Authentication and Traceability Study of Hazelnuts from Piedmont, Italy

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Hazelnut is one of the most important items in high-quality food products from Piedmont, Italy. The 'Tonda Gentile delle Langhe' (TGL) variety is acknowledged all over the world as the best one, and it is particularly appreciated when used to provide flavor in chocolate products. Authentication and/or traceability studies must therefore be developed to safeguard this variety against fraud, which can occur when the product is partially or totally substituted with hazelnuts of lower quality. In this work, a classification of hazelnuts from different countries is presented, showing the possibility to discriminate the TGL from other productions on the basis of the distribution of trace elements as determined by means of inductively coupled plasma−mass spectrometry (ICP−MS), with particular reference to lanthanides. Accuracy of the sample treatment procedure was tested by analysis of biological certified materials. Data from elemental analysis were chemometrically treated with an unsupervised method, such as principal component analysis (PCA), allowing for a good discrimination among groups.

INTRODUCTION

Hazelnut (*Corylus avellana* L.) is a very digestible and high-energy food, rich in fats, proteins, and vitamins, and is therefore an important item in the agricultural market, mainly because of its use to provide flavor in dairy, bakery, confectionery, candy, and chocolate products, among which items such as chocolates, creams, or fancy cakes are famous. Turkey is recognized as the main producing country (up to 80% of world total production), while Italy, Spain, and U.S. A. account for the remaining production. Among all varieties cultivated in the world, there is general agreement on the fact that hazelnuts from Piedmont, Italy, are possibly the most valuable, with particular reference to the 'Tonda Gentile delle Langhe' (TGL) variety, whose fruits are acknowledged to have the best quality. TGL is cultivated in the whole Piedmont region but mostly in the provinces of Cuneo and Asti. In 1996, TGL gained from the European Union (EU) the protected geographical indication (IGP) under the name *Nocciola Piemonte* (*1*). Other important Italian regions for hazelnut production are Latium (Viterbo and Rome provinces), Campania (Avellino and Naples), and Sicily (Messina), where other varieties used by the food industry are cultivated: 'Tonda Gentile Romana', 'Tonda di Giffoni', 'Mortarella', 'S. Giovanni', 'Tonda bianca', 'Tonda rossa', and 'Riccia di Talanico'. In Italy, a large use of hazelnut production is devoted to the confectionery industry.

Because of its limited production if compared to, for example, Turkish varieties, TGL is frequently subjected to fraud practices, such as partial or total substitution with lower quality varieties. It is therefore of great importance to be able to distinguish among different varieties of hazelnuts or to determine their provenance, that is, to perform authentication and/or traceability studies. To the authors' knowledge, though, there are few such studies in the scientific literature. Most of the research on authentication of hazelnuts is devoted to the adulteration of hazelnut oil with other species (*2, 3*). For what concerns authentication of raw nuts, Duncar et al. (*4*) discriminated among three different Turkish varieties according to their mineral content, while Alasalvar et al. (*5*) used an aromatic profile as determined with an electronic nose sensor to discriminate among five Turkish varieties. As can be noted, though, these studies concern discrimination of varieties coming from the same country; no comparison has been attempted, up to know, among hazelnuts from different countries. Moreover, while mineral composition of hazelnuts is well-known in terms of major and minor elements, there is scarce literature on trace and ultra-trace element content (*6, 7*).

In this study, a classification of hazelnuts coming from different countries is presented. Classification is based on the distribution of trace elements as determined by inductively coupled plasma−mass spectrometry (ICP−MS), with particular reference to lanthanides. Data from elemental analysis are chemometrically treated with an unsupervised classification procedure, such as principal component analysis (PCA). Moreover, it is shown that distribution of lanthanides can be a chemical link among soil on which

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hazel trees grow and hazelnuts, allowing us to perform a traceability study.

MATERIALS AND METHODS

Materials. High-purity water from a Milli-Q (Milford, MA) apparatus and TraceSelect hydrogen peroxide, nitric acid, and hydrochloric acid (Fluka, Milan, Italy) were used throughout the work. Polypropylene and polystyrene vials, used respectively for sample storage and analysis with an autosampler system, were kept in 1% nitric acid and then rinsed with high-purity water upon request. Porcelain capsules of 30 mL volume were used for microwave dry ashing. Element stock solutions (Inorganic Ventures, Lakewood, NJ) were used to perform external calibration and internal standardization.

Sample Collection. Hazelnuts of various geographical origins, with raw material for the confectionery industry, have been supplied by Consorzio di Tutela Nocciola Piemonte (Bossolasco, Italy) and Novi-Elah-Dufour SpA (Novi Ligure, Italy). Samples are described in **Table 1**; as can be seen, hazelnuts are mostly of cultivar TGL. Among hazelnut samples from Piedmont, the following were available together with soil samples collected from crops where hazel trees grow: Carrù (Cuneo province), Aimo and Passuera; Cravanzana (Cuneo province); Lu (Alessandria province), Bersano, Castagna, and Rosa.

Samples were shelled and preserved in a cold room before analysis.

Sample Treatment. To develop a simple and contaminant-free procedure, we applied dry ashing to hazelnut samples. Nearly 20 g of crushed hazelnuts in a porcelain crucible were subjected to heat treatment in a Pyro 260 microwave ashing system (Milestone, Sorisole, Italy), with a gradient temperature program of 820 °C. The resulting ash was dissolved in 1 mL of concentrated nitric acid of TraceSelect reagent grade (Sigma-Aldrich, Milan, Italy). Sample solution was then taken up to 100 mL to obtain a nitric acid concentration of 1%. All solutions were prepared from high-purity water.

Soil Treatment. Samples of soil were treated following a standardized procedure, which involves extraction of metal ions with aqua regia. A proper amount of soil was dried at 105 °C for 24 h, after which 1 g was sieved (ø 0.2 mm), and it was taken to be extracted with 20 mL of hot hydrogen peroxide for 20 min and then with 12 mL of aqua regia on a heating plate for 2 h. The resulting solution was taken to 100 mL with high-purity water.

ICP−MS Analysis. Elemental analyses were performed with a Thermo Scientific (Waltham, MA) X5 Series model inductively coupled plasma mass spectrometer. The instrument is equipped with a quartz torch with a PlasmaScreen device, a quadrupole mass analyzer, a lens ion optics based on a hexapole design with a chicane ion deflector and a simultaneous detector with real-time multichannel analyzer electronics, operating in either analogue signal mode or pulse counting mode. A high-efficiency ESI APEX-Q nebulizer (Epond SA, Vevey, Switzerland) was used as a nebulization system. Instrument and accessories are PC-controlled by PlasmaLab software. Instrument and measurement parameters are as follows: forward power, 1400 W; nebulizer gas flow, 0.92 L min^{-1}; auxiliary gas flow, 1.00 L min^{-1}; plasma gas flow, 13.1 L min^{-1}; dual mode detection with peak jumping; dwell time, 10 ms; 25 sweeps; 3 replicates for a total acquisition time of 180 s; and isotopes used ^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, and ^{175}Lu. Interferences were evaluated as follows: $CeO^+/Ce^+ < 1\%$ and $Ba^{2+}/Ba^+ < 1\%$. A stability test was performed before each analysis session by monitoring ^{7}Li, ^{59}Co, ^{115}In, ^{140}Ce, and ^{238}U masses and verifying a precision higher than 2%. The instrumental precision was better than 2% for trace elements, while the overall uncertainty, calculated on the basis of five genuine replicates, was better than 5%. Background signals were monitored at 5, 101, and 220 masses to perform a sensitivity test on the above-reported analyte masses. The limits of detection (LOD),

Table 1. List of Hazelnut Samples

sample	sample code	provenance	cultivar
Akc	A1	Turkey	Levant Akçakoca
Akc	A2	Turkey	Levant Akçakoca
Giresun	G1	Turkey	Giresun
Giresun	G2	Turkey	Giresun
Ord	O1	Turkey	Levant Ord
Ord	O2	Turkey	Levant Ord
Avellino	M1	Campania, Italy	Mortarella
Avellino	M2	Campania, Italy	Mortarella
Viterbo	R1	Lazio, Italy	Tonda Gentile Romana
Viterbo	R2	Lazio, Italy	Tonda Gentile Romana
Piemonte	TGL1	Piedmont, Italy	TGL
Piemonte	TGL2	Piedmont, Italy	TGL
Lu Monferrato, Bersano	LB	Piedmont, Italy	TGL
Lu Monferrato, Castagna	LC	Piedmont, Italy	TGL
Lu Monferrato, Rosao	LR	Piedmont, Italy	TGL
Carrù, Aimo	CA	Piedmont, Italy	TGL
Carrù, Passuera	CP	Piedmont, Italy	TGL
Cravanzana	Cr	Piedmont, Italy	TGL

Table 2. LOD and LOQ for Lanthanides, Expressed as ng/L

element	*m/z*	LOD	LOQ
La	139	0.8	2.8
Ce	140	0.5	2.9
Pr	141	0.48	0.4
Nd	143	1.1	0.4
Sm	147	1.6	2.8
Eu	153	0.8	0.6
Gd	157	0.1	1.2
Tb	159	0.9	0.8
Dy	163	1.0	0.3
Ho	165	0.7	0.6
Er	166	0.7	0.7
Tm	169	0.8	0.8
Yb	172	0.5	0.3
Lu	175	0.8	0.7

calculated as 3 times the standard deviation of 11 blank measurements, and the limits of quantification (LOQ) are shown in **Table 2**. External calibration was employed for quantification, using multi-element standards prepared at five concentration levels in the range of 5−5000 ng/L, by diluting multi-element stock solutions (100 mg/L) in 1% nitric acid solution. Internal standardization was used to correct for instrumental drifts by monitoring signals from ^{103}Rh, ^{115}In, and ^{209}Bi isotopes, which were added to all samples, standards, and blanks at a concentration level of 10 μg/L; responses from the three isotopes were interpolated to yield a better correction.

Analysis of Certified Samples. To check performance and recovery of the proposed sample treatments, three certified samples were analyzed. BCR 668 (mussel tissue) and BCR 670 (duck weed) certified materials from IRMM were analyzed applying the ashing procedure and gave the results reported in **Table 3**, which show good agreement among certified and found values. SRM 2586 (trace elements in soil containing lead from paint) certified material from the National Institute of Standards and Technology (NIST) was analyzed according to the described treatment for soil samples and gave the results reported in **Table 4**, again showing a good agreement among certified and found values.

RESULTS AND DISCUSSION

To develop a classification scheme, elemental concentrations of trace elements were chosen as variables. Such variables can be used as markers for traceability studies of food products, as demonstrated by several works (*8*). In the present case, because of the very low level of element

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Table 3. Analysis of Certified Biological Materials

	BCR 668 mussel tissue				BCR 670 duck weed			
element	certified values (μg/kg)	uncertainty	found (μg/kg)	sd	certified values (μg/kg)	uncertainty	found (μg/kg)	sd
La	80	6	76	2	487	20	476	4.3
Ce	89	7	107	9	990	40	953	34
Pr	12.3	1.1	13.3	0.6	121	6	113	11
Nd	54	4	52.7	0.7	473	15	493	14
Sm	11.2	0.8	11	0.9	94	7	96.3	0.5
Eu	2.79	0.16	3.2	0.13	23.2	1.5	28.6	0.3
Gd	13	0.6	12.9	0.5	98	8	106	6
Tb	1.62	0.12	1.7	0.2	14	1.1	14.2	0.2
Dy	8.9	0.6	8.4	0.6	79	7	80	5
Ho	1.8[a]	0.60[a]	1.5	0.2	15.8	1.8	17	2
Er	4.5	0.5	4.2	0.3	44	2.8	46.4	0.6
Tm	0.48	0.08	0.62	0.02	5.7	0.7	5	1
Yb	2.8[a]	0.5[a]	3.08	0.07	40	4	44	6
Lu	0.389	0.024	0.59	0.04	6.3	0.5	7.5	0.9

[a] Indicative value.

Table 4. Analysis of Certified Soil

element	certified values	uncertainty	found (mg/kg)	sd
La	29.7	4.8	26.2	0.9
Ce	58	8	58	1.6
Pr	7.3[a]		7.6	0.20
Nd	26.4	2.9	26.4	0.8
Sm	6.1[a]		5.1	0.2
Eu	1.5[a]		0.94	0.03
Gd	5.8[a]		4.9	0.2
Tb	0.9[a]		0.58	0.02
Dy	5.4[a]		3.4	0.1
Ho	1.1[a]		0.53	0.02
Er	3.30[a]		1.76	0.04
Tm	0.5[a]		0.13	0.03
Yb	2.64	0.51	1.4	0.05
Lu	not determined		0.10	0.01

[a] Indicative value.

Table 5. Concentration Ranges of Lanthanides (μg/kg) Found in Hazelnuts

element	Turkish	Italian	Piedmont
La	1.15−1.49	4.38−5.07	1.01−5.69
Ce	1.88−2.20	6.07−8.99	1.86−7.73
Pr	0.47−0.53	1.07−1.21	0.24−1.64
Nd	0.79−0.95	2.89−3.26	0.78−5.71
Sm	0.52−0.58	0.81−0.88	0.12−1.11
Eu	1.23−1.90	1.39−2.00	0.18−1.84
Gd	0.59−0.80	0.99−1.08	0.17−1.22
Tb	0.40−0.46	0.43−0.47	0.03−0.19
Dy	0.48−0.53	0.66−0.70	0.10−0.97
Ho	0.41−0.46	0.44−0.50	0.03−0.19
Er	0.45−0.51	0.54−0.61	0.06−0.53
Tm	0.44−0.48	0.44−0.48	0.01−0.07
Yb	0.49−0.70	0.57−0.62	0.07−0.43
Lu	0.45−0.51	0.46−0.51	0.02−0.53

concentrations in the samples, we chose to follow a simplified and contaminant-free sample treatment procedure, which had its main drawback in excluding all volatile and semivolatile elements, such as lead, cadmium, or mercury. It is obvious that all elements with boiling points lower than 820 °C, even if determined, were not used in the classification procedures. Nevertheless, it is important to point out that most of the excluded elements can be considered as mostly anthropogenic and not linked to soil, and, as such they would not be useful as provenance markers.

On the contrary, lanthanides can be considered as potential soil markers. The relation among soil and plants, with reference to the role of lanthanides, has been frequently discussed in the past and recently reviewed by Liang et al. (*9*). There is no general agreement on the role of lanthanides as chemical tracers; it is hypothesized that fractionation occurs, at least among light (La−Sm) and heavy (Gd−Lu) lanthanides. Other works, though, suggest their usefulness (*10*), even in application to food authentication (*11*). Lanthanides were therefore considered as the most important variables, and ICP−MS parameters were tuned to maximize sensitivity in the 139−175 amu range. It was observed that lanthanide distributions in both soil and hazelnut samples were in accordance with the Oddo−Harkins rule, that is, the well-known effect according to which even-numbered nuclides are more stable than odd-numbered nuclides. Moreover, results from hazelnut analysis were comparable to published data, for what concerns all trace elements considered, that is, with exclusion of volatile elements, with particular reference to lanthanides, as seen from **Table 5**. Results will be presented and discussed in two stages: a traceability study and an authentication study.

Traceability Study. The possibility to trace a food chain is strictly bound to the impact of the different passages occurring from soil to the final food product. The easier the chain, the better the possibilities to have a chemical link useful for traceability. The hazelnut chain can be thought of as a relatively easy chain because no heavy anthropogenic interventions occur, so that the original lanthanide distribution in soil should be regulated by the following factors: availability of lanthanide ions in soil, uptake mechanisms of lanthanide ions from plant roots, metabolic role of lanthanide ions in plant physiology, and transport of lanthanide ions from roots to fruits, i.e., hazelnuts.

Lanthanide ions seem to have no role in plant metabolism; they are possibly taken up as substitutes of the Ca^{2+} ion (*12*). It can be hypothesized, therefore, that only slight or no variations of the original distribution occur when passing from soil to fruits. To individuate a link among soil and hazelnut samples, it is important to stress the fact that distributions of concentrations, instead of single absolute concentration values, must be taken into account. The comparison of lanthanide distribution in soil and hazelnut samples is therefore the critical parameter in determining
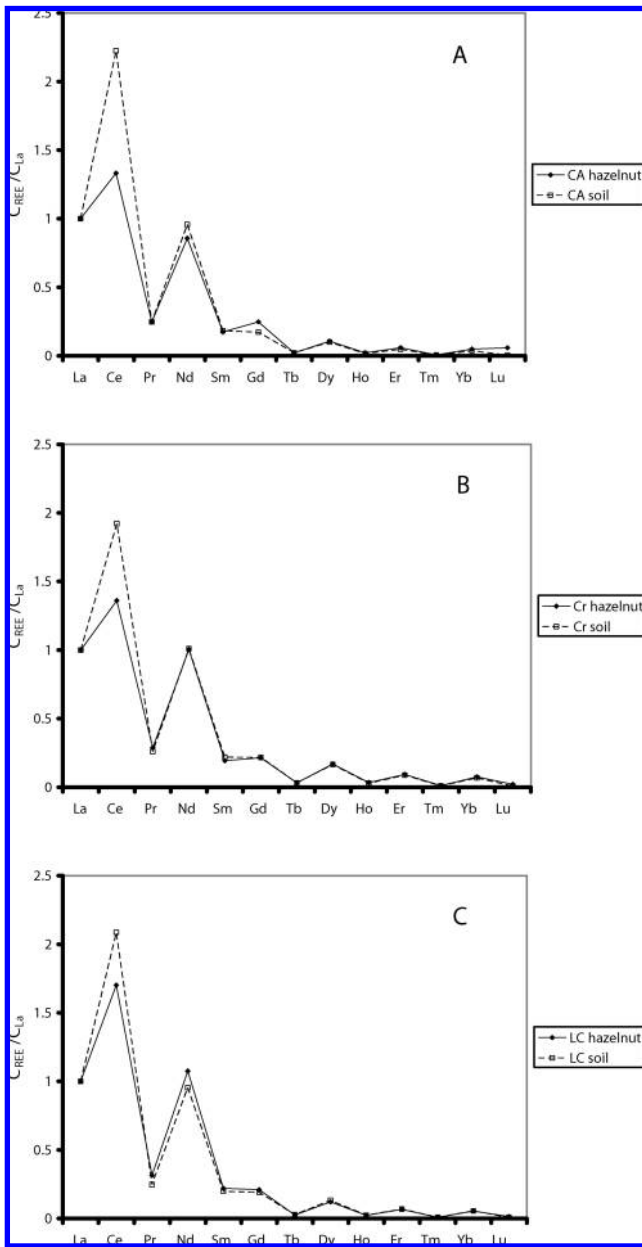
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Figure 1. Lanthanum-normalized lanthanide distributions for soil/hazelnut samples from Piedmont: (A) Carrù, (B) Cravanzana, and (C) Lu.

whether it is possible to find the chemical link, that is, to trace the food product. If the original lanthanide distribution of soil can be recognized in hazelnuts, this means that no fractionation had occurred along the chain and that trace-ability is possible.

First at all, data have to be treated to be comparable, because lanthanide concentrations are higher in soil than in hazelnuts by an average factor of 4 orders of magnitude. In geochemical studies, it is common practice to apply the Coryell−Masuda method of normalizing lanthanide data (*13*, *14*), according to which a ratio of one lanthanide pattern to another, commonly a chondritic abundance pattern, is calculated. In the present study, though, we chose to normalize data by dividing lanthanide concentrations, for every sample of soil or hazelnut, by the respective lanthanum concentration, so that the resulting distributions were easier to be compared inside every soil−hazelnut couple. This comparison was performed on hazelnuts from Piedmont only, because no soil samples were available
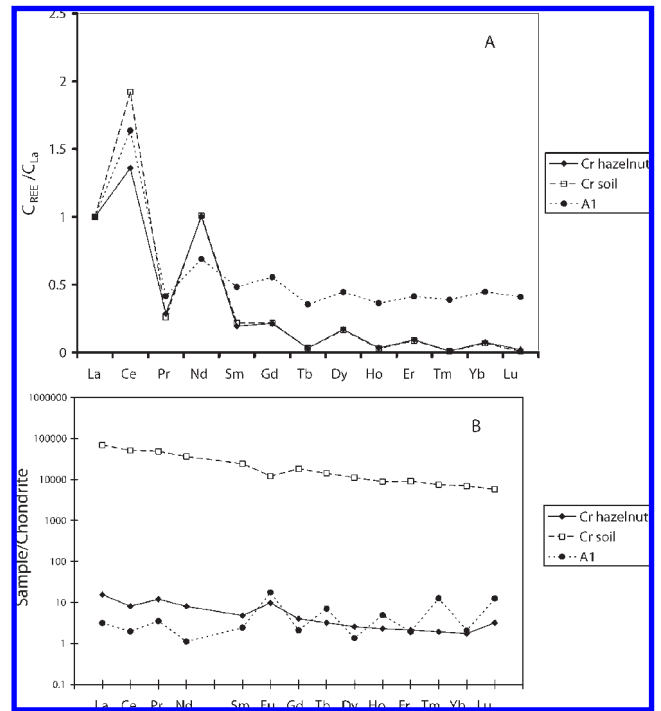

Figure 2. (A) Lanthanum- and (B) chondrite-normalized lanthanide distributions for a hazelnut sample from Turkey (A1, Akc) and a soil/hazelnut sample from Piedmont (Cr, Cravanzana).
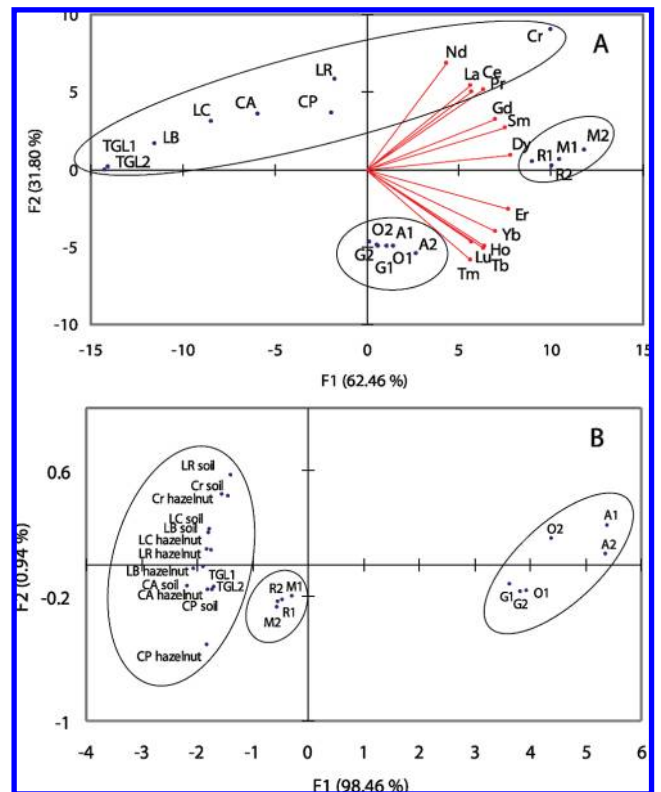

Figure 3. Score plot from PC analysis of (A) hazelnuts (raw data) and (B) hazelnuts and soils (lanthanum-normalized data).

for other provenances. **Figure 1** shows lanthanum-normalized lanthanide distributions for soil/hazelnut samples from Carrù, Cravanzana, and Lu; in all cases, distributions in hazelnuts match well with distributions in soils, in particular for what concerns heavy lanthanides, that is from terbium to lutetium. An exception is europium that seems to be enriched

in hazelnut with respect to soil, but this might be possibly due a proportionally higher content of barium in hazelnuts than in soil, causing positive interference of $^{135}Ba^{16}O$ and $^{137}Ba^{16}O$ polyatomic ions on ^{151}Eu and ^{153}Eu isotopes, respectively.

It is interesting, then, to compare the lanthanide distribution of Piedmont soils to those of non-Piedmont hazelnuts. In **Figure 2A**, a sample from Turkey (Akc) is compared to a soil−hazelnut couple from Piedmont (Cravanzana): the difference in distributions is apparent and highly significant for heavy lanthanides. The same consideration can be performed when comparing chondrite-normalized distributions (**Figure 2B**), in which, apart from the anomalous behavior of Eu explained before, it is apparent the great similarity among soil and hazelnut from Piedmont and their difference with the Turkish hazelnut. These results suggest that no significant fractionation occurs among lanthanides when passing from soil to hazelnut.

Classification of Hazelnuts from Different Countries. One important consequence of the above-reported results is that hazelnuts grown on different soils should have different, recognizable lanthanide distributions, allowing us to discriminate among hazelnuts coming from different countries or different zones. It is highly probable, therefore, that hazelnuts coming from Piedmont had distributions different from foreign hazelnuts. To verify this hypothesis, multivariate methods of analysis were applied to trace element data obtained from ICP−MS analysis; because of spectral interferences on Eu, this element was not included among the variables selected. Results are shown in the following figures. In **Figure 3A**, the score plot resulting from PCA, applied to raw data, shows that hazelnuts from Piedmont can be discriminated from all other samples considered; hazelnuts from Turkey are grouped together, while hazelnuts from Campania and Lazio form a third group. Sample Cr (Cravanzana, Piedmont) is slightly far from the Piedmont group because of its higher content in light lanthanides, as a consequence of soil content. The loading vectors (indicated with red lines) show that the most discriminating variables are, as expected, heavy lanthanides. Interestingly, if we select heavy lanthanides as variables and apply PCA on both hazelnut and soil samples using lanthanum-normalized data, the resulting score plot, reported in **Figure 3B**, shows that hazelnuts from Piedmont are more similar to the respective soil samples than to other hazelnut samples, confirming that lanthanides act as a natural fingerprint of soil, at least for what concerns the chain considered.

Trace metals, especially lanthanides, showed their usefulness in classification of hazelnuts coming from different zones. It was possible to discriminate among hazelnuts from Piedmont and hazelnuts from other regions or countries; moreover, it was possible to individuate a link among hazelnuts and soil through lanthanide distribution. These results make it possible to develop a control procedure to verify the provenance of hazelnuts used in the food industry.

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