CHARACTERIZATION, CLASSIFICATION AND AUTHENTICATION OF HONEY THROUGH NON-TARGETED UHPLC-HRMS CHROMATOGRAPHIC FINGERPRINTS AND CHEMOMETRIC METHODS

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1. INTRODUCTION

Honey is a natural substance produced by bees of the genus Apis, from floral secretions or secretions from sucking insects. Depending on the raw material from which it starts, honeys can be differentiated into two large groups. Blossom honeys, which are the product resulting from the metabolization of nectar extracted from plants, and honeydew honeys, which are produced from plant or insect secretions. Physicochemical characteristics differentiate these two classes. Honeydew honeys are darker in color, directly related to the high content of phenolic acids, unlike blossom honeys, which stand out for their abundance in flavonoids, being light in color. In order to consider whether a honey belongs to a specific floral variety, it must be based on a minimum floral pollen origin. Commonly, the minimum value established to be considered a monofloral honey is 45%, however, fluctuations in this value may occur.

Honey is in the fifth position of consumer products with the highest presence of food fraud. Most of the adulterated honey incurs in ingredient dilution fraud, adding sweet substances, such as syrups, sugar cane, or corn syrup, among others. In the market, this was reflected in the dubious lowering of prices for this product. In the last few years, honey adulteration frauds have come to light, with honeys from China, Portugal and Romania being an exemplary case.

This work aims to develop a non-targeted UHPLC-HRMS fingerprinting method to address the characterization, classification and authentication of Spanish honey.

2. SAMPLES

136 honey samples of different botanical varieties

MULTIFLORAL HONEYS

34 Multifloral (ML)

BLOSSOM-HONEYS

13 Eucalyptus (EU)

26 Rosemary (RO)

7 Thyme (TH)

12 Orange/Lemon Blossom (BL)

18 Heather (ML)

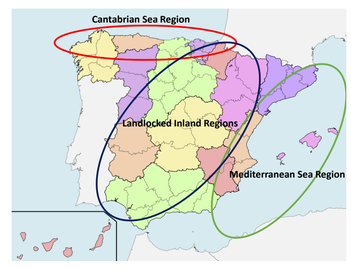
HONEYDEW-HONEYS

6 Mountain (MO)

10 Forest (FO)

10 Holm Oak (HO)

Geographical origin (climatic region)



Cantabrian Sea Region (CSR): 19

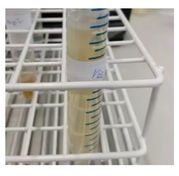
Landlocked Inland Regions (LIR): 67

Mediterranean Sea Region (MSR): 38

3. SAMPLE TREATMENT

~1 g honey + 10 mL water

Vortex
Centrifugation (5 min, 3500 rpm)



1:1 dilution with methanol

Filtration (0.45 µm nylon filters)

UHPLC-HRMS analysis

QUALITY CONTROL (QC): mixing of 50 µL of each aqueous sample extract diluted with methanol.

4. HPLC-LRMS METHOD

➢ Liquid chromatography:

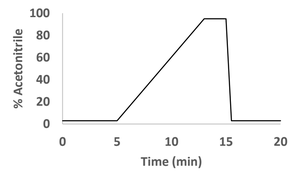
Instrument: Dionex UHPLC (Thermo Fisher Scientific)

Column: Kinetex C18 (100 x 2.1 mm, 2.6 µm) (Phenomenex)

Mobile phase: - Solvent A: water (0.1% formic acid)
- Solvent B: Acetonitrile

Flow rate: 400 µL/min

Gradient program:



➢ High-resolution mass spectrometry:

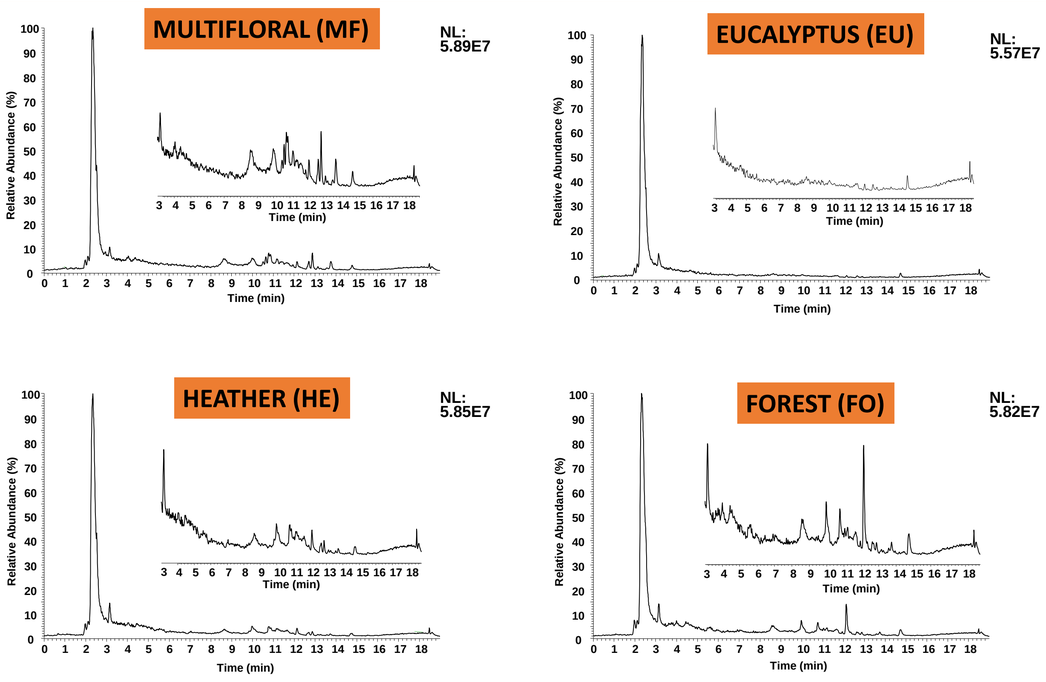
Instrument: FT-MS LTQ-Orbitrap (Thermo Fisher Scientific)

Ionization source: Heated-Electrospray (H-ESI)

Source parameters:
- Capillary voltage: -3.5 kV
- Capillary temperature: 350 ºC
- Sheath gas flow rate: N_2 50 a.u.
- Auxiliary gas flow rate: N_2 20 a.u.
- Sweep gas flow rate: N_2 2 a.u.

Acquisition mode: Full scan MS at *m/z* 110-1,000 at a resolution of 50,000 FWHM (full-width at half maximum at *m/z* 200)

5. NON-TARGETED UHPLC-HRMS FINGERPRINTS

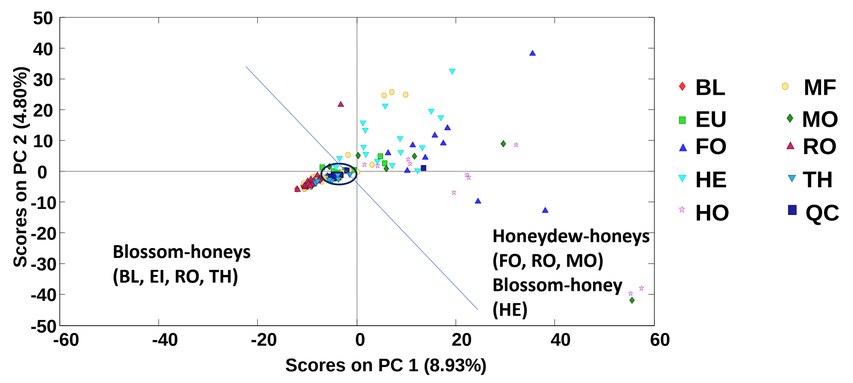


Non-targeted UHPLC-HRMS chromatographic fingerprints (Total ion chromatogram) of selected honey samples

Important differences regarding number of peaks and signal intensities were observed between the analyzed honey samples

Non-targeted UHPLC-HRMS Fingerprints could be good honey chemical descriptors for sample classification

6. PRINCIPAL COMPONENT ANALYSIS

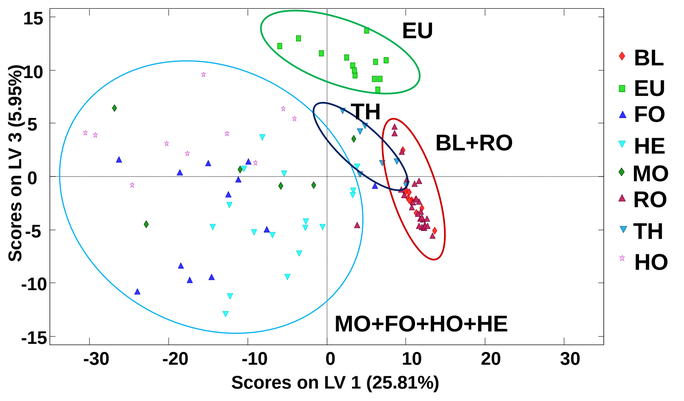


QCs are grouped, showing the reproducibility and robustness of the proposed methodology, and that chemometric results were not affected by instrumental drifts.
Separation between Blossom- and Honeydew-honeys was observed. Heather blossom honeys appeared with the honeydew ones because of their similar physicochemical properties.

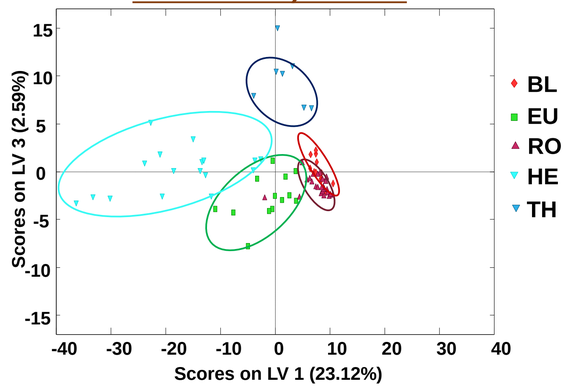
7. PARTIAL LEAST SQUARES-DISCRIMINANT ANALYSIS

Classification based on Botanical variety

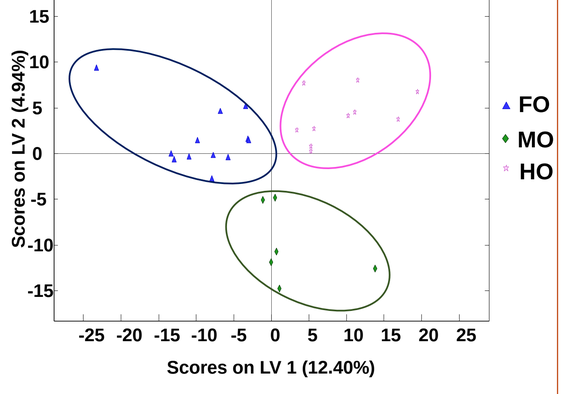
All analyzed honey varieties



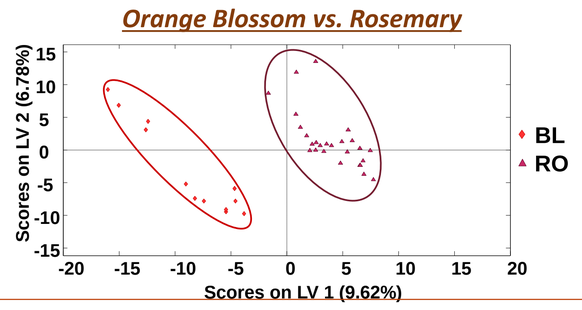
Blossom-honey varieties



Honeydew-honey varieties



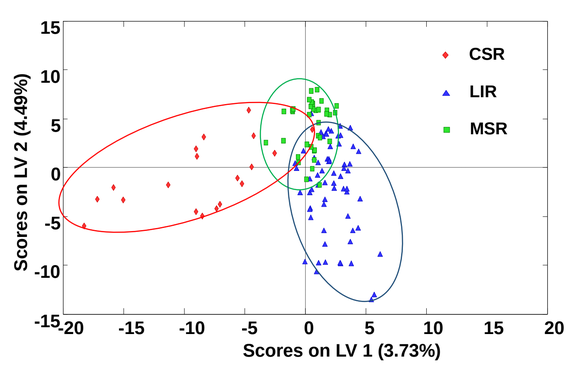
Orange Blossom vs. Rosemary



Discrimination of some honey varieties improved in comparison to PCA. Separation between blossom- and honeydew-honeys was accomplished.

Very acceptable discrimination among the different varieties of blossom- and honeydew-honeys was accomplished by PLS-DA

Classification based on Geographical Climatic region



Certain discrimination was also accomplished based on geographical climatic region, which was complete when studying paired PLS-DA models

8. CONCLUSIONS

- **Non-targeted UHPLC-HRMS chromatographic fingerprints were evaluated as sample chemical descriptors to address the characterization, classification, and authentication of honey samples according to their botanical origin (blossom- and honeydew-honeys) as well as the region of production based on climatic conditions.**
- **Good discrimination capability among botanical varieties was observed between all blossom- and honeydew-honey samples.**
- **Acceptable discrimination was also accomplished regarding honey geographical climatic regions of production.**

9. REFERENCES

- **Chin, N.L.; Sowndhararajan, K. A Review on Analytical Methods for Honey Classification, Identification and Authentication in V. D-A. Arnaut De Toledo and E.M. Chambó (Eds.) Honey Analysis. New Advances and Challenges, Intechopen. 2020, doi:10.5772/intechopen.90232**

10. ACKNOWLEDGEMENTS

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