ALMOND AUTHENTICATION: A COMPARATIVE STUDY OF METABOLITE FINGERPRINTING, ELEMENTAL AND ISOTOPE ANALYSIS

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BACKGROUND

- Almonds are a high-value food. Their quality and sensory traits depend on **their geographical and botanical origin,** which makes the supply chain vulnerable to **origin fraud.**
- **Metabolic fingerprinting** of the **unsaponifiable fraction** by **GC-MS** captures broad chemical information related to cultivar and environment, offering strong potential for origin and varietal authentication. [1]
- **Stable-isotope markers** and **trace-element profiles** provide **complementary** information and can strengthen geographical discrimination when used together.
- Classification models (e.g., **PLS-DA**) are used to evaluate these data streams and assess their performance for authentication.

AIMS

This study aims to evaluate GC-MS metabolic fingerprinting of the unsaponifiable fraction for almond authentication (geographical origin and cultivar) using PLS-DA with external validation, and to assess the contribution of stable-isotope markers (bulk $\delta^{18}O$ and fatty acids' $\delta^{2}H$, $\delta^{13}C$) and trace-element content (B, Rb, Sr) to geographical discrimination.

MATERIALS AND METHODS

GEOGRAPHICAL AUTHENTICATION

$\delta^{2}H$ and $\delta^{13}C$ of fatty acids methyl esters by gas Chromatography-Isotope Ratio Mass spectrometry **(GC-IRMS)** [2]

Bulk $\delta^{18}O$ isotopic analysis by Elemental Analysis-Isotope Ratio Mass Spectrometry **(EA-IRMS)** [3]

100 samples

Gas Chromatography–Mass Spectrometry **(GC–MS)** fingerprinting of the unsaponifiable fraction [1]

Elemental Analysis:
B, Sr and Rb quantification by Inductively Coupled Plasma-Mass Spectrometry **(ICP-MS)**
Performed on 40 samples:10 samples from each country

Samples

A total of 100 almond samples, cultivar Nonpareil, from 4 countries

- AUSTRALIA n = 20
- SPAIN n = 24
- PORTUGAL n = 31
- USA n = 25

A total of 94 almond samples, from **Spain** and 4 different cultivars

- NONPAREIL n = 24
- GUARA n = 31
- FERRAGNES n = 20
- VAIRO n = 19

CULTIVAR AUTHENTICATION

94 samples

Gas Chromatography–Mass Spectrometry **(GC–MS)** fingerprinting of the unsaponifiable fraction [1]

CHEMOMETRICS

Full Sample Sets → **Training set (80%)** → PLS-DA → **PLS-DA** Classification models

Full Sample Sets → **Validation set (20%)** → **External validation** → **% Correct Class** predicted by the training model

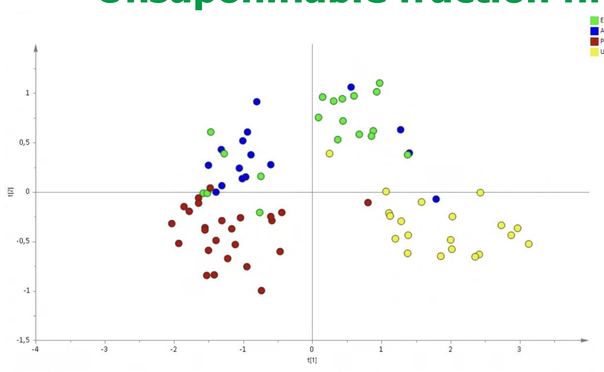
X5 o X7 iterations

PLS-DA classification models
Cross-validation (leave 10%-out) of training models, nº of LV (latent variables) according to RMSEcv and Q^2

RESULTS

GEOGRAPHICAL AUTHENTICATION

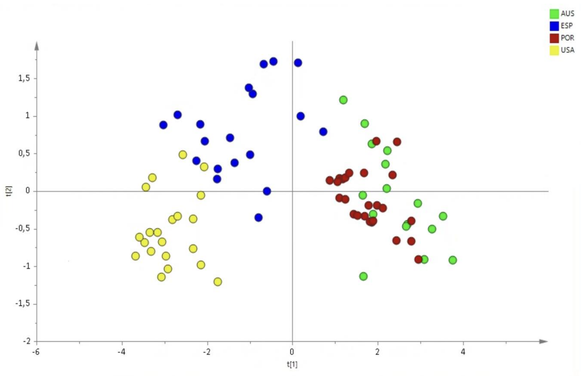
Unsaponifiable fraction fingerprinting analysis



External validation	Correct class (mean, % of assigned)
Australia	**84** ± 17
Spain	**90** ± 14
Portugal	**93** ± 9
USA	**96** ± 9
TOTAL	**91** ± 5

n= 80; 5LV; RMSEcv= 0.227;
Q^2= 0.6818; ANOVA *p value* < 0.05

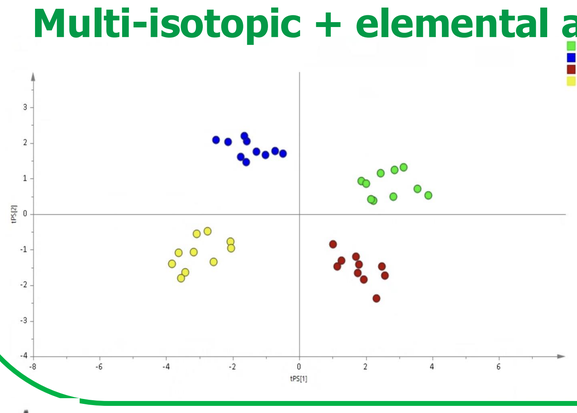
Multi-isotopic analysis: ($\delta^{18}O$; $\delta^{2}H$, $\delta^{13}C$ of fatty acids)



External validation	Correct class (mean, % of assigned)
Australia	**36** ± 20
Spain	**74** ± 22
Portugal	**83** ± 13
USA	**89** ± 9
TOTAL	**71** ± 9

n= 80; 7LV; RMSEcv= 0.229;
Q^2= 0.455; ANOVA *p value* < 0.05

Multi-isotopic + elemental analysis (B, Rb, Sr)

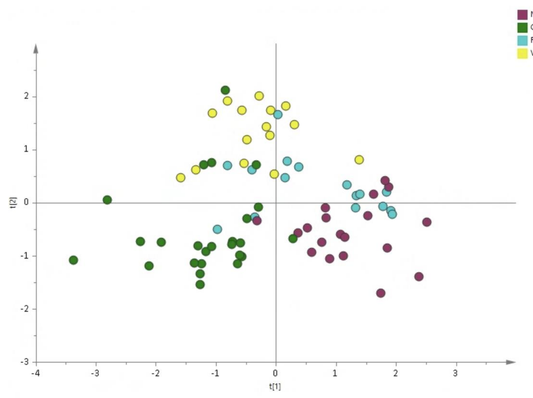


External validation	Correct class (mean, % of assigned)
Australia	**100** ± 0
Spain	**100** ± 0
Portugal	**100** ± 0
USA	**100** ± 0
TOTAL	**100** ± 0

n= 32; 3LV; RMSEcv= 0.181;
Q^2= 0.707; ANOVA *p value* < 0.05

CULTIVAR AUTHENTICATION

Unsaponifiable fraction fingerprinting analysis



External validation	Correct class (mean, % of assigned)
Nonpareil	**92** ± 18
Guara	**87** ± 22
Ferragnes	**100** ± 0
Vairo	**85** ± 22
TOTAL	**90** ± 10

n= 76; 6LV; RMSEcv= 0,25013;
Q^2= 0.744; ANOVA *p value* < 0.05

RMSECV = *Root Mean Square Error of Cross-Validation*
LV = *Latent Variables*
Q^2 = Predictive Ability Coefficient

CONCLUSIONS

Metabolic fingerprinting of the unsaponifiable fraction by GC-MS showed **promising results** for both **geographical (91%)** and **cultivar (90%)** classification, demonstrating its versatility for simultaneous origin and varietal authentication.
Selected isotopic analysis alone achieved 71% accuracy, but its combination with the elemental content of B, Rb, and Sr significantly enhanced the model's performance, resulting in **100% correct classification**.
Overall, integrating **metabolic, isotopic, and elemental data** significantly improves the **reliability** and **robustness** of authentication methods.

References

[1] Torres-Cobos, B. *et al.* (2024). *Food Chem.*, 441, 138294
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[3] von Wuthenau, K. *et al.* (2022). *Food Control*, 134, 108689.

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