OP-238 [Beekeeping Technology and Quality]
Near-infrared spectroscopy and isotope ratio mass spectrometry for Honey authenticity

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Food authenticity and high levels of food safety are challenging goals to achieve today, due to the complexity of globalized supply chains and the multiplicity of stakeholders and participants in the food trade. Honey is a natural food product much appreciated by consumers, as confirmed by the high market demand. However, reduced production and rising costs raise numerous concerns about honey authenticity regarding its botanical or geographical origin, as well as adulteration through sugar addition. Here we present the results of honey authenticity verification through isotope ratio mass spectrometry (IRMS), in order to detect the addition of exogenous sugar. Moreover, we developed a model for the botanical origin characterization using near-Infrared Spectroscopy (NIR) coupled with chemometric approach. 40 honey samples were collected and analyzed, both multifloral and monofloral including chestnut, eucalyptus, rhododendron, cherry and acacia. The IRMS DELTA V hyphenate EA IsoLink system (ThermoFisher) was used for the analysis of carbon isotope ratios δ13C. The samples were analyzed according to the Association of Analytical Communities (AOAC) method 998.12. Approximately 1.0-1.4 mg of honey and extracted proteins were loaded into a tin capsule, which was dropped via an autosampler into the reactor where it was burned in the presence of pure oxygen to form CO2 for isotope analysis. The NIR acquisitions were performed in triplicate in reflectance and transmittance mode. The spectra were acquired with the MicroNIR (Viavi) in a wavelength of 908-1676 nm spectral range and processed with the Unscrambler X. Linear discriminant analysis was used to create a sample classification model.

The results obtained from IRMS revealed a difference between the δ13C values of proteins and honey lower than 1‰, suggesting the absence of adulteration and confirming the authenticity of the samples. Regarding NIR results, a good classification model was developed to discriminate between monofloral and multifloral honey with an accuracy around 80%. Honey adulteration can negatively affect both consumers and honest producers. By using IRMS and NIR spectroscopy it is possible to evaluate the incidence of commercial frauds. Furthermore, these two methods can be used both in the context of real markets and in official control plans.

Near-infrared spectroscopy and Isotope Ratio Mass Spectrometry for Honey authenticity

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Commercial Fraud

To date, honey is in sixth place in the list of products at risk of fraud according to the European Commission and among the most common honey trade frauds are:

Adulteration of honey with sugars (6%)

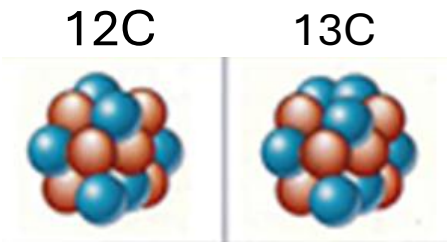
False declaration of botanical origin (7%)

- Develop accurate analytical methods
- Use targeted and/or non-targeted strategies

EA-IRMS

Elemental Analyzer coupled with Isotope Ratio Mass Spectrometry

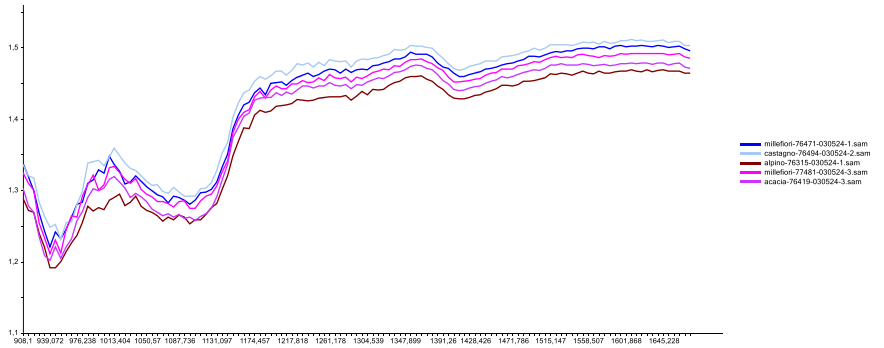
The organic molecules that make up natural products contain different isotopic ratios of the atoms of Hydrogen, Carbon, Oxygen and Nitrogen (2H/1H, 13C/12C, 18O/16O, 15N/14N)



NIR and Chemometrics

Near-infrared spectroscopy coupled with chemometric analysis

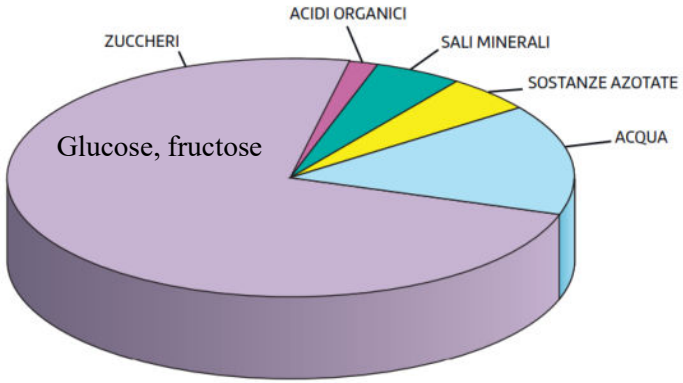
Interaction between electromagnetic radiation (800 nm and 2500 nm) and matter by inducing its characteristic vibrations.



EA-IRMS

Carbon isotopes in plants and the resulting products are primarily influenced by the carbon fixation mechanism.

plants with the Calvin-Benson photosynthetic cycle (C3 plants)
$\delta 13C = -23‰ \div -28‰$



plants with photosynthetic cycle Hatch-Slack (C4 Plants)
$\delta 13C = -9‰ \div -15‰$

δ13C‰ values can be used to detect the handling of honey with C4 vegetable sugars such as corn syrup

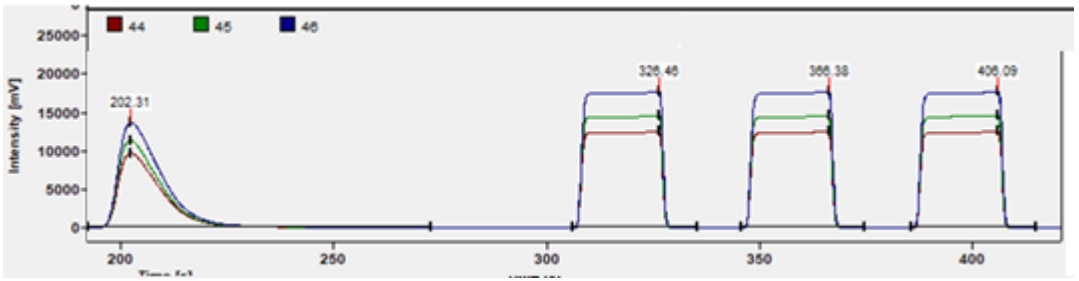
The addition of undeclared sweeteners to honey labeled as genuine is a widespread commercial fraud. A US study reports that out of 144 honey samples analyzed, 10% were non-compliant [1]

[1] https://www.fda.gov/food/economically-motivated-adulteration-food-fraud/fy2122-sample-collection-and-analysis-imported-honey-economically-motivated-adulteration

Materials and Methods

IRMS DELTA V EA
IsoLink (ThermoFisher)

Approximately 1.0-1.4 mg of sample is loaded in a tin capsule



For each sample, δ13C‰ values of protein and honey were determined. USGS-64, USGS-65 and USGS-66 were used as reference materials.

$$\delta \text{ heavy isotope } [‰] = \left(\frac{\left(\frac{\text{heavy isotope}}{\text{light isotope}}\right)_{\text{sample}}}{\left(\frac{\text{heavy isotope}}{\text{light isotope}}\right)_{\text{standard}}} - 1 \right) \times 1000$$

AOAC Official Method 998.12
C-4 Plant Sugars in Honey
Internal Standard
Stable Carbon Isotope Ratio Method
First Action 1998

[Method is used to demonstrate C-4 (corn or cane) sugars in honey at a concentration >7%.]

ID	δ13CH measured (‰)	δ13CP measured (‰)	C4 sugar (%)	δ13CH-δ13CP
77110	-25.57	-26.53	5.71	0.96
77010+10%	-24.56	-26.53	11.72	1.97
77010+20%	-23.77	-26.53	16.41	2.76

$$\text{C4 sugar (\%)} = \frac{\delta\ 13C‰\ \text{protein} - \delta\ 13C‰\ \text{honey}}{\delta\ 13C\text{protein} - (-\ 9.7)} \times 100$$

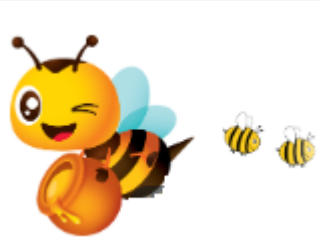
Based on the results obtained, the samples were compliant, at least as regards the addition of corn or cane sugars.

range of C4 sugar: 0-5.71%

Sugar syrups used to increase honey production are made from corn but are gradually being replaced with syrups made from rice, wheat or sugar beet.

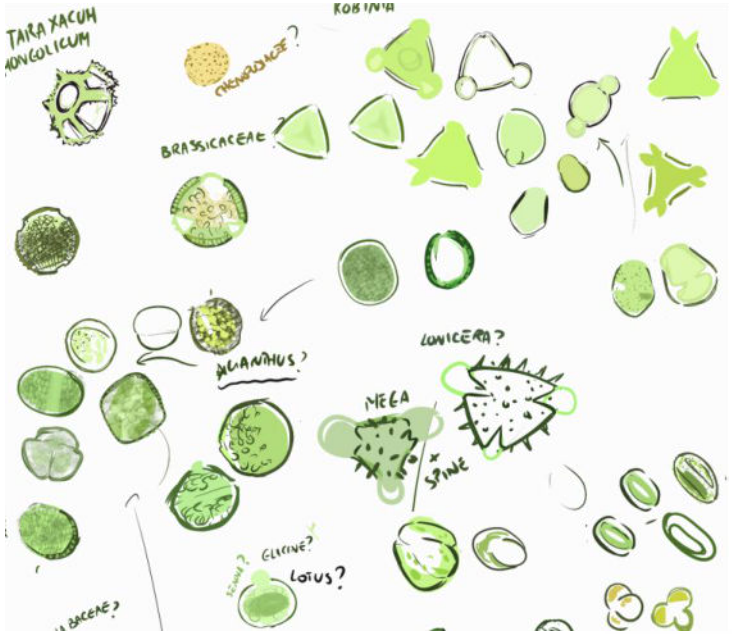
LC-IRMS

NIR and Chemometrics



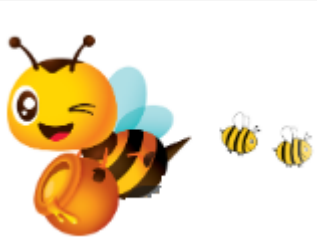
- Currently, there is no analytical system that allows the precise assessment of the origin of a honey.
- A marker for every possible botanical origin would be the optimal strategy
- Melissopalynological analysis is a method still used for this purpose in combination with sensory analysis and chemical-physical analysis

Unifloral or monofloral honey is defined as honey derived solely or mainly from the nectar or honeydew of a single plant

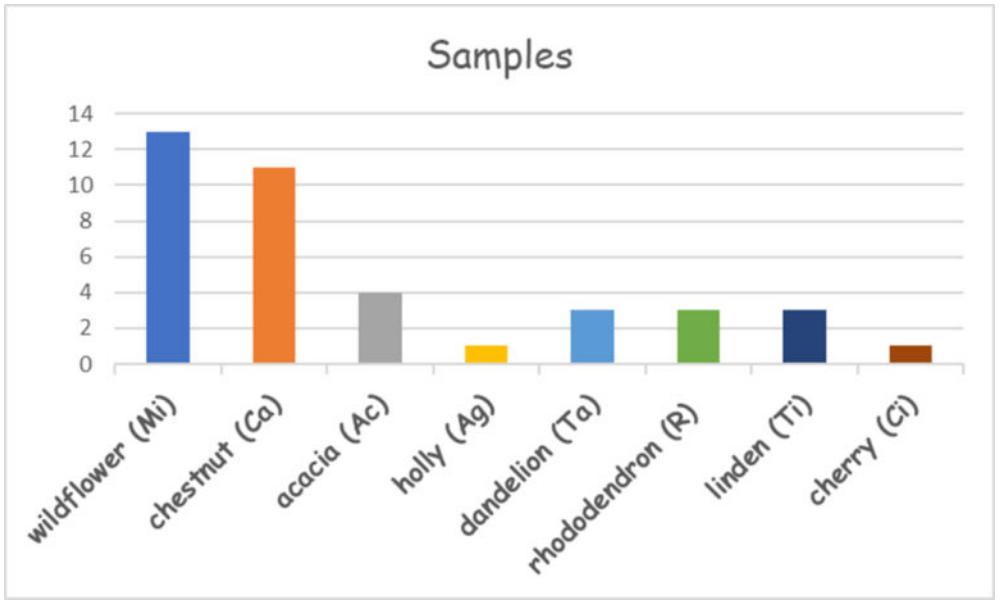


Multifloral or wildflower is defined as the honey that bees produce from the nectar collected from various flowers

Materials and Methods

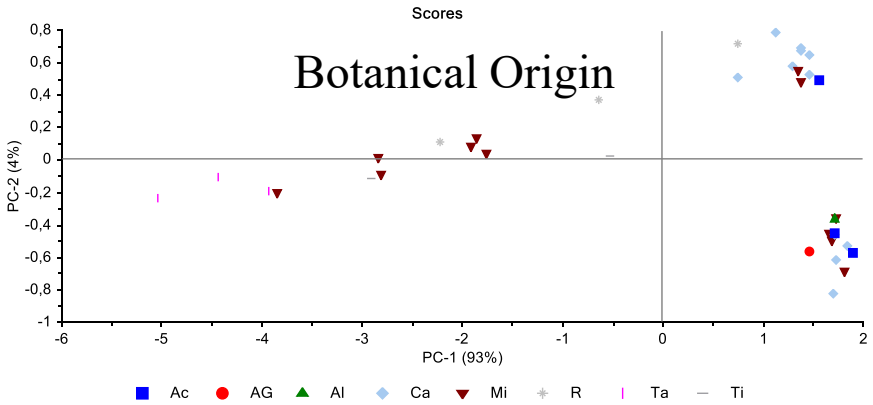


The spectra were acquired with a portable MicroNIR 1700 Pro ES instrument (VIAVI Solutions, San Jose, CA, USA), set to a spectral range 908-1676 nm, resolution 12 cm-1, integration time 12.5 µs, 200 scans at 80 Hz. Chemometric analyses were carried out using the Unscrumbler software



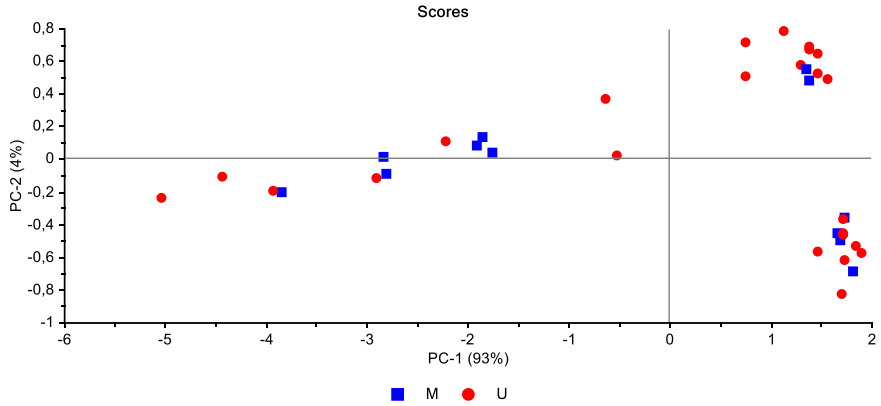
Principal Component Analysis - PCA

Botanical Origin

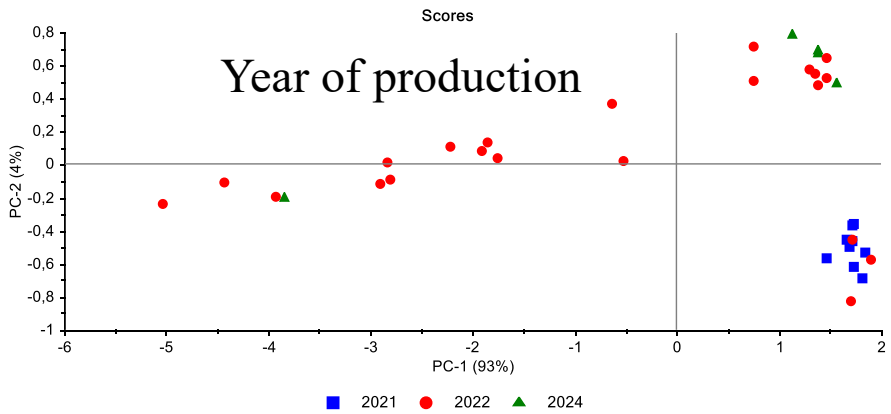


- Acacia (Ac)
- Holly (AG)
- Chestnut (Ca)
- Cherry (Ci)
- Millefiori (Mi)
- Rhododendron (R)
- Dandelion (Ta)
- Linden (Ti)

Multifloral/Unifloral

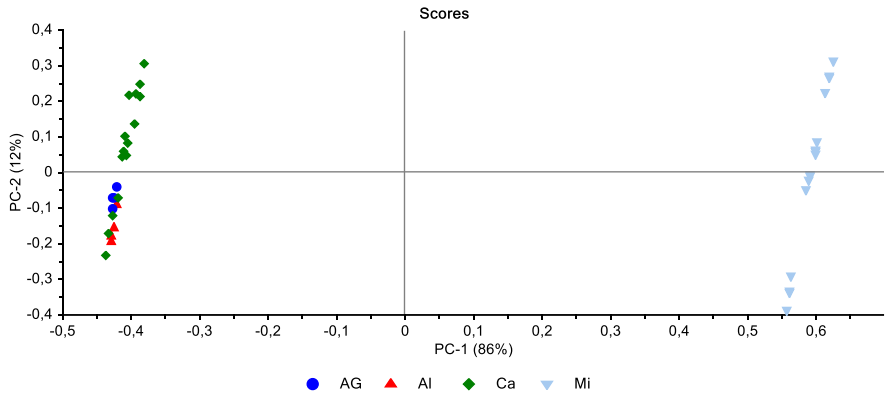


Year of production

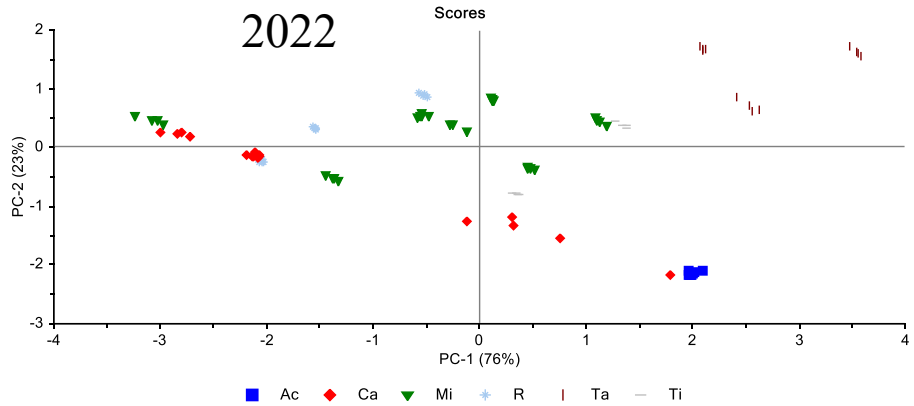


Principal Component Analysis- PCA

2021



2022



- Acacia (Ac)
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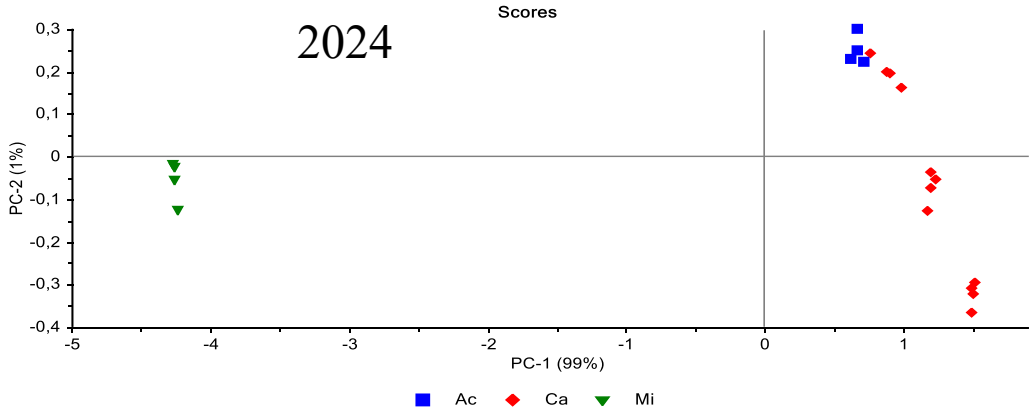
- Millefiori (Mi)
- Rhododendron (R)
- Dandelion (Ta)
- Linden (Ti)

2024

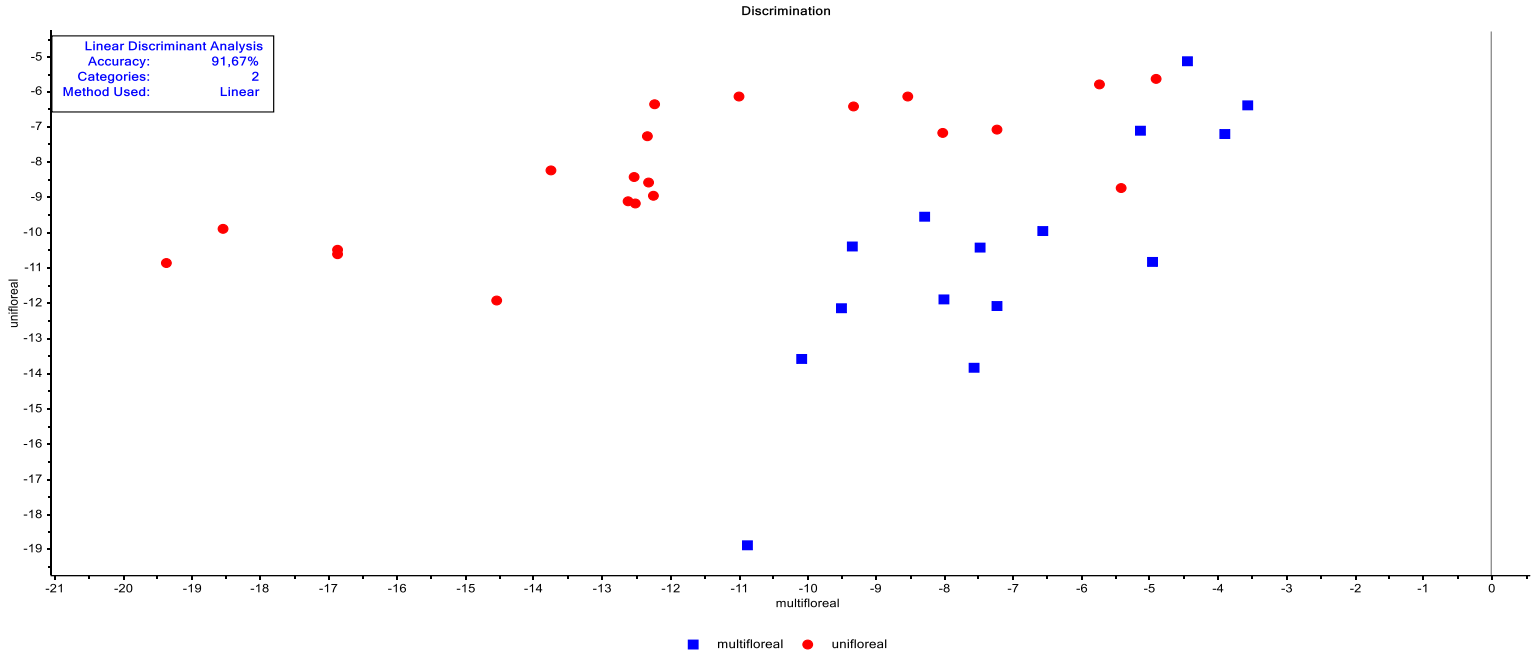


LDA-Linear Discriminant Analysis

2021- Multifloral/Unifloral classification

Data preprocessing: Normalize and SNV

The informative spectral regions selected was from 1300 to 1400 nm (combination of C–H stretching)



Accuracy 91.67%

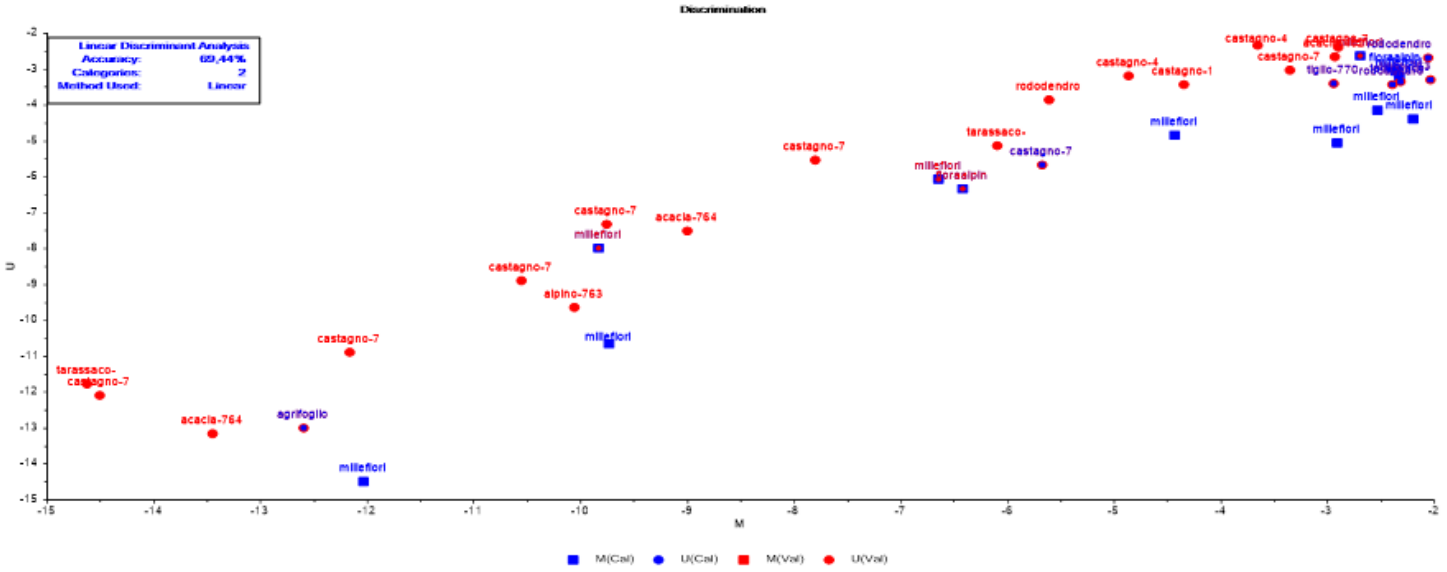
Specificity 100%

Sensitivity 85.71%

Confusion...	Actual	multifloreal	unifloreal
Predicted		1	2
multifloreal	1	15	3
unifloreal	2	0	18

LDA-Linear Discriminant Analysis

2021/2022/2024- Multifloral/Unifloral classification
Data preprocessing: Normalize and SNV
The informative spectral regions selected was from 1300 to 1400 nm (combination of C–H stretching)



Accuracy 69.44%

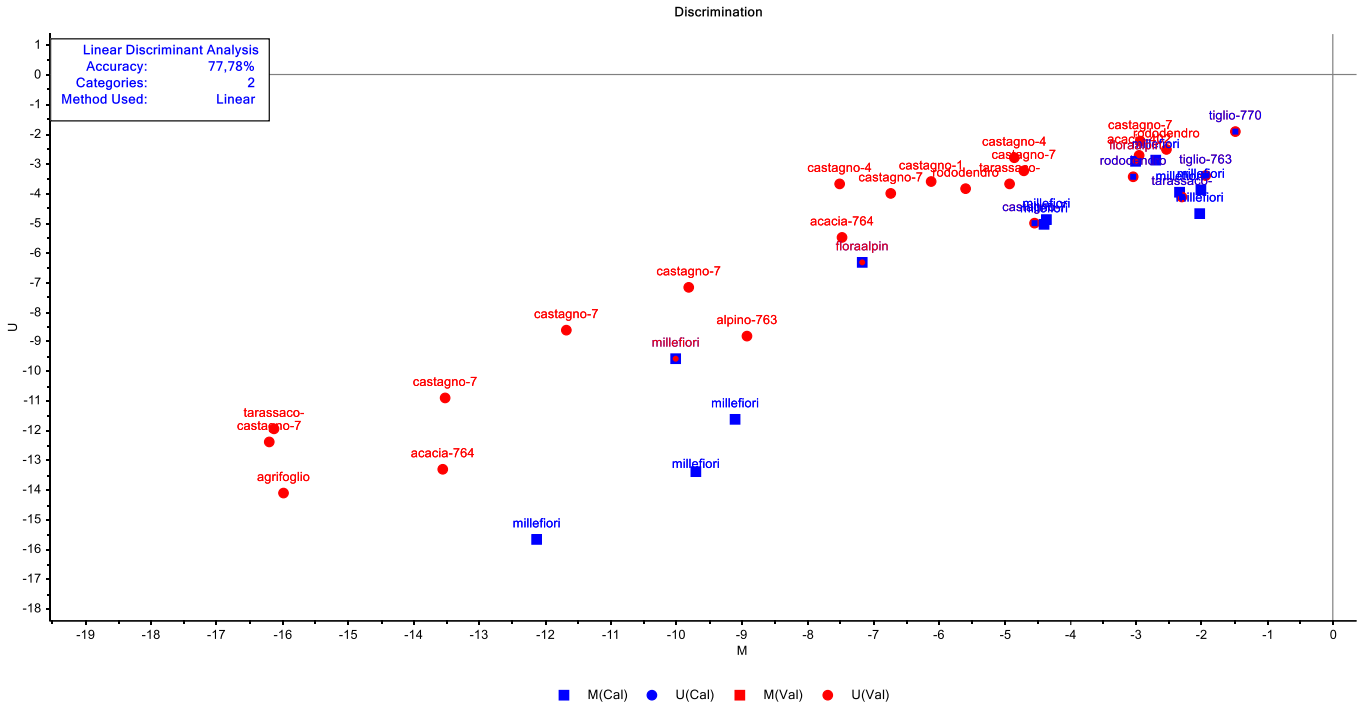
Sensitivity 66.67%

Specificity 70.83%

Confusion...	Actual	M	U
Predicted		1	2
M	1	8	7
U	2	4	17

LDA-Linear Discriminant Analysis

2021/2022/2024- Multifloral/Unifloral classification
Data preprocessing: Normalize and Baseline
The informative spectral regions selected was from 1300 to 1400 nm (combination of C–H stretching)



Accuracy 77.78%

Sensitivity 79.17%

Specificity 75.00%

Confusion...	Actual	M	U
Predicted		1	2
M	1	9	5
U	2	3	19

After?

- Increase sample size
- NIR analysis on an external dataset to evaluate the efficiency of the training model
- Method validation

Acknowledgement

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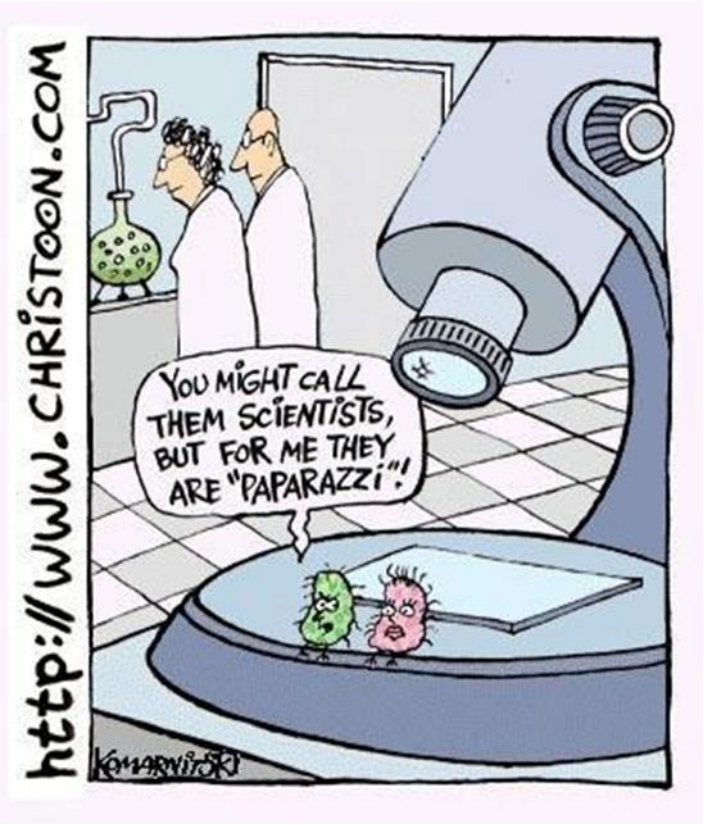

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