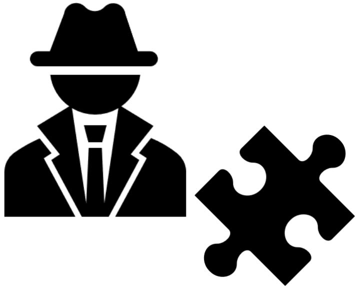
# Nuts about Authentication: Using a Handful of Isotopic Tools to Crack the Hazelnut Origin Mystery

Berta Torres Cobos [1,2*], Mònica Rosell [3], Albert Soler [3], Mercè Rovira [4], Agustí Romero [4], Francesc Guardiola [1,2], Alba Tres [1,2], Stefania Vichi [1,2]

[1] Departament de Nutrició, Ciències de l'Alimentació i Gastronomia, Facultat de Farmàcia, Ciències de l'alimentació i Gastronomia, Universitat de Barcelona, Santa Coloma de Gramenet, Spain.
[2] Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona, Santa Coloma de Gramenet, Spain.
[3] Grup MAiMA, Mineralogia Aplicada, Geoquímica i Hidrogeologia, Departament de Mineralogia, Petrologia i Geologia Aplicada, Institut de Recerca de l'Aigua (IdRA), Universitat de Barcelona, Barcelona, Spain.
[4] Institute of Agrifood Research and Technology (IRTA), Constantí, Spain.



# HAZELNUT ORIGIN AUTHENTICATION


[redacted]

**14€/kg**


[redacted]

**25€/kg**

https://www.compraonline.bonpreuesclat.cat (Accessed on 16th April 2023)

# HAZELNUT ORIGIN AUTHENTICATION

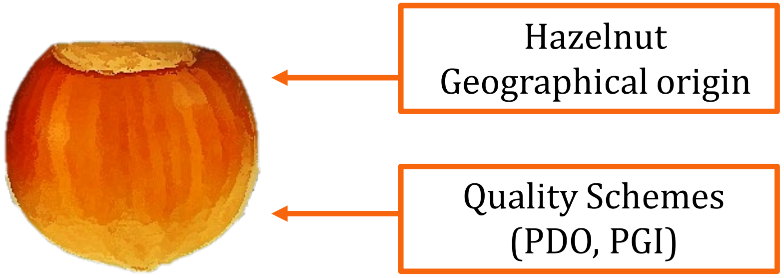
**14€/kg**

**25€/kg**

**SPECIFIC ORIGIN (REUS) & PDO QUALITY SCHEME**

https://www.compraonline.bonpreuesclat.cat (Accessed on 16th April 2023)

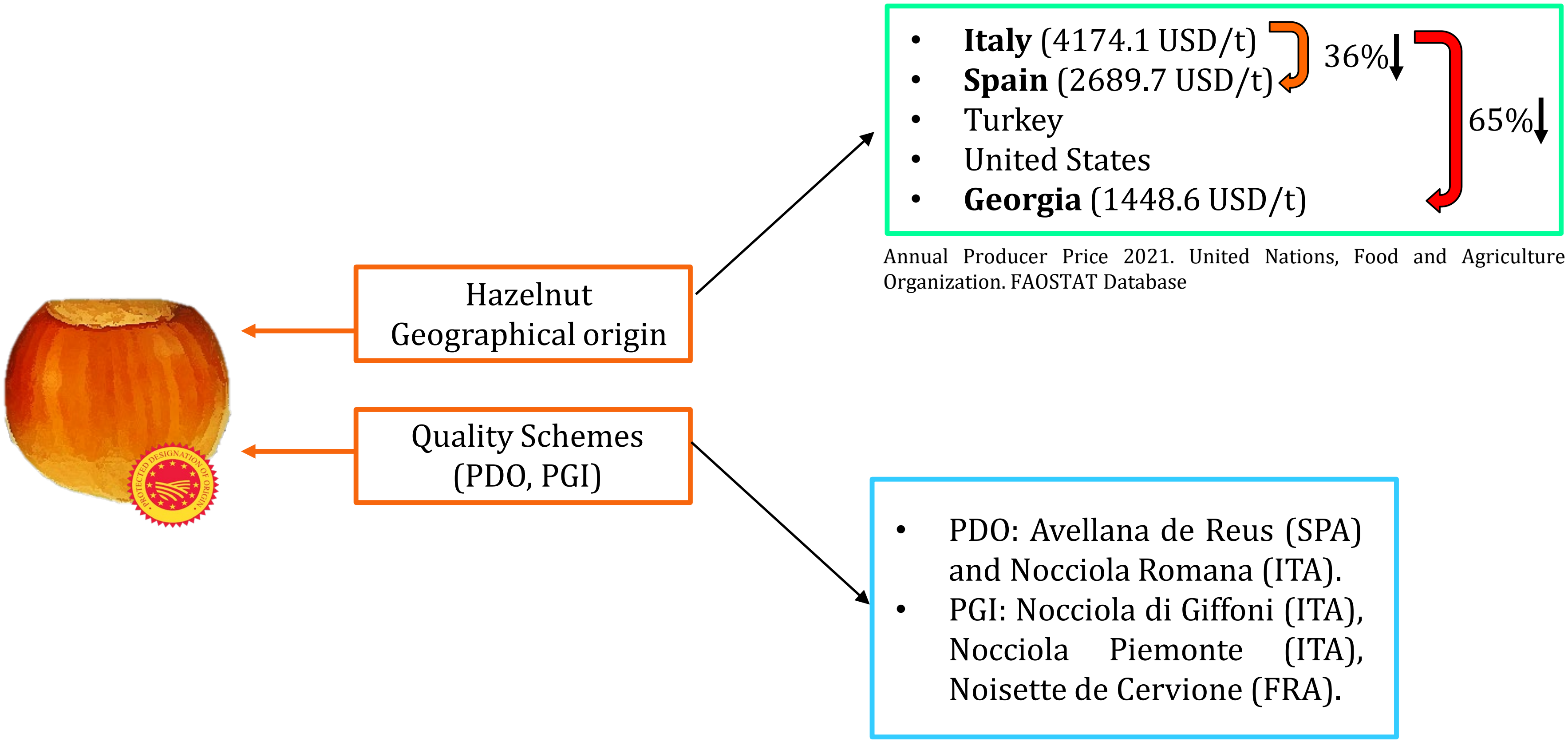
# HAZELNUT ORIGIN AUTHENTICATION



Hazelnut
Geographical origin

Quality Schemes
(PDO, PGI)

# HAZELNUT ORIGIN AUTHENTICATION



Hazelnut Geographical origin

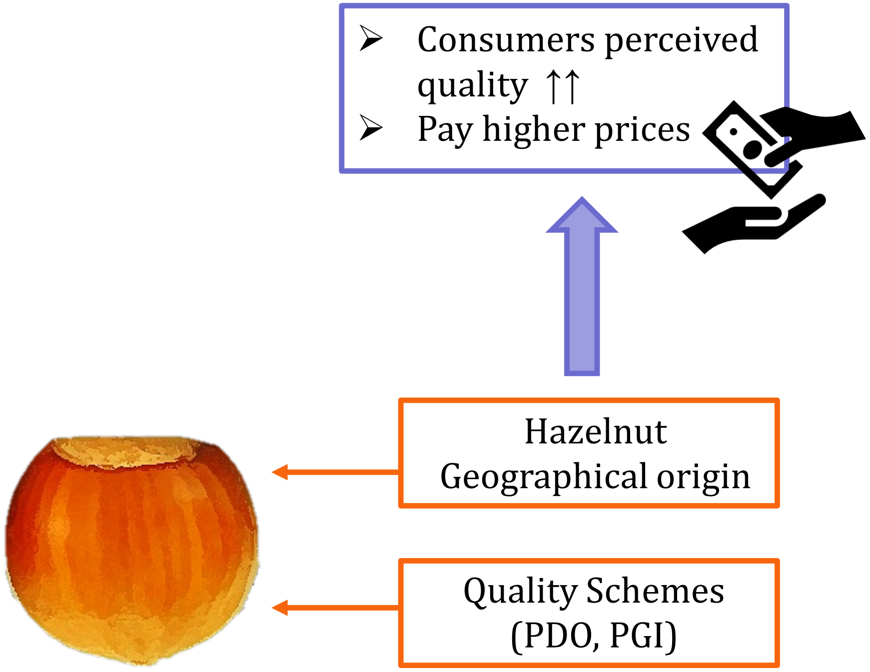
Quality Schemes (PDO, PGI)

- **Italy** (4174.1 USD/t)
- **Spain** (2689.7 USD/t)
- Turkey
- United States
- **Georgia** (1448.6 USD/t)

Annual Producer Price 2021. United Nations, Food and Agriculture Organization. FAOSTAT Database

- PDO: Avellana de Reus (SPA) and Nocciola Romana (ITA).
- PGI: Nocciola di Giffoni (ITA), Nocciola Piemonte (ITA), Noisette de Cervione (FRA).

# HAZELNUT ORIGIN AUTHENTICATION

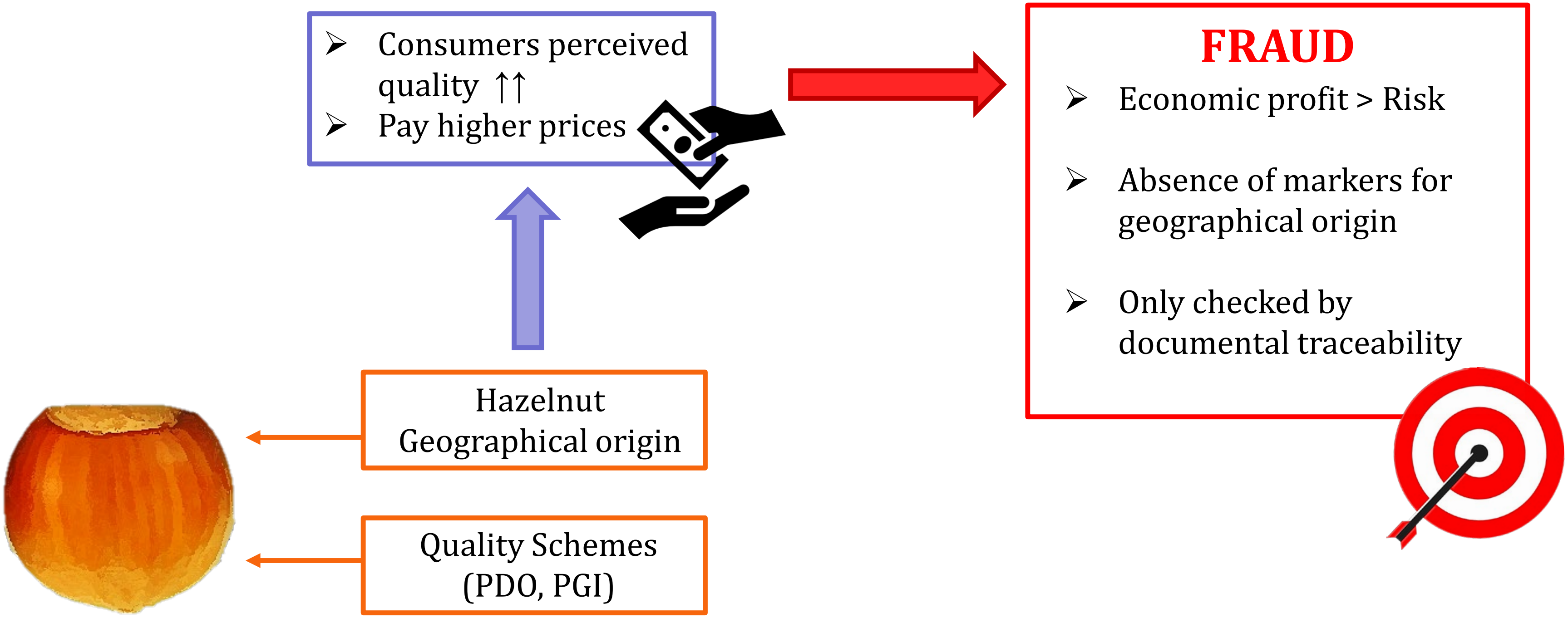


- Consumers perceived quality ↑↑
- Pay higher prices

Hazelnut Geographical origin

Quality Schemes (PDO, PGI)

# HAZELNUT ORIGIN AUTHENTICATION



- Consumers perceived quality ↑↑
- Pay higher prices

**FRAUD**

- Economic profit > Risk
- Absence of markers for geographical origin
- Only checked by documental traceability

Hazelnut Geographical origin

Quality Schemes (PDO, PGI)

# HAZELNUT ORIGIN AUTHENTICATION

- Consumers perceived quality ↑↑
- Pay higher prices

## FRAUD

- Economic profit > Risk
- Absence of markers for geographical origin
- Only checked by documental traceability

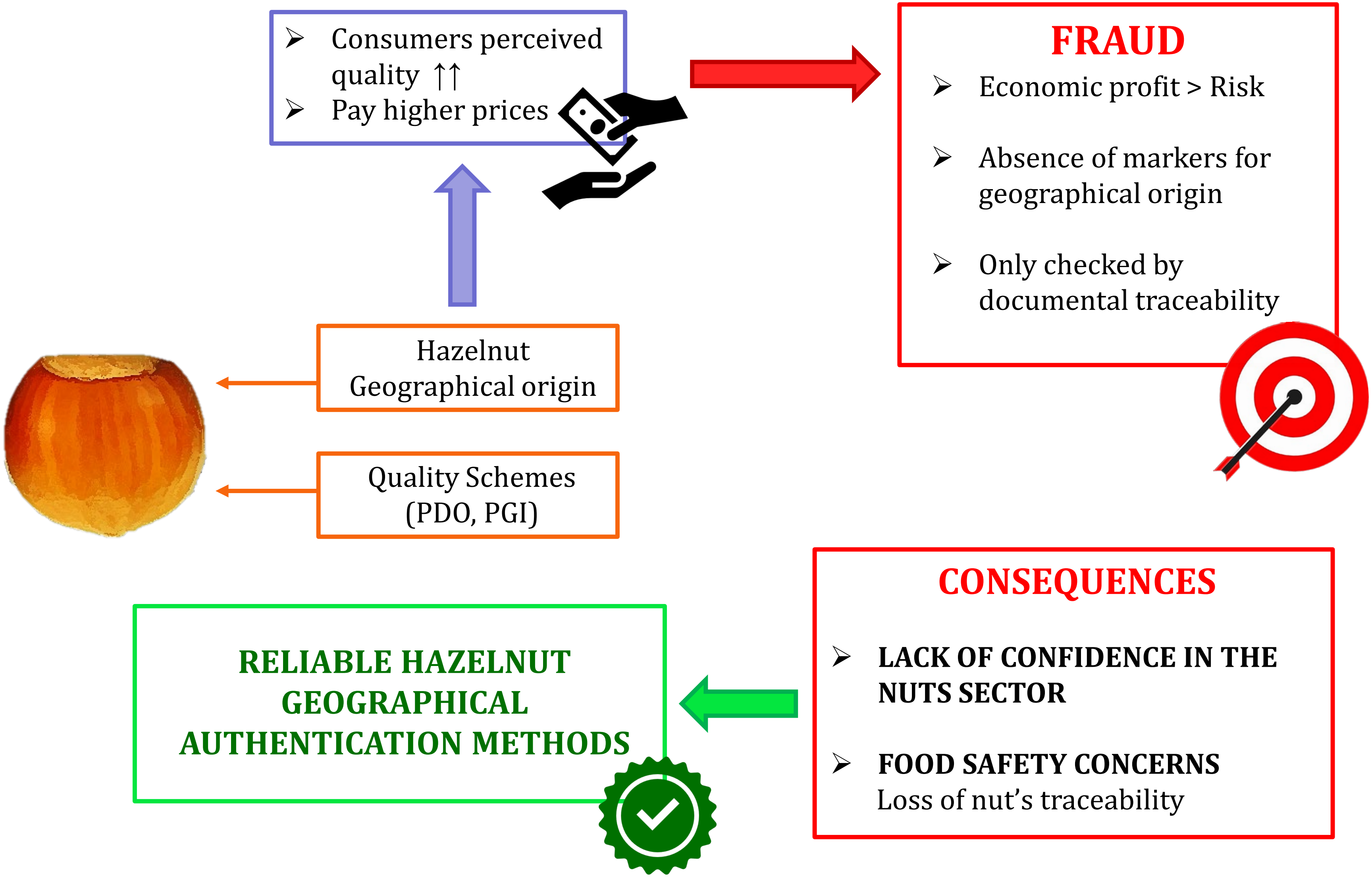
Hazelnut Geographical origin

Quality Schemes (PDO, PGI)

## CONSEQUENCES

- **LACK OF CONFIDENCE IN THE NUTS SECTOR**
- **FOOD SAFETY CONCERNS**
  Loss of nut's traceability

# HAZELNUT ORIGIN AUTHENTICATION



- Consumers perceived quality ↑↑
- Pay higher prices

## FRAUD

- Economic profit > Risk
- Absence of markers for geographical origin
- Only checked by documental traceability

Hazelnut Geographical origin

Quality Schemes (PDO, PGI)

## CONSEQUENCES

- **LACK OF CONFIDENCE IN THE NUTS SECTOR**
- **FOOD SAFETY CONCERNS**
  Loss of nut's traceability

**RELIABLE HAZELNUT GEOGRAPHICAL AUTHENTICATION METHODS**

# HAZELNUT AUTHENTICATION METHODS

## CHARACTERISTICS

- ✓ Geographical markers minimally influenced by other factors.
- ✓ Simple, easy to apply and automatable
- ✓ Combination of multiple variables to have more information.

## ISOTOPIC ANALYSIS

- Light bio-elements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$)
- Heavy geo-elements ($^{87}Sr$/ $^{86}Sr$)
- Compound specific isotope analysis
  Fatty acid $\delta^{13}C$ (linoleic, oleic, palmitic, stearic)
  $\delta^{2}H$ (linoleic, oleic, palmitic)

# AIM

Few studies of isotopic analysis for nuts geographical authentication (1,2)

[1] S. Krauß, A. Vieweg, W. Vetter (2020) J Sci Food Agric, 100, 1625–1634.
[2] K. A. Anderson, B. W. Smith (2016) J. Agric. Food Chem., 54, 1747–1752.

# AIM

Few studies of isotopic analysis for nuts geographical authentication (1,2)

✚

No data are available on the application of isotopic markers for hazelnut geographical authentication

[1] S. Krauß, A. Vieweg, W. Vetter (2020) J Sci Food Agric, 100, 1625–1634.
[2] K. A. Anderson, B. W. Smith (2016) J. Agric. Food Chem., 54, 1747–1752.

# AIM

Few studies of isotopic analysis for nuts geographical authentication (1,2)

+

No data are available on the application of isotopic markers for hazelnut geographical authentication

=

NEED: Test different isotopic analysis as hazelnut geographical origin authentication tools. Determine which isotopic markers are the most promising ones

---

[1] S. Krauß, A. Vieweg, W. Vetter (2020) J Sci Food Agric, 100, 1625–1634.

[2] K. A. Anderson, B. W. Smith (2016) J. Agric. Food Chem., 54, 1747–1752.

# AIM

Few studies of isotopic analysis for nuts geographical authentication (1,2)

✚

<u>No data</u> are available on the application of <u>isotopic markers for hazelnut geographical authentication</u>

=

NEED: Test different isotopic analysis as hazelnut geographical origin authentication tools. Determine which isotopic markers are the most promising ones

→

## PRELIMINARY STUDY

I) **Evaluate the efficacy of the main isotopic analysis applied to food geographical authentication**

- Light bio-elements (($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$), compound specific fatty acid $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)
- Heavy geo-elements ($^{87}Sr$/ $^{86}Sr$)

**as hazelnut geographical origin markers.**

[1] S. Krauß, A. Vieweg, W. Vetter (2020) J Sci Food Agric, 100, 1625–1634.
[2] K. A. Anderson, B. W. Smith (2016) J. Agric. Food Chem., 54, 1747–1752.

# AIM

**COULD BE AFFECTED BY THE FERTILIZERS**

↓

**STUDY HOW THEY ARE RELATED WITH THE SAMPLES AND THE INFLUENCE OF THE FERTILITZATION**

## PRELIMINARY STUDY

I) **Evaluate the efficacy of the main isotopic analysis applied to food geographical authentication**

- Light bio-elements (($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$), compound specific fatty acid $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)
- Heavy geo-elements ($^{87}Sr$/ $^{86}Sr$)

**as hazelnut geographical origin markers.**

[1] S. Krauß, A. Vieweg, W. Vetter (2020) J Sci Food Agric, 100, 1625–1634.
[2] K. A. Anderson, B. W. Smith (2016) J. Agric. Food Chem., 54, 1747–1752.

# AIM

Few studies of isotopic analysis for nuts geographical authentication (1,2)

+

No data are available on the application of isotopic markers for hazelnut geographical authentication

=

NEED: Test different isotopic analysis as hazelnut geographical origin authentication tools. Determine which isotopic markers are the most promising ones

→

## PRELIMINARY STUDY

I) **Evaluate the efficacy of the main isotopic analysis applied to food geographical authentication**

- Light bio-elements (($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$), compound specific fatty acid $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)
- Heavy geo-elements ($^{87}Sr/ ^{86}Sr$)

**as hazelnut geographical origin markers.**

II) **Identify the most promising ones for hazelnut authentication.**

---

[1] S. Krauß, A. Vieweg, W. Vetter (2020) J Sci Food Agric, 100, 1625–1634.

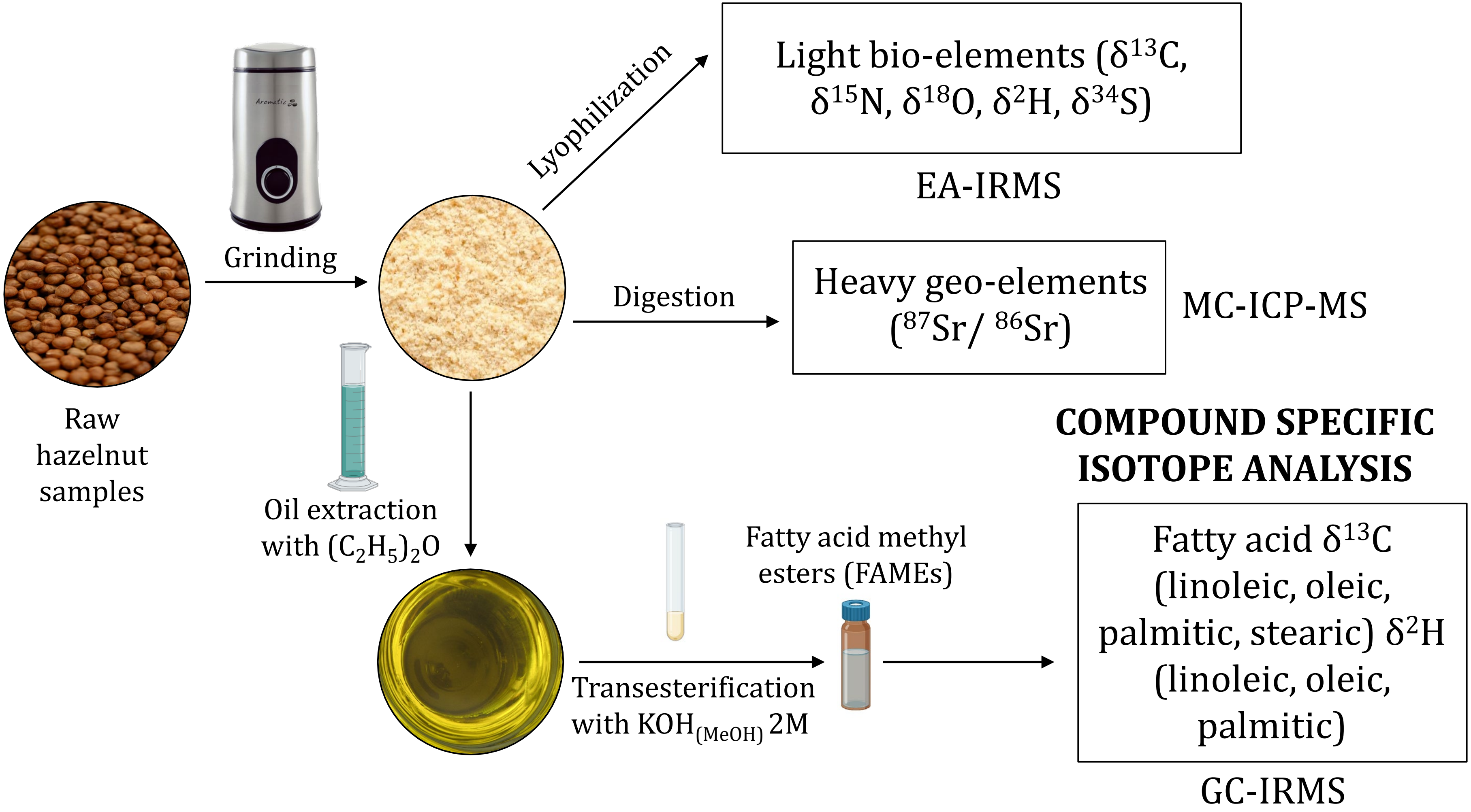
[2] K. A. Anderson, B. W. Smith (2016) J. Agric. Food Chem., 54, 1747–1752.

# SAMPLE SET

**40** Hazelnuts from 2019, 2020 and 2021

- Chile (n= 10)
- Italy (n= 10)
- Georgia (n=10)
- Spain (n= 10)

# SAMPLE PREPARATION & ANALYTICAL METHOD



Raw hazelnut samples

Grinding

Lyophilization

## ELEMENTAL STABLE ISOTOPIC RATIOS

Light bio-elements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$)

EA-IRMS

Digestion

Heavy geo-elements ($^{87}Sr/^{86}Sr$)

MC-ICP-MS

Oil extraction with $(C_2H_5)_2O$

Transesterification with $KOH_{(MeOH)}$ 2M

Fatty acid methyl esters (FAMEs)

## COMPOUND SPECIFIC ISOTOPE ANALYSIS

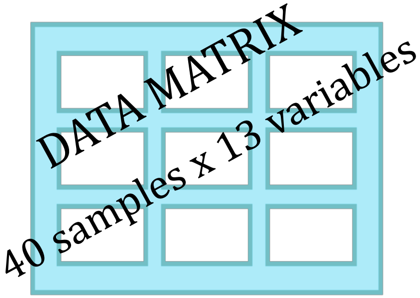
Fatty acid $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)

GC-IRMS

# CHEMOMETRICS

1) Study the relation between the variables and the samples.

## 1) PCA Exploration



DATA MATRIX

40 samples x 13 variables

**Variables:** $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)

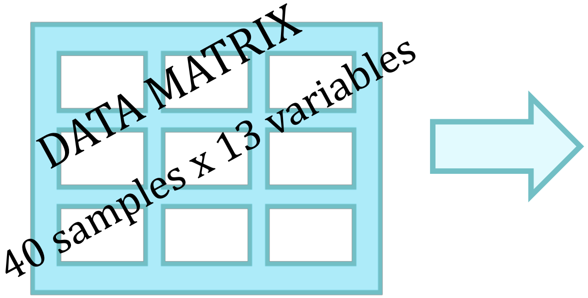
**Outlier's detection:**

(Hotelling's $T^2$ , Q residuals).

# CHEMOMETRICS

1) Study the relation between the variables and the samples.
2) Evaluate the efficacy of the variables as hazelnut geographical authentication markers.

## 1) PCA Exploration



**Variables:** $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)

**Outlier's detection:**

(Hotelling's $T^2$ , Q residuals).

## 2) PLS-DA global origin model

**ALL VARIABLES**

**Internal validation** Cross-validation (leave-10%-out)

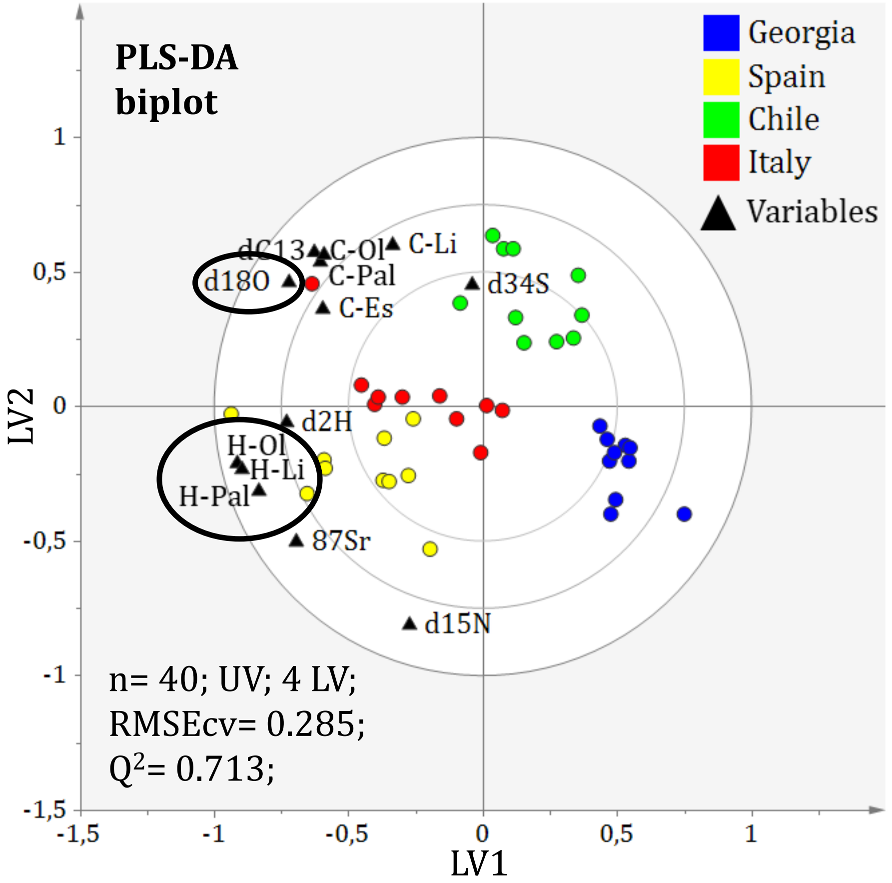
**Overfitting assess**

- Permutation test (n=20)
- RMSEcv
- p-value (ANOVA Cross-Validation)

**Relevant variables**

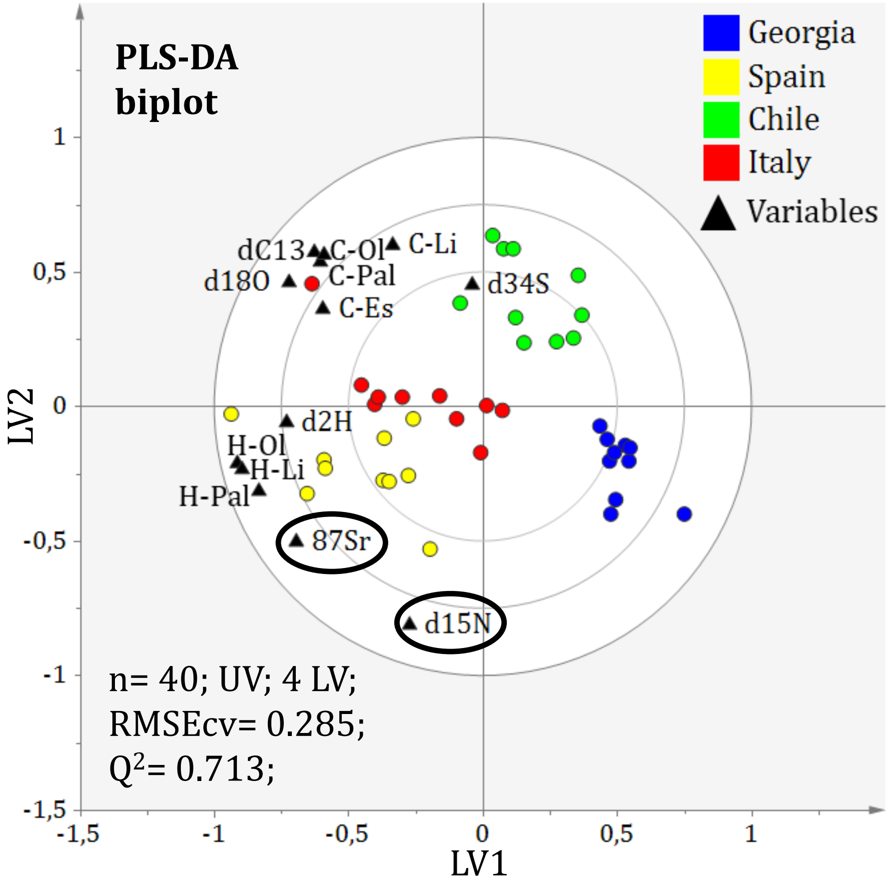
Regression coefficients study

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



Global correct classification= **97.5%**

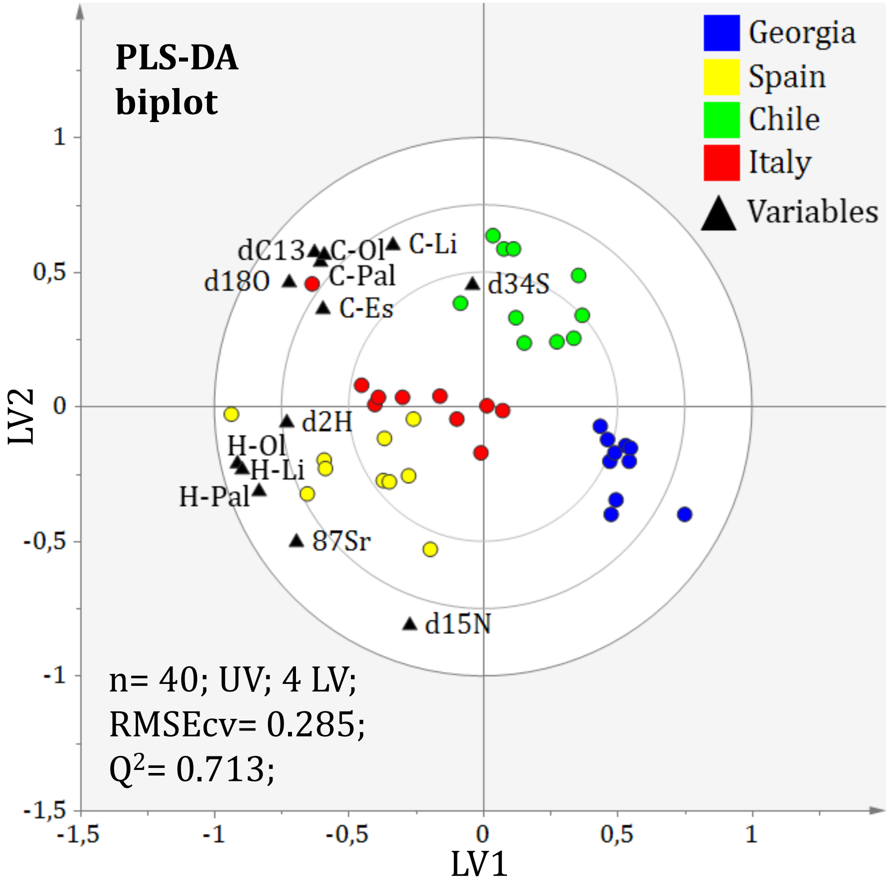
# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



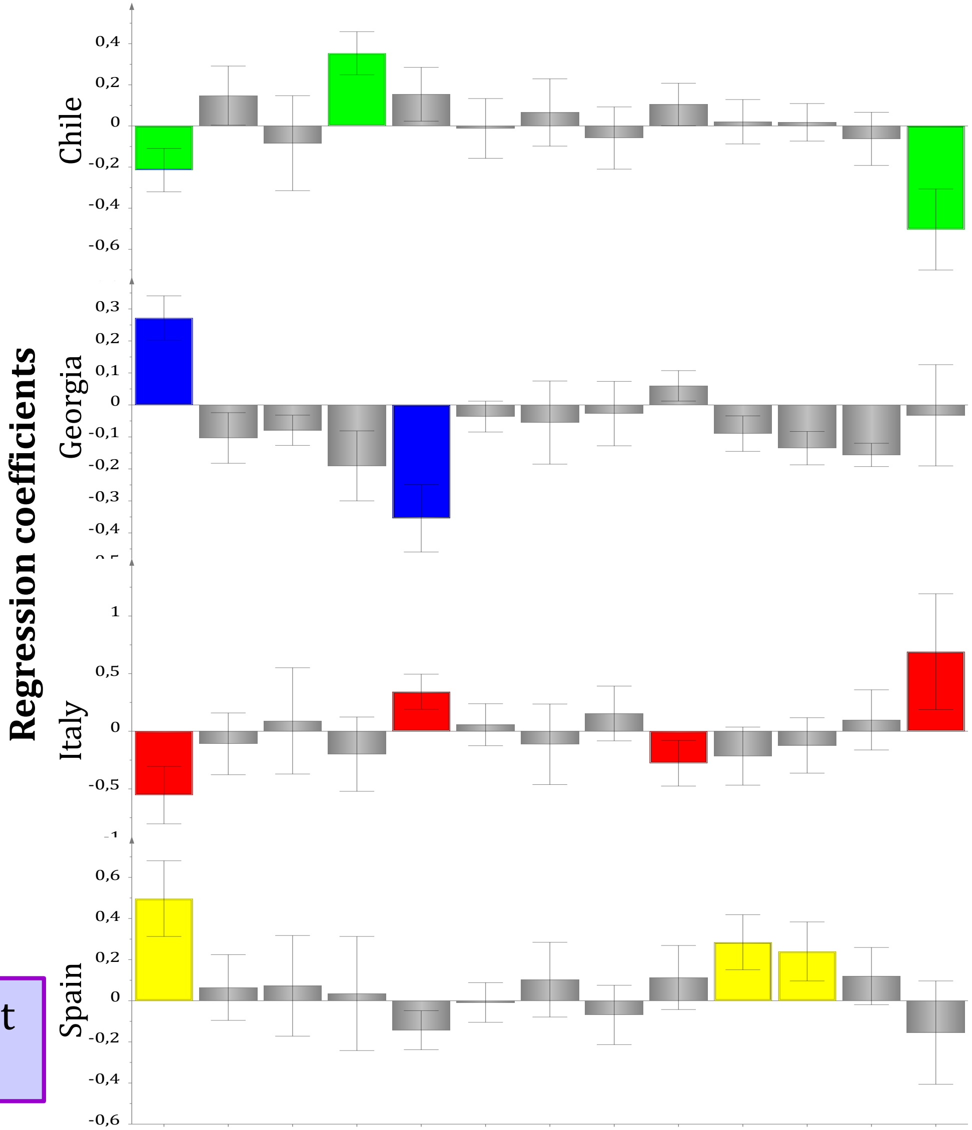
n= 40; UV; 4 LV;
RMSEcv= 0.285;
$Q^2$= 0.713;

Global correct classification= **97.5%**

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



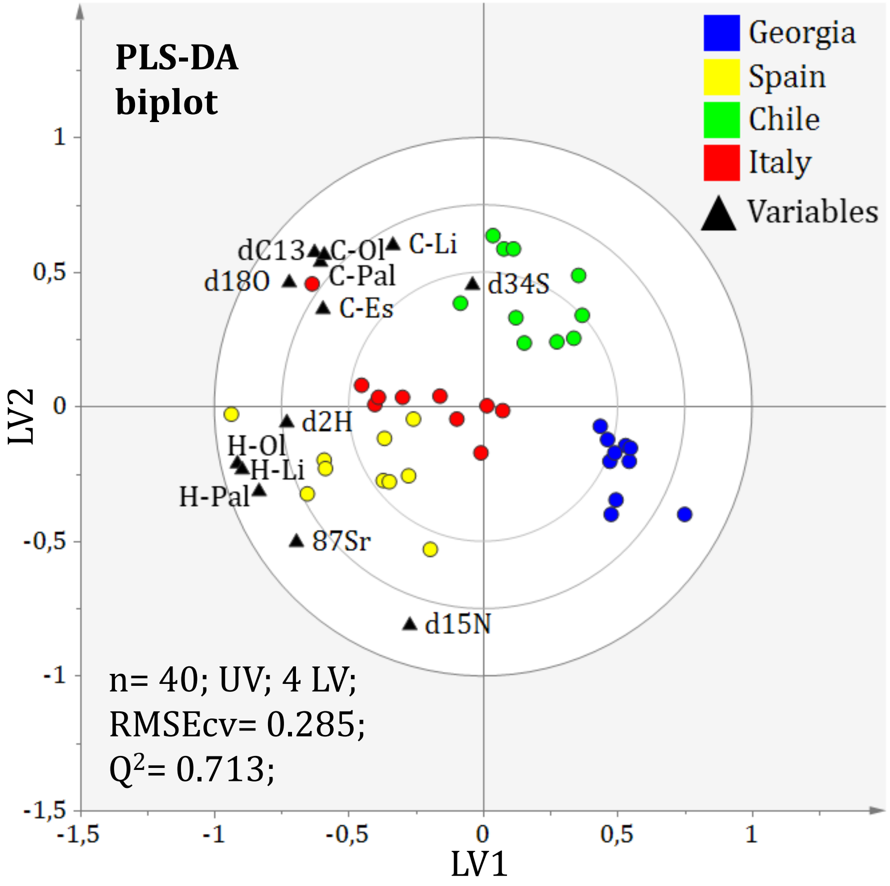
n= 40; UV; 4 LV;
RMSEcv= 0.285;
$Q^2$= 0.713;



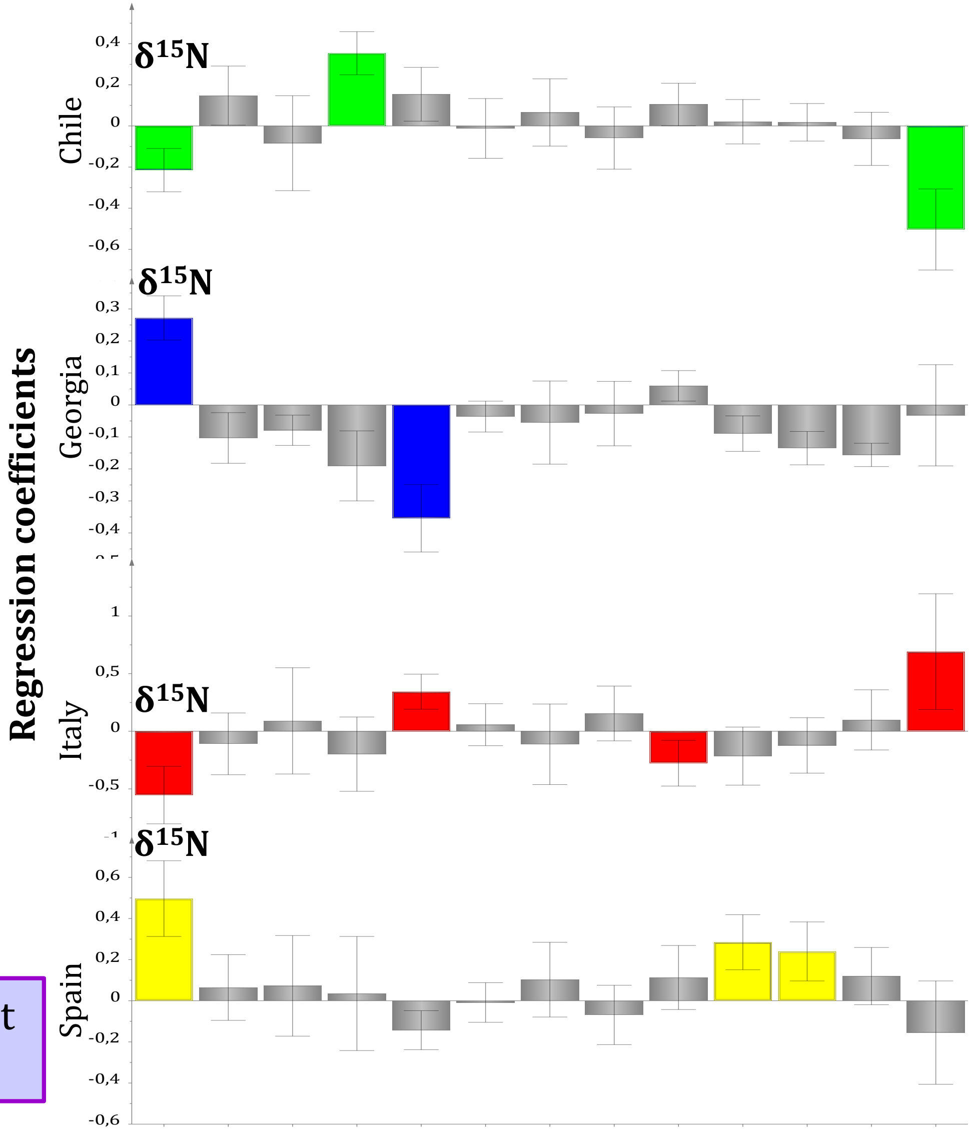
Global correct classification= **97.5%**

Relevant variables criteria: coefcvSE < Coefficient
Coefficient > 0.2

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



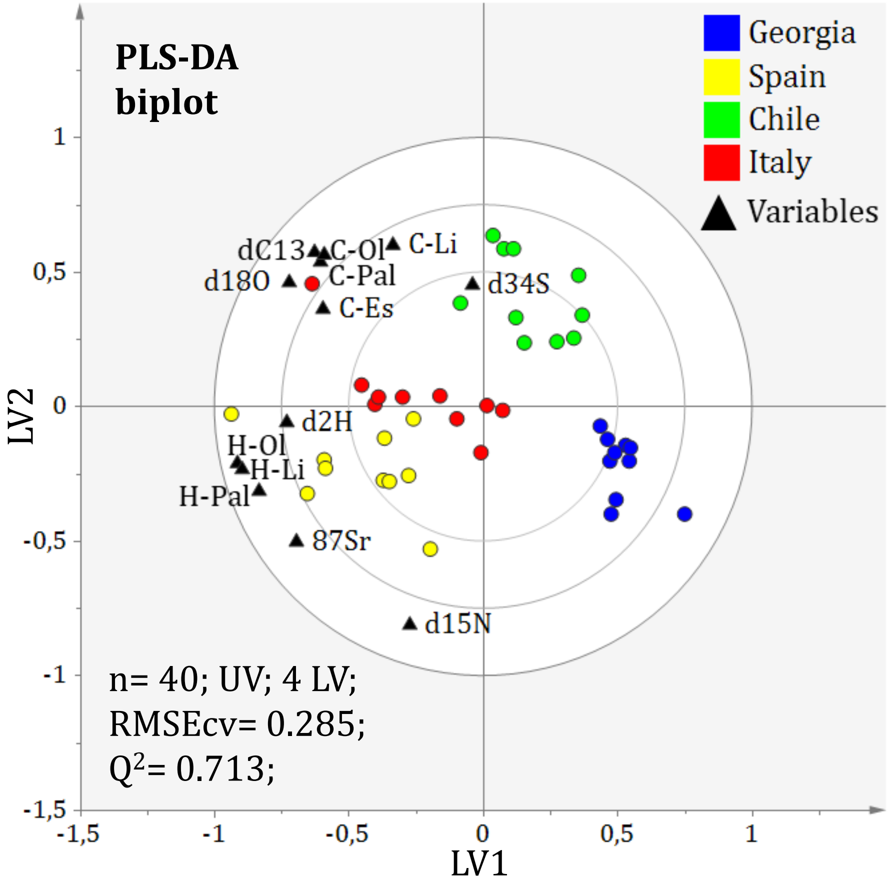
n= 40; UV; 4 LV;
RMSEcv= 0.285;
Q$^2$= 0.713;

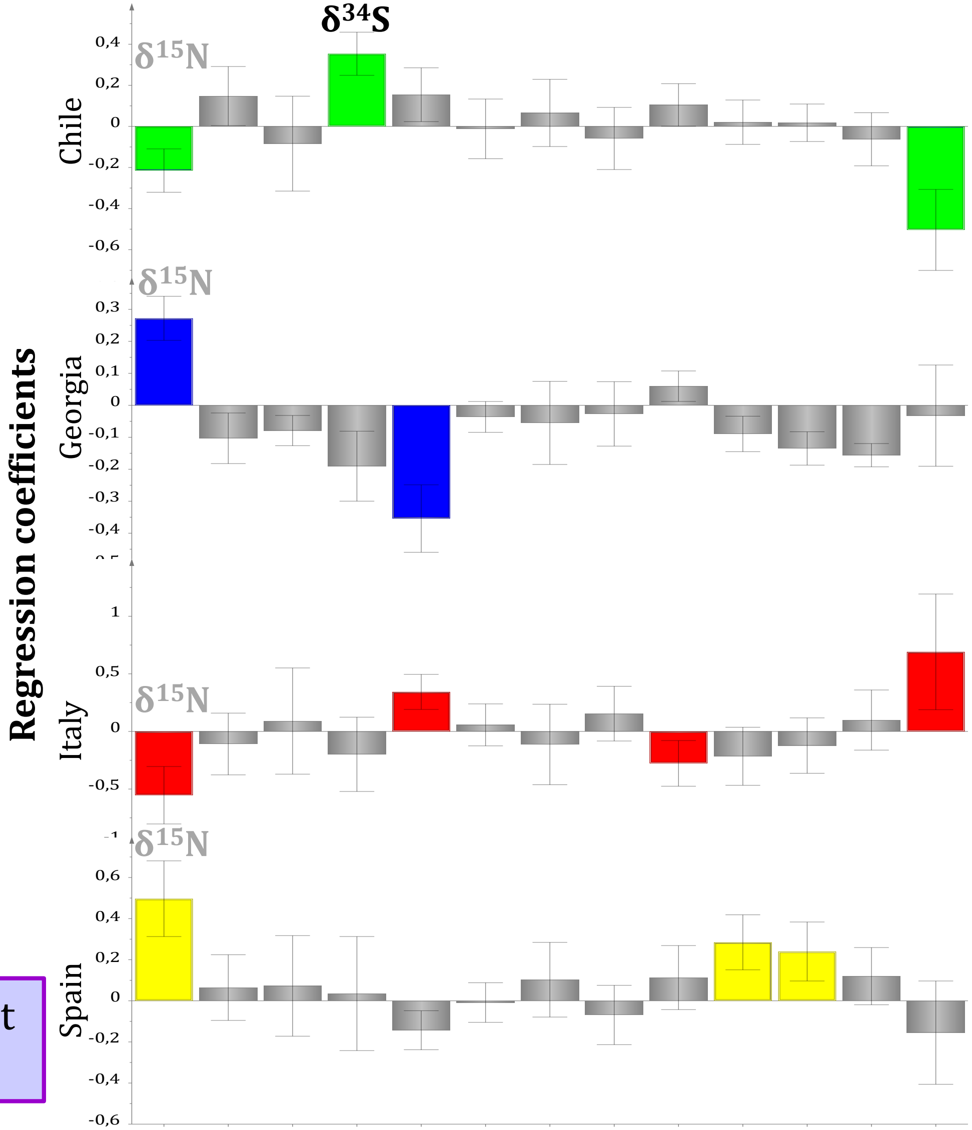


Global correct classification= **97.5%**

Relevant variables criteria: coefcvSE < Coefficient
Coefficient > 0.2

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

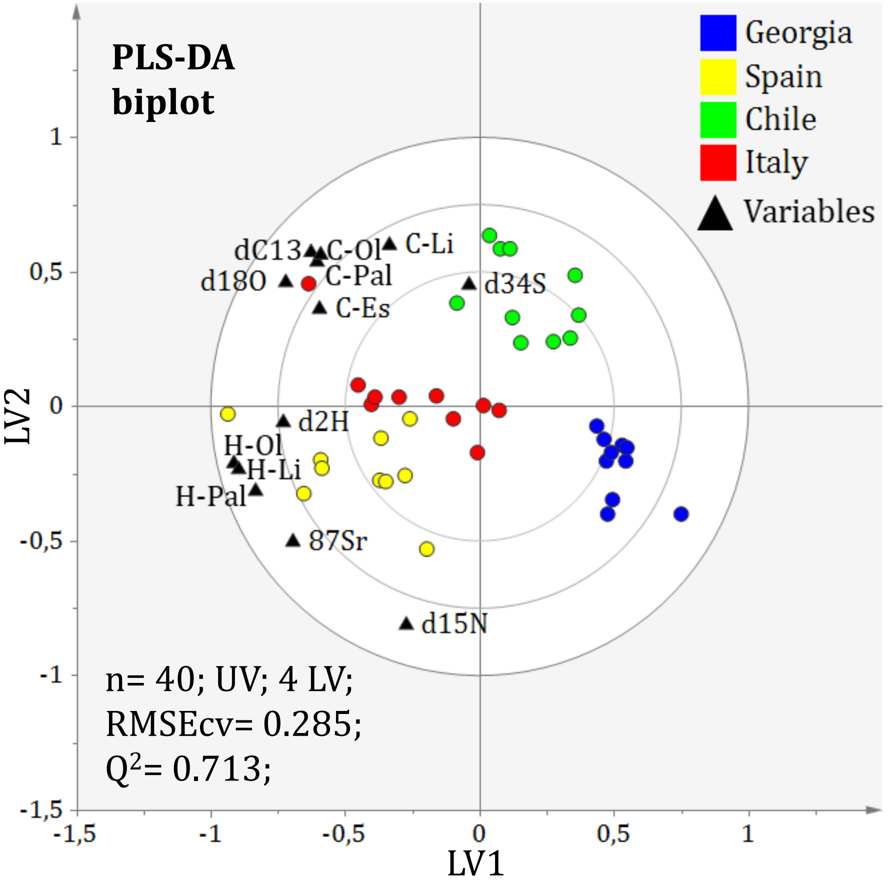


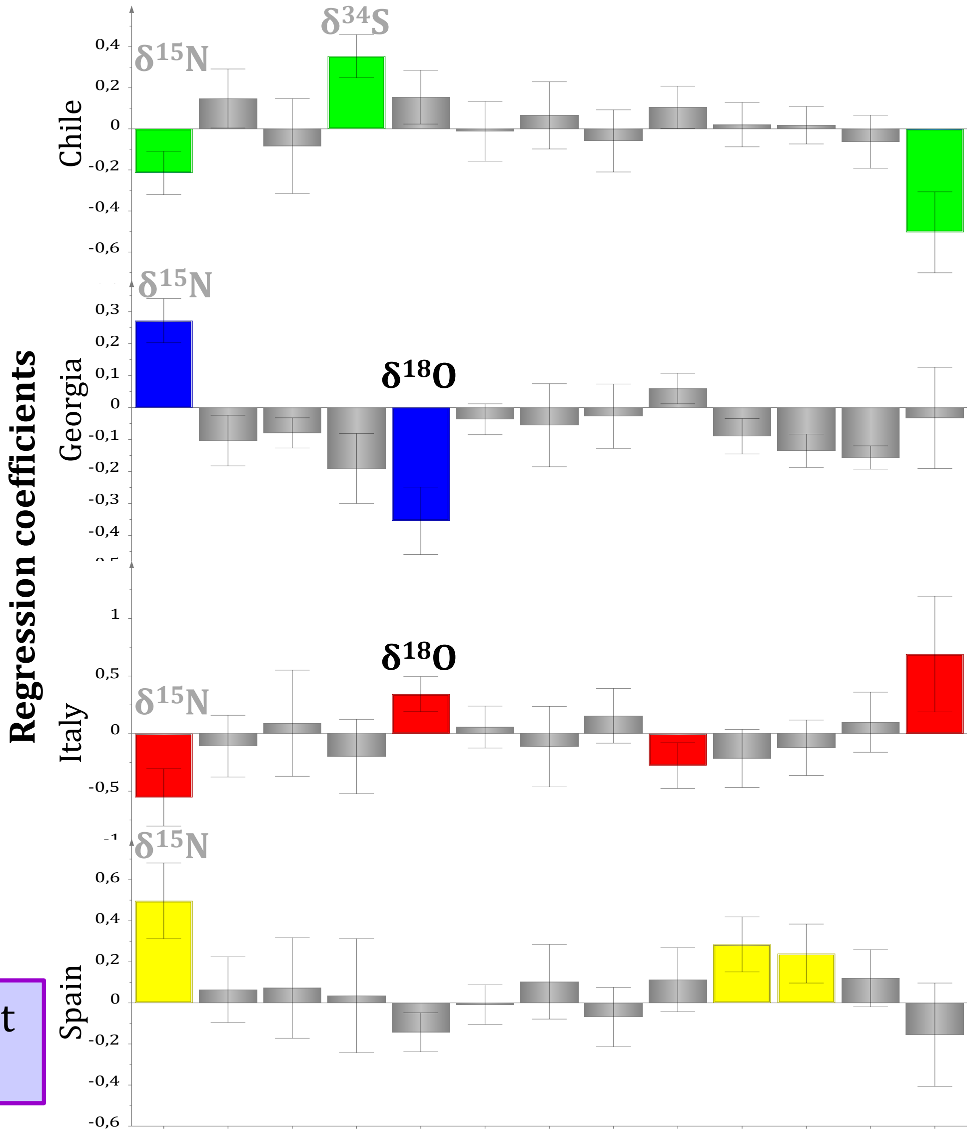


Global correct classification= **97.5%**

Relevant variables criteria: coefcvSE < Coefficient
Coefficient > 0.2

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

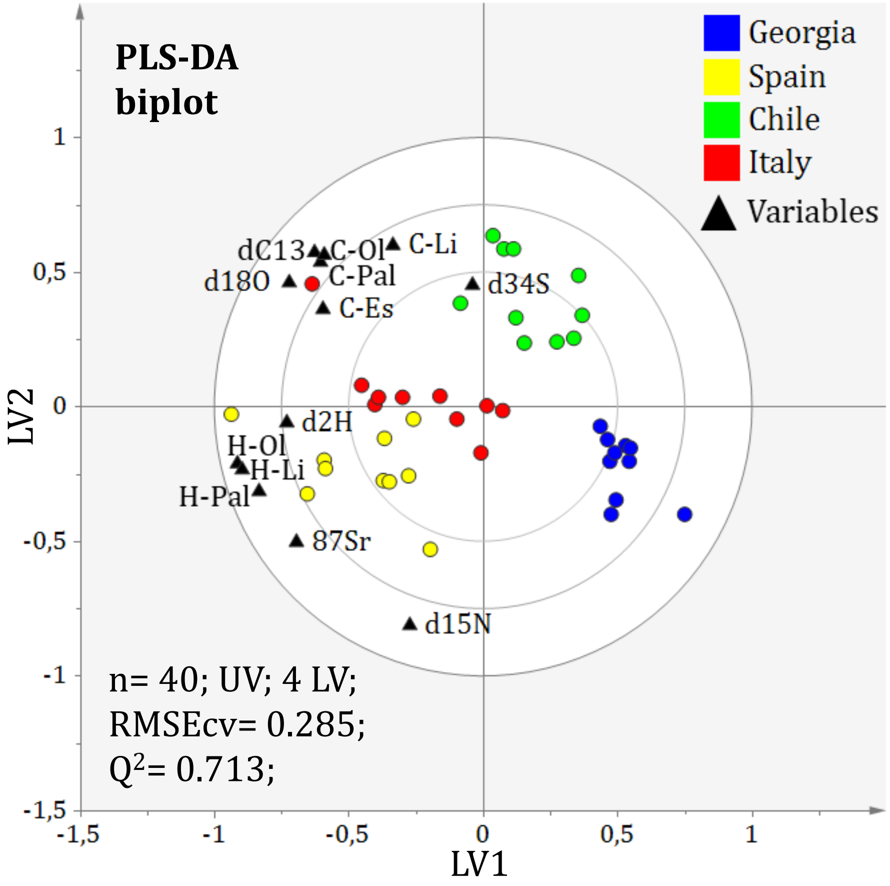




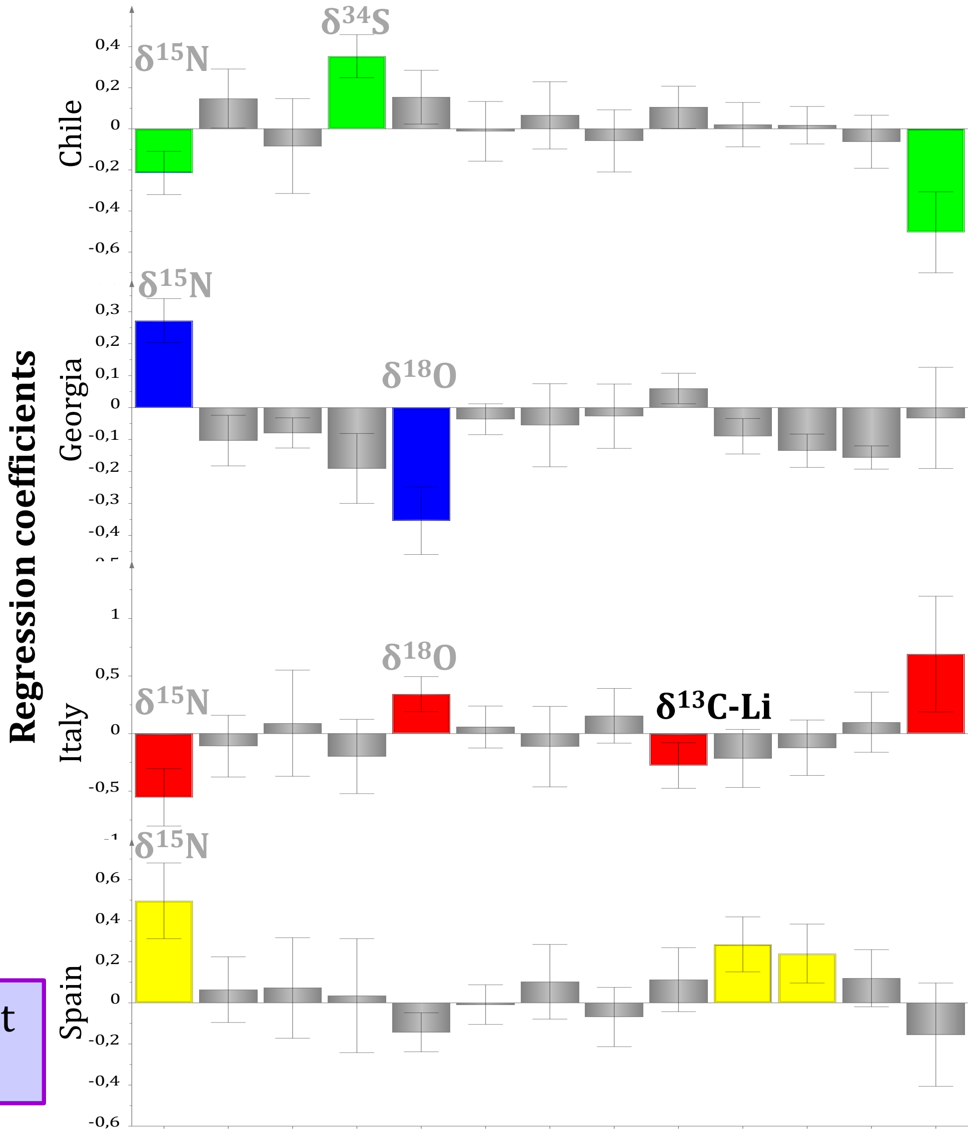
Global correct classification= **97.5%**

Relevant variables criteria: coefcvSE < Coefficient
Coefficient > 0.2

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



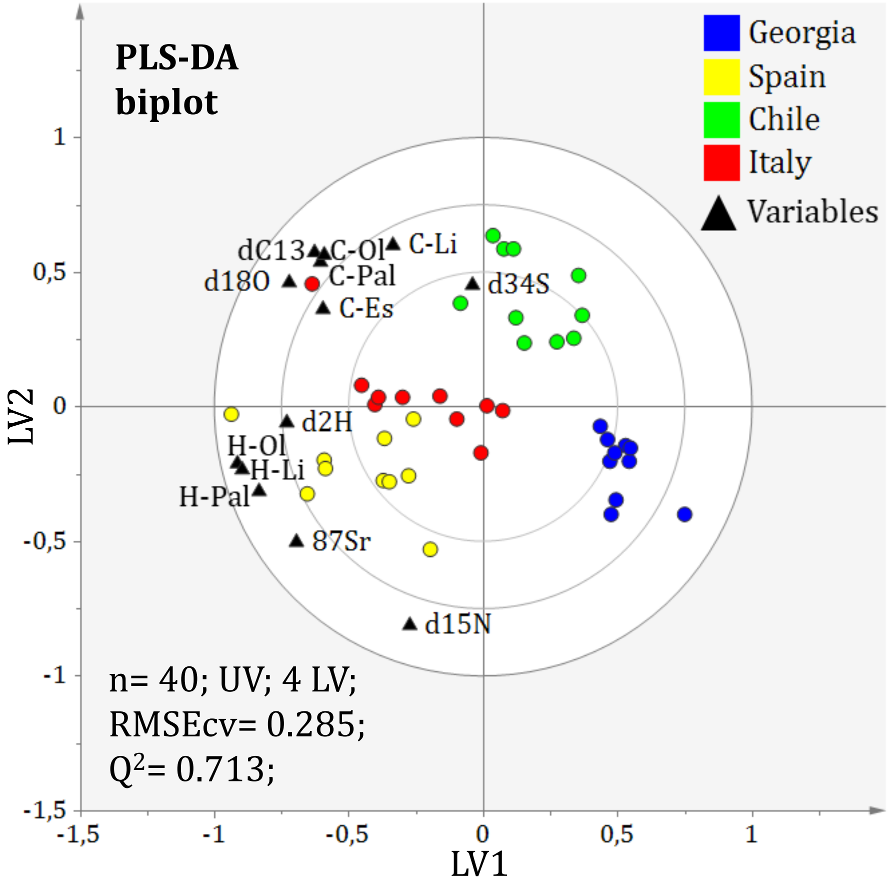
n= 40; UV; 4 LV;
RMSEcv= 0.285;
$Q^2$= 0.713;

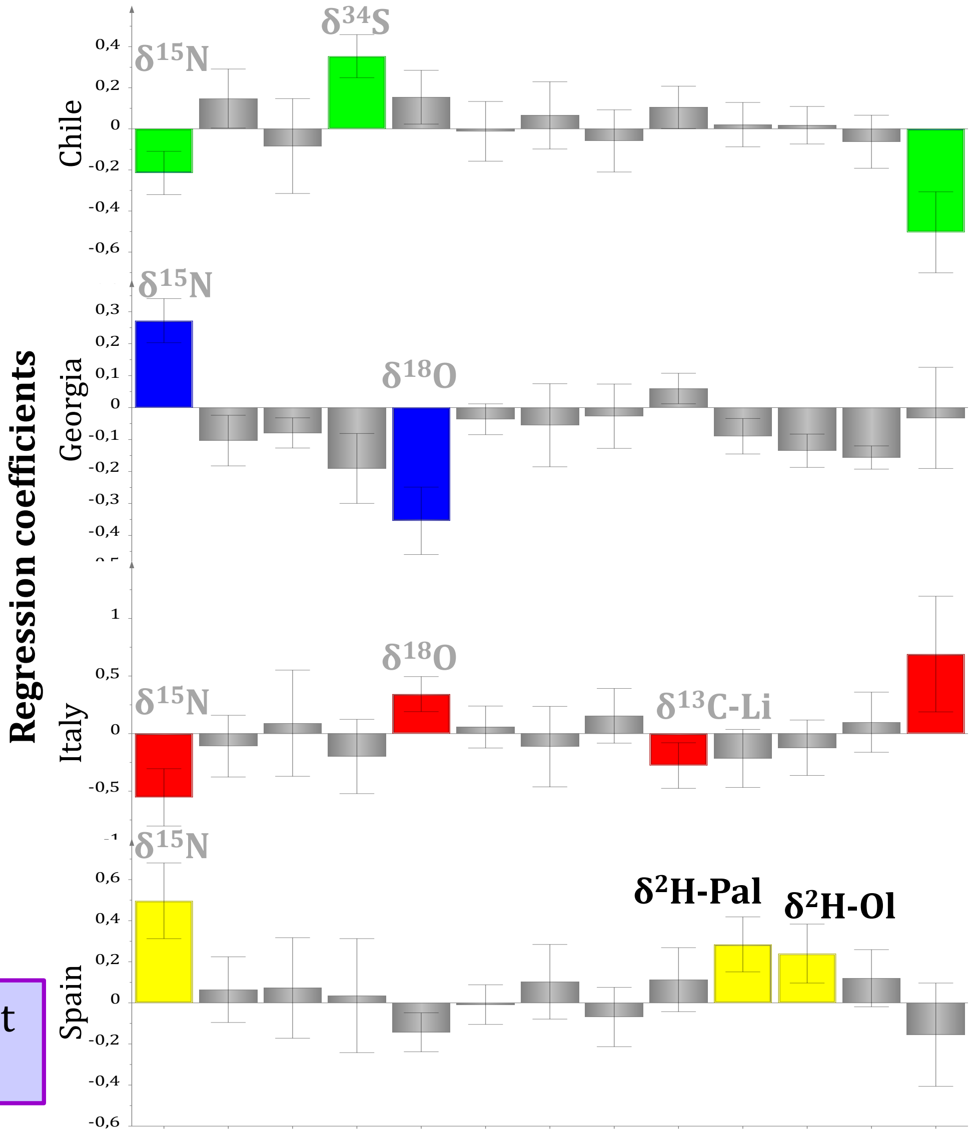


Global correct classification= **97.5%**

Relevant variables criteria: coefcvSE < Coefficient
Coefficient > 0.2

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



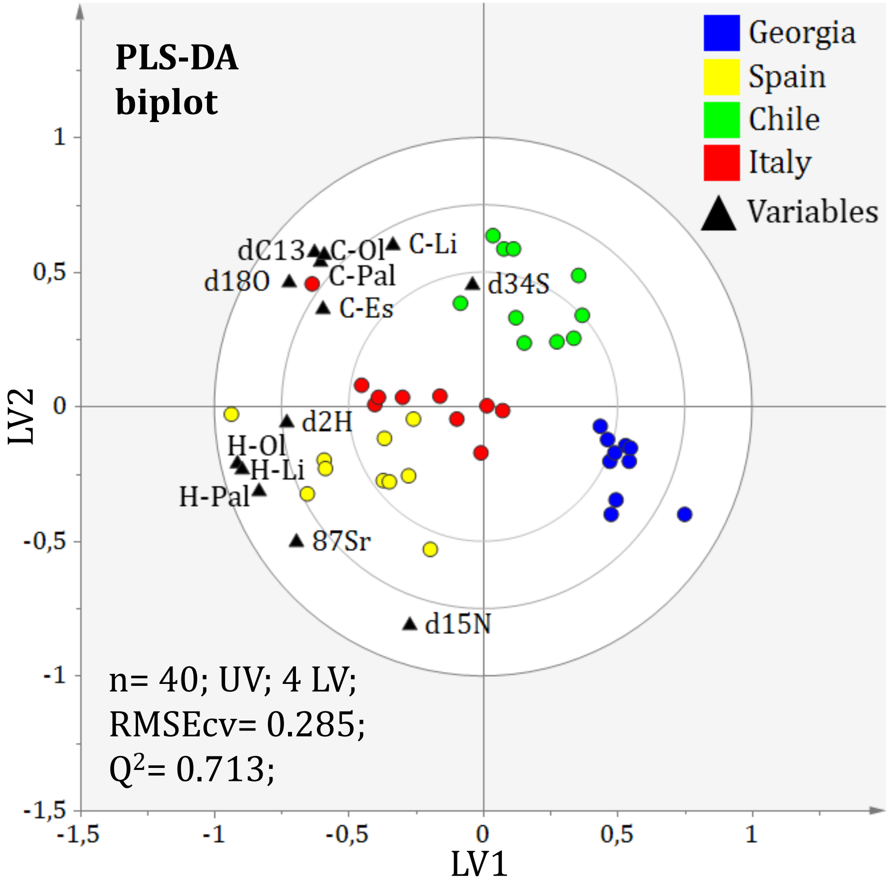


Global correct classification= **97.5%**

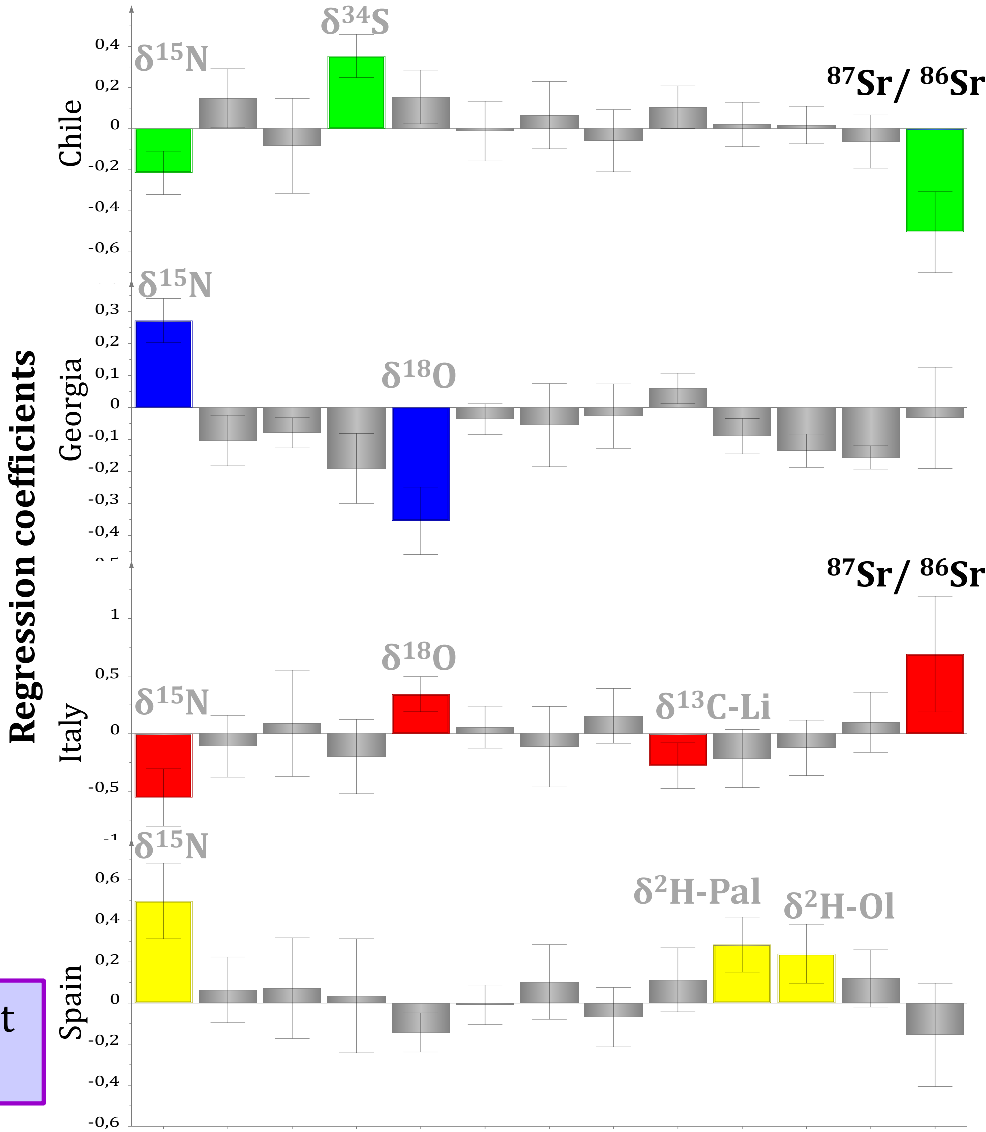
Relevant variables criteria: coefcvSE < Coefficient

Coefficient > 0.2

# GLOBAL RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**



n= 40; UV; 4 LV;
RMSEcv= 0.285;
$Q^2$= 0.713;



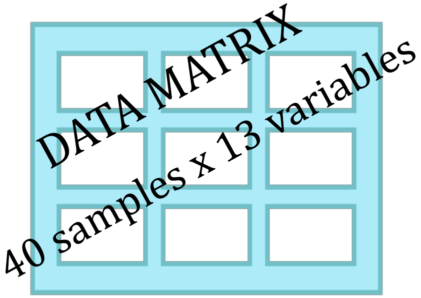
Global correct classification= **97.5%**

Relevant variables criteria: coefcvSE < Coefficient
Coefficient > 0.2

# CHEMOMETRICS

1) Study the relation between the variables and the samples.
2) Evaluate the efficacy of the variables as hazelnut geographical authentication markers.

## 1) PCA Exploration



**Variables:** $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)

**Outlier's detection:**
(Hotelling's $T^2$, Q residuals).

## 2) PLS-DA global origin model

**ALL VARIABLES**

**Internal validation** Cross-validation (leave-10%-out)

**Overfitting assess**

- Permutation test (n=20)
- RMSEcv
- p-value (ANOVA Cross-Validation)

**Relevant variables**

Regression coefficients study

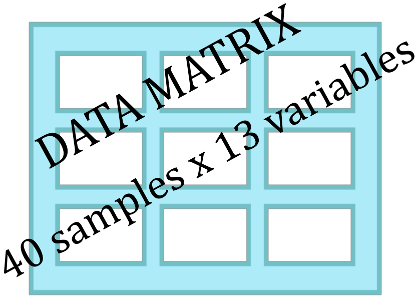
**OPTIMIZE THE METHOD**

**Minimal** number of **analysis**, **keeping** the **information** and **discriminant capacity**

# CHEMOMETRICS

1) Study the relation between the variables and the samples.
2) Evaluate the efficacy of the variables as hazelnut geographical authentication markers.
3) Select the most promising variables not influenced by other factors

## 1) PCA Exploration



**Variables:** $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)

**Outlier's detection:** (Hotelling's $T^2$, Q residuals).

## 2) PLS-DA global origin model

**ALL VARIABLES**

**Internal validation** Cross-validation (leave-10%-out)

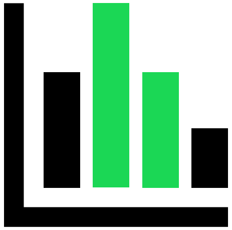
**Overfitting assess**

- Permutation test (n=20)
- RMSEcv
- p-value (ANOVA Cross-Validation)

**Relevant variables**

Regression coefficients study

## 3) Relevant variables PLS-DA origin model



**ONLY SELECTED VARIABLES**

(Significant and NOT influenced by other factors)

Analytical efficiency ↑

**Internal validation** Cross-validation (leave-10%-out)

**Overfitting assess**

**Assess relevant variables**

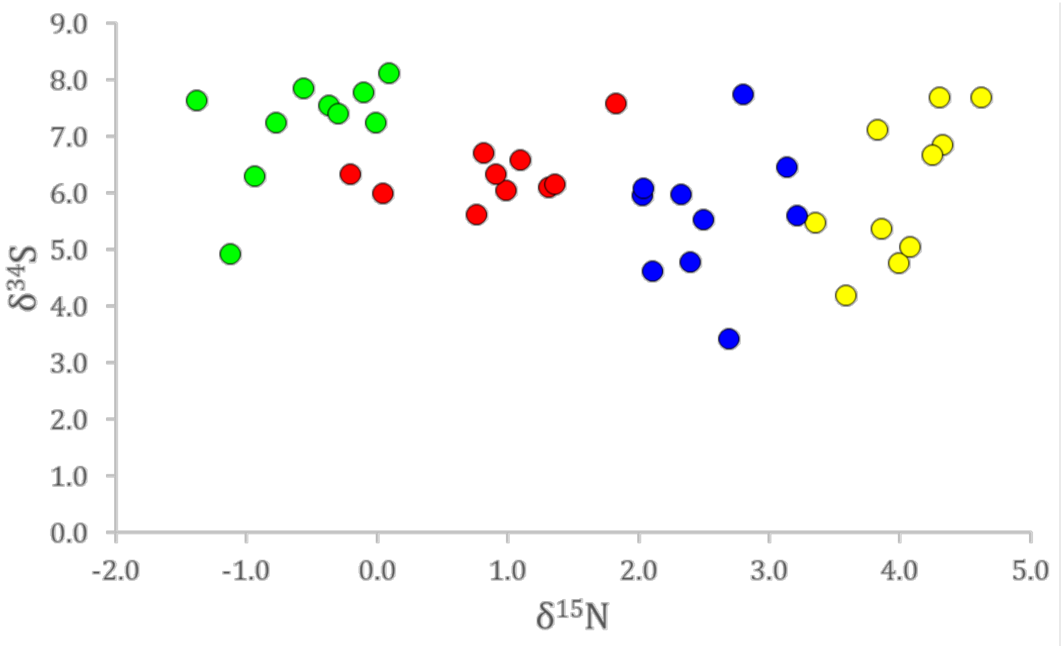
Regression coefficients study

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**

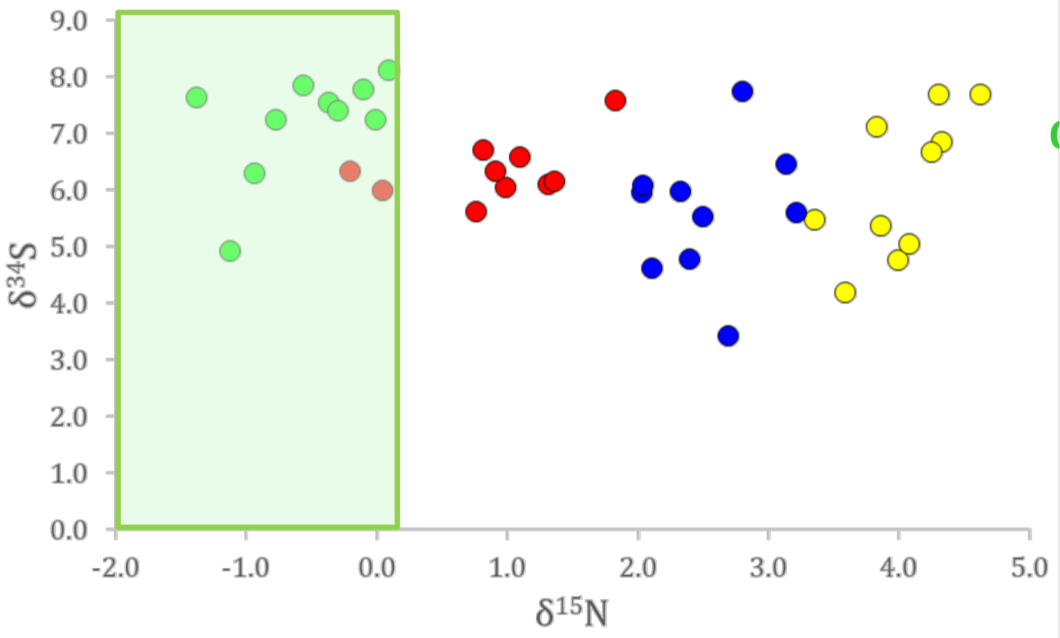


# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



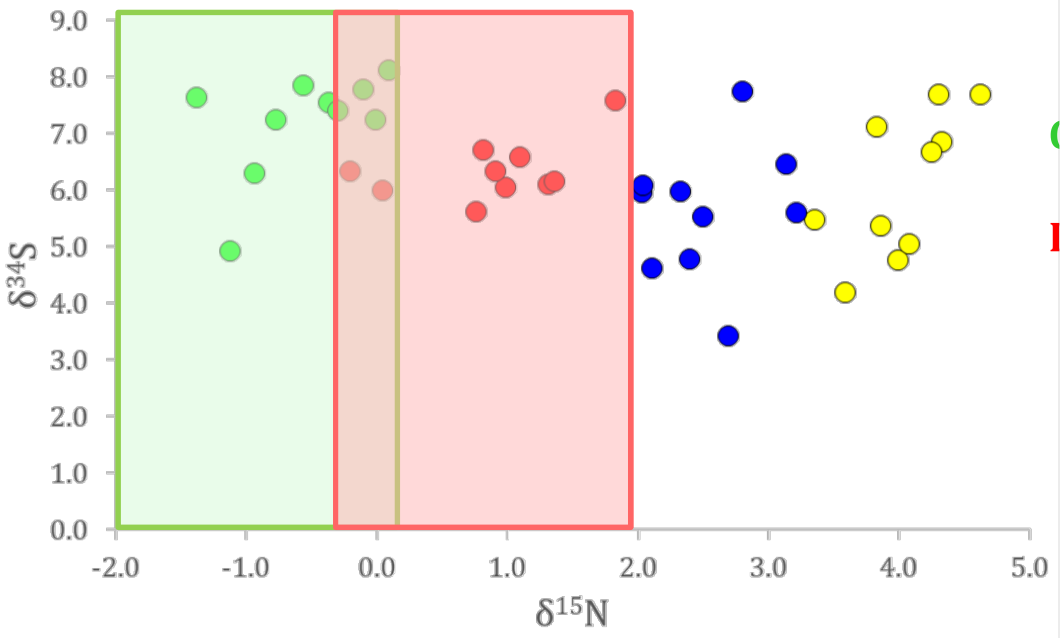
**Chile: chemical fertilizer $\delta^{15}N$ (-2 – 0)**

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



**Chile: chemical fertilizer $\delta^{15}N$ (-2 – 0)**

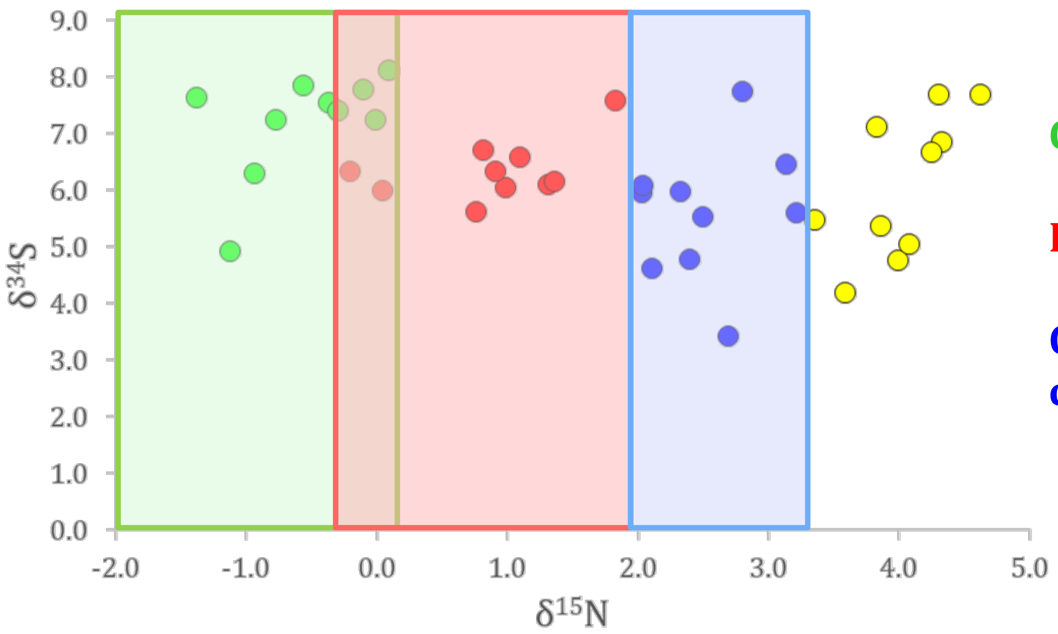
**Italy: not fertilized $\delta^{15}N$ (0 – 2)**

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



**Chile: chemical fertilizer $\delta^{15}N$ (-2 – 0)**

**Italy: not fertilized $\delta^{15}N$ (0 – 2)**

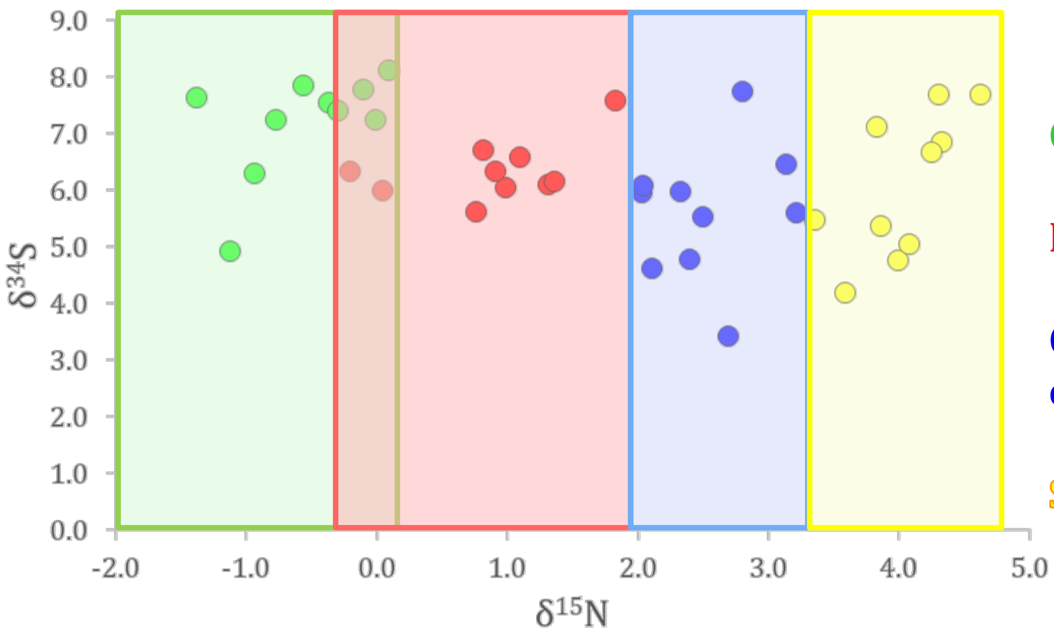
**Georgia: mix fertilizer (organic and chemical) $\delta^{15}N$ (2 – 3)**

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



**Chile: chemical fertilizer $\delta^{15}N$ (-2 – 0)**

**Italy: not fertilized $\delta^{15}N$ (0 – 2)**

**Georgia: mix fertilizer (organic and chemical) $\delta^{15}N$ (2 – 3)**

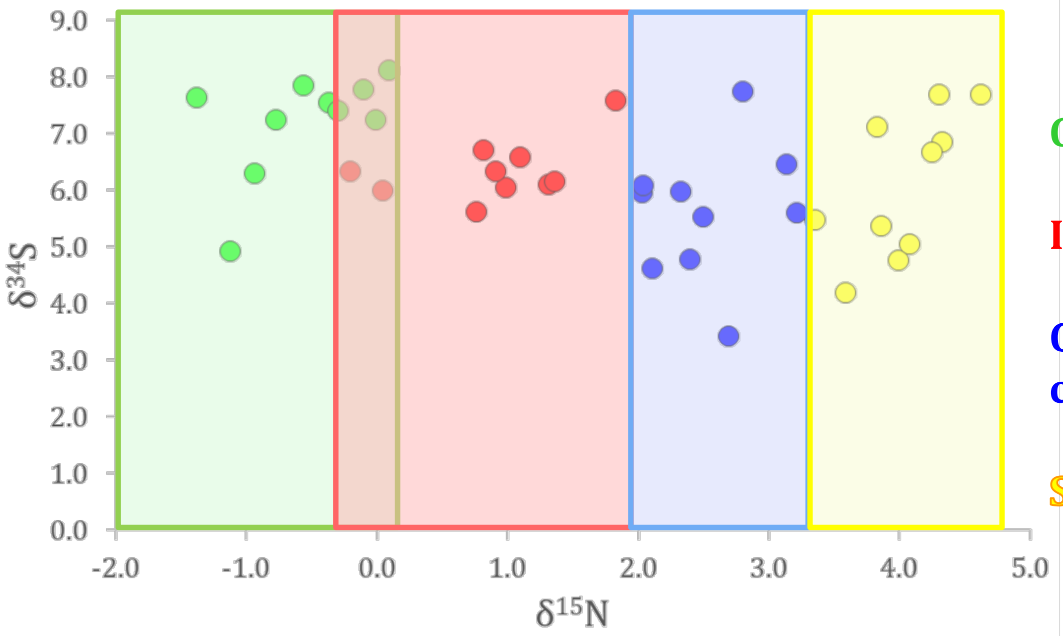
**Spain: organic fertilizer $\delta^{15}N$ (2 – 5)**

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



**Chile: chemical fertilizer $\delta^{15}N$ (-2 – 0)**

**Italy: not fertilized $\delta^{15}N$ (0 – 2)**

**Georgia: mix fertilizer (organic and chemical) $\delta^{15}N$ (2 – 3)**

**Spain: organic fertilizer $\delta^{15}N$ (2 – 5)**

1. **$\delta^{15}N$ values agree with** those reported for **each kind of fertilizer** and its corresponding **values in plant** (3,4). **$\delta^{15}N$ discriminates samples by origin but it is AFFECTED by the fertilizers.**
2. **$\delta^{34}S$ values** does **not** seem to be **influenced by the fertilizer** but do **NOT** provide any **DISCRIMINATION among samples.**

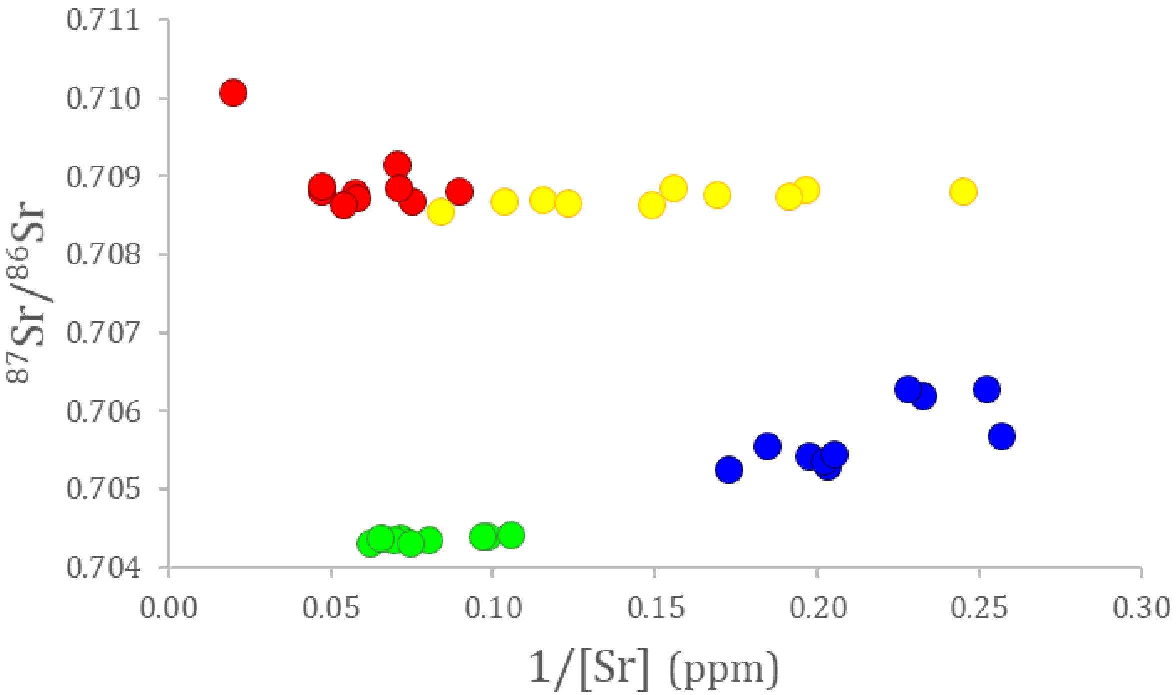
[3]L. Vitòria, et al. (2004) J Environ. Sci. Technol., 38, 3254-3262. [4]K . H. Laursen (2013) J. Food Chemistry, 141, 2812–2820.

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**

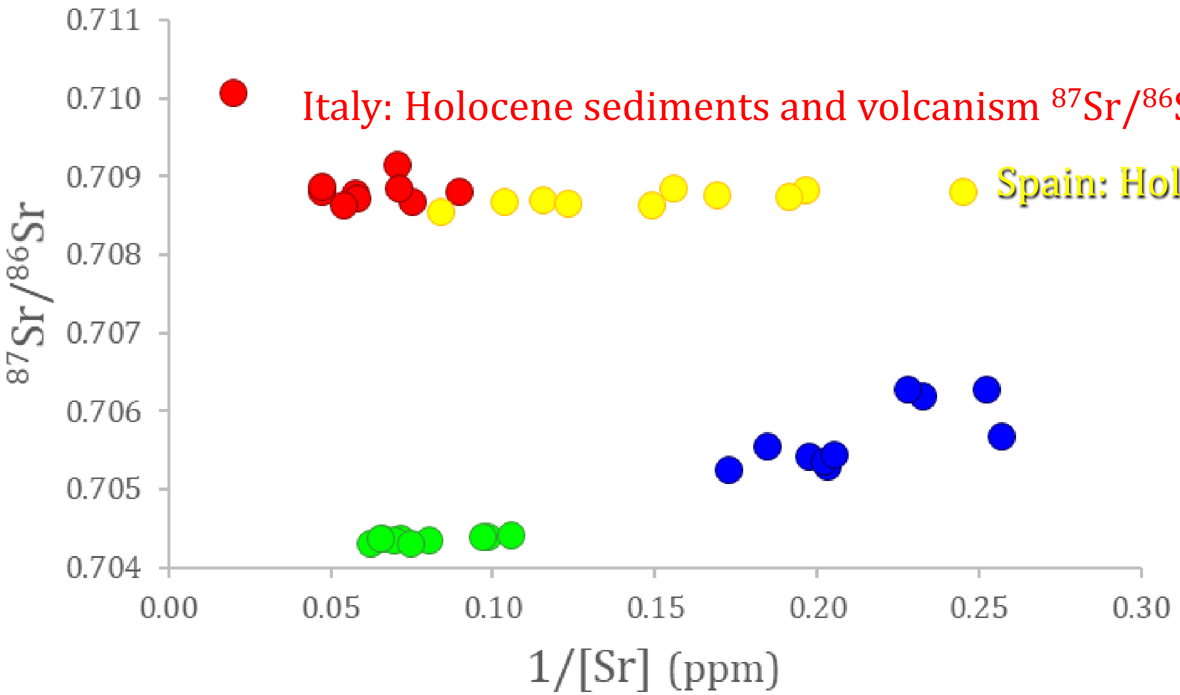


# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



Italy: Holocene sediments and volcanism $^{87}Sr/^{86}Sr \simeq 0.709$

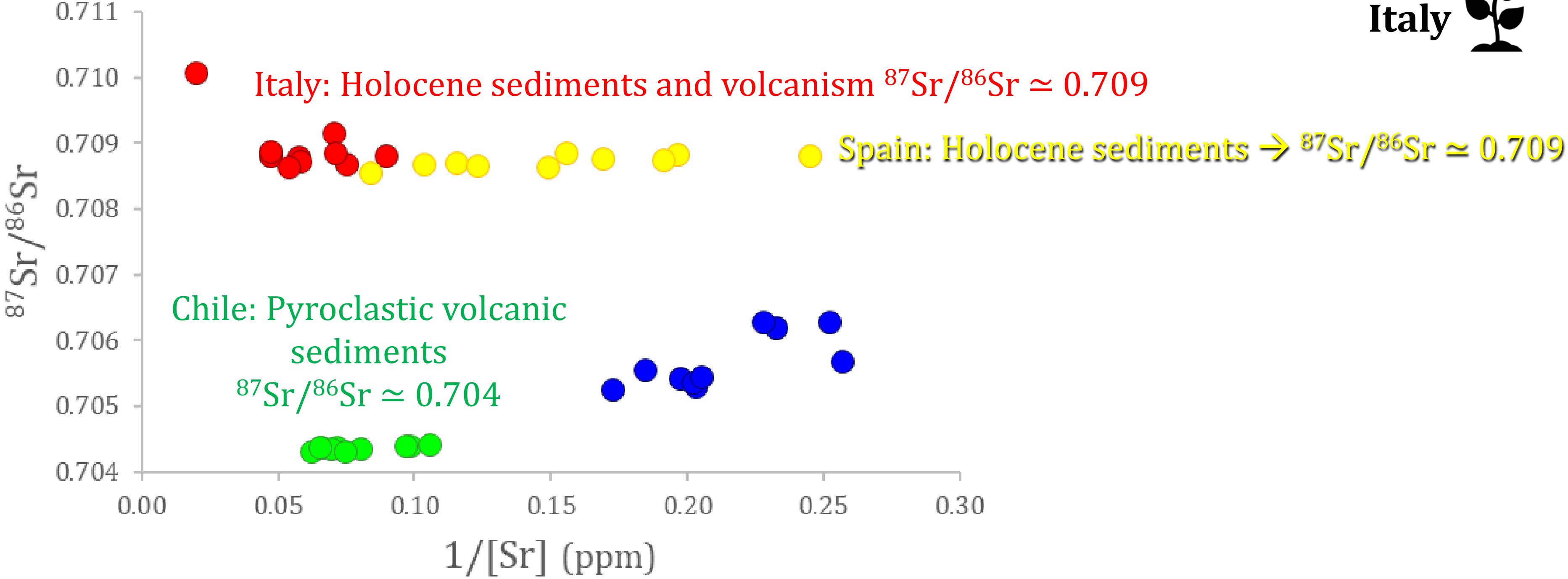
Spain: Holocene sediments → $^{87}Sr/^{86}Sr \simeq 0.709$

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**

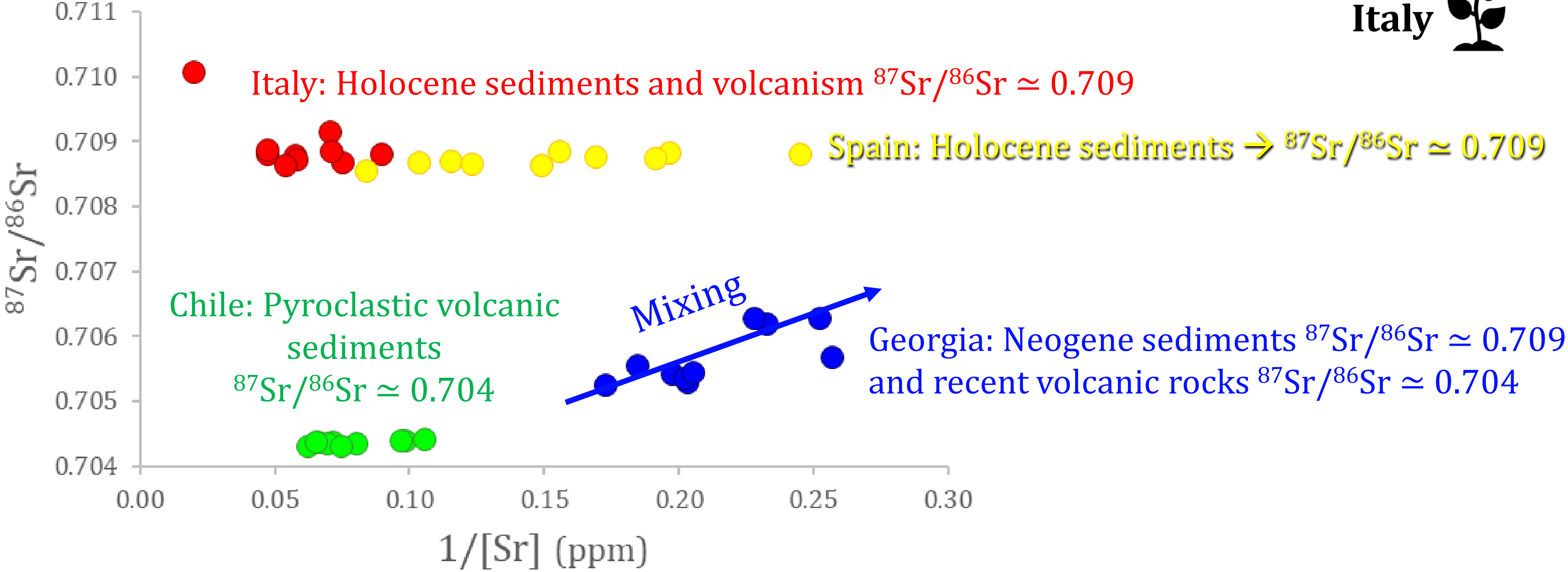


# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



Italy: Holocene sediments and volcanism $^{87}Sr/^{86}Sr \simeq 0.709$

Spain: Holocene sediments → $^{87}Sr/^{86}Sr \simeq 0.709$

Chile: Pyroclastic volcanic sediments $^{87}Sr/^{86}Sr \simeq 0.704$

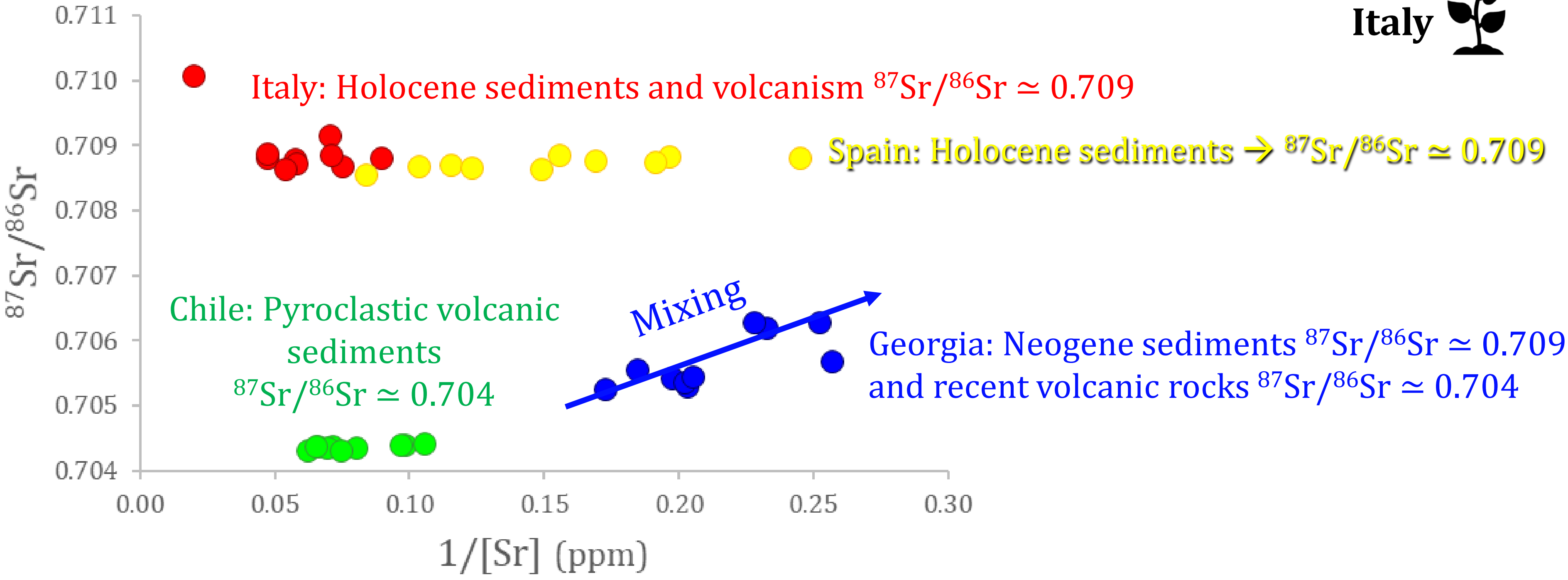
Georgia: Neogene sediments $^{87}Sr/^{86}Sr \simeq 0.709$ and recent volcanic rocks $^{87}Sr/^{86}Sr \simeq 0.704$

# RELEVANT VARIABLES RESULTS

ISSUE: FERTILIZERS can modify **N**, **S** and **Sr** isotope ratios

**Georgia, Chile, Spain**

**Italy**



Italy: Holocene sediments and volcanism $^{87}Sr/^{86}Sr \simeq 0.709$

Spain: Holocene sediments → $^{87}Sr/^{86}Sr \simeq 0.709$

Chile: Pyroclastic volcanic sediments $^{87}Sr/^{86}Sr \simeq 0.704$

Georgia: Neogene sediments $^{87}Sr/^{86}Sr \simeq 0.709$ and recent volcanic rocks $^{87}Sr/^{86}Sr \simeq 0.704$

1. **$^{87}Sr/^{86}Sr$ values** does **not** seem to be **influenced by the fertilizers and agree with the Geogenic factors**.
2. **$^{87}Sr/^{86}Sr$ discriminates Chile** and **Georgia** samples **from Spain and Italy** samples.

# RELEVANT VARIABLES RESULTS

New PLS-DA model including ONLY relevant variables and variables NOT influenced by other factors such as fertilization and affordable for routine analysis.

✗ **$\delta^{15}N$ excluded because it but it is AFFECTED by the fertilizers.**

✗ **$\delta^{34}S$ excluded because it does not discriminate among samples.**

✗ **$\delta^{13}C$ excluded because it does not discriminate among samples.**

✗ **$\delta^{2}H$ excluded because it does not discriminate among samples.**

**$^{87}Sr/^{86}Sr$** → 🪙 → **Can't apply routinely or for screening**

✓ SELECTED VARIABLES: **$\delta^{18}O$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic acid) $\delta^{2}H$ (linoleic, oleic, palmitic acid)**

# VARIABLE SELECTION RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

Selected variables: **$\delta^{18}O$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic acid) $\delta^{2}H$ (linoleic, oleic, palmitic acid)**

**PLS-DA**

n= 40; 4 LV; RMSEcv= 0.376; $Q^2$= 0,448

| | n | Chile | Georgia | Italy | Spain | No Class | % Correct |
|---|---|---|---|---|---|---|---|
| Chile | 10 | 7 | 0 | 0 | 0 | 3 | 70 |
| Georgia | 10 | 0 | 10 | 0 | 0 | 0 | 100 |
| Italy | 10 | 0 | 0 | 9 | 0 | 1 | 90 |
| Spain | 10 | 0 | 0 | 1 | 8 | 1 | 80 |
| Total | 40 | 7 | 10 | 10 | 8 | 5 | **85** |

Relevant variables (positive and negative):

Chile: **$\delta^{18}O$**, **$\delta^{13}C$ oleic**, **$\delta^{2}H$ linoleic**

Georgia: **$\delta^{18}O$**

Italy: **$\delta^{18}O$**, **$\delta^{13}C$ oleic**, **$\delta^{13}C$ linoleic**

Spain:**$\delta^{2}H$ oleic** and **palmitic**

# VARIABLE SELECTION RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

Selected variables: **$\delta^{18}O$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic acid) $\delta^{2}H$ (linoleic, oleic, palmitic acid)**

**PLS-DA** n= 40; 4 LV; RMSEcv= 0.376; $Q^2$= 0,448

| | n | Chile | Georgia | Italy | Spain | No Class | % Correct |
|---|---|---|---|---|---|---|---|
| Chile | 10 | 7 | 0 | 0 | 0 | 3 | 70 |
| Georgia | 10 | 0 | 10 | 0 | 0 | 0 | 100 |
| Italy | 10 | 0 | 0 | 9 | 0 | 1 | 90 |
| Spain | 10 | 0 | 0 | 1 | 8 | 1 | 80 |
| Total | 40 | 7 | 10 | 10 | 8 | 5 | **85** |

1 misclassified sample
5 not classified
34 correctly classified

**97.1% correctly classified from the classified samples**

Relevant variables (positive and negative):
Chile: **$\delta^{18}O$**, **$\delta^{13}C$ oleic**, **$\delta^{2}H$ linoleic**
Georgia: **$\delta^{18}O$**
Italy: **$\delta^{18}O$**, **$\delta^{13}C$ oleic**, **$\delta^{13}C$ linoleic**
Spain:**$\delta^{2}H$ oleic** and **palmitic**

# VARIABLE SELECTION RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

Selected variables: **$\delta^{18}$O, $\delta^{13}$C (linoleic, oleic, palmitic, stearic acid) $\delta^{2}$H (linoleic, oleic, palmitic acid)**

**PLS-DA**

n= 40; 4 LV; RMSEcv= 0.376; $Q^2$= 0,448

| | n | Chile | Georgia | Italy | Spain | No Class | % Correct |
|---|---|---|---|---|---|---|---|
| Chile | 10 | 7 | 0 | 0 | 0 | 3 | 70 |
| Georgia | 10 | 0 | 10 | 0 | 0 | 0 | 100 |
| Italy | 10 | 0 | 0 | 9 | 0 | 1 | 90 |
| Spain | 10 | 0 | 0 | 1 | 8 | 1 | 80 |
| Total | 40 | 7 | 10 | 10 | 8 | 5 | **85** |

1 misclassified sample
5 not classified
34 correctly classified

**97.1% correctly classified from the classified samples**

Relevant variables (positive and negative):
Chile: **$\delta^{18}$O**, **$\delta^{13}$C oleic**, **$\delta^{2}$H linoleic**
Georgia: **$\delta^{18}$O**
Italy: **$\delta^{18}$O**, **$\delta^{13}$C oleic**, **$\delta^{13}$C linoleic**
Spain: **$\delta^{2}$H oleic** and **palmitic**

13% samples not classified

**PLS-DA with $^{87}$Sr/ $^{86}$Sr**

# RELEVANT VARIABLES RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

Selected variables: **$\delta^{18}O$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic acid) $\delta^{2}H$ (linoleic, oleic, palmitic acid), $^{87}Sr/^{86}Sr$**

**PLS-DA**

n= 40; 4 LV; RMSEcv= 0.336; $Q^2$= 0,617

| | n | Chile | Georgia | Italy | Spain | No Class | % Correct |
|---|---|---|---|---|---|---|---|
| Chile | 10 | 10 | 0 | 0 | 0 | 0 | 100 |
| Georgia | 10 | 0 | 10 | 0 | 0 | 0 | 100 |
| Italy | 10 | 1 | 0 | 8 | 1 | 0 | 80 |
| Spain | 10 | 0 | 0 | 0 | 9 | 1 | 90 |
| Total | 40 | 11 | 10 | 8 | 10 | 1 | **92.5** |

Relevant variables (positive and negative):

Chile: **$\delta^{18}O$, $^{87}Sr/^{86}Sr$**

Georgia: **$\delta^{18}O$**

Italy: **$\delta^{18}O$, $^{87}Sr/^{86}Sr$, $\delta^{13}C$ linoleic, $\delta^{2}H$ oleic** and **palmitic**

Spain: **$\delta^{18}O$, $\delta^{2}H$ oleic** and **palmitic**

# RELEVANT VARIABLES RESULTS: **PLS-DA GEOGRAPHICAL ORIGIN MODEL**

Selected variables: **$\delta^{18}O$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic acid) $\delta^{2}H$ (linoleic, oleic, palmitic acid), $^{87}Sr/^{86}Sr$**

**PLS-DA**

n= 40; 4 LV; RMSEcv= 0.336; $Q^2$= 0,617

| | n | Chile | Georgia | Italy | Spain | No Class | % Correct |
|---|---|---|---|---|---|---|---|
| Chile | 10 | 10 | 0 | 0 | 0 | 0 | 100 |
| Georgia | 10 | 0 | 10 | 0 | 0 | 0 | 100 |
| Italy | 10 | 1 | 0 | 8 | 1 | 0 | 80 |
| Spain | 10 | 0 | 0 | 0 | 9 | 1 | 90 |
| Total | 40 | 11 | 10 | 8 | 10 | 1 | **92.5** |

2 misclassified samples
1 not classified
37 correctly classified

**94.9% correctly classified from the classified samples**

Relevant variables (positive and negative):
Chile: **$\delta^{18}O$**, **$^{87}Sr/^{86}Sr$**
Georgia: **$\delta^{18}O$**
Italy: **$\delta^{18}O$**, **$^{87}Sr/^{86}Sr$**, **$\delta^{13}C$ linoleic**, **$\delta^{2}H$ oleic** and **palmitic**
Spain: **$\delta^{18}O$**, **$\delta^{2}H$ oleic** and **palmitic**

**$^{87}Sr/^{86}Sr$ apply to few dubious samples, or as quality control checks**

In this PRELIMINARY study, multiple isotopic analysis have been evaluated as hazelnut geographical origin tools:

**$\delta^{15}N$** can discriminate samples according to their origin but is **influenced by fertilization**.

**$\delta^{34}S$** does not seem to be influenced by the fertilizers but did **not** provide **discrimination** among samples.

**$^{87}Sr/\ ^{86}Sr$** does not seem to be influenced by the fertilizers, can **discriminate** among **samples**, but is too **expensive** to be applied routinely.

**Combining $\delta^{18}O$,** and **fatty acids $\delta^{13}C$, $\delta^{2}H$, promising results** are obtained, achieving **85%** of correct classification in internal validation.

NEXT STEP : **$\delta^{18}O$, $\delta^{13}C$ (linoleic, oleic, palmitic, stearic) $\delta^{2}H$ (linoleic, oleic, palmitic)** of a **large sample set** (> 200 hazelnuts) to prove its suitability as a **hazelnut geographical origin authentication method.**

# Acknowledgements

This work was developed in the context of the project **Project PID2020-117701RB TRACENUTS** funded by **MCIN/AEI/10.13039/501100011033.**

Special thanks to **ISO-FOOD** for the **Young Scientist Support**.

This work was also supported by the Spanish Ministry of Science, Innovation and Universities [grant number FPU20/01454]. Grant RYC-2017-23601 funded by MCIN/AEI/ 10.13039/501100011033 and by “ESF Investing in your future”.

ISO Fd Food ERA Chair
Safety
Quality
Traceability

MINISTERIO
DE CIENCIA
E INNOVACIÓN

AGENCIA
ESTATAL DE
INVESTIGACIÓN

UNIVERSITAT DE BARCELONA

Campus
de l'Alimentació
Universitat de Barcelona

FACULTAT DE FARMÀCIA I
CIÈNCIES DE L'ALIMENTACIÓ

maima
ISÒTOPS ESTABLES I MINERALOGIA

INSA
Institut de Recerca en Nutrició
i Seguretat Alimentària

IRTA
Institut
de Recerca i Tecnologia
Agroalimentàries

libifood
Lípids i compostos bioactius

# THANKS FOR YOUR KIND ATTENTION!

Contact: **bertatorres@ub.edu, stefaniavichi@ub.edu**

**@libifood**

**www.libifood.com**

UNIVERSITAT DE BARCELONA

Campus de l'Alimentació
Universitat de Barcelona

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

maima
ISÒTOPS ESTABLES I MINERALOGIA

INSA
Institut de Recerca en Nutrició i Seguretat Alimentària

IRTA
Institut de Recerca i Tecnologia Agroalimentàries

libifood
Lípids i compostos bioactius