



# Investigating isotopic markers for hazelnut geographical authentication: Promising variables and potential applications

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## ABSTRACT

Hazelnuts' features and price are influenced by their geographical origin, making them susceptible to fraud, especially counterfeit claims regarding their provenance. Stable isotope analysis is a recognised approach to establish the geographical origin of foods, yet its potential in hazelnut authentication remains unexplored. In this prospective study, we assessed multiple isotopic markers in hazelnuts from different origins and evaluated the most promising variables for geographical authentication by chemometric tools. Our findings indicate that bulk  $\delta^{18}\text{O}$ , along with  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  in the main fatty acid methyl esters, exhibit significant potential in discriminating geographical origins, and  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis could serve as a proficient confirmatory tool. Though no single marker alone can differentiate between all the studied origins, employing a multi-isotopic approach based on PLS-DA models achieved up to 92.5 % accuracy in leave-10 %-out cross-validation. These findings will probably lay the groundwork for developing robust models for hazelnut geographical authentication based on larger datasets.

## 1. Introduction

Hazelnuts are a widely used and prized ingredient in both sweet and savoury foods. Their sensory and qualitative characteristics are heavily influenced by their growing region (Król & Gantner, 2020) and their market value fluctuates accordingly. As an example, in 2021, the price range for in-shell hazelnuts from Georgia and Italy was between 1449 USD/T and 4174 USD/T, respectively (FAOSTAT, 2021). Hazelnuts' high economic value makes them particularly susceptible to frauds, including the counterfeiting of geographical origin. Even though the official Regulation (Regulation (EU) No, 1169) defines geographical origin as a label claim that must be verified by official inspection services, when necessary (Mahalovich et al., 2016), the lack of effective methods for detecting this fraud provides counterfeiters with an opportunity to exploit the situation. Fraudulent activities can have a significant impact on the industrial sector that uses these commodities. This holds particular significance for foods that fall under EU

recognition, including Protected Designation of Origin, as the origin of the food product serves as the basis of these quality schemes. Hence, the implementation of effective methods to detect and prevent fraudulent activities related to hazelnut origin claims is necessary to protect the industry and consumers.

Currently, stable isotope composition analysis is among the most acknowledged approaches to establish the geographical origin of foods, as the isotopic composition of both light bio-elements, namely carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulphur (S) and heavy geo-elements, such as strontium (Sr) are strongly influenced by factors that are indicative of the geographical origin, such as geology and hydrogeology (Kelly et al., 2005; Laursen et al., 2016; Podio et al., 2013; De Rijke et al., 2016). Regarding light elements such as H and O, phase changes of water (solid–liquid–vapour) lead to isotopic fractionation because of the different saturation vapour pressures of different isotopologues. This isotopic fractionation resulting from evaporation, condensation, and precipitation of meteoric water is influenced by

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factors like temperature, latitude, altitude, and proximity to the coast. These factors give rise to notable variations in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  within groundwater across different geographical regions. By analysing these isotopic signatures in plant samples, valuable insights can be obtained regarding their geographical provenance (Kelly et al., 2005; De Rijke et al., 2016). On the other hand, the  $\delta^{13}\text{C}$  value in plants is primarily determined by their specific types of photosynthetic carboxylation reactions. However, it has been noted that environmental factors can also exert an influence on carbon isotope fractionation, making of  $\delta^{13}\text{C}$  a potential indicator of the origin of plants (Yoneyama et al., 2000; Branch et al., 2003). The isotopic composition of S and N in plant tissues can be influenced by the application of isotopically different sulphur and nitrogen-containing fertilisers (Vitória et al., 2004), making them more dependent on agricultural practices rather than geographical factors. Nevertheless, the full extent of this impact is not entirely understood and requires evaluation specific to the plant species of interest. For instance, regarding  $\delta^{15}\text{N}$ , it has been observed that it can be influenced by the plant's nitrogen uptake process (Anderson and Smith, 2006). Additionally, nitrogen isotopes are subject to the influence of geoclimatic factors (soil type, temperatures, precipitation), which vary across geographical regions and can significantly contribute to shaping the isotopic signature of plant materials (Yoneyama et al., 2000; Brescia et al., 2002; Anderson and Smith, 2006). Furthermore, the isotopic analysis of heavy geo-elements, like strontium, offers an enhanced connection between soil characteristics and primary agricultural products due to the negligible isotopic fractionation because of the small mass difference between the two main Sr isotopes ( $^{87}\text{Sr}$  and  $^{86}\text{Sr}$ ). Only  $^{87}\text{Sr}$  is radiogenic; it is produced by the long-term decay from the radioactive  $^{87}\text{Rb}$  present in the distinct rock types and geological formations (Kelly et al., 2005; Laursen et al., 2016). Also, in this case, traces of geological Sr found in fertilisers made by phosphate from mining could potentially impact the Sr isotopic composition in crops (Vitória et al., 2004; Techer et al., 2017).

Given these considerations, the isotopic analysis has been extensively utilized for the geographical verification of a diverse range of food products (Bertoldi et al., 2019; Camin et al., 2017; Kelly et al., 2005; Perini et al., 2018; Podio et al., 2013; De Rijke et al., 2016). The consistently satisfactory results obtained from this analysis have prompted the proposal of employing this method even in legal cases (Camin et al., 2017). As demonstrated by previous studies, measuring multiple parameters within a food product enhances the accuracy in determining its geographical origin (Bertoldi et al., 2019; Luykx & Van Ruth, 2008; Podio et al., 2013), especially when multi-isotope-ratio analysis is paired with molecular-specific isotopic data (Bontempo et al., 2019). However, only a limited number of studies focus on single or multi-isotopic composition specifically for nut products. Encouraging results have been obtained from the analysis of bio-element isotopic composition in walnuts (Di Pierro et al., 2018; Krauß et al., 2020), pistachios (Anderson and Smith, 2006), and North American pine nuts (Mahalovich et al., 2016) and from the evaluation of  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopes in pistachios (Zannella et al., 2017) and peanuts (Zhu et al., 2014). No data are available on the application of isotopic markers for hazelnut geographical authentication. Finally, a key element in authentication by multi-isotope-ratio analysis is the data treatment and chemometric analysis (Drivelos & Georgiou, 2012; Podio et al., 2013; De Rijke et al., 2016). When a large number of variables are studied, multivariate techniques such as Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), among others, are crucial to extract all the relevant information.

The objective of this prospective study was to evaluate the efficacy of specific isotopic markers to identify hazelnut geographical origin. To achieve this purpose, we analysed the elemental isotopic profile ( $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ , and  $^{86}\text{Sr}/^{87}\text{Sr}$ ) and the  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  of the fatty acid methyl esters (FAMES) of the hazelnut oil of 40 samples of raw hazelnuts from four different origins. Chemometric tools were used within a multi-isotopic approach to compare and identify the most promising variables for hazelnut geographical authentication.

## 2. Material and methods

### 2.1. Samples

Forty samples of hazelnuts from the 'Tonda di Giffoni' Italian cultivar were produced over one to three harvest seasons in four different countries: Spain ( $n = 10$ ; 2019, 2020, 2021), Italy ( $n = 10$ ; 2019, 2020, 2021), Georgia ( $n = 10$ ; 2021) and Chile ( $n = 10$ ; 2019, 2020). All samples were collected when hazelnuts were ripe (harvested in August–October for Georgia Italy and Spain and in March–April for Chile). Each sample was obtained from one single tree. Geographical coordinates and fertilisation data are reported in Table 1. Samples were provided in the framework of the TRACENUTS project (PID2020-117701RB). Collected hazelnuts were shelled at the laboratory. Kernels were stored under vacuum and refrigerated at 4 °C until analysis.

### 2.2. Bulk isotopic analysis by Elemental Analysis-Isotope ratio Mass spectrometry (EA-IRMS)

#### 2.2.1. Sample preparation

Preliminary tests were conducted to determine the most suitable sample treatment. For this purpose, three aliquots of 30 g each were taken from the same homogeneous hazelnut sample (500 g) and subjected to different grinding methods: cryogenic milling (Cryogenic Mill 6850, SPEX Certiprep, Metuchen, New Jersey, USA), domestic grinder (Aromatic, Taurus, Oliana, Spain) and a combination of grinding followed by cryogenic milling. Then, 5 g were taken in triplicate from each ground sample and lyophilized in a Telstar Cryodos-45 freeze dryer (Telstar, Terrassa, Spain). The lyophilization time until constant weight was assessed, and the amount of water removed from the sample was calculated.

The repeatability of the method chosen for sample treatment was assessed on C/N ratios,  $\delta^{15}\text{N}\text{‰}$ , and  $\delta^{13}\text{C}\text{‰}$ , determined on 10 aliquots obtained from the same ground and lyophilized sample, and extrapolated to the rest of the light elements. Mean values and standard deviation of each parameter were calculated.

The sample treatment selected for the isotope bulk analysis of  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  involved grinding 30 g of the raw hazelnuts into fine powder using a domestic grinder (Aromatic, Taurus, Oliana, Spain), followed by a three-day lyophilizing process.

A dual-water equilibration test was performed to estimate any possible exchange of H between the samples and the ambient according to previous protocols (Sauer et al., 2009; Qi and Coplen, 2011). For this, a set of 10 aliquots extracted from the same ground and lyophilized sample, plus the standards, were weighed and loaded into individual silver capsules (Lüdi Swiss, Flawil, Switzerland). Then, five of them were equilibrated in a glass desiccator with water depleted in  $^2\text{H}$  (Milli-Q water,  $\delta^2\text{H} = -43\text{‰}$ ) and the other five were equilibrated with deuterated water ( $\delta^2\text{H} = +100\text{‰}$ ). In each glass desiccator, a set of standards was also included. Samples were equilibrated for seven days at ambient temperature (25 °C). Prior to analysis, samples equilibrated with light and heavy water were dried in separate desiccators filled with Sicapent ( $\text{P}_2\text{O}_5$ ) for seven days.

#### 2.2.2. EA-IRMS

##### 2.2.2.1. C ( $\delta^{13}\text{C}$ -values in ‰) and N ( $\delta^{15}\text{N}$ -values in ‰) isotope analysis.

About 0.8 mg of the powdered sample was weighed into individual tin capsules for the determination of bulk  $\delta^{13}\text{C}$ - and  $\delta^{15}\text{N}$ -values (‰). Samples were measured in a Flash IRMS™ Elemental Analyser coupled to a Delta V Advantage (IRMS) via ConFlo IV interface (Thermo Fisher Scientific, Waltham, Massachusetts, USA). In the elemental analyser, each sample was combusted with oxygen added to the helium stream at a temperature of 900 °C in a reactor comprised of copper oxide and silvered oxides of cobalt (ThermoFisher Scientific, Waltham,

**Table 1**

Geographical coordinates and the applied fertilization data of the hazelnut production parcels during the studied years, as provided by the producers.

| Geographical coordinates |                            | Fertiliser (as N source)<br>(Units N/ha)    |                                                |                  |                           | Organic N             |
|--------------------------|----------------------------|---------------------------------------------|------------------------------------------------|------------------|---------------------------|-----------------------|
|                          |                            | Straight<br>NH <sub>4</sub> NO <sub>3</sub> | NPK<br>(N: NO <sub>3</sub> ; NH <sub>4</sub> ) | NPK<br>(N: Urea) | NPK<br>(N: not specified) |                       |
| Chile                    | 35°15'36" S, 71°32'60" W   | —                                           | —                                              | —                | 91–115                    | —                     |
| Italy                    | 42°25'23" N,<br>12°4'45" E | —                                           | —                                              | —                | —                         | —                     |
| Georgia                  | 42°27'34" N, 41°51'31" E   | 40                                          | —                                              | —                | 30                        | —                     |
| Spain                    | 41°10'15" N,<br>1°10'09" E | —                                           | 45                                             | 27               | —                         | mulching <sup>a</sup> |

<sup>a</sup> : spontaneous vegetation, mowed 2–3 times per year and left on the ground between trees.

Massachusetts, USA) and then NO<sub>x</sub> gases were reduced at 650 °C with electrolytic copper to produce N<sub>2</sub>. The gases obtained were carried through water traps (granular magnesium perchlorate, Elemental Microanalysis Ltd, Okehampton, UK) and N<sub>2</sub> and CO<sub>2</sub> were separated in a stream of helium N 60 (grade 99.9999 % purity, Air Liquide, Madrid, Spain) at 180 mL/min with a GC column (length 2 m, diameter 6 x 5 mm) set to 40 °C and transferred into the isotope ratio mass spectrometer. The certified international standards used were IAEA CH7 ( $\delta^{13}\text{C}$  = –32.15 ‰) and IAEA N1 ( $\delta^{15}\text{N}$  = 0.4 ‰) both from Vienna (Austria). UCGEMA K (keratin,  $\delta^{13}\text{C}$  = –14.97 ‰,  $\delta^{15}\text{N}$  = 13.52 ‰), UCGEMA CH (chitin,  $\delta^{13}\text{C}$  = –22.08 ‰,  $\delta^{15}\text{N}$  = –4.81 ‰), fructose ( $\delta^{13}\text{C}$  = –10.8 ‰) and UCGEMA P (animal hair,  $\delta^{15}\text{N}$  = +7.6 ‰) were used as internal secondary standards. The reference gases were N<sub>2</sub> ( $\delta^{15}\text{N}$  = –1.0 ‰) and CO<sub>2</sub> ( $\delta^{13}\text{C}$  = –41.2 ‰). For sample combustion, O<sub>2</sub> was injected for 2 s at 250 mL/min and for the  $\delta^{13}\text{C}$  analysis the sample was diluted with the carried gas to 78 %. Samples were analysed in duplicate.

**2.2.2.2. H ( $\delta^2\text{H}$ -values in ‰) isotope analysis.** About 0.1 mg of the powdered sample was transferred into silver capsules for solids. To prevent potential further H exchange, the capsules were sealed immediately after being removed from the desiccator and were analysed right after the encapsulation and pressing of the sample, where the sample remains relatively isolated from the atmosphere. Samples were analysed in a TC/EA-IRMS Delta Plus XP (Thermo Fisher Scientific) equipped with a conventional autosampler (Sample Tray, N° 2, MAS200R autosampler) and a pyrolysis reactor, heated to 1450 °C. Specifically, the filling of this 450 mm ceramic reactor from the bottom to the top is as follows: 20 mm quartz wool (Lüdi Swiss), 30 mm of graphite (IVA Analysentechnik, Meerbusch, Germany). Then, glassy carbon reactor (IVA) is introduced inside the ceramic reactor and filled with 120 mm of graphite (IVA) and then the graphite crucible (IVA); all this procedure is according to the Thermo Fisher Scientific manual. Helium was used as carrier gas (pressure 90 kPa) and the reference gas was hydrogen (H<sub>2</sub>) with a  $\delta^2\text{H}$ -value of –115.6 ‰. The certified standards used were IAEA-CH-7 (polyethylene,  $\delta^2\text{H}$  = –100.3 ‰), coumarin ( $\delta^2\text{H}$  = 82.3 ‰, reference material from Schimmelmann Research: Indiana University Bloomington), icosanoic acid ( $\delta^2\text{H}$  = –166.7 ‰) and biphenyl ( $\delta^2\text{H}$  = –41.2 ‰). Samples were analysed in duplicate in less than 12 h after their sealing.

**2.2.2.3. O ( $\delta^{18}\text{O}$ -values in ‰) isotope analysis.** About 0.3 mg of the powdered sample was introduced into silver capsules (Lüdi Swiss, Flawil, Switzerland). Samples were analysed in the same TC/EA-IRMS Delta Plus XP mentioned above but the pyrolysis reactor temperature was 1445 °C. The filling of the reactor was the same as reported for <sup>2</sup>H analyses. Helium was used as carrier gas (pressure 62 kPa) and the reference gas was carbon monoxide (CO) with a  $\delta^{18}\text{O}$ -value of –8.68 ‰ (pressure 180 kPa). The certified standard IAEA-601 (benzoic acid,  $\delta^{18}\text{O}$  = +23.3 ‰) and the internal secondary standards, UB-YCEM ( $\delta^{18}\text{O}$  = +17.6 ‰) and UB-ASC ( $\delta^{18}\text{O}$  = +13.2 ‰), both barium sulphates, were used fitting the range of the samples. Samples were analysed in

duplicate.

**2.2.2.4. S ( $\delta^{34}\text{S}$ -values in ‰) isotope analysis.** About 8 mg of the powdered sample was weighed into tin capsules (Elemental Microanalyses, Okehampton, UK). V<sub>2</sub>O<sub>5</sub> was added as a catalyser. Samples were analysed in the same TC/EA-IRMS Delta Plus XP with a pyrolysis reactor at 1035 °C. Specifically, the filling of the 450 mm quartz reactor from the bottom to the top consists of: 30 mm of quartz wool (Lüdi Swiss, Flawil, Switzerland), 90 mm of copper wires (Elemental Microanalysis), 45 mm of quartz chips (Lüdi Swiss) and 45 mm of tungsten (VI) oxide (WO<sub>3</sub>) (Elemental Microanalysis). Helium was used as carrier gas (pressure 65.5 kPa) and the reference gas was sulphur dioxide (SO<sub>2</sub>) with a  $\delta^{34}\text{S}$ -value of 1.266 ‰ (pressure 50 kPa). The certified standards, IAEA S-2 (silver sulphide,  $\delta^{34}\text{S}$  = +22.7 ‰), IAEA SO-5 (barium sulphate,  $\delta^{34}\text{S}$  = +0.5 ‰) and IAEA SO-6 (barium sulphate,  $\delta^{34}\text{S}$  = –34.1 ‰), and the secondary standard UB-YCEM (barium sulphate,  $\delta^{34}\text{S}$  = +12.8 ‰) were used. Samples were analysed in duplicate.

### 2.3. Isotopic analysis of FAMES by gas Chromatography-Isotope ratio Mass spectrometry (GC-IRMS)

#### 2.3.1. Sample preparation

**2.3.1.1. Oil extraction.** Lipid fraction was extracted with 50 mL of diethyl ether from 25 g of ground hazelnuts and the organic solvent was evaporated to dryness.

**2.3.1.2. Preparation of FAMES.** An aliquot of 100 mg of hazelnut oil was dissolved in 2 mL of hexane and 200 µL of 2 M methanolic potassium hydroxide solution was added (Hrastar et al., 2009). The mixture was centrifuged, and the supernatant was analysed.

#### 2.3.2. GC-IRMS

The analysis of H and C isotopes of individual FAMES was carried out in duplicate using a Trace GC Ultra gas chromatograph with a Triplus Autosampler coupled to an Isotope Ratio Mass Spectrometer Delta V Advantage through a GC Isolink interface (Thermo Fisher Scientific). A total of 1 µL of sample was injected with a split ratio of 1:5. Analytes were separated on a VF-23ms capillary column (60 × 0.32 mm I.D., 0.15 µm of Agilent Technologies, Santa Clara, California, USA). The initial GC oven temperature was 60 °C and was held for 1 min, then, it was increased to 160 °C at 6 °C/min and held for 10 min. Finally, it was increased to 240 °C at 6 °C/min.

Helium was the carrier gas, at a flow rate of 1.8 mL/min. The temperature of the injector was 240 °C. The commercial NiO/CuO-NiO-Pt combustion reactor (P/N 1255321, Thermo Fisher Scientific) operated at 1000 °C for CO<sub>2</sub>. In the case of H<sub>2</sub>, the commercial high temperature reactor (P/N 1255330, Thermo Fisher Scientific) was set at 1400 °C. This pyrolysis reactor is empty, but an inner layer of carbon is formed that covers the walls of the ceramic when conditioning it, this layer serves to avoid contact with the oxygen of the walls (which is Al<sub>2</sub>O<sub>3</sub>)

and, on the other hand, to catalyse the reaction. The certificated standards, androstane, USGS76, coumarin, and a secondary standard, FAME C19, were used for the  $\delta^2\text{H}$  analysis. Icosane, FAME C16, USGS76, FAME C19, USGS72, phenanthrene 16/0020 were used for the  $\delta^{13}\text{C}$  analysis.

#### 2.4. Isotopic analysis of $^{87}\text{Sr}/^{86}\text{Sr}$ by multi Collector-Inductively coupled Plasma-Mass spectrometry (MC-ICP-MS)

##### 2.4.1. Sample preparation and digestion

A 0.5 g-powdered sample was treated with 3 mL of concentrated  $\text{HNO}_3$  and 1 mL of water, both ultrapure, into a closed quartz reactor using a microwave assisted digestion system Ultrawave ECR (Milestone Srl, Sorisole, Italy) at 240 °C (ramp for 30 min and maintenance for 15 min). After complete digestion, 5 mL of ultrapure water were added, and the solution was weighed. The final volume was calculated by the solution weight and weight/volume ratio.

##### 2.4.2. Preliminary quantification of Sr and Rb by Inductively coupled Plasma-Mass spectrometry (ICP-MS)

Samples were analysed by a Nexion 350d ICP-MS (PerkinElmer Life & Analytical Sciences, Waltham, Massachusetts, USA) in standard mode without collision and/or reaction gas in 1/20 ratio.

Standard solutions of concentrations in the range of the samples, prepared from 1 g/L Rb and Sr certified standards (Inorganic Ventures, Christiansburg, Virginia, USA), were used for calibration.

##### 2.4.3. Sample purification

After digestion, samples were evaporated to dryness in using Saville® PFA beakers using ultra clean evaporation stations (ISO 5) at the LIRA (Laboratori d'Isòtops Radiogenics i Ambientals) ultraclean Lab (UB). Then, samples were brought back into solution in ultrapure (double distilled) 8 N  $\text{HNO}_3$  acid (1 mL). Sr was purified using Sr-SPEC Resin (100–150  $\mu\text{m}$  mesh; Triskem International) packed in 1 mL polypropylene cartridges with porous PFA mesh used as frits and connected into a Triskem24-manifold vacuum box attached to a vacuum pump system. Sr elution started by cleaning the Sr-SPEC resin with 20 mL of 0.05 N  $\text{HNO}_3$  and conditioning with 5 mL of 8 N  $\text{HNO}_3$  before loading the sample in 1 mL of 8 N  $\text{HNO}_3$  (a previous concentration check is made to assure loading around 500 ng of Sr). Next, matrix was eluted with 5 mL of 8 N  $\text{HNO}_3$  to mainly get rid of sample matrix as well as Rb, which presents isobaric interferences during the spectrometric measurements. The purified Sr was finally eluted with 5 mL of ultrapure 0.05 N  $\text{HNO}_3$ , evaporated and dissolved in 2 %  $\text{HNO}_3$  acid for the MC-ICP-MS measurements.

##### 2.4.4. MC-ICP-MS

The determination of Sr isotope composition was performed using a Plasma 3 Multi Collector Inductively Coupled Plasma Mass Spectrometer (Nu Instruments-AMETEK) at Centres Científics i Tecnològics of the Universitat de Barcelona (CCiTUB). Samples and standards were matched in concentration. Procedural blanks contained less than 360 pg of Sr, which was negligible compared to the Sr amounts in the samples (over 250 ng) and were systematically corrected. The contribution of  $^{87}\text{Rb}$  to the  $^{87}\text{Sr}$  signal was mathematically corrected from the measurement of the  $^{85}\text{Rb}$  signal, assuming a  $^{87}\text{Rb}/^{85}\text{Rb}$  ratio of 0.38562. The  $^{86}\text{Kr}$  interference on  $^{86}\text{Sr}$ , caused by Kr impurities in the argon gas, was also corrected by measuring the  $^{83}\text{Kr}$  signal, and assuming a  $^{83}\text{Kr}/^{86}\text{Kr}$  value of 0.66453. Mass bias was corrected with the exponential model, using the traditionally accepted  $^{86}\text{Sr}/^{88}\text{Sr}$  ratio value of 0.1194 (Nier, 1938). A further normalization by the sample-standard bracketing method was performed by analysing the NIST SRM 987 isotopic certified standard before and after each sample. Results were provided relative to this standard, assuming a reference value for the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.710249 (Azmy et al., 1999). The reproducibility of the analysis was typically better than 0.000030 (2SD).

#### 2.5. Data treatment and statistical analysis

First, the possible effect of the fertilisation on the  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  was studied.  $\delta^{15}\text{N}$  values were plotted versus  $\delta^{34}\text{S}$  values and  $1/[\text{Sr}]$  ( $\mu\text{g/g}$ ) was plotted versus  $^{87}\text{Sr}/^{86}\text{Sr}$  values as found in Epova et al. (2019). Then, these values were compared with those reported in the literature according to the type of fertilisers and to the geogenic values of each geographical area. After evaluating the results obtained in our study, the variables  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  were excluded from the development of geographical modelling, while  $^{87}\text{Sr}/^{86}\text{Sr}$  was further considered.

A data matrix was built, consisting of the 40 samples (rows) and 11 variables presumed to be unaffected by agronomic practices (bulk  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ;  $\delta^{13}\text{C}$  of C16:0, C18:0, C18:1, C18:2 FAMES and  $\delta^2\text{H}$  of C16:0, C18:1, C18:2 FAMES;  $^{87}\text{Sr}/^{86}\text{Sr}$ ) (columns).

Principal Component Analysis (PCA) was performed with SIMCA v13.0© (Umetrics AB, Sweden) to explore the data and to identify any potential outliers according to the Hotelling's  $T^2$  range and model residuals parameters. No outliers were detected.

Afterwards, the data matrix was used to develop and validate Partial Least Square-Discriminant Analysis (PLS-DA) classification models to discriminate samples according to their geographical origin (SIMCA v13.0©). In PLS-DA multi-class models, a dummy Y matrix with as many classification vectors as classes was used, each vector had values of 1 for one class (a specific country of origin) and 0 for all the other classes (the other countries). Then, each sample was classified into the class corresponding to the vector leading to the highest PLS predicted value (PV); but samples whose PV did not reach the classification threshold ( $\text{PV} < 0.5$ ) for any vector were not assigned to any country (no class).

The regression coefficients of the global PLS-DA model were studied to determine which variables were more relevant to discriminate among origins. To evaluate the significance of the regression coefficients, the jack-knife standard error of cross-validation (SEcv) was used. Coefficients were considered significant if their values were higher than their corresponding SEcv. The variables exhibiting significant coefficients were used to develop optimized PLS-DA classification models. Two models were built: one based on isotopic profiling of light elements alone, which are suitable for routine analysis, and another model that incorporated also  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic composition, to further evaluate its discriminatory potential as a heavy geo-element.

Models' performance was assessed through internal validation with leave-10 %-out cross-validation, and the optimal number of latent variables (LV) was selected according to the lowest Root Mean Squared Error of Cross Validation (RMSEcv) criteria. The optimal pre-processing for all the models was mean centring and scaling to the unit of variance. Permutation test and ANOVA on the cross-validated predictive residuals (p-value) were carried out to assess the models' overfitting. The suitability of the PLS-DA models was evaluated by the  $Q^2$  values and the percentage of correct classification of each class.

### 3. Results and discussion

#### 3.1. Sample preparation for bulk isotopic analysis

Homogeneity can be a critical factor that limits the precision of bulk isotopic analysis when applied to solid samples with distinct morphological components (Carter & Fry, 2013), such as hazelnuts. Moreover, the high lipid content in hazelnut kernels can pose challenges in obtaining a fine powder necessary for accurately weighing small and uniform amounts of this sample matrix. For this reason, different grinding methods were tested, a small domestic grinder, a cryogenic mill and a combination of both. The lyophilization efficiency achieved after the application of each grinding method was evaluated (Table S1, Supplementary material). All the replicates obtained using a domestic grinder were completely lyophilized within 72 h, while only two of the cryogenic mill replicates achieved the complete lyophilization, after 127 h. The combination of grinder and cryogenic mill led to a paste that

could not be lyophilized within 127 h. Considering these results, the cryogenic mill was excluded.

Repeatability results for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  bulk analysis ( $n = 10$ ) were  $-28.17 \pm 0.05 \text{ ‰}$  and  $6.9 \pm 0.2 \text{ ‰}$ , respectively, confirming that the selected method produces a sufficiently homogeneous sample, ensuring acceptable precision in the isotopic measurements. It was assumed that the observed repeatability also held valid for the remaining elements analysed by EA-IRMS.

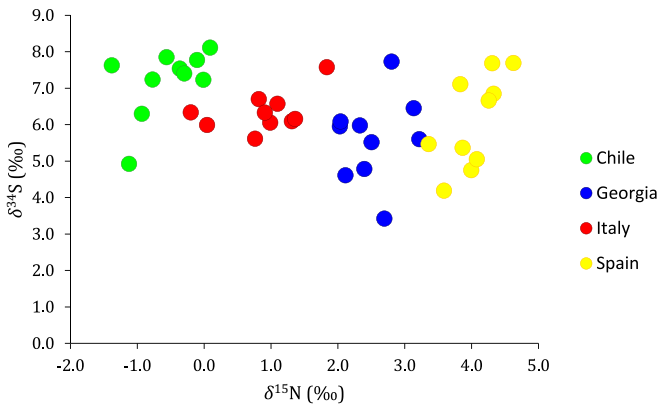
The extent of exchangeable H was evaluated to assess the impact of recent exposure of hazelnuts to water or vapor on the H isotope ratios. The dual-water equilibration assay revealed identical  $\delta^2\text{H}$  ‰ values ( $-149 \pm 2$  and  $-152 \pm 2$ ) between the samples equilibrated with the two isotopically different waters (Milli-Q grade water  $-43 \text{ ‰}$  and deuterated water  $+100 \text{ ‰}$ , respectively). Therefore, labile H appeared not to be isotopically relevant in the hazelnut samples.

3.2. N, S and Sr isotopic profile of hazelnuts and possible impact of fertilisation practices

Mean isotopic compositions of N, S and Sr in hazelnuts from the four origins are reported with the corresponding standard deviation in Table 2. The plot of  $\delta^{15}\text{N}$  ‰ versus  $\delta^{34}\text{S}$  ‰ values (Fig. 1) reveals a lack of discernible patterns in the  $\delta^{34}\text{S}$  values of hazelnuts across the four distinct geographical areas. According to Vitòria et al., 2004, these  $\delta^{34}\text{S}$  values are in the range of fertilisers, so geographical factors that may differentiate these zones could have been masked by the diverse agronomic practices employed in each plantation, thereby losing value as a geographical marker in this study. On the contrary,  $\delta^{15}\text{N}$  differentiates almost completely the samples from each of the four provenances, showing the lowest values in Chilean hazelnuts, followed by Italian, Georgian and Spanish ones. The N isotopic composition of both Chilean and Italian samples aligns with the expected values based on current fertilisation agricultural practices (Table 1) and previous literature (Vitòria et al., 2004; Bateman and Kelly, 2007; Laursen et al., 2013). Specifically, the application of synthetic fertilisers justifies the depletion of  $^{15}\text{N}$  in the Chilean samples compared to the non-fertilized Italian samples. Conversely, also synthetically fertilised Georgian and Spanish samples exhibited enriched  $^{15}\text{N}$  values compared to the latter. These results may be attributed to the specific type of fertilisation employed or the long-term fertilisation history of such soils, given that residual nitrogen from fertilizers, as forecasted by Sebilo et al. (2013), possesses

**Table 2**  
Mean and standard deviation results of all the isotopic markers for the 4 origins (Chile, Georgia, Italy and Spain).

|                                     | Chile                          | Georgia                      | Italy                        | Spain                         |
|-------------------------------------|--------------------------------|------------------------------|------------------------------|-------------------------------|
| $\delta^{15}\text{N}$ (‰)           | $-0.6 \pm 0.5$                 | $2.5 \pm 0.4$                | $0.9 \pm 0.6$                | $4.0 \pm 0.4$                 |
| $\delta^{34}\text{S}$ (‰)           | $7.2 \pm 0.9$                  | $5.6 \pm 1.2$                | $6.4 \pm 0.5$                | $6.1 \pm 1.3$                 |
| $\delta^{13}\text{C}$ (‰)           | $-26.7 \pm 0.8$                | $-27.7 \pm 0.4$              | $-26.9 \pm 0.6$              | $-26.7 \pm 0.5$               |
| $\delta^2\text{H}$ (‰)              | $-169.2 \pm 8.3$               | $-172.4 \pm 3.1$             | $-161.8 \pm 7.8$             | $-159.0 \pm 7.9$              |
| $\delta^{18}\text{O}$ (‰)           | $23.7 \pm 1.0$                 | $20.1 \pm 0.5$               | $24.4 \pm 0.7$               | $23.6 \pm 0.4$                |
| $\delta^{13}\text{C}$ -Palmitic (‰) | $-30.1 \pm 0.6$                | $-30.9 \pm 0.4$              | $-30.1 \pm 0.7$              | $-30.1 \pm 0.5$               |
| $\delta^{13}\text{C}$ -Stearic (‰)  | $-32.2 \pm 1.1$                | $-33.5 \pm 1.2$              | $-32.2 \pm 1.2$              | $-31.8 \pm 1.0$               |
| $\delta^{13}\text{C}$ -Oleic (‰)    | $-28.3 \pm 0.7$                | $-29.0 \pm 0.4$              | $-28.3 \pm 0.8$              | $-28.3 \pm 0.6$               |
| $\delta^{13}\text{C}$ -Linoleic (‰) | $-30.6 \pm 0.7$                | $-31.4 \pm 0.4$              | $-31.2 \pm 0.9$              | $-30.9 \pm 0.9$               |
| $\delta^2\text{H}$ -Palmitic (‰)    | $-183.9 \pm 7.4$               | $-187.5 \pm 5.6$             | $-175.4 \pm 5.4$             | $-164.9 \pm 8.4$              |
| $\delta^2\text{H}$ -Oleic (‰)       | $-195.2 \pm 4.8$               | $-200.8 \pm 2.7$             | $-187.4 \pm 3.1$             | $-179.8 \pm 6.1$              |
| $\delta^2\text{H}$ -Linoleic (‰)    | $-218.4 \pm 3.6$               | $-223.3 \pm 4.9$             | $-207.3 \pm 4.9$             | $-202.2 \pm 6.6$              |
| $^{87}\text{Sr}/^{86}\text{Sr}$     | $0.70436 \pm 4 \times 10^{-5}$ | $0.706 \pm 1 \times 10^{-3}$ | $0.709 \pm 1 \times 10^{-3}$ | $0.7087 \pm 1 \times 10^{-4}$ |

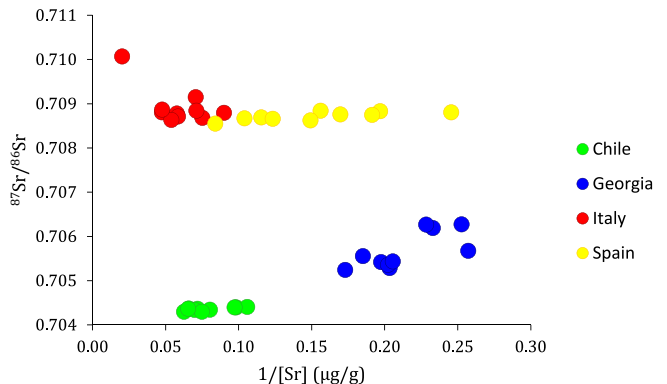


**Fig. 1.** Plot of  $\delta^{15}\text{N}$  ‰ values versus  $\delta^{34}\text{S}$  ‰ values in hazelnuts from Chile (green), Georgia (blue), Italy (red), and Spain (yellow).

the capacity to sustain crop nutrition for a duration extending up to five decades. In Georgia, significant amounts of straight fertiliser  $\text{NH}_4\text{NO}_3$  were used in addition to NPK, which has been reported to yield relatively higher enriched  $^{15}\text{N}$  water-soluble products ( $\delta^{15}\text{N}_{\text{NO}_3} = +5.6 \text{ ‰}$ ) in some cases (Vitòria et al., 2004). On the other hand, the higher  $\delta^{15}\text{N}$  values observed in Spanish samples could not be easily related to the current fertilisation practises, as producers said to apply a mixture of NPK and leave spontaneous vegetation mowed 2–3 times per year as mulch between the rows of trees. This vegetation cover (legumes or non-legumes) would enhance the homogeneity of the soil nitrogen supply and reduce the fertilizer influence on the isotopic composition of hazelnuts (Krauß et al., 2020; Giannioti et al., 2024), so we propose that it should be the result of previous history of soil fertilization.

The findings from this study, along with previous research, indicate that the complexity arising from the variability of synthetic fertilisers' isotopic signatures, plant physiological factors, potential bacterial-mediated reactions resulting in N isotopic fractionation, the potential influence of climate and soil characteristics and remaining fertilizer N still residing in the soil present challenges in elucidating the impact of agricultural practices and, specifically, in predicting N isotopic composition based on geographical origin.

Regarding Sr, combining its concentration and isotopic composition in hazelnuts permitted a clear discrimination of the samples according to their origin (Fig. 2). Despite the reported isotopic differences in trace amounts of Sr present in fertilisers (Vitòria et al., 2004), the  $^{87}\text{Sr}/^{86}\text{Sr}$  composition in hazelnuts align with the geogenic factors of each production area after checking geological maps (Jones et al., 1994). Spanish and Italian samples presented the highest values, consistent with Holocene formations, whereas Georgian and Chilean samples showed lower ones, indicative of more recent Neogene formations and recent



**Fig. 2.** Plot of  $1/[\text{Sr}]$  ( $\mu\text{g/g}$ ) versus  $^{87}\text{Sr}/^{86}\text{Sr}$  values in hazelnuts from Chile (green), Georgia (blue), Italy (red) and Spain (yellow).

volcanism, respectively. Furthermore, the Sr abundance in hazelnuts varied according to their origin, with Italian and Chilean hazelnuts exhibiting higher levels, followed by Spanish and Georgian samples. Although Sr isotopic composition can potentially be influenced by the use of different fertilisers, the impact of these variations does not seem to be critical in the analysed samples. Therefore, it is deemed appropriate to further consider this marker for the development of geographical classification models, while also acknowledging that the practical application of this determination in routine analysis may present challenges.

### 3.3. Evaluating relevant isotopic markers for hazelnut geographical authentication by Partial Least Square-Discriminant analysis (PLS-DA)

#### 3.3.1. Evaluation of light element isotopic composition: Regression coefficients of global PLS-DA model

In Table 2, mean and standard deviation results of all the isotopic markers for the four origins are reported. After excluding  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  for the reasons given above, noticeable differences between the means can be appreciated, especially for the  $\delta^{18}\text{O}$ ,  $\delta^2\text{H}$  of the FAMES and  $^{87}\text{Sr}/^{86}\text{Sr}$ , but no marker, by itself, can differentiate between all the origins. For this reason, it is more advisable to study the complete isotopic profile applying multivariate techniques such as PLS-DA. This approach provides a more comprehensive understanding of the samples by utilizing a broader range of information, thus enabling better discrimination. Additionally, it permits the identification of variables with the highest discriminant power. Indeed, one of the primary objectives of this study was to identify highly promising isotopic markers that can be utilized in future classification models, with a focus on using the minimum number of variables that demonstrate the highest discrimination power. This approach was aimed at streamlining the analytical procedure while maximizing accuracy. To accomplish this, the regression coefficients of the prospective PLS-DA classification models, developed with hazelnut samples from four distinct origins, were evaluated.

A multiclass PLS-DA model was built to discriminate samples according to their geographical origin, including light element bulk  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the FAMES as the variables. Elements whose isotopic value could potentially be influenced by specific agronomic practices or demonstrated negligible variation in relation to hazelnut origin, such as N and S (Section 3.2), were omitted from the model to prevent any potential bias. Moreover, to focus first on light elements alone, Sr was also excluded from modelling at this stage. The PLS-DA model cross-validation results provided an 85 % of global correct classification, with percentages higher than 70 % for each individual class. Only two samples were misclassified, while four samples were not assigned to any category (Table 3a). Permutation tests, which display the prediction capacity of 20 random models, and ANOVA of the cross-validation ( $p < 0.05$ ) indicated the absence of a random classification and of model overfitting.

PLS regression coefficients (Fig. 3a) indicated that among bulk isotopic markers, only  $\delta^{18}\text{O}$  seemed to contribute relevantly to the discrimination between geographical origins, showing significant positive correlation with Chile and Italy classes and significant negative correlation with Georgia and Spain ones. The  $\delta^2\text{H}$  and  $\delta^{13}\text{C}$  of some of the main FAMES ( $\delta^2\text{H}$  of palmitic and oleic acid;  $\delta^{13}\text{C}$  of linoleic acid) were also relevant for the discrimination of Italian and Spanish samples, respectively. Neither bulk  $\delta^{13}\text{C}$  or  $\delta^2\text{H}$  seemed to be relevant for the classification of any of the tested hazelnut origins. The mean values of the bulk  $\delta^2\text{H}$  and  $\delta^2\text{H}$  of the FAMES of each origin (Table 2) were coherent with the isotopic ratios of the precipitations at the specified locations: the Georgian fruits and water ( $\delta^2\text{H}_{\text{water}} = -53$  ‰) exhibited the most depleted values in  $^2\text{H}$ , followed by Chile ( $\delta^2\text{H}_{\text{water}} = -41$  ‰), Italy ( $\delta^2\text{H}_{\text{water}} = -38$  ‰) and Spain ( $\delta^2\text{H}_{\text{water}} = -34$  ‰) (Bowen & Revenaugh, 2003; Bowen, 2024; IAEA/WMO, 2015). Conversely, the  $\delta^{18}\text{O}$  mean values of each origin did not presented a clear correlation

**Table 3**

Results of the leave-10 %-out cross-validation of the: a) Global PLS-DA classification model that included the variables  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES; b) PLS-DA classification model that included the variables  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES and c) PLS-DA classification model that included the variables  $\text{Sr}^{87}/\text{Sr}^{86}$ ,  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES.

| a)                                                                                                  |    |       |         |       |       |                   |           |
|-----------------------------------------------------------------------------------------------------|----|-------|---------|-------|-------|-------------------|-----------|
|                                                                                                     | n  | Chile | Georgia | Italy | Spain | Correct class (%) | No Class* |
| Chile                                                                                               | 10 | 9     | 0       | 0     | 0     | 90                | 1         |
| Georgia                                                                                             | 10 | 0     | 10      | 0     | 0     | 100               | 0         |
| Italy                                                                                               | 10 | 0     | 0       | 7     | 1     | 70                | 2         |
| Spain                                                                                               | 10 | 0     | 0       | 1     | 8     | 80                | 1         |
| Total                                                                                               | 40 |       |         |       |       | 85                |           |
| N = 40, 5 LVs, $Q^2 = 0.38$ , RMSEcv = 0.43, ANOVA p-value < 0.05.* Not assigned samples (PV < 0.5) |    |       |         |       |       |                   |           |
| b)                                                                                                  |    |       |         |       |       |                   |           |
|                                                                                                     | n  | Chile | Georgia | Italy | Spain | Correct class (%) | No Class* |
| Chile                                                                                               | 10 | 7     | 0       | 0     | 0     | 70                | 3         |
| Georgia                                                                                             | 10 | 0     | 10      | 0     | 0     | 100               | 0         |
| Italy                                                                                               | 10 | 0     | 0       | 9     | 0     | 90                | 1         |
| Spain                                                                                               | 10 | 0     | 0       | 1     | 8     | 80                | 1         |
| Total                                                                                               | 40 |       |         |       |       | 85                |           |
| N = 40, 4 LVs, $Q^2 = 0.45$ , RMSEcv = 0.38, ANOVA p-value < 0.05.* Not assigned samples (PV < 0.5) |    |       |         |       |       |                   |           |
| c)                                                                                                  |    |       |         |       |       |                   |           |
|                                                                                                     | n  | Chile | Georgia | Italy | Spain | Correct class (%) | No Class* |
| Chile                                                                                               | 10 | 10    | 0       | 0     | 0     | 100               | 0         |
| Georgia                                                                                             | 10 | 0     | 10      | 0     | 0     | 100               | 0         |
| Italy                                                                                               | 10 | 1     | 0       | 8     | 1     | 80                | 1         |
| Spain                                                                                               | 10 | 0     | 0       | 0     | 9     | 90                | 1         |
| Total                                                                                               | 40 |       |         |       |       | 92.5              |           |

N = 40, 4 LVs,  $Q^2 = 0.62$ , RMSEcv = 0.34, ANOVA p-value < 0.05.\* Not assigned samples (PV < 0.5).

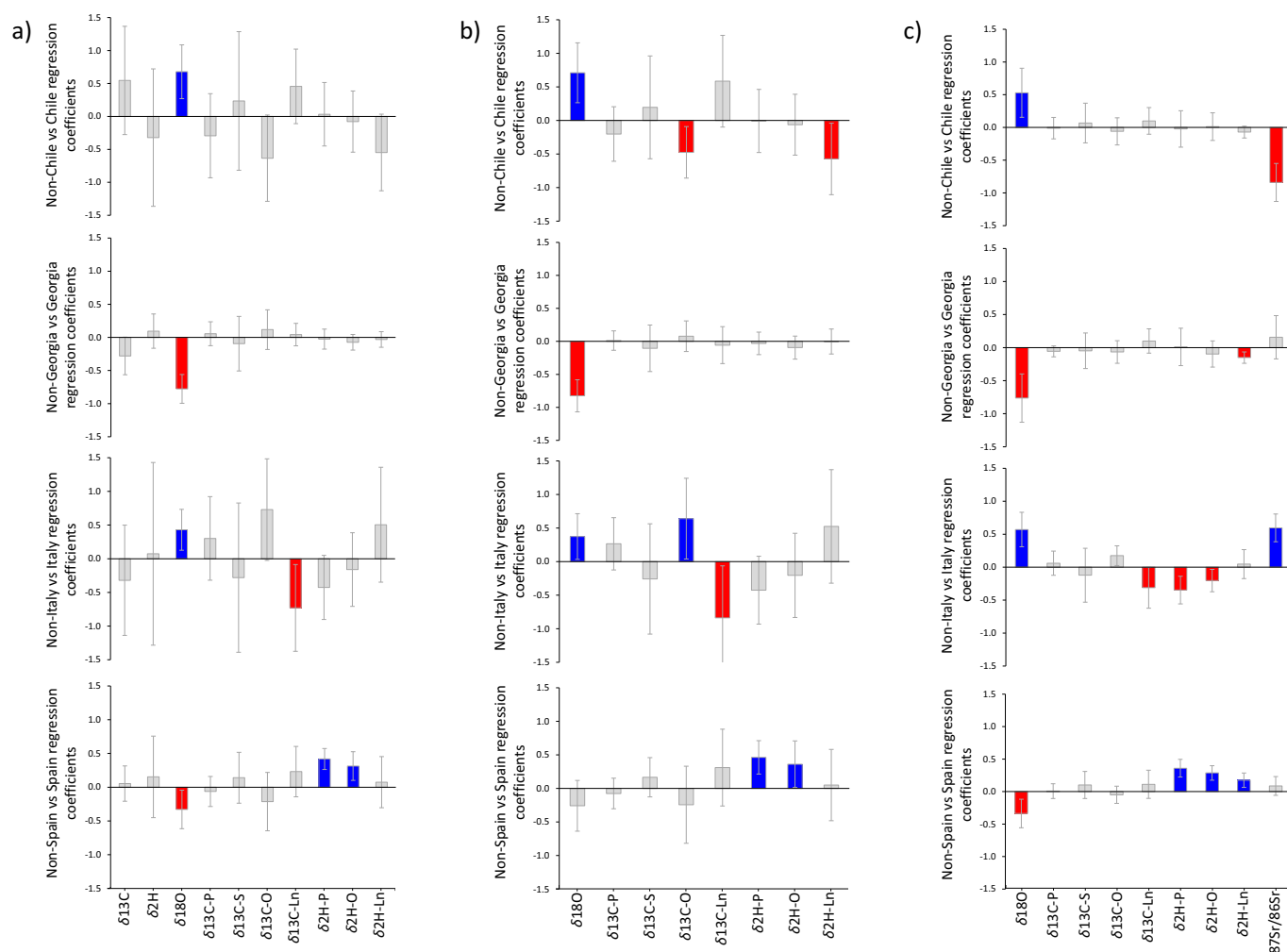
with precipitation (Georgia  $\delta^{18}\text{O}_{\text{water}} = -8.4$  ‰; Chile  $\delta^{18}\text{O}_{\text{water}} = -6.4$  ‰; Italy  $\delta^{18}\text{O}_{\text{water}} = -6.2$  ‰; Spain  $\delta^{18}\text{O}_{\text{water}} = -5.6$  ‰) (Bowen et al., 2024; Bowen and Revenaugh, 2003; IAEA/WMO, 2015). This observation may be attributed to the fact that organic hydrogen exclusively derives from water in the hydrosphere, whereas oxygen can originate from various sources, including atmospheric oxygen and photo-synthesized carbon dioxide (De Rijke et al., 2016).

#### 3.3.2. Optimised PLS-DA model based on light element isotopic composition

A new PLS-DA model was built with the most relevant variables not influenced by other factors:  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the FAMES. The absence of random classification and model overfitting was evidenced by the permutation tests and ANOVA ( $p < 0.05$ ).

The performance of the new simplified model was equivalent to the previous one (Table 3b), yielding slightly better and slightly worse results for Italian and Chilean samples, respectively, but also achieving an 85 % of global correct classification. This confirms that isotopic markers omitted in this simplified model did not play a crucial role in the discrimination. Besides, only one sample was misclassified, and five were not classified.

The assessment of the regression coefficients assessment demonstrated that most of the included variables were relevant in discriminating at least one origin (Fig. 3b). For the classification of Georgian, Chilean and Italian samples, the  $\delta^{18}\text{O}$  was a relevant variable, negatively correlated with the first one and positively correlated with the other two origins. The  $\delta^2\text{H}$  of FAMES was significant for the samples from Spain and Chile. Additionally, for this last origin and for Italian samples,  $\delta^{13}\text{C}$  of the FAMES was also a relevant variable.



**Fig. 3.** Regression coefficients of the: a) Global PLS-DA model developed including the variables  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES ( $\delta^2\text{H}$  of palmitic  $\delta^2\text{H-P}$ , oleic-  $\delta^2\text{H-O}$ , and linoleic acids  $\delta^2\text{H-Ln}$ ;  $\delta^{13}\text{C}$  of palmitic  $\delta^{13}\text{C-P}$ , stearic  $\delta^{13}\text{C-S}$ , oleic  $\delta^{13}\text{C-O}$  and linoleic acids  $\delta^{13}\text{C-Ln}$ ); b) PLS-DA model developed including the variables  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES and c) PLS-DA classification model that included the variables  $\text{Sr}^{87}/\text{Sr}^{86}$ ,  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES. Significant coefficients are highlighted in blue or red colour ('Chile' (blue) vs 'non-Chile' (red), 'Georgia' (blue) vs 'non-Georgia' (red), 'Italy' (blue) vs 'non-Italy' (red) and 'Spain' (blue) vs 'non-Spain' (red)).

### 3.3.3. PLS-DA model including relevant light element and Sr isotopic composition

Isotopic composition of Sr is expected to provide a strong correlation with the geographical origin of agricultural products, as it is influenced by the geological characteristics of the soil. Indeed, the results obtained in the present study (presented in section 3.2), demonstrated that  $\text{Sr}^{87}/\text{Sr}^{86}$  composition enabled to clearly distinguish Chilean and Georgian hazelnuts among them and from Spanish and Italian hazelnuts (Fig. 2). It is important to note that isotopic analysis of Sr involves labour-intensive purification steps and higher operating costs compared to analysing more abundant light elements (Katerinopoulou et al., 2020; Laursen et al., 2016), making this marker difficult to apply for routine analysis. However, it can be evaluated as a confirmatory tool for samples that resulted uncertain or unclassified by the model based on light element isotopic data, thus limited to a smaller number of samples.

To evaluate the suitability of  $\text{Sr}^{87}/\text{Sr}^{86}$  composition as such a confirmatory tool, a classification model was developed including the relevant light isotopic markers and  $\text{Sr}^{87}/\text{Sr}^{86}$  composition. The classification results were improved including the  $\text{Sr}^{87}/\text{Sr}^{86}$  (Table 3c), achieving a 92.5 % of global correct classification and classifying three of the five samples that resulted as not classified in the previous model. In this way, for each individual class, the percentages of classification

were higher than 80 %. ANOVA and permutation tests showed that the model had a high discrimination capacity and was not overfitted.

The evaluation of the model regression coefficients (Fig. 3c) evidenced that most of the included variables were still significant for one or more origins, being the  $\text{Sr}^{87}/\text{Sr}^{86}$  especially relevant for the Chilean (negative correlation) and Italian (positive correlation) hazelnuts.

These results demonstrate the potential use of  $\text{Sr}^{87}/\text{Sr}^{86}$  as confirmatory tool to classify uncertain samples.

## 4. Conclusions

Multiple specific isotopic markers were assessed in this prospective study as potential tools to authenticate hazelnut geographical origin. Based on the obtained results, it can be concluded that the most promising variables are bulk  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main FAMES, since they are minimally influenced by external factors such as fertilisation treatment and have shown to be relevant in discriminating among geographical origins. On the other hand, the analysis of  $\text{Sr}^{87}/\text{Sr}^{86}$  could be valuable to discriminate uncertain samples or as a confirmatory tool. These findings serve as a starting point for the development of efficient and robust models based on large-scale datasets, which should be further validated to assess their efficiency as hazelnut geographical

authentication tools.

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## CRediT authorship contribution statement

**B. Torres-Cobos:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **M. Rosell:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – review & editing. **A. Soler:** Supervision, Writing – review & editing. **M. Rovira:** Conceptualization, Resources, Writing – review & editing. **A. Romero:** Conceptualization, Resources, Writing – review & editing. **F. Guardiola:** Supervision, Writing – review & editing. **S. Vichi:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **A. Tres:** Conceptualization, Methodology, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.139083>.

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