New Insights into Tracing Virgin Olive Oil Provenance: Comparing Isotopic Markers and Sesquiterpene Fingerprints

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Background

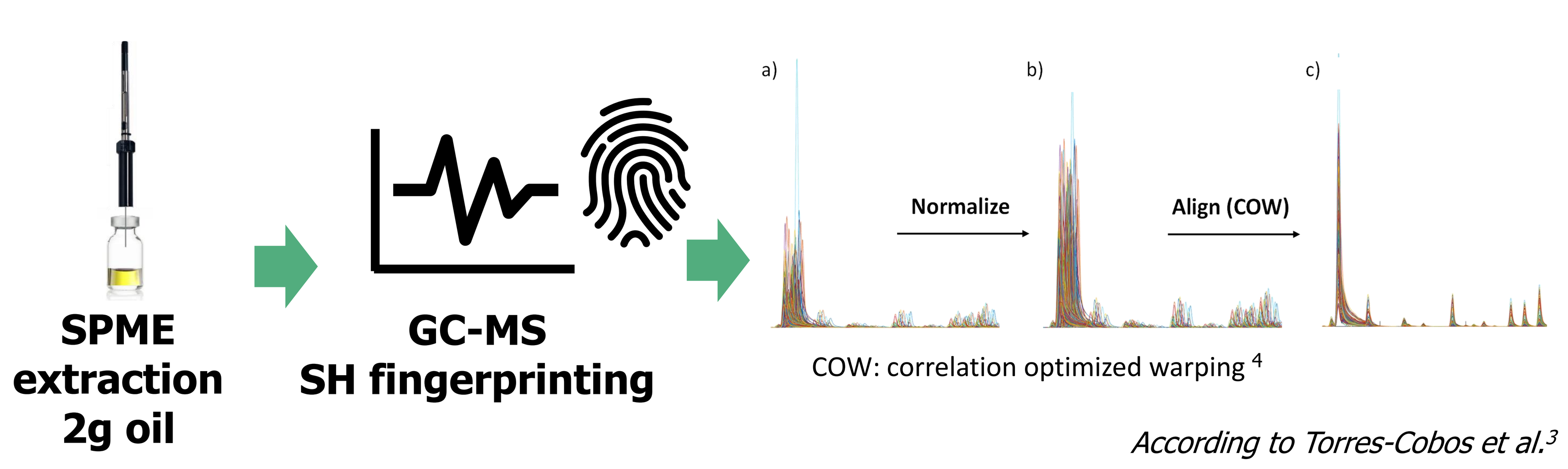
- Authenticating the **geographical origin of virgin olive oil** (VOO) is essential for **preventing fraud** and ensuring compliance with protected designation schemes (PDO/PGI)[1].
- **Stable isotope analysis** is a well-established, standardized technique for verifying the geographical origin of VOO[2].
- **Sesquiterpene hydrocarbon (SH) fingerprinting**, based on untargeted metabolomics and chemometrics, is an emerging method with high potential for VOO authentication[3].
- A **systematic comparison** under identical conditions is needed, as results from existing studies are not directly comparable due to differences in sample sets, analytical protocols, and statistical treatments.

The **AIM** was to **systematically compare** the performance **of stable isotope analysis** and **SH fingerprinting**, using the same VOO sample set and standardized statistical approaches, for differentiating Italian vs. non-Italian oils and among Italian regions.

Samples

Total sample set:
n = 393 VOOs
(2016/17 to 2019/20)
ITA n=242
Non-ITA n= 151

Analytical methods



Calculation of $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$

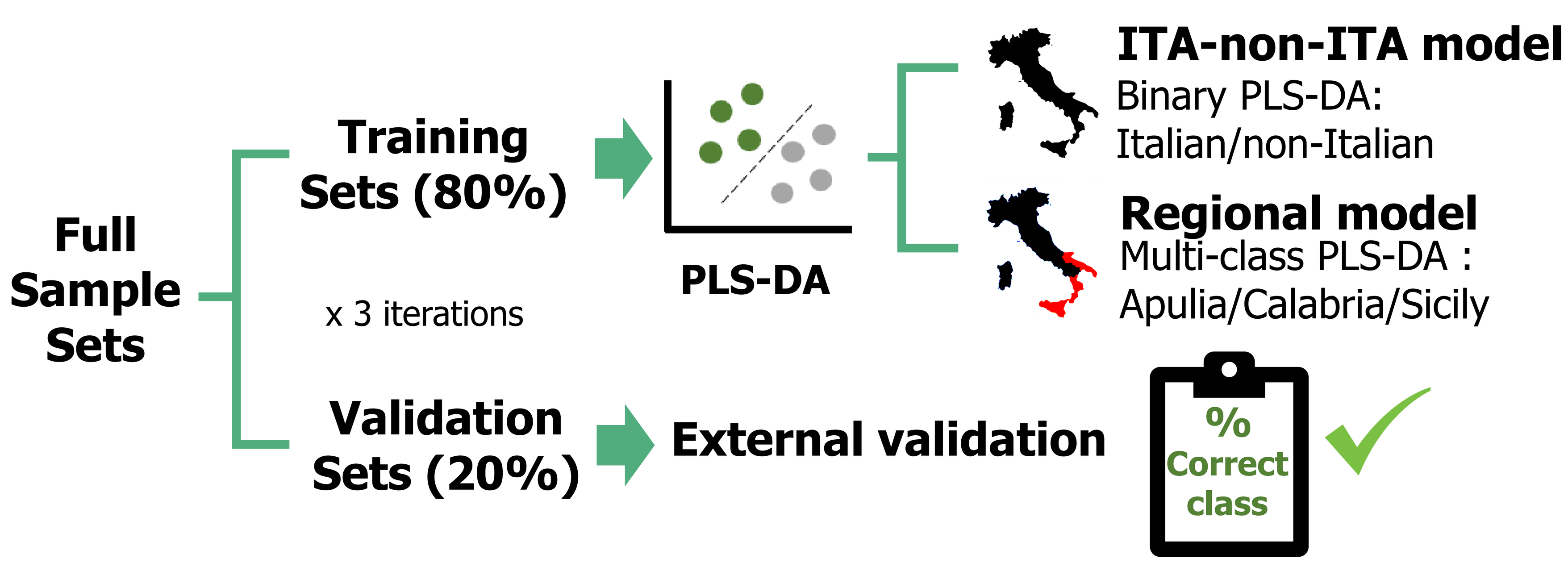
$$\delta^{i}(E_{sample/standard}) = \left(\frac{R(^{i}E/^{j}E)_{sample}}{R(^{i}E/^{j}E)_{standard}} - 1\right)1000$$

$^{i}E/^{j}E$: ratio between heavier and lighter isotopes

According to Camin et al.[2]

Chemometrics

- **Data pre-processing:** 1st derivative and mean centering for SH fingerprint
- **PLS-DA classification models:** Cross-validation (leave 10%-out), nº of LV (latent variables) according to RMSEcv and Q^2
- **Optimisation of classification thresholds** by receiver operating characteristic (ROC) analysis



Results

External validation	Correct class (mean %)
ITA	90.6 ± 6.3
Non-ITA	92.4 ± 7.3
TOTAL	**91.7** ± 6.2

Training model (N= 313, 8-9 LVs). Mean values obtained with the training sets from 3 iterations., Q^2 = 0.721, RMSEcv = 0.228. For all models, ANOVA p-value < 0.05.

External validation	Correct class (mean %)
ITA	80.2 ± 11.8
Non-ITA	72.9 ± 2.1
TOTAL	**75.8** ± 3.8

1Training model (N = 313, 2 LVs). Mean values obtained with the training sets from 3 iterations: , Q^2 = 0.299, RMSEcv = 0.406. For all models, ANOVA p-value < 0.05

External validation	Not assigned (mean, % of total)	Correct class (mean, % of total)
Apulia	0.7 ± 12	82.2 ± 13.9
Calabria	0.3 ± 17	83.3 ± 8.3
Sicily	0.0± 0.0	79.2 ± 7.2
TOTAL		**81.9** ± 5.9

Training model (N = 136, 13-14 LVs) parameters: mean values obtained with the training sets from 3 iterations: Q^2 = 0.627, RMSEcv =0.292. For all models, ANOVA p-value < 0.05

External validation	Not assigned (mean, % of total)	Correct class (mean, % of total)
Apulia	0.3 ± 0.6	75.6 ± 3.8
Calabria	0.0 ± 0.0	36.1 ± 21.0
Sicily	0.3 ± 0.6	83.3 ± 19.1
TOTAL		**63.8** ± 11.5

Training model (N = 136, 3 LVs) parameters: mean values obtained with the training sets from 3 iterations: Q^2 = 0.225, RMSEcv = 0.460. For all models, ANOVA p-value < 0.05

Conclusions

While targeted **isotopic methods** offer **satisfactory accuracy and greater applicability and versatility**, as they can be more easily adapted and implemented across various contexts, **SH fingerprinting** demonstrated **greater potential for VOO geographical differentiation, despite its challenges in method transferability**.
This comparison lays the groundwork for future research, guiding future research efforts aimed at enhancing the effectiveness and reliability of the tested methods for VOO geographical authentication.

References:
[1] Casadei et al., 2021, Food Contr., 124, 107902.
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[3] Camin et al, 2010, J. Agric. Food Chem., 58, 570-577.
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Acknowledgments: INSA-UB Maria de Maeztu Unit of Excellence (Grant CEX2021-001234-M) funded by MICIU/AEI. Unaprol - Italian Olive Consortium - for assistance in samples of Italian Virgin olive oil collecting and financial support.

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