- $^{87}Sr/^{86}Sr$ ratio of soil bioavailable Sr is useful to trace geographical origin;
- Geochemical profiling improves the discrimination power of Sr isotopic signature;
- Geochemical profiles were more homogeneous in soils than in plants.

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 et al., 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 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 (Swoboda et al., 2008, Brunner, Katona, Stefánka, & Prohaska, 2010). 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 et al., 2007), grape or wine (Marchionni et al., 2013) and tomato (Trincherini et al., 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 samples were collected. More details of samples and sampling sites are given in Table 1. Cultivation soils had an acidic pH (4.7 – 5.9). Carbonates contents were below 4 g/kg and cation exchange capacity ranged from 17.1 – 31.5 $cmol_+$/kg. Soils were predominantly sandy with organic carbon content of 24 to 50 g/kg. In all farms, the pistachio cultivation was managed in full compliance with the PDO *Green Pistachio from Bronte* production guidelines (European Community Council Regulation UE 21/2010; GUUE 8/2010; Dossier number IT/PDO/0005/0305). Pistachio is cropped without fertilization.

2.2 Plant and soil preparation

Asparagus turions were cleaned from adhering soil, washed with MilliQ water, dried and cut into pieces of approximately the same size. Pistachio seeds were cleaned manually by the husk immediately after their collection and were divided into three sub-samples: whole sample, peel and pulp. All asparagus and pistachio samples were placed in Falcon and freeze-dried in a DELTA 1–24 LSC freeze−dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), until complete dehydration. Asparagus turions were pulverized using agate mill jars, while pistachio materials were milled in a mortar with the use of liquid nitrogen. The freeze-dried samples were stored at -20 °C. All cleaning operations and management of the plant material were performed

under a glove box to avoid contamination. The geochemical surveys were conducted on samples of surface soil (0-30 cm). Soils were air-dried and 2 mm sieved and subsequently the residual moisture was determined at 105 °C.

2.3 DNA extraction

DNA was extracted from asparagus turions of BSN and seeds of BRNT. Included in the analysis were also turions of four commercial varieties (*Argenteuil*; *Green Asparagus from Maremma Tosco-Laziale*; *Connovers Colossal* and a *White Peruvian Asparagus,* hereafter coded ARG, MTL, CON and PER, respectively) and seeds of four commercial pistachio products (Marketed Pistachio flour "*Sicilia perfetta*"; Pistachios from Iran; Pistachios from Turkey; Pistachios from USA hereafter coded SCL, IRN, TRK and USA, respectively). In particular, the marketed Pistachio SCL was included in the study because its geographical area of production (Bronte), corresponds to that of BRNT, and this makes it as "*Green Pistachio from Bronte*"-like. DNA was isolated from asparagus turions and pistachio seeds using the *DNeasy* plant mini kit (Qiagen, Valencia, CA, USA), properly modified to optimize the extraction from tissues different from leaves. For each variety three samples were analysed and a reference DNA was extracted from leaves of each of them. DNA concentration and quality was estimated through NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and electrophoretic run on agarose/TAE gel 0.8%. Extracted DNA have been diluted to a concentration of 15 ng/µL and stored at -20°C.

2.4 SSR amplification and analysis

Fifteen primer pairs for asparagus amplification were chosen from two sources: 2 (AA01, AA03) from Aceto et al. (2003) and 13 (TC1, TC2, TC7, AG2, AG3, AG5, AG7, AG8, AG10, AG11, AG12, AAT1, AGA1) from Caruso, Federici, & Roose (2008) (Supplementary Table 1). As for pistachios, 14 primer pairs (Ptms11, Ptms12 and Ptms47, Ptms3, Ptms42, Ptms41, Ptms7, Ptms31, Ptms10, Ptms33 and Ptms45, Ptms9, Ptms14 and Ptms40) were chosen from Ahmad, Ferguson & Southwick (2003) (Supplementary Table 2). Asparagus SSR were designed on expressed sequences while pistachio SSR came from genomic sequences. The forward primer of each set was M13-tailed to facilitate subsequently labelling with a fluorophore (Oetting et al. 1995). PCR amplification was carried out with a Veriti^{TM} Thermal Cycler (Applied Biosistems, Foster City, CA, USA) under varying annealing temperatures (T*a*), depending on the primer pair. Each PCR reaction included the M13-tailed forward primer, the reverse primer and a M13 forward primer 5'-end-labeled with a fluorophore. PCR reactions were performed in a 20 µL volume containing 1× reaction buffer with 1.6 mM $MgCl_2$, 0.2 mM of each dNTP, 1 unit of go*Taq*polymerase (Promega, Madison, WI, USA),

20 pM of each primer and 30 ng of genomic DNA. Cycling conditions consisted in two different cycles for the different species analyzed (Supplementary Table 3). Amplification products were checked and quantified on 2% agarose/TAE gel using 1 Kb plus ladder (Life Technologies, Carlsbad, CA, USA) and displayed at the trans illuminator. Each gel was first visually examined, and amplicons were then separated with the ABI PRISM® 3130 DNA Analyzer system (Life Technologies). Size calibration was performed with the molecular weight ladder GenScan® 500 ROX™ Size Standard (Life Technologies). Electropherograms were analyzed using the software Peak scanner ver. 1.0 (Applied Biosystems) following the factory default sizing algorithm modified in values of quality flags (pass range 0.1-1; low quality range 0-0.05). Polymorphisms of useful primer pairs were surveyed in all the individuals and several parameters of genetic diversity, including the number of alleles per locus (Na) and the number of alleles (Ne), allele size range, number of private alleles and observed heterozygosity (Ho) were calculated using GenAlex v. 6.5 (Peakall & Smouse, 2012). Also, microsatellite effectiveness for differentiating among species was based on the following parameters: Power of Discrimination (PD $=1-\Sigma p_i^2$, where p_i is the frequency of the i^{th} genotype calculated for each SSR) and the Polymorphic Information Content (PIC, calculated according to the formula above, but with genotypic frequency replaced by the allele frequency). For each variety, data were scored for the presence or absence of each allele in all genotypes. A cluster analysis was performed based on a similarity matrix calculated using the Dice's coefficient (Sneath & Sokal 1973) and using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) hierarchical clustering method. The analysis were carried out through a server running the program Dendro_UPGMA (http://genomes.urv.es/UPGMA/) (Garcia-Vallve, Palau, & Romeu, 1999).

2.5 Multielement analysis

Multielement analysis of soil and plant materials was carried out at Acme Analytical Laboratories Ltd (Vancouver, Canada) by Perkin Elmer Elan 6000 ICP-MS. As for soils, Acme's Group 1EX package (HNO_3-$HClO_4$-HF digestion) for 41 elements (Ag, Al, As, Au, Ba, Be, Bi, Ca, Ce, Cd, Co, Cr, Cu, Fe, Hf, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, S, Sb, Sc, Sn, Sr, Ta, Th, Ti, U, V, W, Y, Zn, Zr) was used. As for plant materials, Acme's Group 1VE – MS package (HNO_3 digestion followed by *aqua regia*) for 53 elements (Au, Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hf, Hg, In, K, Ge, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Pt, Rb, Re, S, Sb, Sc, Se, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn, Zr, Pd) was employed.

The degree of similarity among geochemical profiles of soils and plants collected in the same production areas was evaluated by single element and overall coefficient of variation (CV),

expressed as percentage, and defined as the ratio of the standard deviation to the mean.

2.6 Sr isotope ratio

For the determination of total Sr in soil, microwave (Anton PaarMultiwave 3000, Anton Paar, Graz, Austria) assisted acid (HF-HCl-HNO_3) digestion of milled soil was carried out into Teflon vessels. The digested samples were evaporated to almost dryness. *Aqua regia* and double sub-boiled HNO_3 were added and again evaporated at 90°C to a volume of 0.5 mL. The volume was finally filtered (0.45 µm) and filled up with 8 mol/L HNO_3. The bioavailable Sr fraction was extracted from the soil by 1 mol/L NH_4NO_3 following the extraction procedure proposed by the German national standard DIN 19730 (Prueb, 1997). To obtain accurate results with minimum uncertainty, a Sr/matrix separation prior to measurement by MC-ICPMS was done, using a Sr specific resin (ElChrom Industries, Inc., Darien, IL, USA) with a particle size of 100 µm – 150 µm according to a standard procedure (Swoboda et al., 2008). The Sr isotope ratios in the obtained solutions were measured with a double-focusing sector field MC ICP-MS instrument (Nu Plasma HR, Nu Instruments, Wrexham, Wales, UK) with a desolvating membrane nebulizer (DSN 100, Nu Instruments, Wrexham, Wales, UK) using optimized operational parameters (Swoboda et al., 2008). An SRM 987 solution (cert. $^{87}Sr/^{86}Sr = 0.71034 \pm 0.00026$) was used to calibrate the instrument for instrumental isotopic fractionation by applying a standard – sample - bracketing approach. The 'measure zero' method of the NU Plasma software was used for the blank correction-using instrument (Nu Plasma HR, Nu Instruments, Wrexham, Wales, UK) measuring separation blanks within the measurement sequence

3. Results and discussion

3.1 Genetic signature

3.1.1 Asparagus

The initial screening for the selection of markers was conducted on 15 SSRs. Five loci were excluded because of their pattern profiles: AA03, AG11 and AG5 were affected by the presence of unamplified (null) alleles for BSN, while TC2 band profiles were not easy to interpret because of the excess of stutter bands. Finally, AGA1 amplified bands larger than 600bp, as also reported in Caruso, Federici, & Roose (2008), and for this reason it was not included. The remaining 10 SSR loci (TC1, AAT1, AG2, AG3, AG7, AG8, AG10, AG12, TC7 and AA01) showed reliable polymorphisms, confirming previous data (Caruso, Federici, & Roose, 2008). Overall, we identified 40 alleles, with an average of 4 alleles per locus (Table 2). Primers TC1, AAT1 and AG2 produced the highest number of alleles (respectively 8, 5 and 5 alleles per locus). Out of ten loci, seven were

useful to identify variety-specific alleles (hereafter named private alleles). These are alleles that are found only in a single sample among a broader collection (Szpiech & Rosenberg, 2011). Private alleles are important not only in traceability studies (Adamo et al., 2012) but also in molecular ecology and conservation genetics (Kalinowski, 2004) as well as human evolutionary genetics (Schroeder et al., 2007). Our results revealed four private alleles for BSN (three from locus TC1 and one from AG10). Private alleles were also found for the other varieties. Locus TC1 produced the highest number of private alleles (Supplementary Figure 1), with informative results in three different varieties. AAT1, AG2 and AG10 produced two private alleles each, whereas AG3, AG7 and TC7 gave one private allele. Loci AAT1 and TC1 confirmed their power to discriminate unambiguously varieties from each other as shown in Caruso, Federici, & Roose (2008). The level of informativeness of SSR was estimated as PIC and PD (Table 2), two indices commonly used to evaluate informativeness degree of microsatellites. PIC is based on allele frequencies, whereas PD is based on banding pattern or genotype frequencies at a given locus. The highest PIC value for polymorphic loci was observed in TC1 (0.74) while the lowest in AA01 (0.35). The average PIC value (0.55) indicates a relatively high genetic similarity among varieties studied here. This was expected since cultivated asparagus has a narrow genetic base derived from breeding (Moreno et al., 2006). It is reported that nearly all existing varieties derive from the "Violet Dutch" (s XVIII) (Knaflewski, 1996). In spite of the high similarity between varieties, our results did identify a core set of highly informative primers (TC7, TC1, AAT1, AG2 and AG10) with PIC values higher than 0.60. The PD index, which can range between 0 (monomorphism) and 1 (highly informative), varied from 0.91 to 0.96. It should be pointed out that all samples showed values of discriminant power (PD) higher than 0.90, indicating a good strength of the selected markers. To clarify the genetic relationship between BSN and the outgroup varieties, an UPGMA (unweighted pair groups method using arithmetic averages) dendrogram was built using the similarity coefficient of Dice (Supplementary Table 4 and Fig. 1a). According to the genetic distances, two main groups were distinguished. All varieties were grouped together with the only exception of MLT, which clustered apart. Within the main cluster, the distribution of the varieties highlighted the genetic difference between BSN and PER, the latter often confused with the former PDO.

3.1.2 Pistachio

Only few studies are available that analyse the genetic structure of pistachio varieties. For this reason, a limited number of SSR markers is available for *P. vera,* such as those identified by Ahmad, Ferguson & Southwick (2003) and Zaloğlu, Kafkas, Doğan, & Güney (2015). In our study, three loci (Ptms11, Ptms12 and Ptms47) did not produce any amplification so they were considered

null alleles and as such were excluded. The remaining 11 microsatellites produced 31 alleles and gave easily interpretable and reproducible profiles. Three of them (Ptms9, Ptms14 and Ptms40) were monomorphic, the others produced polymorphisms, with a number of alleles ranging from two to five (on average 3.9 alleles per locus) (Table 3). Similarly, Ahmad, Ferguson & Southwick (2003) obtained an average of 3.6 alleles per locus from the analysis of 17 varieties with 14 SSR primer pairs. Zaloğlu, Kafkas, Doğan, & Güney (2015) reported a value of 3.6 alleles per locus analyzing 47 polymorphic loci in 7 varieties. Table 3 also reports alleles identified for each analyzed marker and values of the main genetic parameters. Locus Ptms7 showed a band pattern more complex than expected, with more than two alleles for each sample analyzed. This result is in accordance with those previously reported in pistachio (Ahmad, Ferguson & Southwick, 2003; Arabnezhad, Bahar, & Pour, 2011). Since pistachio is diploid (2n=2x=30), there is no possibility of tetraploidy. Hence, amplification of more than two loci was explained by assuming the presence of two different copies of the same locus, probably as the result of a past event of duplication of the chromosome fragment containing the locus (Sánchez-Pérez et al., 2005). The presence of two alleles in all the samples made us also assume that one of the two loci was subjected to a stronger selective pressure and therefore had no variability. These two alleles were excluded from the analysis, while the remaining alleles were considered in the count of polymorphic alleles. Primers Ptms42and Ptms10 produced the highest number (i.e. 5) of alleles per locus. Six loci (Ptms3, Ptms14, Ptms42, Ptms41, Ptms7 and Ptms10) gave private alleles. Eight of them were identified in our PDO variety BRNT at four different loci. Among these, three loci (Ptms3, Ptms7 and Ptms10) provided the highest percentage of polymorphic fragments, allowing us to distinguish BRNT from the other varieties. The highest and lowest PIC values belonged to Ptms7 (0.66) and Ptms33 (0.46) loci, respectively. The average PIC value (0.57) indicated the presence of high genetic similarity among genotypes. However, the relatively low level of genetic diversity in the studied samples was expected because of the dioecius and outbreeding nature of the cultivated pistachio as well as the high level of heterozygosity due to the cross pollinating nature of the plant established during evolution and domestication (Kebour, Boutekrabt, & Mefti, 2012). It could also be the result of the high pressure for commercially important traits such as nut size and productivity (Ahmad, Ferguson & Southwick, 2003). Table 3 also reports the percentage of polymorphic profiles (82%), the average number of alleles per locus (3.9) and the average PIC value (0.57). These values confirmed that SSR markers employed here are useful tools for genetic studies in pistachio. In spite of the low level of genetic diversity among our *P. vera* varieties, the similarity values of DNA profiles computed using Dice coefficient (Supplementary Table 5) and presented in the UPGMA dendrogram showed a clear separation of all the genotypes (Fig. 1b), with BRNT clustering apart

from the other varieties. This cluster is supported by an high bootstrap value (95%). Our results are consistent with those by Hormaza, Dollo, & Polito (1994) and Zur, Heier, Blaas, & Fauhl-Hassek (2008), who reported that American pistachios are closely related to Iranian ones, while Turkish genotypes show more differences.

3.2 Geochemical signature

3.2.1 Asparagus

The total concentration of single elements in soils and turions from the five production farms of BSN are given in Supplementary Tables 6 and 7. In Supplementary Figure 2 the chemical composition of soils and turions are compared graphically as farm geochemical profiles. Elements present in all samples with concentrations below LOD are not reported. A high degree of similarity was found among geochemical profiles of the soils collected in the different farms (overall coefficient of variation 13%). As shown in Supplementary Table 6 (and Supplementary Figure 2), the chemical elements whose content in soils from different farms was characterized by a CV < 10% were 20; among them Bi, Ti, Na, Zr, Tl. On the other hand, a greater inhomogeneity was observed among the geochemical profiles of turions samples taken from the five different farms (Supplementary Table 7 and Supplementary Figure 2). Indeed, overall CV for turions was 36%, with only five elements showing a CV lower than 10% (P, Mg, B, K and S). While many authors dealt with element content in asparagus edible parts (Amaro-López, Cosano, Rojas, & Garcia-Gimeno, 1996; Makus, 1994), only few papers consider the element content in relation to geographical origin (Hopkins & Eisen, 1959). Moreover, most papers are restricted to several elements of known human nutritional value (Amaro-López, Cosano, Rojas, & Garcia-Gimeno, 1996) and none of them investigate the soil-plant relationship (Gonzálvez, Armenta, & De La Guardia, 2011). According to Gonzálvez, Armenta, & De La Guardia (2011), a 'good' discriminant element requires large between-groups variability compared with within-groups variability. In our study, the elements P, Mg, B, K and S showed the lowest variability within production farms (CV < 10%) and might be considered potential candidate as discriminant elements for authentication of BSN. All of them are essential elements for metabolic processes (Kabata-Pendias, 2010), and only few of them were considered by Mercurio et al. (2014) as good soil origin markers.

The Sr concentration in the investigated asparagus samples ranged from 1.27 and 2.37 mg/kg (dry weight), in agreement with the values reported by Swoboda et al. (2008) in asparagus samples from Slovakia, Hungary and Austria. The $^{87}Sr/^{86}Sr$ isotopic ratios of total and NH_4NO_3 extractable Sr in soils and total Sr in asparagus are given in Fig. 2. The Sr isotope ratio of asparagus (n=15, range 0.7078 – 0.7096, 2σ 0.0006) is in good agreement with the Sr isotope ratio of bioavailable fraction

in soils (n=15, range 0.7064 – 0.7095, 2σ 0.0011). This was true in all five investigated farms (Fig. 2). By contrast, the total Sr isotope ratio in soils (*n*=15, range 0.7265 – 0.7324, 2σ 0.0020) was significantly different compared to the Sr taken up by asparagus, showing much higher values than those in asparagus and consequently in the bioavailable fraction in soils. These results prove the necessity of assessing the Sr bioavailable fraction because the total Sr cannot be taken as proxy for provenance. This observation is in good agreement with previous studies (Swoboda et al., 2008; Brunner, Katona, Stefánka, & Prohaska, 2010). In addition, similar to the observations by Swoboda et al. (2008), the variation of the Sr isotope composition of total Sr in soil was remarkably larger compared to the NH_4NO_3 extractable fraction. This result is likely related to the geological heterogeneity of the sediments brought by Brenta river and filling the plain where soils formed, in comparison with the more homogeneous composition of the ground water of the whole area. The median ± 2 times the standard deviation of Sr isotope ratio of asparagus from Bassano del Grappa was 0.7087±0.0006. This range reflects the probability of 95% that a sample from the area can be identified as an asparagus sample from Bassano del Grappa. Values measured for Bassano del Grappa asparagus lied between the ranges measured by Swoboda et al. (2008) for Hungary (0.7069±0.0011) and Marchfeld (0.7095±0.0008) asparagus, with no overlapping within the total range for asparagus from Hungary but with a certain overlapping for asparagus from Austria. Peruvian asparagus, which is commonly a fraud product for BSN, could be clearly distinguished (0.7079±0.0002), although only a limited number of samples were analyzed by Swoboda et al. (2008) to represent statistically relevant data. The range of the Sr isotope composition of total soil from different Bassano del Grappa asparagus farms was significantly higher than that found for soils from the Marchfeld region. This likely reflects the different elemental composition and geologic history of the two geographical areas. On the contrary, the ranges of Sr isotope composition of the NH_4NO_3 extracts were comparable. This explains the observed overlapping between the median ± 2 times the standard deviation of Sr isotope ratio of asparagus from Bassano del Grappa and Marchfeld area.

3.2.2 Pistachio

The concentration of single elements in soils and corresponding pistachio nuts (whole sample) from the five production farms of BRNT are given in Supplementary Tables 8 and 9. In Supplementary Figure 3 the chemical composition of soils and whole nuts are compared graphically as farm geochemical profiles. Elements present in all samples with concentrations below LOD are not reported. An overall 16% CV existed among geochemical profiles of pistachio soils. Chemical elements showing a CV < 10% were 12, among them Zn, Mn, Fe, V, Ti and Al. A slightly higher

degree of similarity characterized the geochemical profiles of the whole nuts (overall CV 19%). Elements with CV < 10% were 10, among them P, Cr, Mg, K, Sc and S.

Differences in terms of element concentration were observed between peel and pulp nut components (Supplementary Tables 10 and 11 and Supplementary Figure 4). Some elements tended to accumulate more in the pulp than in the peel, such as Cu (pulp 15.3±3.3, peel 7.1±1.4 mg/kg), Zn (pulp 26.7±4.9, peel 16.3±1.8 mg/kg) and S (pulp 5004±432, peel 2513±382 mg/kg). Some others accumulated more in the peel than in the pulp. Among them Mo (peel 0.97±0.29, pulp 0.40±0.06 mg/kg), Ag (peel 15.1±5.2, pulp 5.5±0.7 mg/kg), Fe (peel 124±10, pulp 84±11 mg/kg) and Ca (peel 2005±233, pulp 1275±229 mg/kg). The overall CV of pulp subsamples was similar to that of the whole nuts (21%). Only six elements showed a CV < 10% (P, Cr, Mg, B, K and S). A slightly higher dissimilarity was observed among geochemical profiles of the peel subsamples, showing an overall CV of 26%, with five elements having CV < 10% (Fe, P, Cr, K and Sc). According to Anderson & Smith (2005), Sr is the most discriminating element among the Iranian, Turkish and US (California) pistachios. Iranian pistachios had high Sr concentration (> 20 μg/g); one set of samples from Turkey also had a high Sr (24.5 μg/g), whereas the other Turkey samples and all Californian samples had a Sr concentration near or below the detection limit (< 1 μg/g). Our pistachios from Bronte had a very low Sr concentration (1.03 to 2.17 μg/g), significantly different from the Iranian and Turkish pistachios. The calcium/strontium ratio proposed by Kabata-Pendias (2010) for better understanding source and uptake of cations provided an additional discriminating power compared to Sr concentration. Indeed, the Iranian, Turkey and USA samples reported by Anderson & Smith (2005) showed a Ca/Sr ratio generally much lower than that of samples from Bronte (Iranian 42-129; Turkey 103-1038; USA 197-532; Bronte 622-1193). The comparison between data from Anderson & Smith (2005) and ours was also performed for the elements Mn, Fe, K, Ca and Mg having low variability between the production farms. Iranian, Turkey and USA pistachios had Mn concentration ranging between 6.7-17.5, 8.4-13.1 and 11.6-11.8 μg/g, respectively. Pistachios from Bronte displayed a Mn concentration of 6.7-8.7 μg/g, hence a value generally lower than all the other pistachios. Iron was generally lower in the Iranian, Turkey and USA samples (overall range 24 – 55 μg/g) than in pistachios from Bronte (64.0 – 82.3 μg/g), suggesting a high discriminating power also of this element for Italian products. By contrast, there were not remarkable differences in the concentrations of macroelements such as K, Ca and Mg.

The Sr isotope composition in soils and pistachio nuts (whole sample) is given in Fig. 3. Since the isotopic fraction in plant proved to be highly correlated to the bioavailable fraction in soil, only the bioavailable Sr isotopic ratio was assessed. A direct correspondence between plant (nuts) and soil at each site was observed. The Sr isotope ratio for nuts ranged from 0.7057 to 0.7072 (n=15; 2σ

0.0005), while for the soil bioavailable fraction the range was 0.7059 – 0.7071 (n=15; 2σ 0.0004). This reinforces the evidence that the Sr in the NH_4NO_3 solution corresponds to the soil Sr source, which is taken up by pistachio plants. The geographic origin of pistachio from Iran, Turkey and California using stable isotope exploration (δ15N‰ and δ13C‰) was studied by Anderson & Smith (2006). Geographic regions were well separated, but seasonal differences were found to affect the discriminating power of isotopes. Sr isotope ratio has the advantage to vary only as a function of the age of the surface geology (Marchionni et al., 2013) and does not undergo significant fractionation during plant uptake due to biological processes (Baroni et al., 2011).

4. Conclusions

The results of this work show DNA fingerprints, geochemical profiling and Sr isotope ratio as promising tools for authentication studies of the high-quality Italian products "*White Asparagus from Bassano del Grappa"* and "*Green Pistachio from Bronte"* according to their geographical area of origin. We have confirmed the usefulness of microsatellites in the identification and discrimination of asparagus and pistachios varieties, even within a small sample with high genetic similarities caused by previous breeding activities. A molecular catalogue is therefore available that can help to compare the molecular patterns of PDO products investigated here with those of other samples. A representative soil and plant geochemical and Sr isotope composition database for authentic "*White Asparagus from Bassano del Grappa"* and "*Green Pistachio from Bronte"* was also produced. The high correlation between the Sr isotopic ratio in plant and in the bioavailable fraction of soil as assessed by 1 mol/L NH_4NO_3 suggests using bioavailable Sr instead of total Sr to distinguish products originating from different geographical areas. The use of trace elements may improve the discrimination power of Sr isotopic signature. In our study, Zn, P, Cr, Mg, B and K, were identified as the less variable elements in plant samples, showing a potential for authentication of "*White Asparagus from Bassano del Grappa"*. For "*Green Pistachio from Bronte"* a similar or even better potential was observed for Mn, P, Cr, Mg, Ti, B, K, Sc and S. The validity of the multidisciplinary approach used in this study deserves future research involving a larger sample size, replicated growing seasons and more samples or data of similar agricultural products for comparisons.

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Tables

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' 3,907"
		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°40’1,695’’
		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°49’6,372’’

Table 2. Locus name, allele size, private alleles identified in bold and genetic parameters (Na: number of alleles; Ne: effective number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content; PD: discriminant power) for each analysed locus of asparagus varieties.

Loci	Allele sizes	Na	Ne	Ho	PIC	PD
TC1	**234**, 238, 241, 243, 244, **245**, 248, **250**	8.00	3.84	0.77	0.74	0.96
AAT1	234, 235, 239, 240, 241	5.00	2.91	0.46	0.66	0.94
AG2	166, 169, 171, 175, 177	5.00	2.49	0.69	0.60	0.93
AG3	237, 239, 241	3.00	2.00	0.77	0.50	0.92
AG7	186, 194, 196, 198	4.00	2.27	0.62	0.56	0.92
AG8	247, 249	2.00	1.83	0.08	0.45	0.91
AG10	179, 196, 202, **296**	4.00	1.74	0.54	0.43	0.92
AG12	249, 250, 253	3.00	2.70	0.54	0.63	0.92
TC7	228, 231, 235, 241	4.00	2.70	0.46	0.63	0.92
AA01	257, 258	2.00	1.55	0.00	0.35	0.91
Mean		*4.00*	*2.40*	*0.49*	*0.55*	*0.93*

Table 3. Locus name, allele size, private alleles identified in bold and genetic parameters (Na: number of alleles; Ne: effective number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content; PD: discriminant power) for each analysed locus of pistachio varieties.

Loci	Allele sizes	Na	Ne	Ho	He	PIC
PTMS3	**153,** 159, 160, 162	4.00	2.70	0.95	0.63	0.63
PTMS7	**185**, 191, 195, **200**	4.00	2.97	0.55	0.66	0.66
PTMS9	147	1.00	1.00	0.00	0.00	0.00
PTMS10	**174**, **163**, **153,** 158	5.00	2.04	0.20	0.51	0.51
PTMS14	123, 128	2.00	1.05	0.05	0.05	0.56
PTMS31	159, 142, 170	3.00	2.29	1.00	0.56	0.56
PTMS33	163, 174	2.00	1.88	0.75	0.47	0.47
PTMS41	**225**, 230, 234, **236**	4.00	2.80	0.70	0.64	0.64
PTMS42	149, 193, 203, 205, 215	5.00	2.56	0.95	0.61	0.61
PTMS40	221	1.00	1.00	0.00	0.00	0.00
PTMS45	193, 222	2.00	2.00	1.00	0.50	0.50
Mean		3.00	2.00	0.68	0.52	0.57

Figures

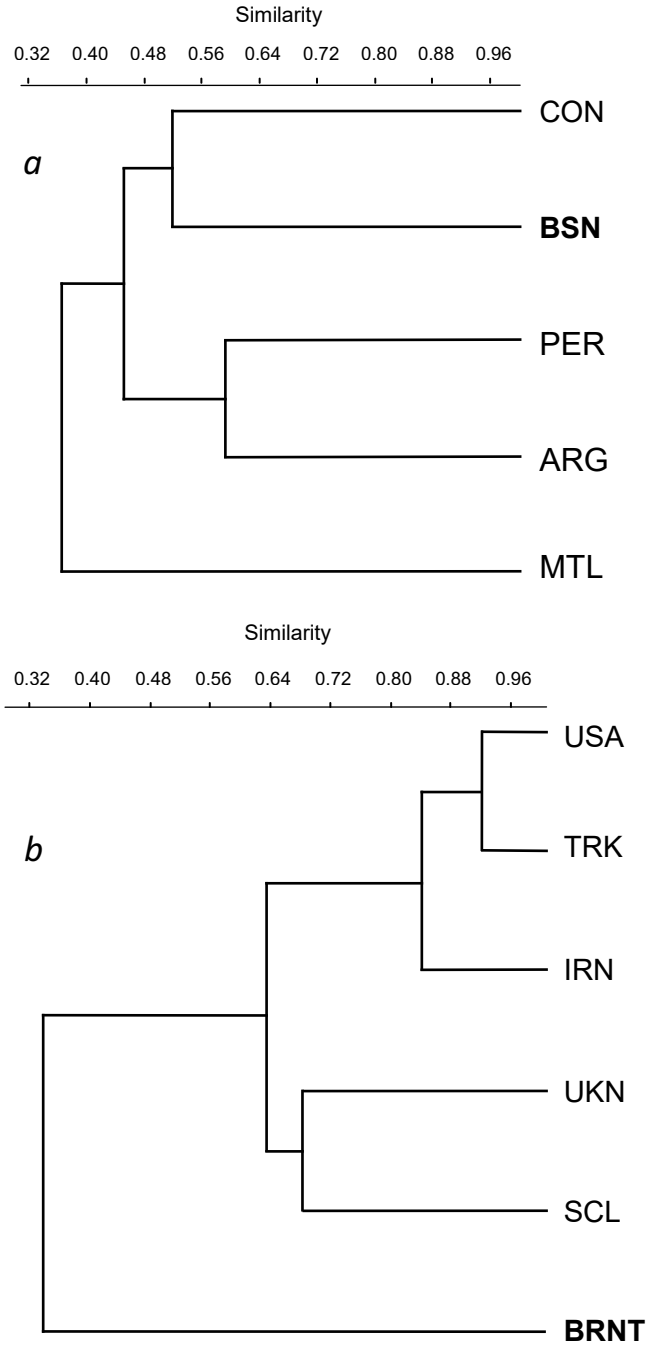


Figure 1. UPGMA dendrogram of genetic relationship, among different genotypes of *Asparagus officinalis* (*a*) *and Pistacia vera* (*b*) using DICE coefficient. White Asparagus from Bassano del Grappa (BSN) and Green Pistachio from Bronte (BRNT) are bolded. Asparagus from Maremma Tosco-Laziale: MTL; Argenteuil: ARG; Connovers Colossal (London): CON; White Peruvian Asparagus: PER. Pistachios from USA: USA; Pistachios from Turkey: TRK; Pistachios from Iran: IRN; Unknown: UKN; Sicilia Perfetta: SCL.

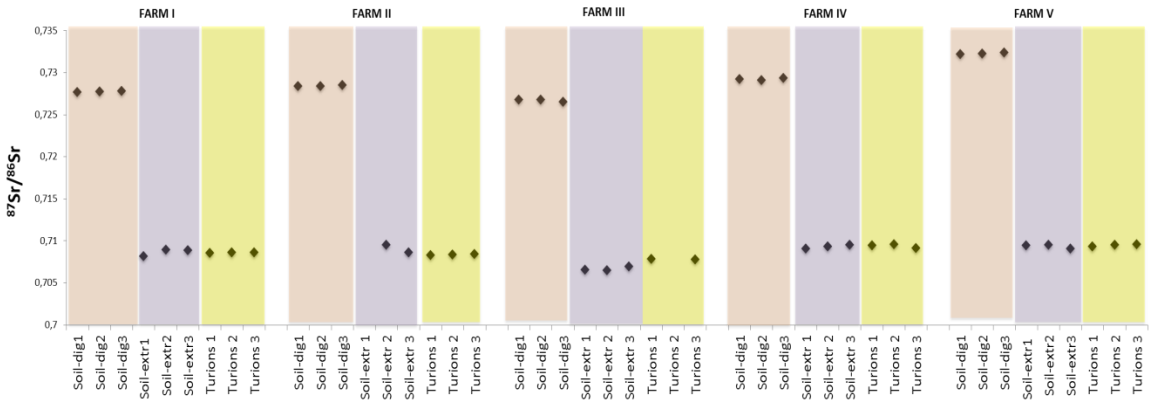


Figure 2. Sr isotope composition in soils and asparagus turions collected from five different production farms of White Asparagus from Bassano del Grappa. Soil-dig= total Sr in soil; Soil-extr= NH_4NO_3-extractable Sr in soil.

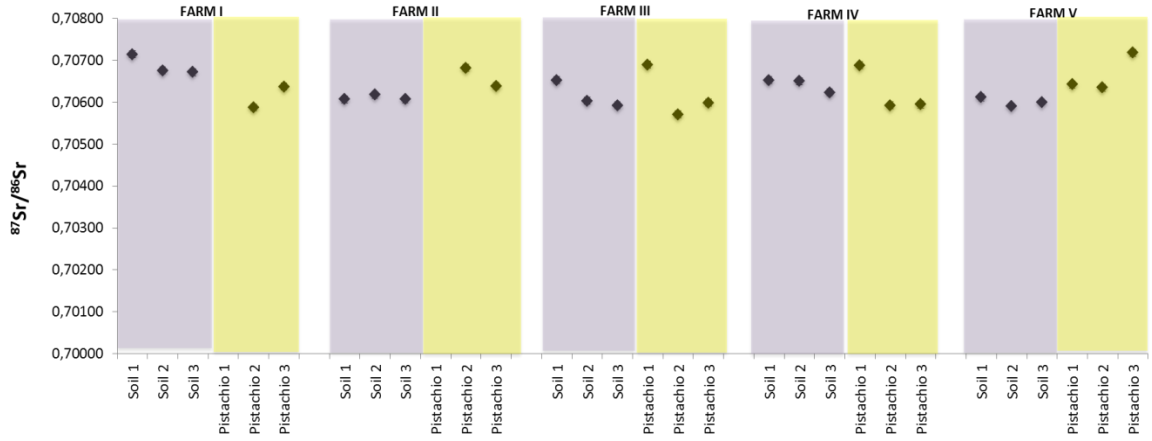


Figure 3. Sr isotope composition in soils (NH_4NO_3-extractable Sr) and nuts collected from five different production farms of Green Pistachio from Bronte.

Supplementary material

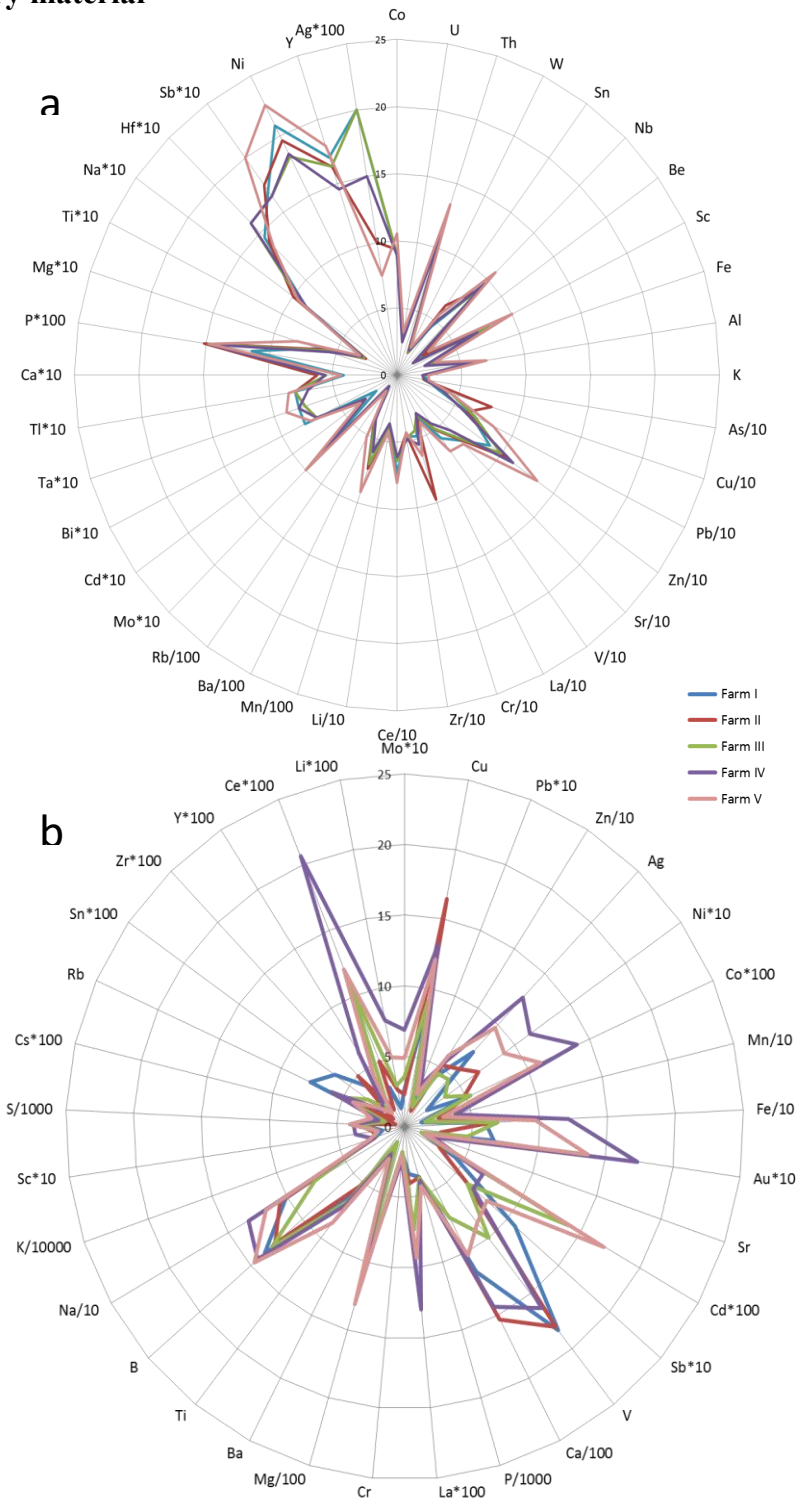


Figure S1. Multielement profiles of soils (a) and turions (b) collected from five different production farms of White Asparagus From Bassano Del Grappa

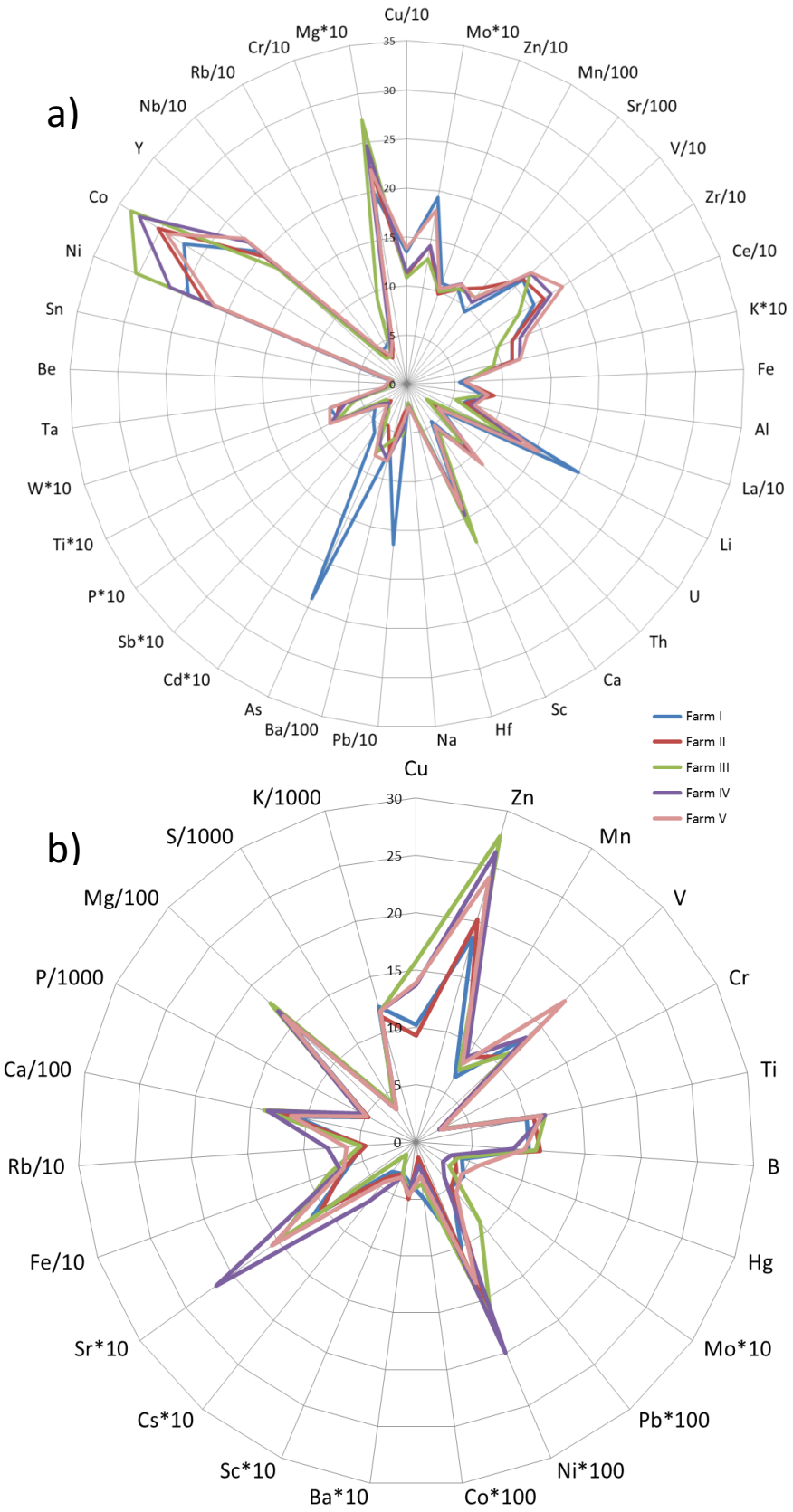


Figure S2. Multielement profiles of soils (a) and whole pistachios (b) collected from five different production farms of Green Pistachio from Bronte

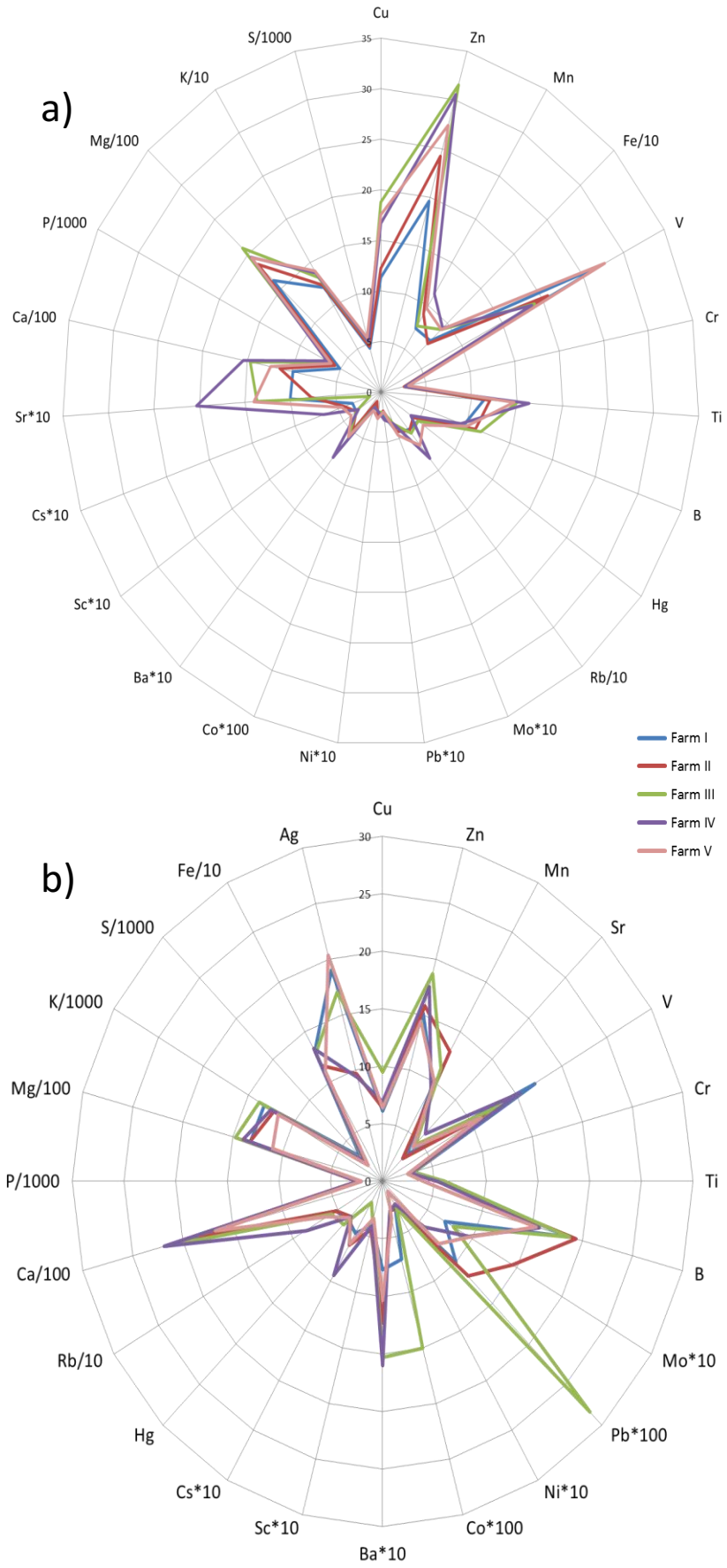


Figure S3. Multielement profiles of separated pulp (a)and peel (b) of Green Pistachio from Bronte

Table S1. Locus name, repeat motif, primer sequences and annealing temperature of each microsatellite locus analyzed in *Asparagus officinalis*

Locus	Repeatmotif	Primersequences	Annealing temperature (°C)
TC1	$(TC)_{12}$	Fw_AGGTGGAGAACAAATGGCTG Rv_CGAGCTCAATTGAAATCCATAA	55
TC7	$(TC)_{15}$	Fw_CGCCCCGAATCAACTAATAA Rv_TACTGCGGAGGTATGTGGGT	55
AG2	$(GA)_9$	Fw_CCTCCTCGGCAATTTAATCA Rv_CAGCTGCATCACGTTCTTGT	55
AG3	$(AG)_{10}$	Fw_TCCACCCCACAAAAAGAAAG Rv_AGAAGTTGACGCCGTTGTCT	55
AG7	$(AG)_{10}$	Fw_TTTTGCTCCGATCATTTTCA Rv_CCTCTTCGTCTTCATCAGCC	55
AG8	$(GA)_9$	Fw_GATTGGGACCAACACAAACA Rv_AGCAATGACTTGATCCCCAG	55
AG10	$(AG)_9$	Fw_CGCCCTTGTTCTTCTTCTTG Rv_CAGTTGTCTGCCGTCTTCAA	55
AG12	$(AG)_9$	Fw_GACTAGCGCCATGAGAAAGG Rv_TTTTAGGGCATTTTAAACGCAT	55
AA01	$(GA)_{10}$	Fw_GAGCGGAGAGGGTGTCCTCGAC(Rv_GACGGATAAGAGTTTGACCGTAC	59
AAT1	$(AAT)_9$	Fw_CTTTTGCTTCTGAACGCTCC Rv_TTGAAGGAGCCGTAAACTGG	55

Table S2. Locus name, repeat motif, primer sequences and annealing temperature of each microsatellite locus analyzed in *Pistacia vera*

Locus	Repeatmotif	Primersequences	Annealing temperature (°C)
PTMS3	$(CA)_{16}$	Fw_TGATGAACAAGTCCAAAAGGG Rv_AAAACAGCACAGCATGCATC	55
PTMS7	$(CA)_{15}$	Fw_TGATGCTCTTGGTGTTGCTC Rv_CCTGAGTAGCTCCAGTTCCG	55
PTMS9	$(CA)_{7}$	Fw_TTGACCGTGGACTTGAAGC Rv_AACCTCCTCTCTTCTCTTTGCC	55
PTMS10	$(CT)_{15}$	Fw_CAGGATGCTTGTTGGTGATG Rv_ACAGTGGATACAAACATGCTGC	55
PTMS14	$(CA)_{46}$	Fw_GGGAAACACAAACATGCAAA Rv_GGCCTCTGGAGAACATGGTA	55
PTMS31	$(CT)_{20}$	Fw_CTGATCATGGGGCCTTTG Rv_GGAAGCACACACATGCAAAC	60
PTMS33	$(CA)_{12}$	Fw_TTCTGCTGGTCATGGGC Rv_TGCCATTTAACCCAAAGGAG	55
PTMS40	$(CTTT)_{4}$	Fw_CAGCTCTCACTGATCCGATTC Rv_TTCGAAAGCCAGTCTCAGGT	55
PTMS41	$(CT)_{11}$	Fw_AGAAGAGGGGAACAGGGAG Rv_CTGAGGACTGGGCAGAATGT	55
PTMS42	$(CTT)_{10}$	Fw_AAACAGGTGTTCCCGTTCAG Rv_ACGACAGGATTGGATGATGG	55
PTMS45	$(CAAA)_3(CA)_4$	Fw_GCTTGTGTGTTTTAGCTCGAAAT Rv_AGCAATGCTTAACATTTTCCAA	55

Table S3. Amplification protocols performed for each species

Specie	N. cycles	First denaturing	Cycles touchdown -1°C for each cycle	Denaturing	Annealing	Extension	Final extension
A. officinalis	35	95°C[5']	NO	94°C[1']	55°C[1']	72°C[1'30"]	72°C [15']
P.vera	25	94°C[5']	5 cycles: Denat.4°C[45"] Ann.63°C[45"] Ext.72°C[1']	94°C[45"]	55°C[45"	72°C[1']	72°C [7']

Table S4.Element content (mg kg^{-1}; Fe, Al, K %) and relative coefficient of variation (CV %) in soils collected from five different production farms of *White Asparagus from Bassano del Grappa*. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.75±0.07	0.85±0.07	1.00±0.14	0.8±0.00	1.0±0.14	0.88	**13**
Cu	32±0.5	77±2.6	44±2.6	37±0.9	44±0.4	47	**38**
Pb	64±2.3	60±2.2	68±0.9	58±1.0	84±3.0	67	**16**
Zn	89±0.0	105±3.5	100±0.7	111±2.8	134±0.0	108	**16**
Ag	0.20±0.00	0.10±0.00	0.20±0.00	0.15±0.07	0.08±0.04	0.15	**39**
Ni	21±1,8	20±0,1	18±0,1	19±0,3	23±1,2	20	**9**
Co	8,9±0,6	9,4±0,1	9,0±0,3	8,9±0,1	10,6±0,4	9.3	**8**
Mn	725±13	731±10	698±1.0	602±37	914±2.0	734	**15**
Fe	2,7±0,0	2,5±0,0	2,4±0,0	2,2±0,0	3,1±0,0	2.6	**13**
As	25±0,7	21±0,7	21±0,0	22±0,7	25±0,0	23	**9**
U	3.0±0.06	2.85±0.07	2.85±0.07	2.50±0.14	3.25±0.21	2.89	**9**
Th	12.8±0.4	11.7±0.4	11.2±0.5	11.3±0.4	13.3±0.1	12.1	**8**
Sr	69±2,8	68±0,7	66±2,1	58±4,2	73±2,1	67	**8**
Cd	0.20±0.00	0.35±0.07	0.30±0.00	0.30±0.00	0.35±0.07	0.30	**20**
Sb	1.70±0.14	1.75±0.07	1.65±0.07	1.65±0.07	2.00±0.0 0	1.75	**8**
Bi	0.80±0.0	0.70±0.0	0.70±0.0	0.70±0.0	0.75±0.1	0.73	**6**
V	58±2.8	51±4.9	50±0.7	45±2.1	70±1.4	55	**18**
Ca	4150±212	6200±283	5500±0	5550±354	4350±71	5150	**17**
P	1140±14	1515±49	1480±28	1470±14	1475±64	1416	**11**
La	37±0.3	32±0.6	33±1.7	32±1.1	39±0.4	35	**10**
Cr	48±0.0	98±3.5	44±0.7	55±9.2	63±4.2	61	**35**
Mg	6400±283	6250±71	5850±212	5550±71	8150±71	6440	**16**
Ba	397±5.7	379±4.2	379±19.1	364±9.9	520±2.8	408	**16**
Ti	0.28±0.0	0.27±0.0	0.28±0.0	0.30±0.0	0.32±0.0	0.29	**7**
Al	5.9±0.1	6.5±0.2	6.1±0.3	5.9±0.4	7.0±0.2	6.3	**8**
Na	8765±78	9920±198	9145±290	8675±21	9475±35	9196	**6**
K	2.06±0.0	2.07±0.1	2.05±0.1	1.97±0.0	2.46±0.0	2.12	**9**
W	1.95±0.1	1.85±0.1	1.90±0.0	2.05±0.1	2.30±0.0	2.01	**9**
Zr	46±0.4	44±1.9	48±1.2	48±3.7	43±0.3	46	**5**
Ce	73±2.8	63±1.4	64±1.4	61±7.1	81±2.1	68	**12**
Sn	4.5±0.2	6.4±0.4	4.8±0.2	4.7±0.1	5.6±0.4	5.2	**16**
Y	17±0.1	16±0.1	16±0.2	15±0.4	18±0.2	16	**8**
Nb	9±0.1	9±0.5	9±0.2	10±0.1	11±0.1	10	**8**
Ta	0.80±0.0	0.75±0.1	0.75±0.1	0.80±0.1	0.90±0.0	0.80	**8**
Be	2.0±0.0	2.5±0.7	1.5±0.7	1.5±0.7	3.0±0.0	2.10	**31**
Sc	7±0.0	7±0.0	8±0.0	7±0.0	10±0.0	8	**17**
Li	40±0.2	40±0.1	39±0.1	36±1.9	43±0.1	40	**6**
Rb	117±0.8	118±4.2	116±0.9	100±5.2	144±6.5	119	**13**
Hf	1.45±0.2	1.40±0.1	1.60±0.0	1.60±0.0	1.35±0.1	1.48	**8**
Tl	0.80±0.1	0.80±0.0	0.80±0.0	0.70±0.0	0.85±0.1	0.79	**7**
					Overallcoefficient of variation		**13**

Table S5. Element content (mg kg^{-1}) and relative coefficient of variation (CV %) in turions collected from five different production farms of White Asparagus from Bassano del Grappa. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.26±0.05	0.23±0.04	0.35±0.07	0.69±0.07	0.49±0.05	0.40	***46***
Cu	8.3±0.6	16.4±0.7	9.1±1.1	13.2±0.3	12.1±0.4	12	***28***
Pb	0.18±0.06	0.12±0.01	0.15±0.01	0.32±0.02	0.25±0.02	0.20	***40***
Zn	43±1.3	52±0.5	45±3.4	55±1.4	60±1.2	51	***14***
Ag	7.3±0.6	5.7±0.6	4.7±0.6	12.7±1.5	9.7±0.6	8.0	***40***
Ni	0.20±0.00	0.67±0.06	0.37±0.06	1.13±0.06	0.90±0.10	0.65	***58***
Co	0.05±0.00	0.05±0.01	0.05±0.01	0.14±0.00	0.11±0.00	0.08	***53***
Mn	13±0.6	26±1.2	16±0.6	39±0.6	29±0.6	24	***43***
Fe	60±1.5	62±3.6	69±5.2	121±10.1	97±4.6	82	***32***
Au	0.67±0.6	0.27±0.2	0.47±0.2	1.73±0.7	1.37±0.2	0.90	***69***
Sr	1.43±0.1	2.37±0.1	1.27±0.1	2.27±0.1	1.53±0.1	1.77	***29***
Cd	0.04±0.0	0.03±0.0	0.14±0.0	0.07±0.0	0.17±0.0	0.09	***69***
Sb	1.08±0.1	0.61±0.0	0.62±0.1	0.67±0.0	0.80±0.0	0.75	***26***
V	18±15	18±11	10±1	16±13	9±3	14	***32***
Ca	1156±37	1535±52	725±62	1433±17	1023±18	1174	***28***
P	3676±31	3732±43	3762±210	3961±85	4365±40	3899	***7***
La	0.03±0.01	0.04±0.01	0.07±0.01	0.13±0.00	0.09±0.01	0.07	***54***
Cr	1.93±0.49	2.03±0.31	1.80±0.62	2.37±0.29	2.03±0.31	2.03	***10***
Mg	1177±26	1188±17	1163±79	1147±45	1309±21	1197	***5***
Ba	1.87±0.06	1.87±0.06	1.17±0.06	2.20±0.10	2.50±0.10	1.92	***26***
Ti	6.0±0.0	5.3±0.6	6.7±0.6	7.0±0.0	8.7±0.6	6.7	***19***
B	14±1.2	13±0.6	13±1.7	14±0.6	15±0.6	14	***6***
Na	101±4	106±4	77±4	133±4	118±4	107	***20***
K	22743±291	23263±403	25501±2220	24509±329	26821±129	24567	***7***
Sc	0.17±0.06	0.23±0.06	0.20±0.10	0.37±0.12	0.20±0.00	0.23	***34***
S	3353±193	3427±142	3369±354	3770±302	4046±68	3593	***8***
Cs	0.01±0.0	0.01±0.0	0.02±0.0	0.02±0.0	0.03±0.0	0.02	***47***
Rb	7.7±0.2	5.9±0.1	5.0±0.3	5.9±0.1	4.2±0.0	5.7	***23***
Sn	0.06±0.06	0.01±0.00	0.03±0.01	0.02±0.01	0.02±0.01	0.03	***68***
Zr	0.04±0.02	0.05±0.00	0.02±0.01	0.02±0.01	0.01±0.01	0.03	***55***
Y	0.02±0.0	0.01±0.0	0.03±0.0	0.06±0.0	0.04±0.0	0.03	***60***
Ce	0.03±0.0	0.05±0.0	0.11±0.0	0.21±0.0	0.12±0.0	0.10	***67***
Li	0.01±0.0	0.03±0.0	0.03±0.0	0.08±0.0	0.05±0.0	0.04	***63***
				Overallcoefficient of variation			***36***

Table S6. Element content (mg kg^{-1}; Fe, Ca, Al %) and relative coefficient of variation (CV %) in soils collected from five different production farms of *Green Pistachio* from Bronte. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	1.93±0.3	1.43±0.2	1.30±0.1	1.43±0.4	1.80±0.0	1.58	***17***
Cu	135±0.3	112±0.2	109±0.1	114±0.4	139±0.0	122	***11***
Pb	164±8	29±15	52±11	46±20	37±3	66	***85***
Zn	109±2	98±11	100±19	104±5	103±10	103	***4***
Ni	24±0.9	23±4.3	30±3.1	26±0.5	22±5.8	25	***14***
Co	27±2.3	30±0.4	34±3.0	33±0.7	29±3.9	31	***8***
Mn	1097±3	1114±9	1115±23	1170±36	1166±54	1132	***3***
Fe	5.5±0.2	6.0±0.0	6.1±0.3	6.1±0.1	6.1±0.6	5.9	***4***
As	24±4.4	4.7±0.6	7.7±5.7	6.7±2.3	8.0±2.0	10.2	***77***
U	4.6±0.4	3.3±0.7	2.5±0.4	4.0±0.4	4.1±1.1	3.7	***22***
Th	10.5±0.9	9.9±0.7	8.2±0.4	10.8±0.1	11.4±0.2	10.2	***12***
Sr	949±81	1264±91	1138±179	1074±21	1129±77	1111	***10***
Cd	0.60±0.0	0.47±0.29	0.33±0.06	0.47±0.15	0.47±0.21	0.47	***20***
Sb	0.50±0.1	0.23±0.06	0.27±0.06	0.27±0.06	0.30±0.1	0.31	***34***
V	161±5	162±5	170±16	171±3	172±15	167	***3***
Ca	4.6±0.2	5.5±0.1	6.0±0.6	5.2±0.1	5.1±0.5	5.3	***10***
P	4103±549	2917±990	2657±798	3423±272	3800±719	3380	***18***
La	62±4.3	64±2.9	53±2.5	70±1.9	74±3.6	65	***12***
Cr	53±3.5	43±10.7	92±34	54±4.4	45±12.1	57	***35***
Mg	19700±1253	22467±462	27400±3666	24633±1193	22167±3215	23273	***12***
Ba	695±52	715±51	587±25	798±24	810±63	721	***13***
Ti	7960±510	8313±258	8083±304	8880±197	8897±601	8427	***5***
Al	8±0.5	9±0.2	8±1.1	8±0.3	9±0.4	8.45	***6***
Na	19397±1652	22903±1561	19330±2233	22680±780	23160±2818	21494	***9***
K	11133±416	11200±265	9200±265	12000±200	12033±1401	11113	***10***
W	0.83±0.06	0.73±0.06	0.57±0.06	0.67±0.12	0.83±0.12	0.73	***16***
Zr	155±11	167±17	137±8	176±7	190±10	165	***12***
Ce	118±8	118±7	102±4	127±2	135±3	120	***10***
Sn	1.83±0.06	1.47±0.06	1.47±0.21	1.53±0.21	1.67±0.15	1.59	***10***
Y	21±0.2	20±0.4	18±1.3	22±1.1	22±0.8	20	***9***
Nb	42±4.8	42±5.1	34±1.1	46±1.3	50±2.2	43	***14***
Ta	2.0±0.2	1.90±0.2	1.63±0.06	2.27±0.06	2.37±0.12	2.03	***14***
Be	2.0±0.0	1.67±0.58	1.67±0.58	2.0±0.0	2.0±0.0	1.87	***10***
Sc	13±0.6	14±1.0	18±1.2	15±0.6	14±1.7	15	***12***
Li	20±1.1	13±1.3	12±2.4	13±0.9	15±2.4	15	***22***
Rb	44±1.6	30±7.5	33±10	34±2.7	33±5.2	35	***15***
Hf	3.5±0.3	3.7±0.4	3.3±0.2	4.0±0.1	4.2±0.2	3.7	***9***
					Overallcoefficient of variation		**16**

Table S7.Element content (mg kg^{-1}, Ag, Hg µg/kg) and relative coefficient of variation (CV %) in whole pistachios collected from five different production farms of *Green Pistachio* from Bronte. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.52±0.02	0.44±0.12	0.36±0.03	0.29±0.07	0.49±0.10	0.42	***22***
Cu	10±2.1	9±2.4	16±0.9	14±2.7	14±0.7	13	***22***
Pb	0.05±0.03	0.05±0.03	0.09±0.08	0.04±0.01	0.06±0.02	0.06	***33***
Zn	19±2.2	20±3.6	28±1.1	26±4.6	24±3.7	23	***17***
Ag	1.67±0.2	1.0±0.1	1.67±0.1	3.0±0.1	2.67±0.3	2.00	***41***
Ni	0.10±0.0	0.17±0.1	0.17±0.1	0.20±0.0	0.13±0.1	0.15	***25***
Co	0.02±0	0.01±0	0.04±0	0.01±0	0.01±0	0.02	***57***
Mn	6.7±1.2	8.7±0.6	7.3±0.6	8.7±1.5	8.0±1.7	7.9	***11***
Fe	64±12	68±6	82±9	72±5	68±3	71	***10***
Sr	1.13±0.5	1.03±0.1	1.50±0.4	2.17±0.5	1.57±0.2	1.48	***30***
V	13±0.6	11±1.0	11±0.6	13±4.5	18±1.0	13	***21***
Ca	1099±172	1233±77	1379±210	1347±79	1132±151	1238	***10***
P	4753±251	4746±51	5254±184	5417±786	4915±261	5017	***6***
Cr	2.37±0.21	2.37±0.06	2.60±0.10	2.37±0.12	2.47±0.06	2.43	***4***
Mg	1582±93	1683±69	1768±212	1671±146	1606±65	1662	***4***
Ba	0.37±0.12	0.50±0.10	0.43±0.12	0.43±0.15	0.47±0.21	0.44	***11***
Ti	10±1.0	11±0.6	12±1.2	12±1.2	11±1.2	11	***7***
B	10±1.0	11±1.0	11±0.6	9±1.2	10±0.6	10	***9***
K	12239±563	11336±747	11868±674	11871±156	11857±1218	11834	***3***
Sc	0.30±0.1	0.30±0.0	0.30±0.0	0.33±0.1	0.33±0.1	0.31	***6***
S	3593±292	3657±187	3834±117	3328±117	3363±23	3555	***6***
Hg	4.33±0.58	3.67±0.58	4.00±1.00	3.33±1.53	6.0±3.61	4.3	***24***
Cs	0.33±0.09	0.40±0.05	0.13±0.05	0.67±0.49	0.4±0.29	0.40	***49***
Rb	48±4.4	45±8.3	49±11	79±14	62±6.9	56	***25***
					Overallcoefficient of variation		**19**

Table S8. Element content (mg kg^{-1}, Ag, Au, Hg µg/kg) and relative coefficient of variation (CV %) in pulp samples of pistachios collected from five different production farms of Green Pistachio from Bronte. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.43±0.09	0.43±0.11	0.34±0.02	0.34±0.06	0.47±0.11	0.40	***14***
Cu	11±1.5	12±2.8	19±1.5	17±2.4	18±1.6	15	***22***
Pb	0.24±0.04	0.19±0.02	0.20±0.02	0.28±0.04	0.20±0.03	0.22	***17***
Zn	20±1.9	24±6.4	32±0.6	31±4.1	27±5.1	27	***18***
Ag	6.0±1.7	4.3±1.5	5.7±1.5	5.7±1.5	6.0±1.7	5.5	***12***
Ni	0.20±0.10	0.27±0.06	0.20±0.10	0.20±0.0	0.27±0.06	0.23	***16***
Co	0.03±0.01	0.02±0.0	0.02±0.01	0.02±0.01	0.02±0.0	0.02	***21***
Mn	7.3±0.6	9.0±1.0	7.7±0.6	11.3±1.5	9.7±2.1	9	***18***
Fe	74±5	70±8	91±3	93±5	91±10	84	***13***
As	0.12±0.1	0.10±0.0	0.17±0.1	0.15±0.0	0.07±0.0	0.12	***33***
Au	0.27±0.0	0.13±0.0	0.43±0.1	0.27±0.1	0.47±0.2	0.31	***44***
Sr	1.0±0.40	0.77±0.06	1.37±0.55	2.03±0.49	1.40±0.26	1.31	***37***
Sb	0.02±0.01	0.01±0.0	0.02±0.0	0.02±0.0	0.02±0.0	0.02	***22***
V	27±14	21±1.2	19±1.7	19±0.6	28±14	23	***19***
Ca	989±207	1138±78	1466±175	1543±187	1240±245	1275	***18***
P	5084±171	5699±269	6396±279	6682±664	6140±323	6000	***10***
Cr	2.80±0.69	2.57±0.12	2.67±0.15	2.67±0.06	3.10±0.53	2.76	***8***
Mg	1614±94	1851±233	2085±242	1954±156	1948±190	1890	***9***
Ba	0.50±0.0	0.50±0.10	0.57±0.06	0.83±0.15	0.57±0.12	0.59	***23***
Ti	11.3±1.2	12.0±1.0	15.0±3.0	16.3±1.2	14.7±1.5	13.9	***15***
B	9.7±0.6	11.0±1.0	11.7±1.2	9.3±1.5	10.0±1.0	10.3	***9***
K	12114±211	12354±504	13242±506	13773±569	14007±797	13098	***6***
Sc	0.33±0.23	0.33±0.06	0.33±0.06	0.30±0.00	0.40±0.17	0.34	***11***
S	4471±1085	4700±281	5102±251	5170±123	5580±1345	5004	***9***
Hg	5.00±3.5	4.33±1.2	5.00±1.0	4.00±1.0	5.67±3.8	4.80	***14***
Se	0.10±0.0	0.08±0.0	0.07±0.0	0.10±0.0	0.10±0.0	0.09	***17***
Cs	0.33±0.10	0.43±0.07	0.13±0.06	0.67±0.53	0.45±0.27	0.40	***49***
Rb	48±4	49±10	53±12	85±14	68±11	61	***26***
Li	0.15±0.26	0.04±0.03	0.02±0.00	0.05±0.00	0.05±0.07	0.06	***79***
					Overallcoefficient of variation		**21**

Table S9. Element content (mg kg^{-1}, Ag, Au, Hg µg/kg) and relative coefficient of variation (CV %) in peel samples of pistachios collected from five different production farms of Green Pistachio from Bronte. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.70±0.14	1.45±0.60	0.79±0.03	0.96±0.38	0.97±0.07	0.97	***30***
Cu	6.1±0.3	6.4±0.9	9.5±1.7	6.9±1.3	6.4±0.8	7.06	***20***
Pb	0.10±0.07	0.12±0.04	0.28±0.22	0.06±0.03	0.08±0.02	0.13	***71***
Zn	15±3.4	16±2.4	19±3.0	18±2.7	14±2.3	16	***11***
Ag	19±14	10±2	17±6	9±2	20±13	15	***35***
Ni	0.23±0.06	0.27±0.12	0.27±0.12	0.23±0.06	0.10±0.0	0.22	***31***
Co	0.07±0.10	0.03±0.01	0.15±0.12	0.03±0.02	0.03±0.02	0.06	***86***
Mn	9.7±1.53	13.0±1.0	11.3±2.08	9.3±2.52	10.0±1.73	10.7	***14***
Fe	131±1	116±10	129±7	133±4	110±27	124	***8***
Au	2.63±2.25	0.90±0.44	2.67±1.46	1.13±1.01	2.37±1.44	1.94	***44***
Sr	3.27±1.12	2.77±0.31	4.50±1.40	5.87±0.38	4.17±0.40	4.11	***29***
Sb	0.02±0.0	0.03±0.0	0.02±0.0	0.03±0.0	0.02±0.0	0.02	***29***
V	17±1	12±8	13±4	15±1	11±6	14	***18***
Ca	2144±293	1843±115	2177±213	2185±103	1673±75	2005	***12***
P	2258±298	2381±139	2405±215	2415±79	2029±81	2298	***7***
Cr	3.10±0.35	2.87±0.12	3.13±0.32	2.77±0.31	2.50±0.26	2.87	***9***
Mg	1325±189	1328±68	1472±53	1398±230	1100±24	1325	***11***
Ba	0.77±0.15	1.23±0.47	1.53±0.45	1.60±0.79	1.03±0.49	1.23	***28***
Ti	5.67±1.2	5.33±0.6	6.00±1.0	5.33±0.6	4.00±0.0	5.27	***14***
B	19±2.1	19±4.0	19±1.5	16±1.5	15±1.2	18	***11***
Na	16±4.0	23±5.5	18±1.5	12±0.7	11±0.6	16	***29***
K	13229±1095	12155±732	13755±2598	12455±1132	11629±1225	12645	***7***
Sc	0.40±0.1	0.43±0.1	0.37±0.1	0.40±0.0	0.33±0.1	0.39	***10***
S	3005±458	2567±227	2600±324	2449±318	1941±306	2513	***15***
Hg	4.67±0.58	4.33±0.58	5.33±1.53	4.33±0.58	3.17±0.71	4.37	***18***
Cs	0.52±0.16	0.64±0.10	0.21±0.05	0.94±0.64	0.63±0.41	0.59	***45***
Rb	52±6	51±14	57±10	86±9	62±7	62	***23***
Sn	0.02±0.0	0.05±0.0	0.04±0.0	0.01±0.0	0.02±0.0	0.03	***59***
Zr	0.01±0.0	0.02±0.0	0.02±0.0	0.03±0.0	0.03±0.0	0.02	***38***
					Overallcoefficient of variation		**26**

Chapter 2

Elemental and isotopic fingerprint of Altamura wheat flour: matching soil and crop composition to differentiate geographical provenance

1. Introduction

Consumers are increasingly aware to the choice of brands and ingredients selection, with greater predisposition for typical products with quality certifications. They have the right to make informed decisions about the food they purchase and have to be ensured that food products are protected for their provenance. Therefore, suitable analytical techniques are needed for determining the geographical origin of agroproducts. Ensuring complete food security is a difficult and complex task that requires penetrating control systems: in this context European Union set out several regulations regarding food authenticity, food quality and geographical origin (Council Regulation n. 510/2006), such as Protected Designation of Origin (PDO), Protected Geographic Indication (PGI) and Traditional Speciality Guaranteed (TSG) brands. These products represent the excellence of European agro-food production and are the result of a unique combination between human and environmental factors of their territories.

The use of geochemical and mineralogical markers is a valuable tool since the mineralogical composition and the concentration of macro and microelements in the soil are the result of the combined influence of lithological matrix and all active factors in the pedosphere, with particular reference to weathering, leaching and pedogenesis processes. Mineralogy and geochemistry factors reflect the interactions between soil formation factors: bedrock, climate, time and biotic entities. Therefore, soils formed in different geographic areas tend to be characterized by different quality and quantity of minerals whose identification and chemical characterization can provide valuable information on the type and properties of different soil. The same concentrations of trace elements in plant tissues are controlled by their content in the soil, in which form they are found and the factors influencing their mobility, as well as the capacity absorption by the plants (Adamo et al., 2012).

Different works have been proposed to authenticate wheat flour. A lot of studies focused on the authentication related to the variety, since the processing procedure differs significantly depending of the different grain; the methods to identify wheat varieties are mainly phenol and rapid tyrosinase test, electrophoresis by protein composition, techniques for grain hardness and digital imaging (Lees et al., 2003). Furthermore, the authentication and traceability of wheat flour based on geographic origin has been studied by the use of DNA microsatellites (Pasqualone et al., 2010) and

the combination of $^{87}Sr/^{86}Sr$ isotope ratio, light stable elements (C, N, O, S) and multielemental profiling (Asfaha et al. 2011; Brescia et al., 2002; Li et al., 2016; Liu et al., 2015).

Our investigation focused on the identification of geochemical markers to authenticate the first bread in Europe which has gained the PDO certificate in its category *"Bread from Altamura PDO"*, made from durum flour in Apulia region (south Italy). Multielement composition along with the $^{87}Sr/^{86}Sr$ isotope ratio (analyzed by MC ICP-MS) of wheat flour matched with the cultivation soil proved to be a potential tool in this respect. In the specific case of the soils was analyzed both the total $^{87}Sr/^{86}Sr$ isotope ratio and the bioavailable fraction assessed by 1 mol/L NH_4NO_3. For comparison, the same analyses were carried out also on wheat and soil samples from another Italian area (Grottaminarda, Campania Region) and all data were compared with literature data of wheat and soil samples from different geographical areas, Argentina and China.

2. Materials and methods

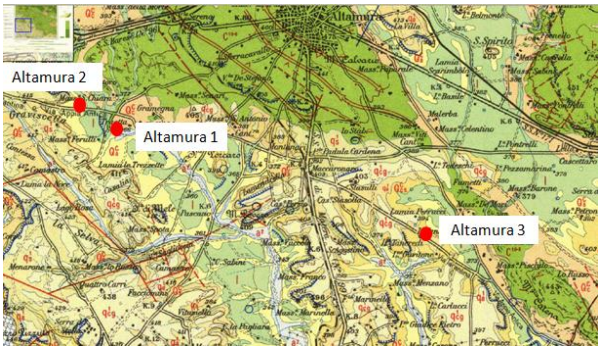
2.1 Plant and soil sampling strategy and preparation

At harvest, wheat was in the full-ripe stage. A sampling strategy with three replications per farm was applied. A total of 15 soil and wheat samples were collected. More details of samples and sampling sites are given in Table 1.

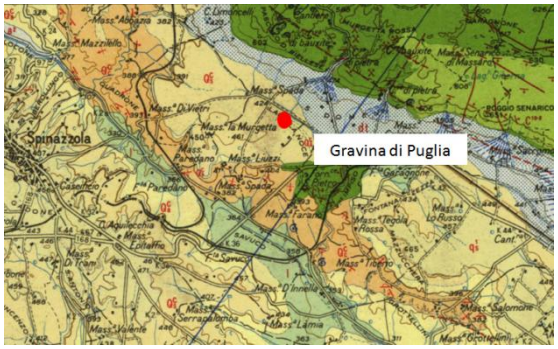
Wheat grain was separated manually from the chaff, dried at 40 °C and pulverized using agate mill jars to obtain the flour. All cleaning operations and management of the plant material were samples of surface soil (0-30 cm). Soils were air-dried and 2 mm sieved and subsequently the residual moisture was determined at 105 °C.

For comparison wheat flour and soil were also collected from another Italian geographical area (not covered by PDO label), Grottaminarda (AV), from two sites and with the same sampling strategy of three replications per site (Table 1).

2.2 Environmental setting of Apulia sites



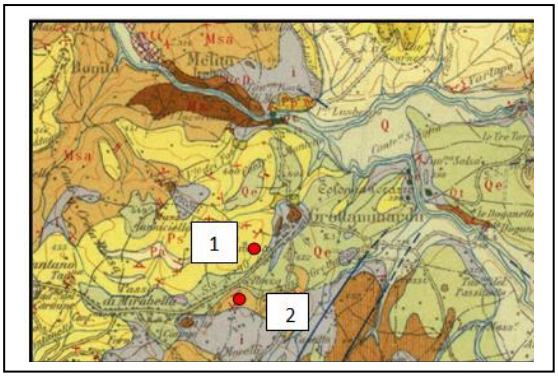
The geographical area devoted to the production of PDO *Bread from Altamura* is located in a landscape of high and medium-high hill plateau, generally at the foothill of the Murge lands (Altamura 1, 2 and 3) and rarely within the Murge (Gravina di Puglia). The geological substrate is always Pleistocenic (Quaternary), but quite different in the analyzed sites. Indeed, fine yellowish calcarenites (a type of limestone composed



predominantly of detrital sand-size carbonate grains), named Tufo di Gravina formation, are found in the site Altamura 1, whereas gray-blue clays and marl clays, named Argille di Gravina formation, in Altamura 2 and then clays and silty gray marls, having calcareous concretions and named Argille Calcigne formation, are present at Altamura 3. The site Gravina di Puglia has a geological substrate made by yellowish calcareous-quartz sands, named Sabbie di Monte Marano formation. Due to the variable geological substrate, soils show very different in the landscape, in terms of texture (from sandy to clayey), carbonate contents, soil depth and degree of development (from shallow low developed to very deep highly developed), fertility and water retention. Therefore, following the Soil Map of Italy at 1:1000.000 scale (Costantini et al., 2012), all the sites from Altamura (Altamura 1, 2 and 3) are part of the Soil Region G, named "Soils of the hills of Central and Southern Italy on neogene marine deposits and limestone", province 36. Soils are classified as Eutric, Calcaric, Vertic and Fluvic Cambisols; Haplic Calcisols; Calcaric Regosols; Haplic, Luvic, Leptic and Skeletic Phaeozems; Luvic Kastanozems; Chromic and Cutanic Luvisols. In the case of the sites Gravina di Puglia, it belongs to the Soil Region I, "Soils of the hills and marine terraces of Southern Italy on calcareous sediments", province 44. Soils are classified as Leptic and Luvic Phaeozems; Leptic and Chromic Luvisols; Haplic Calcisols; Calcic Chernozems; Calcaric Regosols; Calcaric Cambisols; Calcic Kastanozems; Calcaric Leptosols; Calcaric Arenosols.

2.3 Environmental setting of Campania sites

The landscape of the sites Grottaminarda 1 and 2 is part of the marly limestones and marly sandstones inner hills of Irpinia and Sannio areas. The geological substrate (Geological map 1:100.000 scale, Ariano Irpino sheet 174) (Servizio Geologico d'Italia, 1960) at Grottaminarda 1 is made by pliocenic sand and sandstone, having lens of polygenic puddingstone and sandy clays,



while in Grottaminarda 2 miocenic molasses and clay sands, locally having microfauna of the late Miocene, are found. In terms of soils, the site Grottaminarda 1 is located in D3.3 sub-system, at the boundary with H1.1, while the site Grottaminarda 2 is in H1.1 sub-system, both sub-systems identified in the Sistemi di Terre della Campania (di Gennaro et al., 2002).

D3.3 is the landscape of the hills built on the repeated sedimentary cycles of marly limestones and marly sandstones, having discontinuous pyroclastic covers. Different soil types characterize this subsystem. Indeed, there are: 1) soils on weak to moderate slopes on marls are deep, have moderately fine texture, good oxygen availability, classified as Haplic Calcisols; 2) soils on weak to moderate slopes on volcanic ash deposits, moderately coarse in texture, with good oxygen availability, classified as Vitric Andosols; 3) soils on moderately steep to steep slopes, deep to moderately deep, having moderately fine texture but generally skeletric, with good oxygen availability, classified Calcaric Cambisols; 4) soils on steep to very steep slopes, shallow to moderately deep, stony, having moderately fine texture and good oxygen availability, classified Calcaric Regosols. The land use is destined to arable lands, arboretum (olive groves, vineyards, hazelnuts), arborated gardens, natural vegetation areas (chestnut woods, deciduous wood forests, bushes).

The landscape of the site Grottaminarda 2 is that of the alluvial terraces of the upper and middle course of the Volturno river, and in general of the Apennine rivers. Generally, the alluvial terraces are characterized by flat to slightly sloping morphology and are bordered by high gradient slopes bordered by watercourses. Terraces altitude ranges from 60 to 450 m asl. Different soil types occur in this subsystem. Indeed, there are: 1) soils on flat surfaces to very steep slopes, deep, over ancient terraced alluvial deposits, with moderately fine or fine texture, good or moderate oxygen availability and presence of gravel in depth, classified as Cutanic Luvisols; 2) soils on flat surfaces, very deep, over ash deposits, having moderately coarse texture on the surface but moderately fine in depth, with good oxygen availability, classified Pachi-Vitric Andosols (Luvic); 3) soils on flat surfaces to gentle slope, on recent alluvial deposits, with moderately fine or fine texture, with good or moderate oxygen availability, classified Eutric Cambisols; 4) soils on steep to very steep slopes, shallow to moderately deep, skeletric, on ancient terraced alluvial deposits, with moderately fine or medium texture, very skeletric, with good oxygen availability, classified Eutric Cambisols and Skeletic Regosols. The main land use is agricultural: herbaceous species are widely spread (65% of the surface of the system), cereal crops, industrial crops from the field, fodder crops. There are also woody and promiscuous ordinances (23%), with fruit trees, olive groves, vineyards, hazelnuts, arbored orchards.

2.3 Multielement analysis

Multielement analysis of soil and wheat flour was conducted on soils and wheat flour from Altamura and Grottaminarda sites and was carried out at Acme Analytical Laboratories Ltd (Vancouver, Canada) by Perkin Elmer Elan 6000 ICP-MS. As for soils, Acme's Group 1EX

package (HNO_3-$HClO_4$-HF digestion) for 41 elements (Ag, Al, As, Au, Ba, Be, Bi, Ca, Ce, Cd, Co, Cr, Cu, Fe, Hf, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, S, Sb, Sc, Sn, Sr, Ta, Th, Ti, U, V, W, Y, Zn, Zr) was used. As for wheat flour, Acme's Group 1VE – MS package (HNO_3 digestion followed by *aqua regia*) for 53 elements (Au, Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hf, Hg, In, K, Ge, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Pt, Rb, Re, S, Sb, Sc, Se, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn, Zr, Pd) was employed.

The degree of similarity among geochemical profiles of soils and wheat collected in the same production areas was evaluated by single element and overall coefficient of variation (CV), expressed as percentage, and defined as the ratio of the standard deviation to the mean.

2.4 Sr isotope ratio

For what concern the Sr isotopc ratio, the determination was conducted only for samples from Altamura and was carried out at VIRIS Laboratory, University of Natural Resources and Life Sciences, Vienna (Tulln an der Donau, Austria).

For the determination of total Sr in soil, microwave (Anton PaarMultiwave 3000, Anton Paar, Graz, Austria) assisted acid (HF-HCl-HNO_3) digestion of milled soil was carried out into Teflon vessels. The digested samples were evaporated to almost dryness. *Aqua regia* and double sub-boiled HNO_3 were added and again evaporated at 90°C to a volume of 0.5 mL. The volume was finally filtered (0.45 μm) and filled up with 8 mol/L HNO_3. The bioavailable Sr fraction was extracted from the soil by 1 mol/L NH_4NO_3 following the extraction procedure proposed by the German national standard DIN 19730 (Prueb, 1997). To obtain accurate results with minimum uncertainty, a Sr/matrix separation prior to measurement by MC-ICPMS was done, using a Sr specific resin (ElChrom Industries, Inc., Darien, IL, USA) with a particle size of 100 μm – 150 μm according to a standard procedure (Swoboda et al., 2008). The Sr isotope ratios in the obtained solutions were measured with a double-focusing sector field MC ICP-MS instrument (Nu Plasma HR, Nu Instruments, Wrexham, Wales, UK) with a desolvating membrane nebulizer (DSN 100, Nu Instruments, Wrexham, Wales, UK) using optimized operational parameters (Swoboda et al., 2008). An SRM 987 solution (cert. $^{87}Sr/^{86}Sr = 0.71034 \pm 0.00026$) was used to calibrate the instrument for instrumental isotopic fractionation by applying a standard - sample - bracketing approach. The 'measure zero' method of the NU Plasma software was used for the blank correction-using instrument (Nu Plasma HR, Nu Instruments, Wrexham, Wales, UK) measuring separation blanks within the measurement sequence.

2.5 Statistical analysis

The comparison of our data with those ones published by literature was carried out by means a Factorial Discriminant Analyses (FDA) performed by XLSTAT.

3. Results and discussion

3.1 Chemical and physical properties of soil

Altamura soils were characterized by neutral and sub-alkaline pH ranging between 6.9 and 8.0 and total carbonates ranging from 1.6 and 176 g/kg, sandy-clay and exhibited a moderately high organic carbon content (values ranging from 10.8 to 22.1 g/kg) and an high cation exchange capacity as measured by $BaCl_2$ pH 8,1 (between 13.7 and 31.0 $cmol_+$/kg) (ISO 13536, 1995). In all farms, the wheat cultivation was managed in full compliance with the PDO *Bread from Altamura* production guidelines (European Community Council Regulation EC 1291/2003; GUCE 181/2003; Dossier number IT/PDO/0005/0136). The official production protocol requires that the PDO bread has to be prepared from the durum wheat cultivars *Appulo, Duilio, Arcangelo* and *Simeto* (single or in combination, accounting for minimum 80%) and eventually other cultivars diffused in the production area.

Grottaminarda soils were characterized by sub-acids and neutral pH ranging between 6.3 and 7.2 and total carbonates ranging from 3 and 134 g/kg, sandy-clay and exhibited an high organic carbon content (values ranging from 16.2 to 29.2 g/kg) and an high cation exchange capacity as measured by $BaCl_2$ pH 8,1 (between 17.3 and 33.4 $cmol_+$/kg) (ISO 13536, 1995).

3.2 Multi-element fingerprint

The total concentration of single elements in soils and wheat from the five production farms of Altamura are given in Tables 2 and 3. In Figure 1 the chemical composition of soils and wheat are compared graphically as farm geochemical profiles. Elements present in all samples with concentrations below LOD are not reported. A low degree of similarity was found among geochemical profiles of the soils collected in all different farms (overall coefficient of variation 33%). As shown in Table 2 (and Figure 1 a), only 8 chemical elements have content in soils from different farms characterized by a CV < 20% (Cu, Zn, Cr, Mg, Al, Na, K, Sc). Inhomogeneity was mostly due to Farms 3 and 4. Indeed, excluding them the overall CV decreases to 17%. On the other hand, a greater homogeneity was observed among the geochemical profiles of wheat samples taken from the five different farms (Table 3 and Figure 1 b). Indeed, overall CV for wheat was 26%, with seven elements showing a CV lower than 10% (Co, Fe, P, Cr, Mg, Ti, K).

In Figure 2 the chemical composition of soils and wheat from the two Grottaminarda sites are compared graphically as farm geochemical profiles. The geochemical profile of soil is more identical than wheat samples, for which the elements that showed a differences in terms of concentration are Zr, Sc and Ba. Even though the Grottaminarda sites were only two, a geochemical comparison was done both for soil and for wheat flour with the five Altamura sites (Figure 3). The main elements which showed a great diversity were Co, As, Sr, Zr, Ce, Mn, Tl and Ta for soil, while Mo, Pb and Ba for wheat.

To better strengthen Altamura's wheat flour authentication, a comparison with other data reported in literature was made. Both wheat flour and soil multielement data were compared with other geographical area: two Chinese provinces, Hebei and Henan (Zhao et al., 2013) and three different Argentinian regions, Buenos Aires, Córdoba and Entre Ríos (Podio et al., 2013).

Manganese, Fe, Sr and Rb were the chemical elements that were able to discriminate Altamura wheat flour from wheat flour of other geographical area.

Manganese was lower in wheat flour from Altamura (27.4 ± 4.3 μg/g) and Grottaminarda (25.0 ± 2.6 μg/g) than in wheat flour from two Chinese provinces, Hebei (39.4 ± 4.2 μg/g) and Henan (31.6 ± 3.7 μg/g), and from Buenos Aires (40 ± 12 μg/g), Córdoba (42 ± 12 μg/g) and Entre Ríos (41 ± 10 μg/g). Iron was higher in wheat flour from Altamura (30.7 ± 2.8 μg/g) and Grottaminarda (36.7 ± 5.8 μg/g) than in wheat flour from Hebei (19.3 ± 5.2 μg/g) and Henan (18.6 ± 8.6 μg/g). Strontium was lower in in wheat flour from Altamura (1.85 ± 1.16 μg/g) and Grottaminarda (1.90 ± 0.30 μg/g) than in wheat flour from Hebei (7.66 ± 1.6 μg/g) and Henan (6.01 ± 1.1 μg/g), and from Buenos Aires (2.8 ± 0.9 μg/g), Córdoba (4.7 ± 1.5 μg/g) and Entre Ríos (3.5 ± 0.8 μg/g). Rubidium was lower in in wheat flour from Altamura (3.05 ± 1.40 μg/g) and Grottaminarda (4.13 ± 0.99 μg/g) than in wheat flour from Hebei (7.23 ± 3.06 μg/g) and Henan (5.39 ± 1.68 μg/g), and from Buenos Aires (2.0 ± 0.005 μg/g), Córdoba (1.3 ± 0.4 μg/g) and Entre Ríos (12 ± 5 μg/g).

Many differences in terms of element concentration were found by comparing cultivation soils from Altamura and Grottaminarda and Chinese provinces. Manganese, Fe, Cr, Mg, Na and Rb were the chemical elements that were able to discriminate Altamura wheat flour from wheat of the other geographical area.

Manganese was much higher in cultivation soil from Altamura (1346 ± 898 μg/g) and Grottaminarda (1184 ± 38 μg/g) than in subsoil from Hebei (624 ± 50 μg/g) and Henan (608 ± 71 μg/g) provinces. Iron was lower in soil from Altamura (28000 ± 7804 μg/g) than subsoil from Hebei (35688 ± 2682 μg/g) and Henan (33569 ± 4493 μg/g) provinces. Strontium was lower in soil from Altamura (170 ± 42 μg/g) than subsoil from Hebei (199 ± 23 μg/g) and Henan (183 ± 28 μg/g) provinces, and much lower than soil from Grottaminarda (416 ± 55 μg/g). Chromium was lower in

soil from Altamura (55 ± 9.3 μg/g) than soil from Grottaminarda (72 ± 7.5 μg/g) and subsoil from Hebei (77.3 ± 18.1 μg/g) and Henan (69.1 ± 6.67 μg/g) provinces. Magnesium was much lower in soil from Altamura (5920 ± 841 μg/g) than soil from Grottaminarda (14467 ± 1305 μg/g) and subsoil from Hebei (13502 ± 1390 μg/g) and Henan (14094 ± 2934 μg/g) provinces. Sodium was lower in soil from Altamura (6688 ± 1710 μg/g) than soil from Grottaminarda (9180 ± 2073 μg/g) and subsoil from Hebei (10248 ± 2521 μg/g) and Henan (7768 ± 1515 μg/g) provinces. Rubidium was higher in soil from Altamura (139 ± 35 μg/g) than subsoil from Hebei (96 ± 5.7 μg/g) and Henan (95 ± 13.5 μg/g) provinces, but lower than soil from Grottaminarda (179 ± 8.6 μg/g).

For a better understanding of source and plant uptake of cations the use of the Ca/Sr ratio (provides an additional discriminating power in addition to the single element concentration.

The Ca/Sr ratio for Altamura wheat flour was similar to Grottaminarda but higher than wheat flour from the Chinese provinces and Argentinian regions (Altamura 216, Grottaminarda 211, Hebei 66, Henan 80, Buenos Aires 150, Córdoba 83, Entre Ríos 129). In contrast, a greater disparity was found for the Ca/Sr ratio in soils; indeed Altamura Ca/Sr ratio (188) was higher than Grottaminarda (101) and Hebei (170) but lower than Henan (279).

A further discrimination has been assessed by a Factorial Discriminant Analysis, both for soils (Figure 4, a and b) and wheat flour (Figure 5, a and b) from different geographical area based on multielement fingerprint, taking into account only the elements which were in common with all the evaluated sites.

In case of soil (Figure 4a), considering the Factor 1 that explains the percentage of the total variance (77.35%), Altamura is well distinguished from Henan and Hebei Chinese provinces (oriented towards the left-hand side), and from Grottaminarda site (right-hand side). Cadmium, Mn and Co are the main elements which distinguish Altamura from the others (Figure 4b) toward the Factor 1, while Cr, Ti, Na, Y as the elements contributing more to distinguish the two Chinese provinces, and Sr, Cu, Zn, V, Be, Rb mostly characterized Grottaminarda soil.

Also in case of FDA analisys describing the relative distribution of elements and geographical area concernig wheat flour (Figure 5a) Altamura (left-hand side of the plot) was well separated (Factor 1 47.45%) from Henan and Hebei Chinese provinces (oriented towards the right-hand side), with a minimum overlapping with Grottaminarda. Although a certain nearness could be observed between the Argentinian sites (Buenos Aires, Córdoba and Entre Ríos) and Altamura, no significant distribution was showedd for the Argentinian sites. As showed by FDA for soils, Co still represented the principal element able to discriminate Altamura from the other sites (Figure 5b), confirming that this element can be considered a provenance indicator.

3.3 Sr isotopic ratio fingerprint

The Sr isotope ratio of Altamura wheat flour (*n*=15, range 0.7079 – 0.7086, 2σ 0.0002) is in good agreement with the Sr isotope ratio of the Sr bioavailable fraction in soils (n=15, range 0.7083 – 0.7089, 2σ 0.0014). This was confirmed in all the investigated farms (Figure 6). By contrast, the total Sr isotope ratio in soils (*n*=15, range 0.7102 – 0.7141, 2σ 0.0012) was significantly different compared to the Sr taken up by wheat, showing much higher values than those in wheat and consequently in the bioavailable fraction in soils. These results prove the necessity of assessing the Sr bioavailable fraction because the total Sr cannot be taken as proxy for provenance.

In Figure 7 are reported the data of $^{87}Sr/^{86}Sr$ vs δ ‰ by means the comparison of Altamura soil and wheat flour with those ones from literature.

The δ ‰ of the isotopic ratio $^{87}Sr/^{86}Sr$ was calculated by the algorithm:

$$\delta^{87}Sr(‰) = (^{87}Sr/^{86}Sr_{analysed} / {}^{87}Sr/^{86}Sr_{standard} - 1) \times 1000$$

where the standard is the certified reference material NIST SRM 987.

In Figure 7a the $^{87}Sr/^{86}Sr$ vs δ ‰ of wheat flour from Altamura were compared with data reported by Podio et al. (2013) from three different Argentinian regions, Buenos Aires, Córdoba and Entre Ríos, and by Liu et al. (2016) from three Chinese regions of Xinxiang (Henan province), Yangling (Shaanxi province) and Shijiazhuang (Hebei province). In this latter case data of Sr isotopic ratio were averaged from two years of experiment (2012/2013 and 2013/2014) and three wheat genotype types (Han, Heng, Zhoumai). The plot shows that wheat flour from Altamura can be well discriminated from all the Chinese flour, indeed all the wheat flour from China are grouped together, while wheat flour from Argentina are grouped near Altamura samples.

In Figure 7b the $^{87}Sr/^{86}Sr$ vs δ ‰ of soil from Altamura were compared with data reported by Baroni et al. (2011) from three different Argentinian regions, Buenos Aires, Córdoba and Entre Ríos, and by Liu et al. (2016) from three Chinese regions of Xinxiang (Henan province), Yangling (Shaanxi province) and Shijiazhuang (Hebei province). Also in this latter case data of Sr isotopic ratio were averaged from two years of experiment (2012/2013 and 2013/2014) and for different sampling depth (0-20, 20-40, 40-60). The same differences have been found for soil (Figure 7b) with a clear distinction between Altamura and the Chinese samples, except for Argentina whereby Buenos Aires and Córdoba are slightly far from the Altamura site.

4. Conclusions

Many studies highlight the efficiency of the use of multielement fingerprint and Sr isotopic ratio for geographical food provenance assessment but only few of them are focused on the real relationship between the agro products and their cultivation soils. This study, indeed, investigates the association

between Altamura wheat flour and the soil of its territory of origin in order to find elements and Sr isotopic ratio values authenticating Altamura wheat flour and therefore potentially able to discriminate Altamura wheat flour from wheat flours of different geographical provenience.

The elements whose content in Altamura wheat flours was less variable and, therefore, potential discriminators of geographical origin, were Co, Fe, P, Cr, Mg, Ti and K. From a comparison with Literature and FDA analysis, two of these elements (Co and Fe) along with Mn, Sr and Rb were able to differentiate Altamura wheat flour from wheat flours coming from China and Argentina.

As regard to the Sr isotopic ratio, the values of Altamura wheat flour were in good agreement with the values of the bioavailable Sr fraction in soils, while much lower of the values of the total Sr in soils. This outcome was true for all the Altamura investigated farms and suggests that the $^{87}Sr/^{86}Sr$ ratio can authenticate the wheat flour used to prepare the PDO *"Bread from Altamura"*. The $^{87}Sr/^{86}Sr$ ratio was also able to well distinguish both Altamura soils and wheat flours from the Argentinean and Chinese samples.

Our results, although promising, cannot discard the possible inter annual variation of chemical composition of wheat. So, further investigation should take this aspect into consideration along with the necessity to analyse a large number of samples from Altamura and not Altamura production areas in order to validate the discriminating power of both element content and Sr isotopic ratio.

TABLES

Table 1. Code and geographical location of the studied samples

Region/ Municipality	Sample Code	Sample	Locality	Latitude N	Longitude E
Apulia/ Altamura	Farm I	soil wheat flour	Altamura 1	40° 48' 11,359"	16° 29' 39,208"
	Farm II	soil wheat flour	Altamura 2	40° 48' 29,350"	16° 29' 8,045"
	Farm III	soil wheat flour	Altamura 3	40° 46' 59,703"	16° 34' 50,531"
	Farm IV	soil wheat flour	Altamura 4	ND	ND
	Farm V	soil wheat flour	Gravina di Puglia	40° 58' 33,584"	16° 9' 51,249"
Campania/ Grottaminarda	Farm I	soil wheat flour	Grottaminarda 1	41° 03' 53,29"	15° 03' 03,74"
	Farm V	soil wheat flour	Grottaminarda 2	41° 03' 31,77"	15° 02' 49,44"

Table 2. Element content (mg kg^{-1}) and relative coefficient of variation (CV %) in soils collected from five different production farms of wheat from Altamura. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.40±0.00	0.47±0.06	1.53±0.06	1.07±0.15	0.70±0.17	*0.83*	***56***
Cu	20.5±1.2	20±1.9	25±1.3	24±2.3	23.5±0.1	*22.6*	***11***
Pb	23.8±1.1	30±2.2	71±4.6	41±3.1	25.0±3.6	*38.3*	***51***
Zn	45±2.3	54±4.9	67±2.5	73±3.0	72±0.6	*62*	***20***
Ni	19.4±1.4	25.5±0.4	33.1±1.2	35.1±2.1	27.4±0.1	*28.1*	***22***
Co	8.3±0.71	11.2±1.2	29.9±1.7	18.2±1.5	12.0±0.5	*15.9*	***54***
Mn	652±13.6	942±154	2817±201	1623±170	855±79	*1378*	***64***
Fe	19300±1389	23733±513	39033±2084	33100±889	28600±1323	*28753*	***27***
As	10.7±1.1	11.3±0.6	23.0±3.0	15.7±2.5	10.3±1.1	*14.2*	***38***
U	1.30±0.10	1.63±0.12	2.47±0.15	2.30±0.30	1.77±0.06	*1.89*	***25***
Th	10.4±0.5	14.2±0.8	21.3±1.0	18.5±1.0	12.4±1.8	*15.4*	***29***
Sr	143±3.2	204±21.2	125±3.5	212±17.7	152±18.0	*167*	***23***
Cd	0.17±0.06	0.37±0.12	0.27±0.06	0.70±0.10	0.20±0.00	*0.34*	***63***
Sb	0.53±0.06	0.67±0.12	1.40±0.17	0.83±0.06	0.53±0.15	*0.79*	***45***
Bi	0.23±0.06	0.30±0.00	0.43±0.06	0.40±0.00	0.27±0.06	*0.33*	***26***
V	57±2.9	73±2.6	126±1.0	101±6.1	85±3.5	*88*	***30***
Ca	34633±3320	31800±10168	6633±513	18600±9398	47533±32801	*27840*	***56***
P	440±44	383±61	373±42	417±49	660±132	*455*	***26***
La	28.4±1.1	39±1.6	62±2.9	54±4.8	33.7±2.5	*43*	***33***
Cr	40±0.6	52±5.0	62±1.0	67±4.0	58±3.6	*56*	***18***
Mg	4833±321	7200±265	5767±153	6633±379	6367±231	*6160*	***15***
Ba	421±5.1	505±53.7	766±42.4	686±79.8	426±67	*561*	***28***
Ti	1943±136	2500±89	3040±125	3563±67	2827±78	*2775*	***22***
Al	51200±1735	55067±3001	78100±2700	71267±802	58433±4989	*62813*	***18***
Na	7740±255	6793±261	4363±158	7447±1054	6803±1019	*6629*	***20***
K	19867±306	20233±551	19267±551	25133±1563	19367±2301	*20773*	***12***
W	1.60±0.20	3.10±2.18	4.53±0.84	3.20±0.40	1.80±0.26	*2.85*	***42***
Zr	77±5.7	110±10.5	190±9.8	164±4.6	89±16.4	*126*	***39***
Ce	59±1.5	85±9.3	237±17.4	137±7.0	69±7.0	*117*	***63***
Sn	1.83±0.06	2.27±0.12	3.63±0.12	3.33±0.59	2.57±0.15	*2.73*	***27***
Y	14.0±0.7	18.1±0.7	24.0±1.6	25.2±1.5	17.0±1.1	*19.6*	***24***
Nb	12.3±0.9	17.0±1.7	26.4±1.0	26.2±1.0	15.1±1.9	*19.4*	***33***
Ta	0.70±0.10	1.00±0.17	1.47±0.06	1.47±0.06	0.93±0.15	*1.11*	***31***
Be	2.00±0.00	3.67±0.58	5.67±0.58	5.00±1.00	2.33±0.58	*3.73*	***43***
Sc	5.00±0.00	6.00±0.00	8.00±0.00	8.00±0.00	7.67±0.58	*6.93*	***20***
Li	23±2.0	29±0.3	45±2.2	36.4±1.3	31.8±0.9	*33.0*	***25***
Rb	113±4.8	131±4.2	180±7.5	166±5.7	114±15.0	*141*	***22***
Hf	2.00±0.20	2.57±0.23	4.53±0.21	3.73±0.25	2.37±0.35	*3.04*	***35***
Tl	0.83±0.06	1.00±0.10	1.83±0.06	1.40±0.00	0.90±0.17	*1.19*	***35***
				Overall coefficient of variation			***33***

Table 3. Element content (mg kg^{-1}) and relative coefficient of variation (CV %) in wheat flour collected from five different production farms of wheat from Altamura. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0.86±0.08	1.51±0.76	0.64±0.45	0.77±0.38	0.98±0.21	*0,95*	***35***
Cu	5.40±0.47	5.58±0.27	4.74±0.21	5.05±0.51	3.51±0.41	*4,86*	***17***
Pb	0.06±0.02	0.05±0.01	0.04±0.02	0.05±0.03	0.22±0.31	*0,08*	***87***
Zn	28.0±2.0	27.1±4.7	21.4±1.3	24.5±1.9	19.5±2.15	*24,1*	***15***
Ni	0.30±0.00	0.23±0.06	0.20±0.00	0.20±0.00	0.20±0.00	*0,23*	***19***
Co	0.02±0.01	0.02±0.01	0.01±0.01	0.02±0.01	0.02±0.01	*0,02*	***9***
Mn	27.0±3.6	22.0±1.7	24.7±4.5	31.3±2.5	32.0±1.7	*27,4*	***16***
Fe	33.3±5.8	26.7±5.8	30.0±0.0	33.3±5.8	30.0±0.0	*30,7*	***9***
Sr	2.07±0.12	3.73±0.31	1.47±0.06	1.30±0.53	0.70±0.10	*1,85*	***62***
Cd	0.03±0.01	0.02±0.01	0.02±0.00	0.03±0.01	0.03±0.01	*0,03*	***22***
Ca	467±58	433±58	333±58	367±58	400±0	*400*	***13***
P	2997±515	2980±480	3417±227	2803±457	3320±148	*3103*	***8***
Cr	1.90±0.10	1.73±0.12	1.67±0.12	1.60±0.10	1.67±0.15	*1,71*	*7*
Mg	927±92	990±96	1043±71	957±71	1010±62	*985*	***5***
Ba	1.57±0.55	2.07±0.81	3.13±0.45	2.50±0.17	1.63±0.42	*2,18*	***30***
Ti	12.0±2.0	12.0±1.7	13.0±1.0	11.0±1.7	13.0±1.0	*12,2*	*7*
Na	40.0±26.5	23.3±5.8	10.0±0.0	13.3±5.8	16.7±5.8	*20,7*	***58***
K	5433±321	4867±289	4833±351	4733±208	4333±153	*4840*	***8***
S	1333±115	1033±58	900±100	1000±173	1133±58	*1080*	***15***
Rb	3.33±0.61	2.67±0.38	4.63±0.15	3.73±0.38	0.90±0.53	*3,05*	***46***
				Overall coefficient of variation			**26**

FIGURES

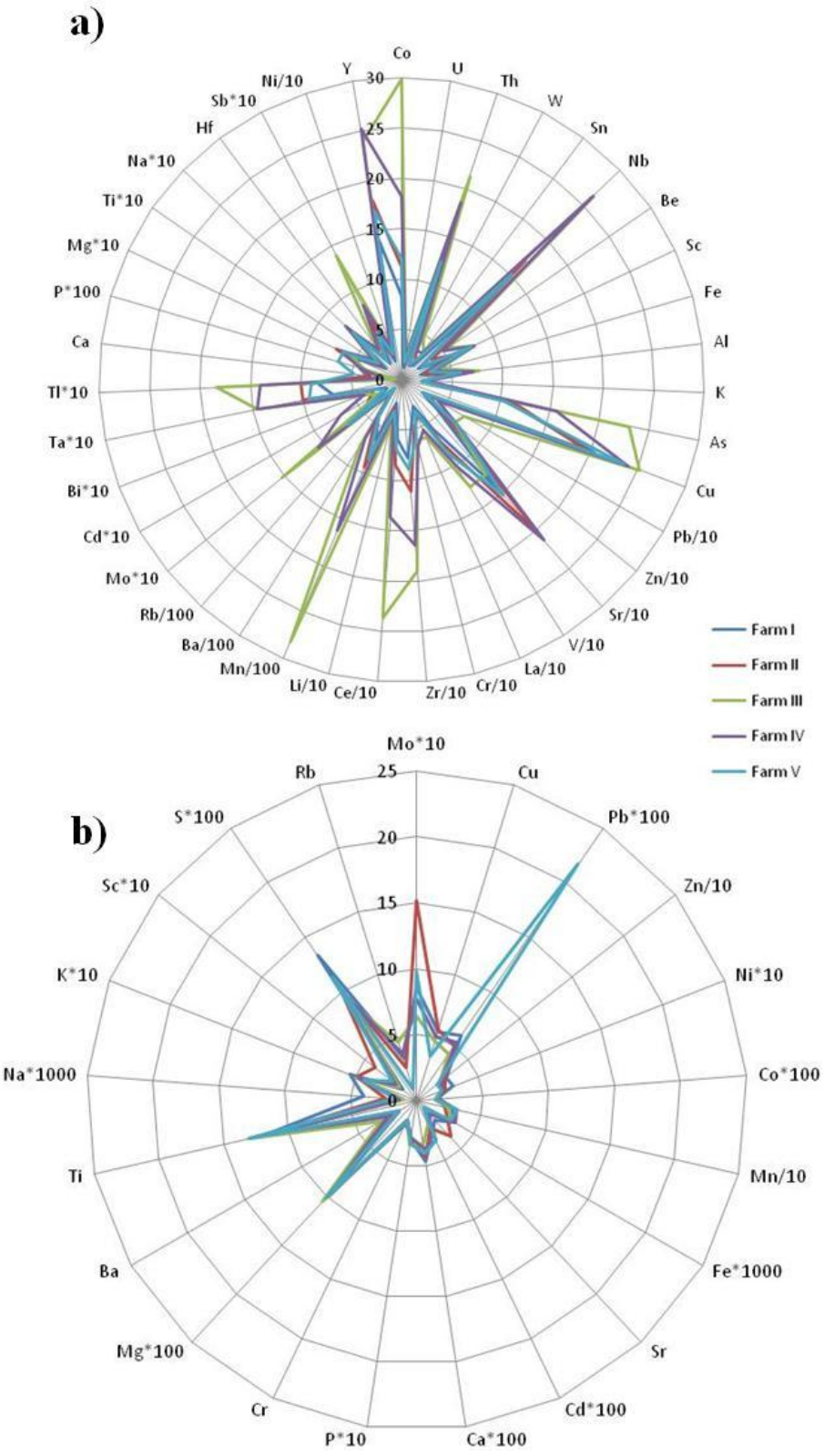


Figure 1. Multielement profile of soils (a) and wheat (b) collected from five different production farms of wheat from Altamura.

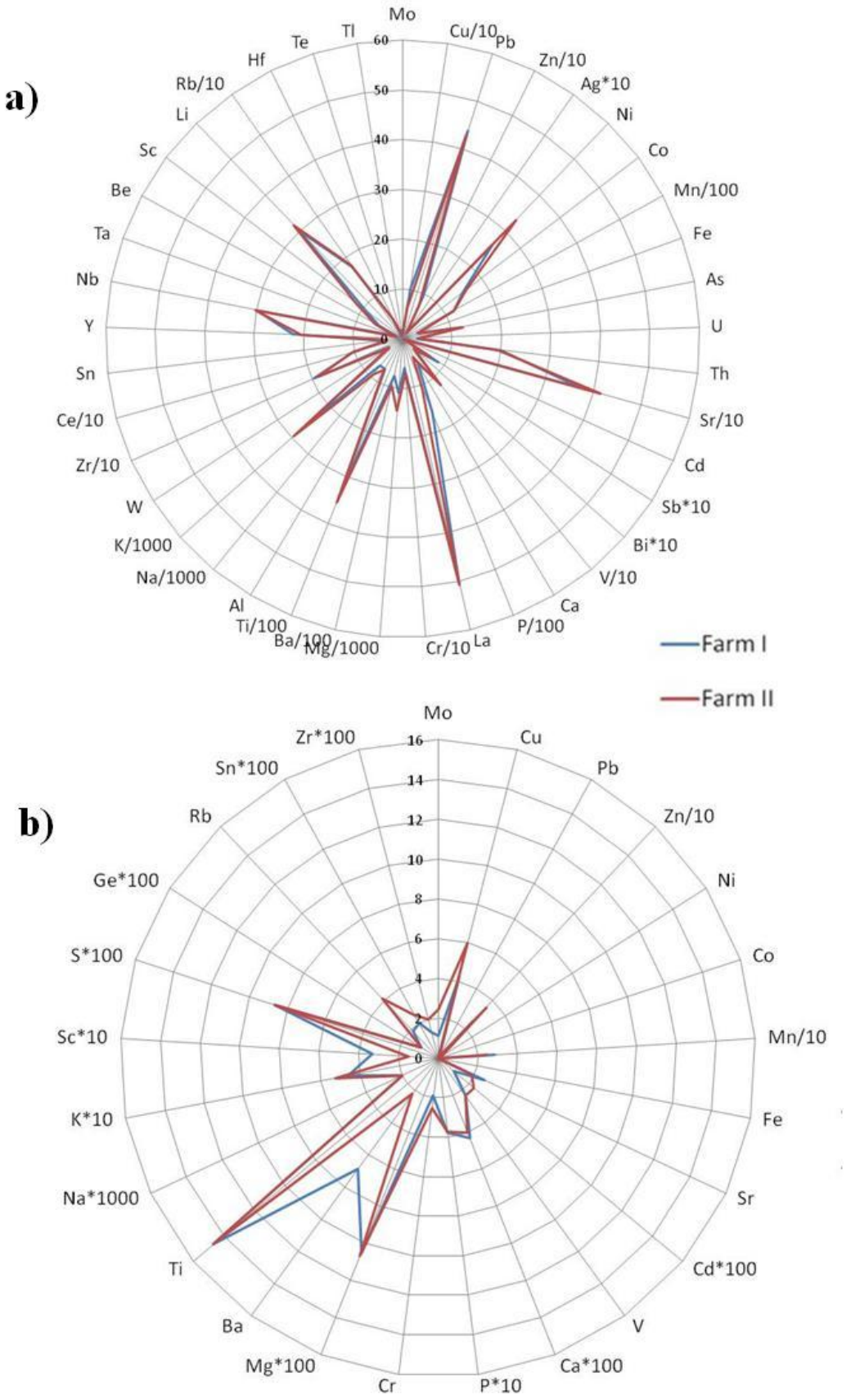


Figure 2. Multielement profile of soils (a) and wheat (b) collected from two production farms of wheat from Grottaminarda.

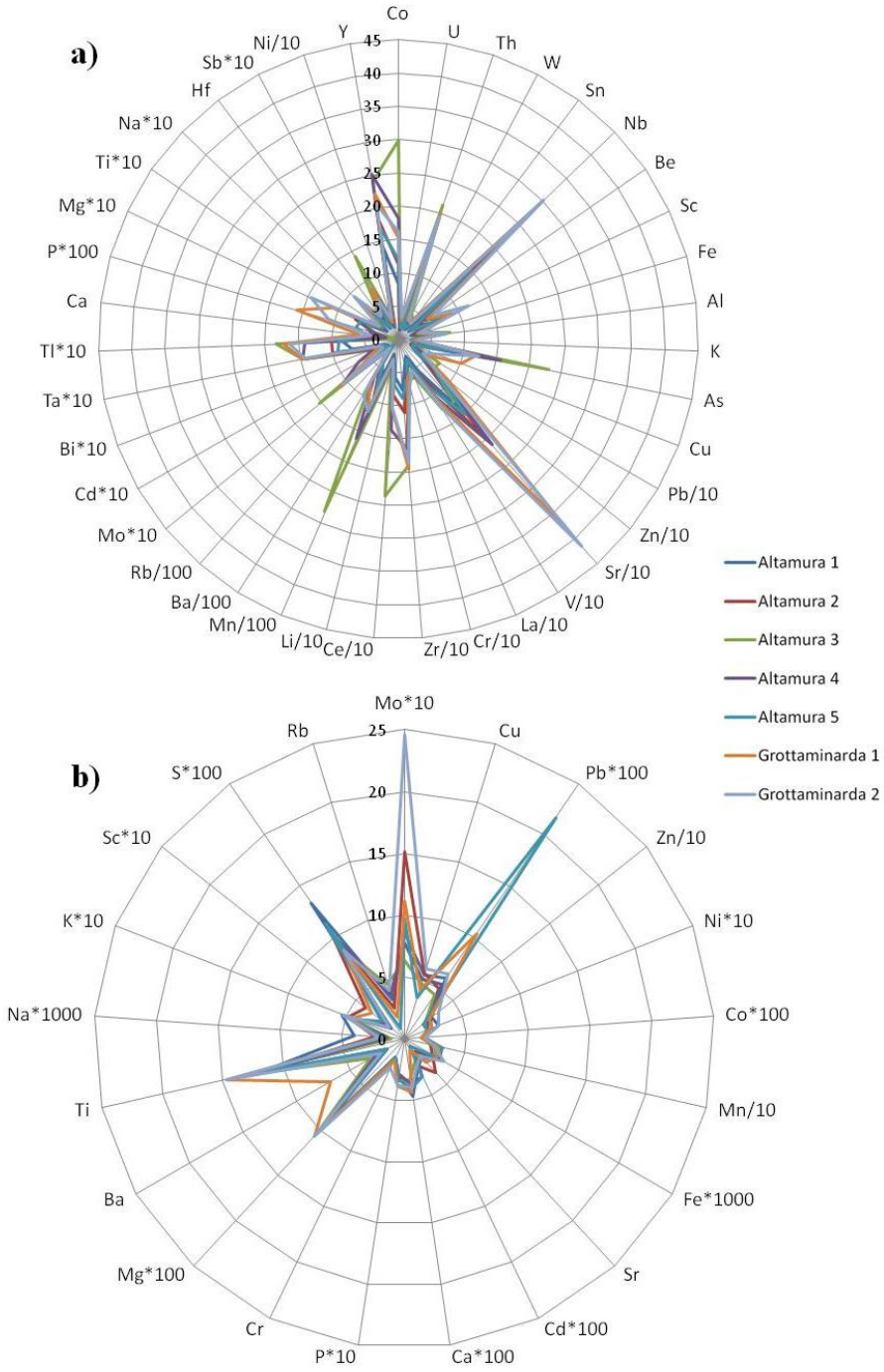


Figure 3. Comparison between the multielement profile of soils (a) and wheat (b) collected from the farms of wheat from Altamura and Grottaminarda.

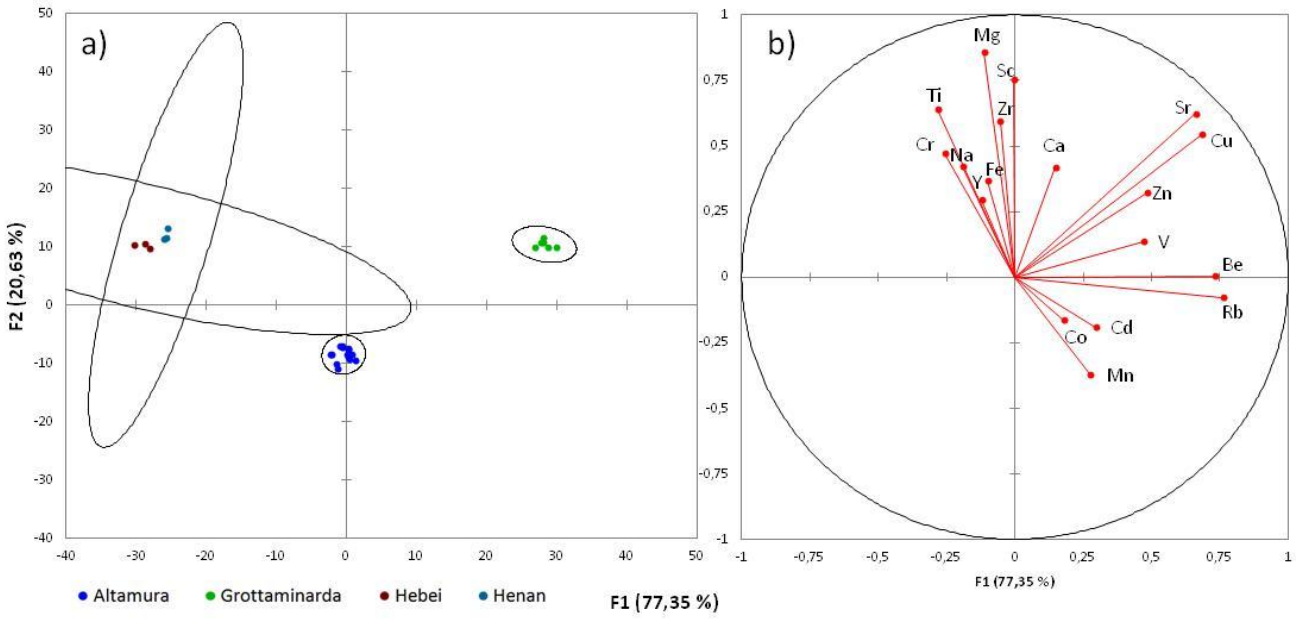


Figure 4. Factorial Discriminant Analysis from comparison of different geographical area based on multielement composition in soil. a) Projection of the cases; b) projection of the variables.

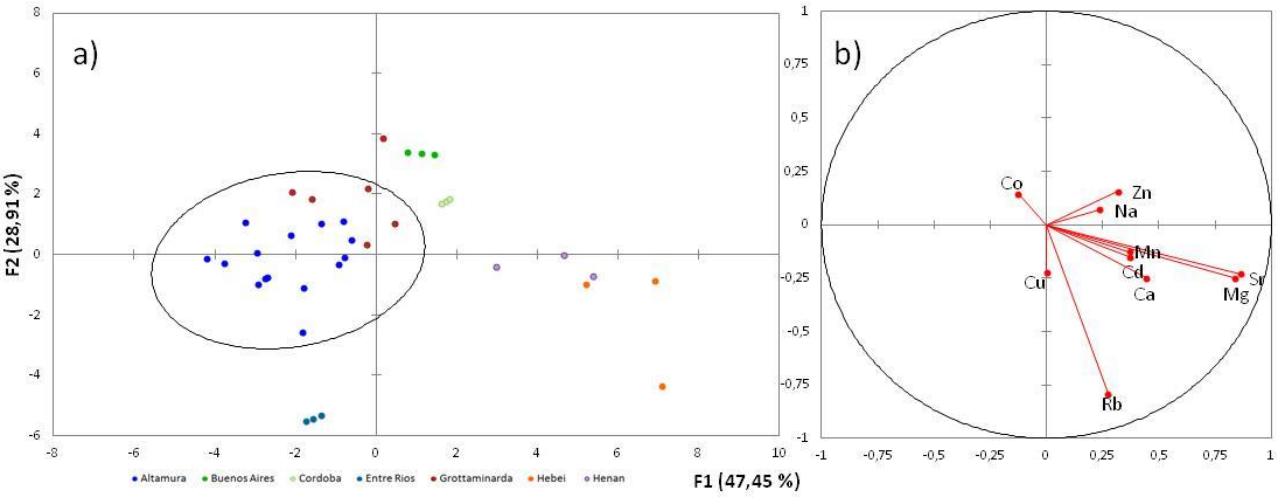


Figure 5. Factorial Discriminant Analysis from comparison of different geographical area based on multielement composition in wheat flour. a) Projection of the cases; b) projection of the variables.

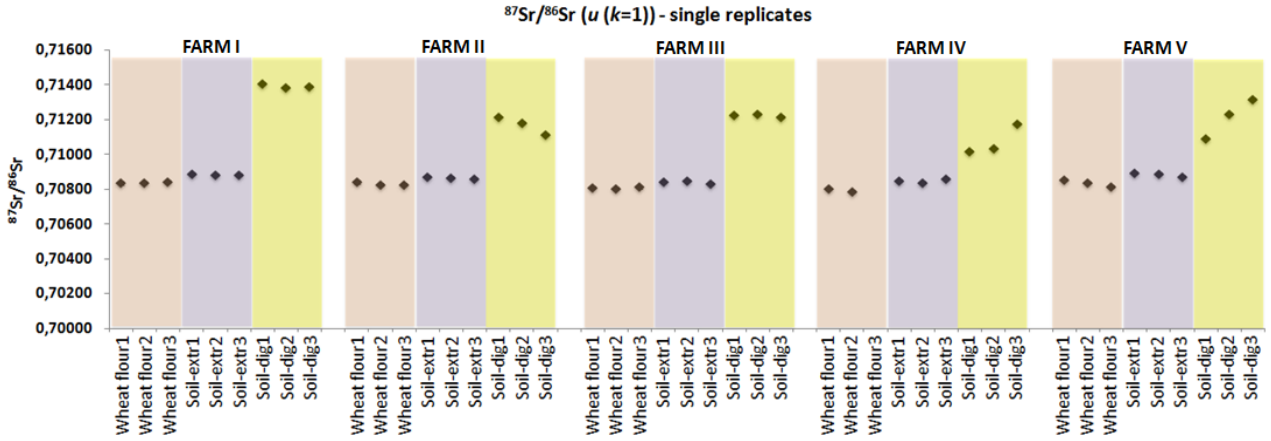


Figure 6. Sr isotope composition in soils and wheat flour collected from five different production farms. Soil-dig= total Sr in soil; Soil-extr= NH_4NO_3-extractable Sr in soil.

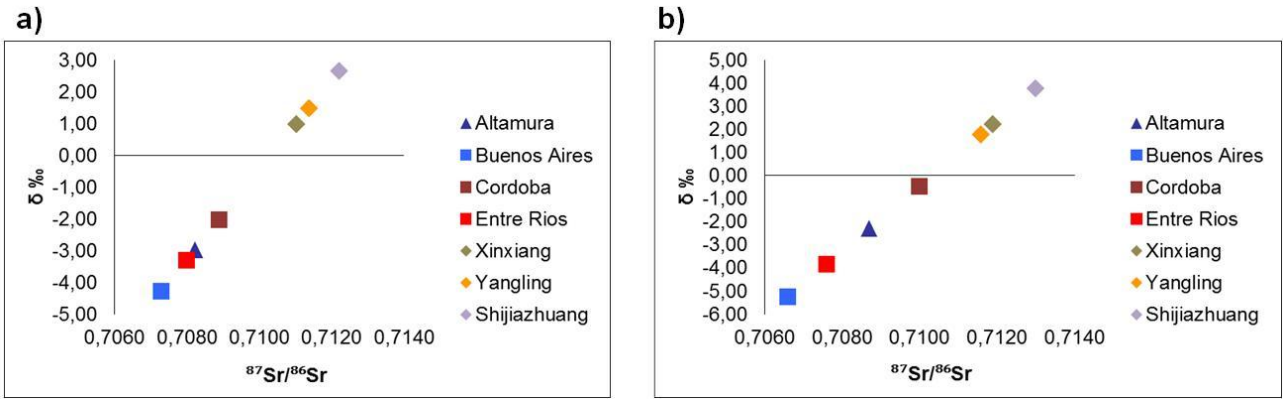


Figure 7. $^{87}Sr/^{86}Sr$ ratio vs. *δ‰* in wheat (a) and soils (b) were compared between Altamura and literature data from China, with Xinxiang, Yangling and Shijiazhuang provinces (Liu et al, 2016), and Argentina, with Buenos Aires, Córdoba and Entre Rios regions (Baroni et al., 2011). *δ‰* = 0 is for SRM 987 standard certified sample.

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Chapter 3

Multielement fingerprint for the geographical authentication of *PGI Tropea Red Onion:* a case study to enhance the quality of agricultural productions

1.0 Introduction

Nowadays the authentication of agrifood products remains a major issue, especially for those products that receive excellent labels from European Union (i.e. PDO, PGI, TSG) due to the high price compared to all other non-branded products. The defense of these products help to prove product authenticity, to combat fraudulent practices and to control adulteration, which are important issues for economic and cultural reasons, as well as valorize a territory in which they are grown.

Many works on food authentication and traceability are based on the use of multielement analysis, alone or in combination with other techniques, by means the use of inductively coupled plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS), which can acquire a considerable amount of data simultaneously (Ariyama et al., 2007).

The elemental content of agrifood grown in a specific area has two sources, natural and anthropogenic. Natural sources of elements are determined by the movement of these elements from rocks to soil and by the uptake from soil to plant (Almeida & Vasconcelos 2003). The weathering of parent rock and climatic changes determine the elemental movement from rock to soil and the uptake of elements from soil to plant is determined by the solubility of inorganic compounds in the soil as well as the type of cultivar, plant age, health, depth of roots, soil pH, rainfall pattern, drainage and temperature (Koreňovská & Suhaj, 2005; Castiñeira et al., 2004; Rossano et al.,2007).

Onion (*Allium cepa* L.) is one of the oldest cultivated plants, and the *PGI Tropea Red Onion* it seems that has been introduced in Calabrian region by the Phoenicians about 4000 years ago. It constitutes an important social and cultural resource for the territory and is largely appreciated for its organoleptic characteristics, because is rich in anthocyanins (in fact the color of the bulbs is red), and has some nutritional benefits (antiseptic, anesthetic, emollient and many others).

These organoleptic characteristics depend on the genetic heritage and the interaction with the environment, such as soil properties, temperature, humidity and the proximity to the sea. For all these reasons it is widely used both as a food and for medical purposes.

For all these interesting characteristics the *PGI Tropea Red Onion* is often counterfeited by other onions that do not have a brand name, improperly sold under a false name to enhance the organoleptic characteristics that are unique for the *PGI Tropea Red Onion.*

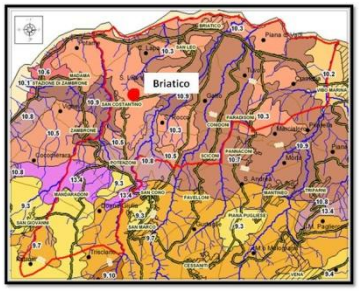
Taking in consideration the large geographical scale of the production area of the *PGI Tropea Red Onion,* the prime aim of our work was to investigate the differences between soil chemical properties affecting the uptake of elements from plants and therefore the resulting elemental composition of onions. This information is crucial to assess the possibility of discriminating *PGI Tropea Red Onion* samples on the basis of their multielemental profile in relation to the geological substrate and soil.

In this work we applied multi-element analysis to determine the geographic origin of *PGI Tropea Red Onion (Allium cepa* L.*)* grown in Calabria region, in south of Italy. Our results were then compared with those reported in Literature, to better understand the main differences in terms of element composition with onions from other geographical areas.

2.0 Materials and methods

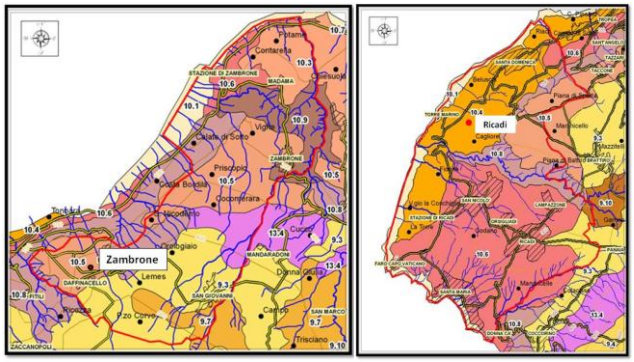
2.1 Geo-pedological properties of the cultivation areas

Most of the sites investigated in the Calabria region (southern Italy), where the *PGI Tropea Red Onion* cultivation originates, are located on the peninsula of Capo Vaticano, except the site of Amantea. Details on samples and sampling sites are given in Table 1. Different geological and pedological characteristics characterize the different sites. By the geological point of view, the peninsula of Capo Vaticano is a structural high located along the Tyrrhenian slope of the Calabrian arch. The geological substrate of the peninsula consists mainly of granite and gneiss of the paleozoic basement, which is covered by the discontinuous remains of carbonates and terrestrial deposits of Miocene and Pliocene (Burton, 1971), on top of which eight orders of erosion surfaces and well preserved Quaternary marine terraces are identified.

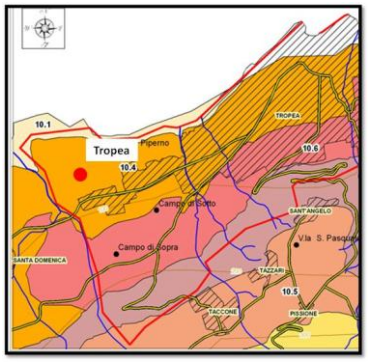


The site of Briatico is located on marine terraces at elevations between 145 and 210 m asl, where the soil parent material is made by moderately fine sediments over calcarenites. For this area, the Soil map of the coast between Capo Vaticano and Vibo Marina (1:25.000 scale) (ARSSA, 1995) reports a consociation of soils: 1) very deep soils, scarcely skeletric, with moderately fine texture, sub-acid to sub-alkaline reaction, from scarce to non-calcareous, with high water reserve, slow drainage and clear evidence of cracks during the dry season, classified as Chromic Haploxerents, fine, mixed, thermic (following USDA, 1992) and Eutric Vertisols (following FAO, 1988); 2) very deep soils, scarcely skeletric, with moderately fine texture, sub-acid to sub-alkaline reaction, from scarce to non-calcareous, with high water reserve, slow drainage, but absence of cracks, classified as Vertic Xerochrepts, fine, mixed, thermic (following USDA, 1992) and Vertic Cambisols (following FAO,

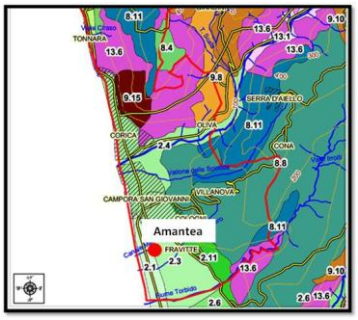
1988); 3) very deep soils, non-skeletric, with moderately fine texture, sub-acid reaction, non-calcareous, with high water reserve, moderately low drainage, classified as Pachic Argixerolls, fine, mixed, thermic (following USDA, 1992) and Luvic Phaeozems (following FAO, 1988).



The sites of Zambrone and Ricadi are located in a colluvial area, which means an accumulation area characterized by moderate slope having concave profile, with local anthropic terraces. The soil map reports very deep soils, having common to abundant skeletal, with moderately coarse to moderately fine texture, acid to subacid reaction, non-calcareous, with high water retention and good drainage, classified as Fluventic Xerochrepts, coarse loamy, mixed, thermic (following USDA, 1992) and Eutric Cambisols (following FAO, 1988). The main land use is arable land and olive trees.



The site of Tropea is located at elevation between 40 and 70 m a.s.l., on a quaternary marine terrace that is bordered by slopes and covered by continental sediments. In the soil map are reported very deep soils, having scarce to common skeletal, with medium to coarse texture, acid to subacid reaction, with moderate water retention and good drainage, classified as Typic Xerochrepts, coarse loamy, mixed, thermic (following USDA, 1992) and Eutric Cambisols (following FAO, 1988). The land use in this very anthropized area is citrus trees and arable lands.



The site of Amantea extends along the Tyrrhenian coast, from the mouth of the river Angitola to Campora San Giovanni. The landscape consists of mobile and vegetated coastal dunes, located close to the beach. The soils are characterized by low developed profile, made by an Ap surface horizon, with sandy to sandy loam texture, neutral reaction, weakly structured, overlaying a C horizon made by uncoherent sands, non-calcareous, classified as Typic Xeropsamments (following USDA, 1992). The land use consists of Eucalyptus and pine reforestation, citrus trees, arable and vegetable lands.

2.2 Soil and plant sampling

In all farms, the onion cultivation was managed in full compliance with the *PGI Tropea Red Onion* production guidelines (European Community Council Regulation EC 264/2013; Dossier number IT/PGI/0005/0369).

Onions and related cultivation soils (0-30 cm) were sampled from five different farms. At harvest, onion were in the full-ripe stage. A sampling strategy with three replications per farm was applied, hence a total of 15 soil and 15 onion samples were collected. More details of samples and sampling sites are given in Table 1.

2.3 Plant and soil preparation

The onions were cleaned from adhering soil, washed with MilliQ water, dried and cut into pieces of approximately the same size. The samples were placed in Falcon and freeze-dried in a DELTA 1–24 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), until complete dehydration and then pulverized using agate mill jars. The freeze-dried samples were stored at -20 °C. All cleaning operations and management of the samples were performed under a glove box to avoid contamination. The geochemical surveys were conducted on samples of surface soil (0-30 cm). Soils were air-dried and 2 mm sieved and subsequently the residual moisture was determined at 105 °C.

2.4 Multielement analysis

Multielement analysis of soil and onion was carried out at Acme Analytical Laboratories Ltd (Vancouver, Canada) by Perkin Elmer Elan 6000 ICP-MS. As for soils, Acme's Group 1EX package (HNO_3-$HClO_4$-HF digestion) for 41 elements (Ag, Al, As, Au, Ba, Be, Bi, Ca, Ce, Cd, Co, Cr, Cu, Fe, Hf, K, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Rb, S, Sb, Sc, Sn, Sr, Ta, Th, Ti, U, V, W, Y, Zn, Zr) was used. As for onion, Acme's Group 1VE – MS package (HNO_3 digestion followed by *aqua regia*) for 53 elements (Au, Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Ga, Hf, Hg, In, K, Ge, La, Li, Mg, Mn, Mo, Na, Nb, Ni, P, Pb, Pt, Rb, Re, S, Sb, Sc, Se, Sn, Sr, Ta, Te, Th, Ti, Tl, U, V, W, Y, Zn, Zr, Pd) was employed.

Multielement analysis of adhering soil was carried out at VIRIS Laboratory, University of Natural Resources and Life Sciences (Vienna, Austria) by NexIon 350D ICP-QMS (PerkinElmer,
Ontario, Canada). The milled soil was HF-HCl- HNO_3 digested for 31 elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sr, Te, Tl, U, V, Zn).

The degree of similarity among geochemical profiles of soils and onions collected in the same production areas was evaluated by single element and overall coefficient of variation (CV), expressed as percentage, and defined as the ratio of the standard deviation to the mean.

2.5 Statistical analysis

The comparison of our data with those ones published by literature was carried out by means a Factorial Discriminant Analyses (FDA) performed by XLSTAT.

3. Results and discussion

3.1 Soil properties and multielemental profile

Cultivation soils were characterized by pH ranging between 4.3 and 8.1. Soils were poor of carbonates (always below 8 g/kg) and exhibited a low organic carbon content (values ranging from 1.9 to 8.8 g/kg) and a low to high cation exchange capacity measured by $BaCl_2$ pH 8,1 (between 2.4 and 23.6 $cmol_+/kg$) (ISO 13536, 1995).

The total concentration of single elements in soils and onions from the five production farms of Tropea are given in Tables 2 and 3. In Figure 1 the chemical composition of soils and onion are compared graphically as farm geochemical profiles. Elements present in all samples with concentrations below LOD are not reported. A low degree of similarity was found among geochemical profiles of the soils and the onions collected in the different farms (*overall* CV for soils 31%). As shown in Table 2 and Figure 1a, the only chemical elements whose content in soils from different farms was characterized by a CV < 20% were eight, Al, Ba, Be, K, P, Rb, Sc, Sr. This inhomogeneity between geochemical profiles of soils from different onion production farms is likely related to the different physical and chemical properties of the soils.

In Figure 2 are showed the main chemical soil properties (pH, organic carbon, cation exchange capacity and total carbonates) for soils collected from the five production farms. It is clear demonstrated the great disparity of soil properties among the different sites, so these differences affect the elemental content among the soils. For example, the most variable elementst among the soils are Zn (from 39 mg/kg Ricadi and 89 mg/kg for Zambrone), Ni (from 6.0 mg/kg Ricadi and 29 mg/kg Amantea), Mn (from 426 mg/kg Ricadi and 889 mg/kg for Zambrone), Sr (from 426 mg/kg Amantea and 273 mg/kg for Ricadi), V (from 56 mg/kg Amantea and 120 mg/kg for Zambrone), Ca (from 7500 mg/kg Zambrone and 27000 mg/kg for Amantea), Cr (from 20 mg/kg Ricadi and 62 mg/kg for Amantea), Mg (from 2400 mg/kg Ricadi and 9300 mg/kg for Amantea), Zr (from 14.3 mg/kg Amantea and 119 mg/kg for Zambrone), Rb (from 71 mg/kg Briatico and Amantea and 113 mg/kg for Tropea).

3.2 Onion multielemental profiles

The geochemical profiles of onion samples taken from the five different farms (Table 3 and Figure 1b) were characterised by a high level of inhomogeneity, even higher than that observed for soils.

Indeed, *overall* CV for onions was 54%, with again eight elements showing a CV lower than 20% (Fe, P, Cr, Mg, Ti, K, Sc, Se).

As for the soil, also in the onions the element content among the samples is variable. Among the most variables there are Zn (from 7.9 mg/kg for Amantea to 20 mg/kg for Ricadi), Mn (from 7.0 mg/kg for Briatico to 139 mg/kg for Ricadi), Sr (from 9.5 mg/kg for Amantea to 26 mg/kg for Briatico), Mg (from 710 mg/kg for Briatico to 1190 mg/kg for Tropea), Ba (from 0.5 mg/kg for Amantea to 47.3 mg/kg for Briatico), Na (from 270 mg/kg for for Zambrone to 1120 mg/kg for Ricadi), Cd (from 0.01 mg/kg for Amantea to 0.42 mg/kg for Ricadi).

3.3 Comparison with Literature data

Our data of multielement analysis were compared with literature data in order to show the main differences in terms of element composition with onion coming from other geographical areas. Since no literature data was found about the element composition of onion cultivation soil, only data of onion samples were compared.

Our *PGI Tropea Red Onion* was compared with:

- data reported by Furia et al. (2011) on Italian onions from Calabria (*PGI Tropea Red Onion* from Capo Vaticano, Amantea, Nocera Terinese, Briatico), Sicily (Agrigento), Campania (Salerno) and Piemonte;
- data reported by Furia et al. (2011) on onions from Holland, by Ariyama et al. (2007) on onions from Japan (Hokkaido, Saga, Hyogo, and imported into Japan), by Ariyama et al. (2004) on onions from Japan and China (Shandong, Shanghai, Amoy Fujian).

Comparison with other Italian data was done for 8 common elements: Zn, Ni, Mn, Sr, Cd, Mg, Ba, Na. Two sites from Calabria were exactly the same site of provenience of our *PGI Tropea Red Onions* (i.e., Amantea and Briatico).

Comparison with non Italian data was done for 13 common elements: Mo, Cu, Zn, Ni, Co, Mn, Sr, Cd, P, Mg, Ba, Na, Rb.

The comparison has been assessed by a Factorial Discriminant Analysis (Figure 3). As already discussed, given the great variability of the elemental composition of *PGI Tropea Red Onion*, it was not possible to discriminate with the other onions coming from different geographical areas. The plot showed an overlapping with both the Chinese, Holland and the other Italian samples (right side of the plot) (Figure 3a). The site Ricadi is the only one that is well separeted (left side of the plot), probably due to the high content of Mn, Na, Cd (Figure 3b), as already discussed.

4. Conclusions

The mineral composition of agricultural products is the result of various factors such as fertilization, climatic conditions in the cultivated year, differences in soil types, history of fields, and variety within a single farm field. In the case of the *PGI Tropea Red Onion*, due to the large geographical scale of the production area (both in latitude and in altitude), the chemical composition for soil and onions was found highly variable among all the analyzed sites, not allowing to create a unique geochemical signature for the products. As expected, due to the great variability existing among the geochemical profiles of the onions it was not possible to discriminate the *PGI Tropea Red Onion* from other onions grown in other geographical areas.

TABLES

Table 1. Code and geographical location of the studied samples

Region/ Municipality	Sample Code	Sample	Locality	Latitude N	Longitude E
Calabria/ Tropea	Farm I	soil onion	Ricadi	38° 38' 59,5336"	15° 51' 12,3009"
	Farm II	soil onion	Tropea	38° 40' 8,1755"	15° 52' 35,5738"
	Farm III	soil onion	Zambrone	38° 40' 44,7335"	15° 56' 44,823"
	Farm IV	soil onion	Briatico	38° 42' 23,2698"	16° 0' 38,1725"
	Farm V	soil onion	Amantea	39° 3' 13,31"	16° 5' 56,3494"

Table 2. Element content (mg kg^{-1}) and relative coefficient of variation (CV %) in soils collected from five different production farms of onion from Tropea. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0,53±0,06	1,17±0,06	1,60±0,00	1,27±0,12	0,30±0,00	*0,97*	**56**
Cu	35,6±0,26	52,1±0,96	79,6±1,96	34,6±0,50	31,8±1,00	*46,7*	**43**
Pb	25,5±0,7	38,7±1,8	35,1±0,6	34,5±1,1	17,5±0,6	*30,2*	**28**
Zn	40,7±1,5	71,3±1,5	85,0±3,6	48,7±1,1	60,0±2,0	*61,1*	**26**
Ni	6,5±0,4	11,2±0,3	16,9±0,9	12,2±0,9	28,7±0,3	*15,1*	**50**
Co	7,9±0,3	12,1±0,4	15,2±0,2	9,5±0,4	9,5±0,4	*10,8*	**23**
Mn	437±15,9	825±16,6	874±18,6	523±20,3	716±13,7	*675*	**25**
Fe	1,87±0,07	2,96±0,11	3,87±0,15	2,70±0,08	2,49±0,01	*2,78*	**24**
As	4,00±0,00	7,67±0,58	10,67±0,58	8,00±0,00	5,67±0,58	*7,20*	**31**
U	2,03±0,06	2,60±0,10	3,57±0,06	1,67±0,06	0,87±0,06	*2,15*	**42**
Th	17,3±1,0	16,9±0,7	17,2±0,7	12,2±0,4	6,2±0,2	*14,0*	**31**
Sr	266±6,4	238±3,5	189±7,4	194±4,0	160±4,2	*209*	**18**
Cd	0,10±0,00	0,33±0,06	0,33±0,06	0,30±0,10	0,15±0,07	*0,24*	**41**
Sb	0,17±0,06	0,50±0,00	0,43±0,06	0,43±0,06	0,53±0,06	*0,41*	**31**
Bi	0,13±0,06	0,40±0,00	0,50±0,00	0,30±0,00	0,15±0,07	*0,30*	**48**
V	67±3,5	77±1,7	115±6,1	89±2,1	59±2,5	*81*	**24**
Ca	1,35±0,08	0,97±0,02	0,76±0,01	1,31±0,08	2,57±0,13	*1,39*	**45**
P	0,06±0,00	0,06±0,00	0,08±0,00	0,09±0,00	0,07±0,00	*0,07*	**12**
La	69,8±5,6	60,3±2,4	59,4±2,3	37,6±0,8	20,2±1,3	*49,5*	**36**
Cr	21,0±1,0	39,7±5,5	40,7±1,5	32,0±0,0	57,0±4,6	*38,1*	**31**
Mg	0,24±0,01	0,42±0,01	0,47±0,02	0,36±0,01	0,91±0,03	*0,48*	**47**
Ba	833±27,6	814±5,9	761±25,2	828±17,7	457±17,3	*739*	**19**
Ti	0,25±0,01	0,35±0,01	0,45±0,00	0,32±0,01	0,25±0,01	*0,32*	**24**
Al	7,6±0,4	8,3±0,5	8,0±0,1	7,2±0,1	6,1±0,1	*7,4*	**10**
Na	2,25±0,02	1,83±0,10	1,30±0,02	1,36±0,02	1,28±0,02	*1,60*	**24**
K	2,17±0,09	2,15±0,08	1,70±0,04	1,74±0,03	2,08±0,03	*1,97*	**10**
W	0,60±0,00	1,10±0,10	1,40±0,10	1,10±0,00	0,90±0,00	*1,02*	**26**
Zr	46±1,7	85±1,4	118±1,4	87±0,1	14,9±0,5	*70,0*	**51**
Ce	130±8,7	113±3,8	115±4,4	76±0,6	39±2,1	*95*	**35**
Sn	1,40±0,10	2,43±0,06	2,50±0,10	4,03±0,23	1,60±0,17	*2,39*	**39**
Y	16,6±0,5	19,2±0,2	19,7±0,5	13,2±0,6	10,7±0,3	*15,9*	**22**
Nb	11,6±0,3	19,7±0,1	24,3±0,3	16,4±0,4	6,5±0,3	*15,7*	**40**
Ta	0,60±0,00	1,00±0,00	1,20±0,00	0,87±0,06	0,47±0,06	*0,83*	**32**
Be	2,33±0,58	2,67±0,58	3,00±0,00	2,00±0,00	2,00±0,00	*2,40*	**16**
Sc	6,7±0,6	8,0±0,0	10,0±0,0	7,7±0,6	8,0±0,0	*8,1*	**13**
Li	12,7±0,4	26,1±0,4	31,3±1,2	17,5±0,9	32,5±0,4	*24,0*	**32**
Rb	86±1,8	112±1,6	88±2,9	71±1,4	78±1,1	*87*	**16**
Hf	1,07±0,06	1,93±0,12	2,63±0,12	1,97±0,06	0,47±0,06	*1,61*	**47**
Tl	1,07±0,06	1,73±0,12	1,87±0,06	1,17±0,06	0,50±0,00	*1,27*	**39**
				Overall coefficient of variation			***31***

Table 3. Element content (mg kg^{-1}) and relative coefficient of variation (CV %) in onion collected from five different production farms of red onion from Tropea. The overall coefficient of variation is the mean of the single element CV.

	Farm I	Farm II	Farm III	Farm IV	Farm V	*mean*	***CV***
Mo	0,01±0,00	0,04±0,01	0,02±0,00	0,22±0,02	0,15±0,01	*0,09*	**103**
Cu	3,90±0,13	7,50±0,31	4,07±0,26	3,71±0,29	4,03±0,38	*4,64*	**35**
Pb	0,04±0,01	0,07±0,06	0,04±0,01	0,05±0,02	0,05±0,01	*0,05*	**22**
Zn	19,3±0,95	14,3±0,96	13,4±0,89	8,9±0,06	8,7±0,68	*12,9*	**34**
Ni	1,10±0,10	0,40±0,00	0,20±0,00	0,40±0,00	0,27±0,06	*0,47*	**76**
Co	1,02±0,09	0,03±0,01	0,03±0,00	0,02±0,01	0,03±0,01	*0,22*	**199**
Mn	131±6,8	15,0±0,00	15,0±0,00	7,7±0,58	11,7±0,58	*36,1*	**148**
Fe	26,7±5,8	33,3±5,8	30,0±0,0	33,3±5,8	30,0±0,0	*30,7*	**9**
Sr	23,2±1,5	18,3±0,9	18,2±0,8	25,1±1,2	9,9±0,4	*19,0*	**31**
Cd	0,41±0,01	0,07±0,01	0,15±0,01	0,07±0,01	0,01±0,01	*0,14*	**112**
Ca	1500±0,0	1500±0,0	1467±58	2300±100	1700±100	*1693*	**21**
P	2227±156	2373±59	1977±15,3	1983±64	1987±101	*2109*	**9**
Cr	1,37±0,12	1,57±0,29	1,50±0,20	1,50±0,17	1,37±0,06	*1,46*	**6**
Mg	770±10,0	1113±68	823±55	763±68	743±23	*843*	**18**
Ba	3,40±0,10	8,6±0,59	6,1±0,21	34,6±11,1	0,57±0,06	*10,7*	**129**
Ti	2,67±0,58	2,67±0,58	1,67±1,15	2,50±0,71	2,00±0,00	*2,30*	**19**
B	16,7±0,58	20,3±2,31	11,7±1,53	13,3±1,15	7,7±0,58	*13,9*	**35**
Na	1073±45	350±10	277±12	330±17	337±31	*473*	**71**
K	13367±252	14600±520	12900±557	11400±458	11833±709	*12820*	**10**
Sc	0,20±0,00	0,20±0,00	0,23±0,06	0,13±0,06	0,17±0,06	*0,19*	**20**
S	3867±153	4000±361	3700±721	2000±361	3200±200	*3353*	**24**
Se	0,23±0,06	0,27±0,06	0,27±0,06	0,33±0,06	0,33±0,06	*0,29*	**16**
Rb	24,7±0,57	4,73±0,12	5,83±0,21	7,3±0,21	2,93±0,15	*9,1*	**97**
				Overall coefficient of variation			***54***

FIGURES

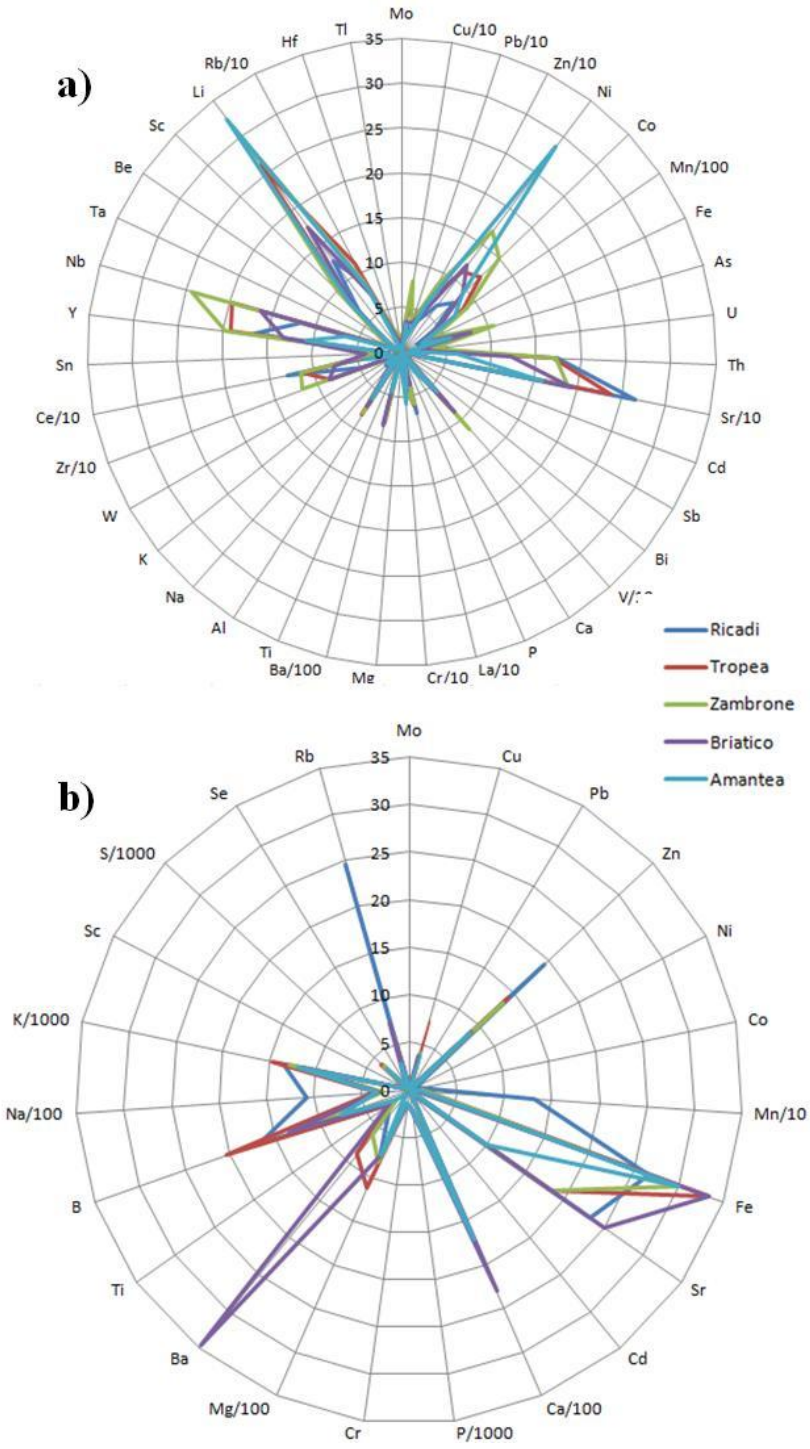


Figure 1. Multielement profile of soils (a) and onions (b) collected from five different production farms of *Red Onion from Tropea.*

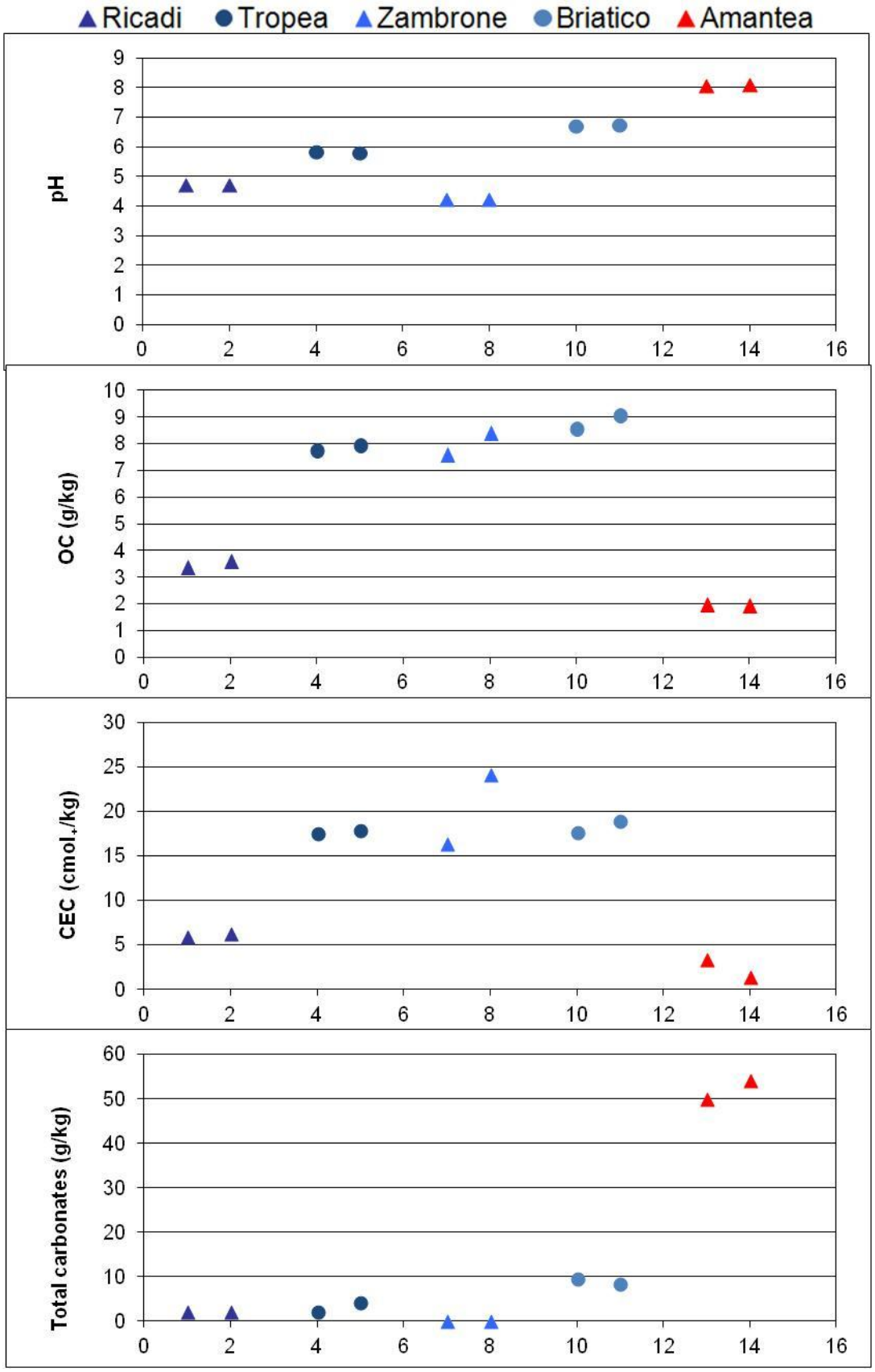


Figure 2. Main soil properties of soils collected from five different production farms of *Red Onion from Tropea.*

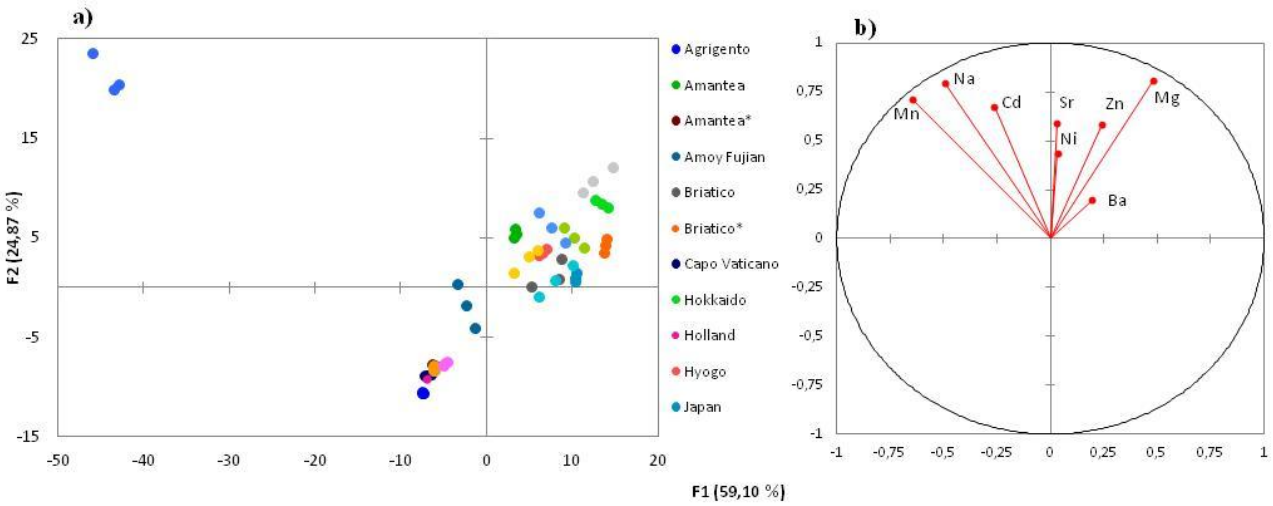


Figure 3. Factorial Discriminant Analysis from comparison of different geographical area based on multielement composition in onions. a) Projection of the cases; b) projection of the variables.

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Chapter 4

Isotopic and elemental analysis of fish tissues for provenance determination

1. Introduction

The reliable tracing of food products through the entire supply chain is an essential requirement for all types of food commodities qualified by origin, composition and quality. Proof of provenance and authenticity supports food safety, enhances consumer confidence and countervails fraudulent practices (Luykx & Van Ruth, 2008). Fish and fish products are amongst the most important food commodities and reliable methods to trace the origin of fish have become of high importance. For this reason, it has become essential to identify markers that can provide reliable indications of the geographic origin of food products (Zitek et al., 2016). Different methods have been proposed for the authentication and traceability of fish and fish products, such as proteomics (Mazzeo & Siciliano, 2016), FT-NIR and FT-IR (Alamprese & Casiraghi, 2015), stable isotopes (δ^{15}N and δ^{13}C), fatty acids and carotenoids (Molkentin et al., 2015), DNA (Fernandes et al., 2017) and multi-element analysis (Li et al., 2016).

Also the $^{87}Sr/^{86}Sr$ stable isotope ratio has been used to authenticate food products and have gained substantial importance to trace the origin of fish, as well (Zitek et al., 2010). The $^{87}Sr/^{86}Sr$ isotopic composition varies depending on the geographical area, because it is linked to the rocks and mineral composition of soils (Baffi & Trincherini, 2016). Through weathering, Sr is released to the water and thus the isotopic information of the geographic area is transferred to the water and as a consequence into living organisms within ecosystems, such as fish (Nakano, 2016). Therefore, the $^{87}Sr/^{86}Sr$ isotope ratio can be used as provenance indicator based on the assumption that it is taken up by fish without fractionation and thus represents the fingerprint of the natural habitat (Dufour et al., 2007). Strontium is stored in different hard and soft tissues of fish such as otoliths, fin rays, backbones as well as fish meat. The content in the latter is by orders of magnitude lower as compared to hard tissues. Moreover, hard tissues like otoliths, fin rays and backbones have the ability to store time resolved information. Otoliths are the most widely investigated fish tissue for Sr isotope ratios. Otoliths are concretions of calcium carbonate, generally aragonite, located in the fish head and used for balance and hearing. Otoliths grow incrementally over the life span of a fish and have a three dimensional concentric lamellar structure. During the growth, the environmental information is stored incrementally. Many studies confirmed that fish otoliths are valuable monitors for identifying habitat changes but also environmental variability (e.g. chemical changes of water via e.g. anthropogenic impact). Therefore, the correlation between the chemical fingerprint of an

otolith region with different water bodies allows to assess migration behavior of fish (Muhlfeld et al., 2012; Irrgeher et al., 2014; Lill et al., 2014; Brennan et al., 2015).

Strontium in fish tissues was investigated for archaeological research questions, as well (Elliott et al., 1998; Kennedy et al., 2000; Dufour et al., 2007). Fish meat appears to be a good source of strontium, compared to other agricultural products. This assumption is confirmed by the fact that the level of content of Sr in bones is correlated with the dietary of humans (Horwood et al., 1989; Burton et al., 1999). As a consequence, strontium continues to be examined as a potential indicator of seafood in the human diet.

Even though otoliths are the most important recorders to trace the provenance of fish, in food commodities, these otoliths are only available if the fish is commercialized as a whole. Therefore, it has become of substantial importance to develop an adequate method in order to use geochemical markers such as elemental and isotopic fingerprints to trace the provenance of fish via other tissues, especially fish meat and fish bones.

The current investigation focused on the development of an analytical approach (including sampling, digestion and analysis) to assess the usage of Sr isotopes and multi-element fingerprints as tracer of origin in fish meat. In the next step, the acquainted information will to be linked to the water bodies, which has been tested on a limited number of samples as preliminary study. Since the Sr/Ca ratio along with the $^{87}Sr/^{86}Sr$ isotope ratio analyzed by (multi collector) inductively coupled plasma mass spectrometry (MC ICP-MS) proved to be the most potential tools in this respect (Prohaska et al., 2016; Zitek et al., 2010), why the further focus was set on these markers.

The analysis was carried out at the VIRIS Laboratory of the University of Natural Resources and Life Sciences Vienna (BOKU) in Tulln, Austria, during a 6 months research stay. The work was accomplished within the scope of the project "*CSI: TRACE YOUR FOOD*" (Sparkling Science Project funded by the Austrian Ministry of Science, Research and Economy (SPA 05/052 – CSI:TRACE your FOOD!) led by Dr. Andreas Zitek, BOKU, Tulln, Austria).

2. Materials and methods

2.1 Sample collection and preparation

Fifteen fish samples of different species were collected at each of the six different sampling sites in Austria, two sites in Lower Austria (carp, *Cyprinus carpio,* brook trout, *Salvelinus fontinalis*), Upper Austria (whitefish, *Coregonus spp*.), Tyrol (brook trout, *Salvelinus fontinalis*), Styria (carp, *Cyprinus carpio*), Carinthia (brook trout, *Salvelinus fontinalis*) in within the above-mentioned project and provided for further analysis. In the next step, the sample preparation was optimized in order to retrieve the limited amount of Sr in the fish meat.

Fish samples were dissected to gain fish meat. The stomach was opened by scissors to take out the organs and the filet was separated from the skin with a ceramic knife, cut into small pieces, freeze-dried (Christ Beta1-8 LD plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and finally milled using an agate mortar.

The powder was digested by applying an optimized microwave-assisted acid digestion procedure (Multiwave 3000, Anton Paar, Graz, Austria) using 5 mL of concentrated HNO_3 (65 % *w*/*w*, Merck-Millipore, Darmstadt, Germany) and 1 mL of H_2O_2 (30 % *w*/*w* suprapur, Merck), prior to the determination of element concentration and Sr isotopic composition.

2.2 *Chemical analysis*

All laboratory consumables (polyethylene bottles, tubes, pipette tips) used for sampling, preparation and dilution of samples were pre-cleaned in a two-stage washing procedure using nitric acid (10 % *w*/*w* and 1 % *w*/*w*).

Elemental analysis was accomplished using single collector quadrupole ICP-MS (NexIon 350D, Perkin Elmer, Waltham, US) for 31 elements (Ag, Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sr, Te, Tl, U, V, Zn).

The instrument was operated in standard mode using the optimized conditions given in Table1. Quantification was accomplished via external calibration (multi elemental ICP-MS standard VI, CertiPur, Merk, Darmstadt, Germany) using ^{115}In as internal normalization standard.

For the subsequent analyte/matrix separation prior to isotopic analysis by MC ICP-MS, all matrix separations were performed using the fully automated low-pressure chromatography sample preparation system prepFAST-MC™ (Elemental Scientific), as described by Retzmann et al. (2017). Separation is a prerequisite to remove interfering Rb and Ca species and possible matrix-induced mass discrimination effects. The samples were diluted using 2 % (*w*/*w*) nitric acid and analyzed for $^{87}Sr/^{86}Sr$ by MC ICP-MS (Nu Plasma HR, NU instruments, Wrexham, UK) using a desolvation nebulization unit with a PFA nebulizer (Aridus II, Cetac Technologies, Omaha, Nebraska).

Correction for external instrumental isotopic fractionation (aka mass bias) was accomplished by measuring a solution of SRM 987 (NIST, Gaithersburg, MD, USA). Procedural blanks were processed and measured as samples. Measurement was accomplished by applying on peak correction. The correction strategies for instrumental isotopic fractionation, for the isobaric interference of ^{87}Rb on ^{87}Sr are further described by Horsky et al. (2016).

2.3 *Statistical analysis of the data*

Data elaboration related to the Sr/Ca and $^{87}Sr/^{86}Sr$ ratios was accomplished using Microsoft Office Excel® 2010. The significance of the differences among the element concentrations in fish meat samples collected in the six sites was performed by One-Way ANOVA and Games-Howell test as *post-hoc* ($p < 0.05$) with SPSS® software.

3. Results and discussion

The developed analytical protocol allowed for the assessment of Sr/Ca and $^{87}Sr/^{86}Sr$ ratios at a level of quality in order to be used for further statistical data evaluation to monitor provenance of fish meat.

Figure 1 depicts the Sr/Ca ratio of fish meat from the investigated sites. Samples from Tyrol had a higher ratio (0.0025-0.0044) compared to the other geographical areas. Low standard deviations showed that the results of individual fish within one region were in good agreement with each other supporting the validity of the sample preparation protocol. Moreover, the Sr/Ca ratios showed distinct differences. Therefore, the Sr/Ca ratio has a high potential to be used as tracer of fish meat. The results were in agreement with prior studies, where the Sr/Ca ratio was confirmed to be a good indicator of food provenance (Faure & Mensing, 2004; Phillis et al., 2011; Nakano et al., 2016).

The multielement content of the analyzed samples is displayed in Figure 2. Elements present in all samples with concentrations below LOD are not reported. Even though it is premature to select adequate elements that can be used as chemical markers to authenticate the fishes in relation to the geographical area, in general, the levels of trace elements were above LOD proving the quality of the sample preparation approach, as well. Significant differences (One-Way ANOVA + *post-hoc;* $p < 0.05$) were observed among the elemental content in the fish meat from the different sampling sites. In particular the site Lower Austria 1 differenced from all the other sites, regarding As, Fe and V. For Ca, Cu, Li, Mn and Rb, no significant differences (One-Way ANOVA + *post-hoc;* $p < 0.05$) were observed comparing the site Upper Austria with the other sites.

Figure 3 illustrates the $^{87}Sr/^{86}Sr$ ratio in fish meat from Tyrol, one site from Lower Austria, Styria and Upper Austria. The analyzed samples from Styria had a higher $^{87}Sr/^{86}Sr$ ratio. The samples from Lower Austria showed inhomogeneities within the same site.

In order to assess the potential to attribute Sr/Ca and $^{87}Sr/^{86}Sr$ to different water bodies, the data for water for two sites, Tyrol (aquaculture) and Lake Constance (natural environment), was provided within the research project CSI: TRACE your FOOD! as comparative data for this study.

Figure 4 shows the Sr/Ca ratio of water and fish meat from these two different aquatic systems. The Sr/Ca ratio of the water differed between the two aquatic environments. In a previous study, the

Sr/Ca ratio of the fish otoliths was found to correlate with the water bodies (Zitek et al., 2010; Prohaska et al., 2016). In this study the relative difference in the Sr/Ca ratio of the water bodies is not directly reflected in the fish meat. It has to be taken in consideration that the Sr/Ca ratio in fish tissues is affected by several factors such as species, size, age, life history, sex, sexual maturity, food source, water chemistry, salinity, climate, contaminants, sampling procedures and etc. (Kafemann et al., 2000; Alasalvar et al., 2002; Roy & Lall, 2006; Yamashita et al., 2006). This first result cannot be used to validate the potential of Sr/Ca in fish meat as provenance tracer since more statistical data need to be acquainted. Moreover, it has to be underlined that in this study, a natural habitat was compared to an aquaculture system. In the latter, both the elemental composition as well as the Sr isotopes are influenced by fish feed substantially (Tchaikovsky et al., 2017). As a consequence, the Sr/Ca ratio in fish meat did not show the same pattern as the respective water sources.

Figure 5 shows the Sr isotopic composition of the water body and fish meat from Tyrol (aquaculture) and Lake Constance (natural environment). The water bodies showed significant differences with respect to the Sr isotopic composition as the values did not overlap within their uncertainties (analytical significance). The $^{87}Sr/^{86}Sr$ isotope ratio measured in the water from the natural environment (0.7084±0.0002) was higher than that of pool water (0.7079±0.0002).

The $^{87}Sr/^{86}Sr$ isotope ratio of fish meat (0.7084±0.0001) from Lake Constance was in good agreement with the respective water body. Therefore there was a direct link from the isotopic information of the natural water habitat to the fish meat. This is evident since Lake Constance is a natural system and the natural feed of the fish reflects the same Sr isotopic composition as the water.

In contrast, the aquaculture of Tyrol showed another picture, which can be expected to be typical for an aquaculture system: the Sr isotopic information was both influenced by the water and the feed, which mostly differ in their Sr isotopic composition (Tchaikovsky et al., 2017).

4. Conclusions

Within this study we proved that the developed and optimized analytical protocol is fit for the intended use to assess multi-element fingerprints as well as the $^{87}Sr/^{86}Sr$ isotope ratios at a level, where these data sets can be further applied for fish provenance via the fish meat. The potential of Sr/Ca ratios has to be further evaluated on a broader data set of natural fish samples in order to evaluate the influence of fish feed with different composition. Nonetheless, it might be that the information content of Sr/Ca in fish meat is less useful for origin determination as compared to the Sr/Ca ratios assessed in otoliths. The Sr isotopic composition could be successfully assessed in fish

meat in order to test its potential for provenancing fish. It could be shown that in a natural habitat the Sr isotopic composition of the water was well reflected in the fish meat, whereas the Sr isotopic composition in fish meat of fish from aquacultures was influenced by both the water as well as by the fish feed. This was in agreement of findings of fish otoliths. Nonetheless, further evaluation by using isotope pattern deconvolution is needed in order to assess the contribution of fish feed and water to the final Sr isotope ratio in fish meat.

The successful accomplishment of an analytical protocol will now be used in order to assess the data on additional sites represented in the CSI: TRACE your FOOD! project. This unique set of data will allow having a clear view of the differences among geographical areas and how to link the different fish tissues to the environment of fishes.

TABLES:

Table 1. Instrumental settings of the ICP-MS NexION 350D

Setting	**Type**
Sample introduction	spray chamber
Nebulizer	ESI µFlow
Interface	Ni sampler, skimmer, hyper-skimmer
RF Power [W]	1300
Carrier gas [L min^{-1}]	0.92
Make-up gas [L min^{-1}]	0.75

FIGURES:

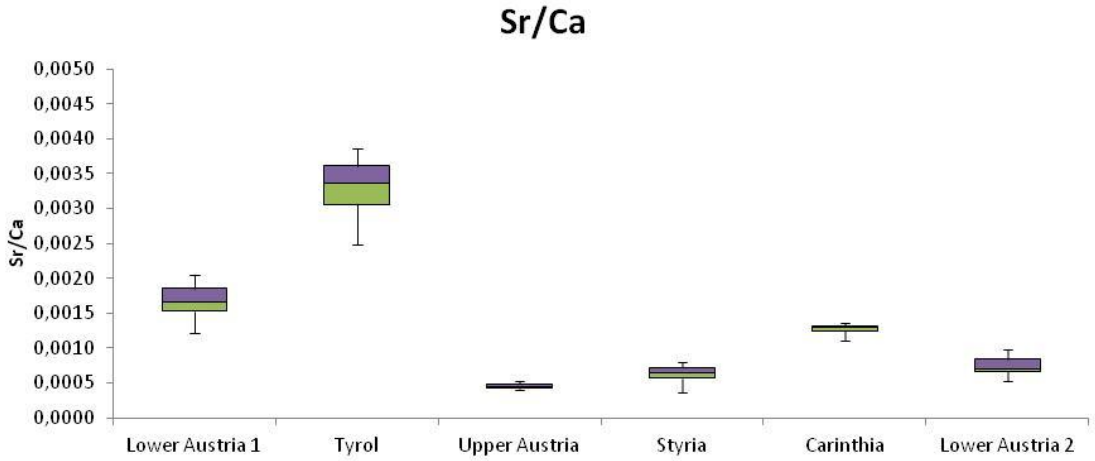

Figure 1. Sr/Ca ratio of fish meat from different Austrian geographical areas; each plot represents minimum and maximum (whiskers) and median (bar) values. The box ranges from 25 to 75th percentile. *U* (k=2).

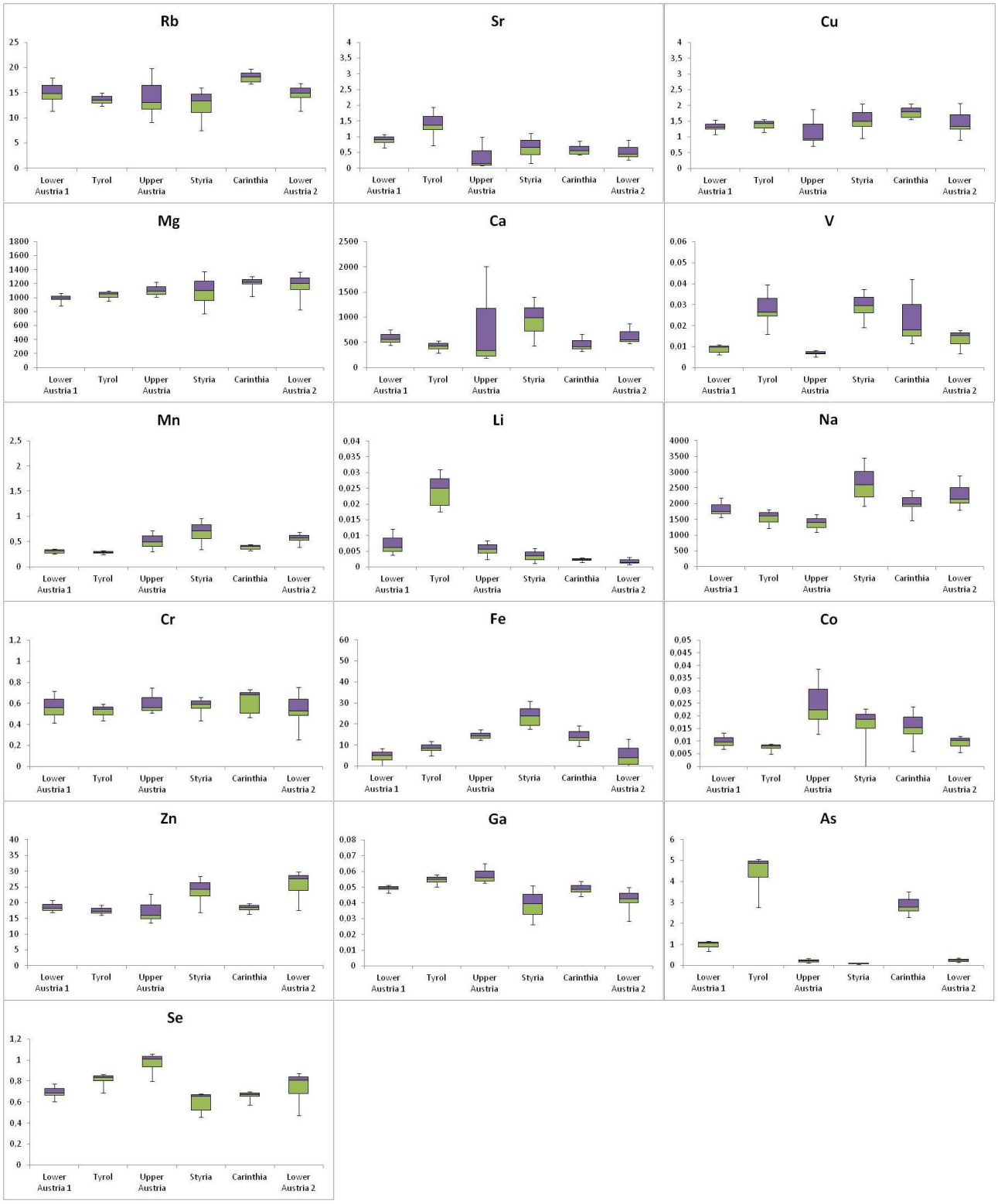

Figure 2. Elemental content (expressed in ng/g) in fish meat from different geographical areas; each plot represents minimum and maximum (whiskers) and median (bar) values. The box ranges from 25 to 75th percentile. *U* (k=2).

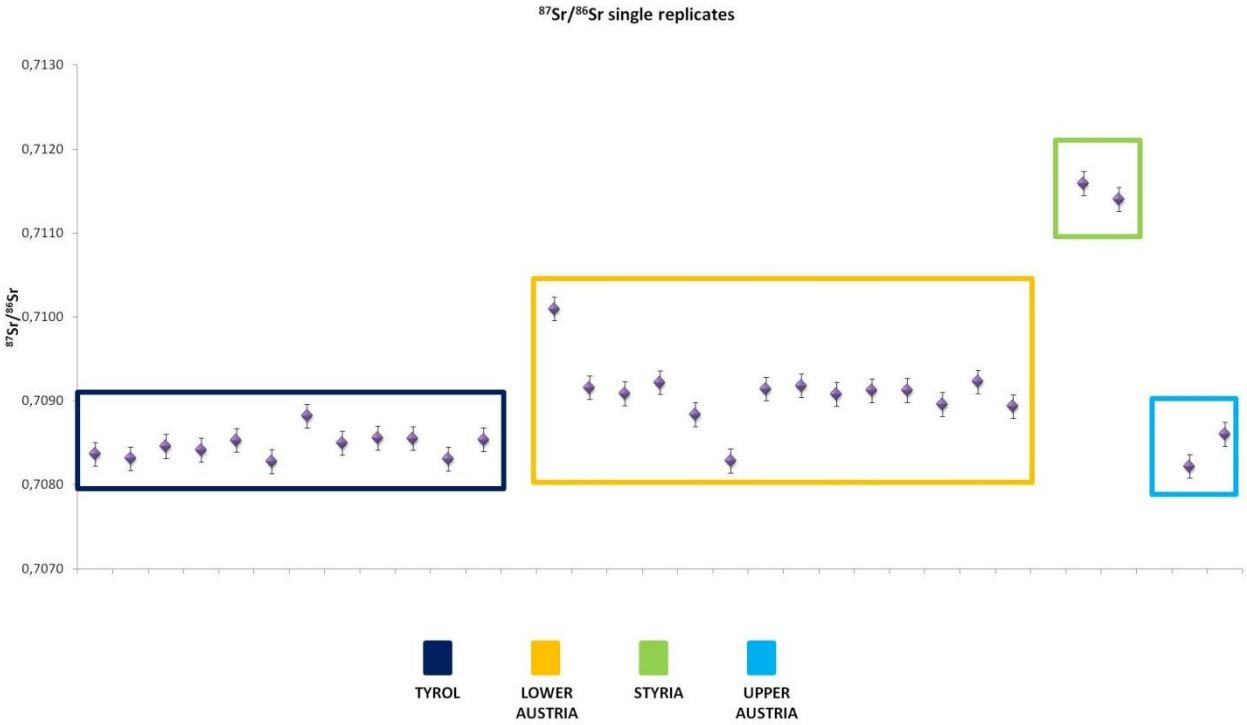

Figure 3. $^{87}Sr/^{86}Sr$ ratio of fish meat from different Austrian geographical area, error bars are expanded total combined uncertainties *U* (k=2).

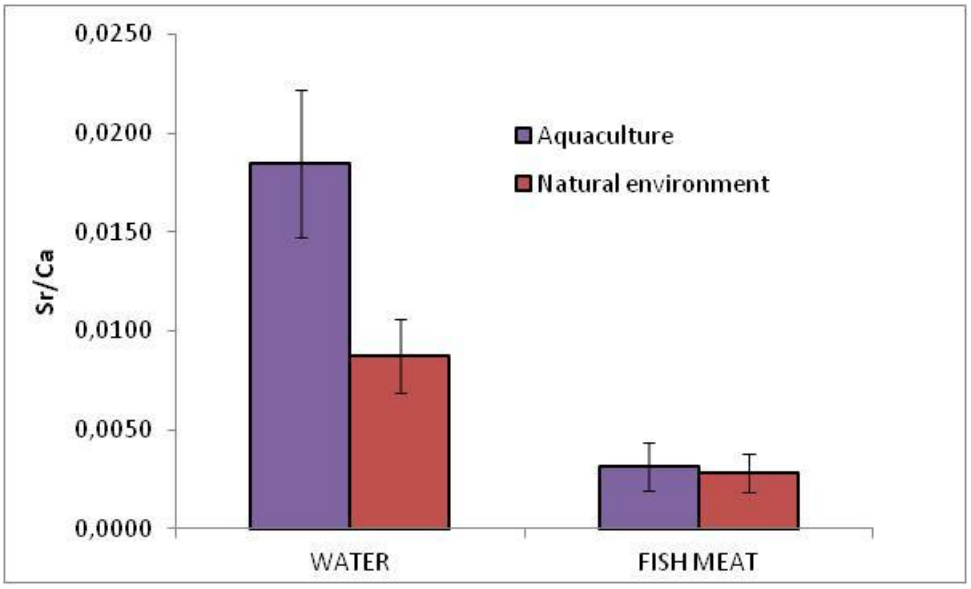

Figure 4. Sr/Ca ratio of water and fish meat from two different Austrian geographical area: Tyrol and Lake Constance, error bars are expanded total combined uncertainties *U* (k=2).

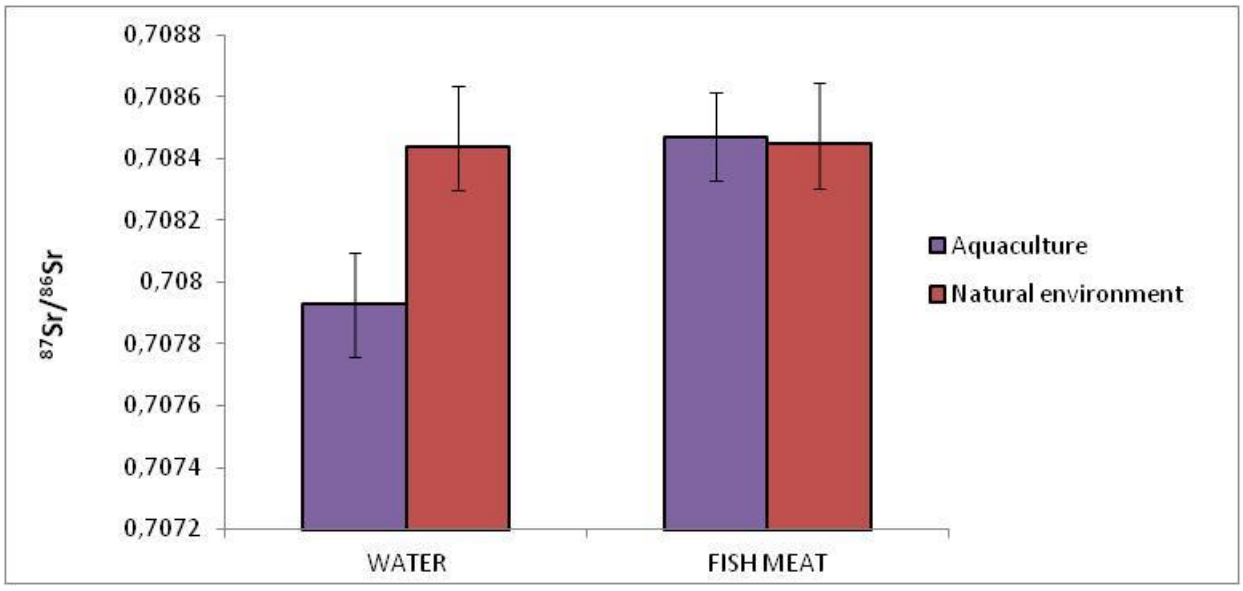

Figure 5. $^{87}Sr/^{86}Sr$ of water and fish meat from two different Austrian geographical area: Tyrol and Lake Constance, error bars are expanded total combined uncertainties *U* (k=2).

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GENERAL CONCLUSIONS

The importance to give a “geochemical identity” to some high-quality products for their authentication and traceability was one of the main objectives of this thesis. In the three years of PhD investigation this was achieved for the following Italian agro-products: "PDO White Asparagus from Bassano del Grappa", "PDO Green Pistachio from Bronte ", “durum wheat flour from Altamura” and "PGI Red Onion from Tropea". Moreover, a geochemical fingerprint able to trace the origin of fish meat from different Austrian regions was investigated.

Our results demonstrated that, by classifying the soils on the basis of the geographical and pedological characteristics, a dissimilar geochemical fingerprint of the products could occur, even if they are cultivated in the same area.

The use of multi-element analysis, both for soils and for plant material, helped us to build a typical geochemical fingerprint for each product, through the identification of the elements that were less variable among the different production areas. By comparison with Literature data, we identified the most significant elements useful to differentiate the investigated products from those cultivated in different geographical areas, especially from those not covered by Geographical Indication labels or considered as potential fraudulent products.

The Sr isotopic ratio was highly promising for products authentication and traceability, since it is not affected by climatic or interannual/seasonal variation and by agricultural management. The findings of our research are quite convincing, especially for the coincidence of the isotopic ratio of bioavailable Sr in the soil with the isotopic ratio of Sr uptaken by the plant, and this important outcome has been confirmed in all the analyzed products for all the investigated farms (except for *Tropea Red Onion* for which isotopic ratio investigation is still ongoing). This successful result suggests that the Sr isotopic ratio has a great potential to be used as a geochemical tracer for the high quality agro-products.

The multielement analysis and the Sr isotopic ratio approach was also applied to determine the geographical provenance of Austrian fishes, proving that the method is applicable to many other kinds of matrices. In this case we concluded that the potential of Sr/Ca ratios has to be further evaluated on a broader data set of natural fish samples in order to evaluate the influence of fish feed with different composition. The Sr isotopic composition could be successfully assessed in fish meat in order to test its potential for provenancing fish.

Further studies based on a larger number of samples of both products covered by Geographical Indication label and similar products with no labels (e.g. agro-products from PDO or PGI areas and similar products outside PDO or PGI areas) might improve the robustness of identified

‘geochemical signatures’ and hence the potential of soil-based indicators for authenticity and geographical provenience traceability.

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