Foods 2024 Conference

The 5th International Electronic Conference on Foods

28–30 October 2024 | Online

MDPI

Potential of HPLC-UV Fingerprints to Assess Honey Geographical Production Region

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INTRODUCTION & AIM

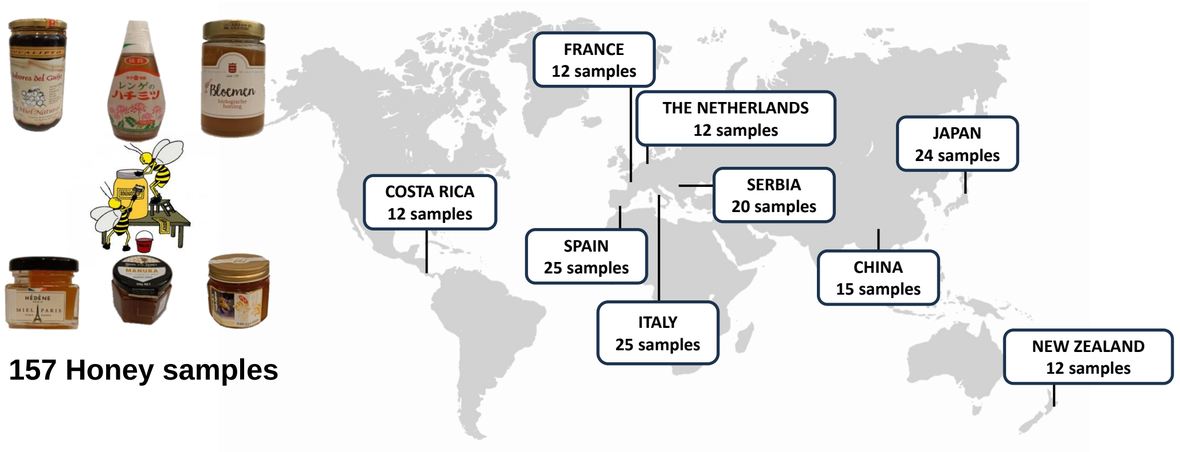
Honey is a traditional food sweetener with a very complex composition, produced naturally by bees (*Apis mellifera*), highly consumed and appreciated by society not only because of its nutritional value and taste but also due to its beneficial properties on human health. Composed mainly of sugars (80-85%), water (15-17%), and proteins (0.1-0.4%), it also contains other bioactive substances such as vitamins, enzymes, macro- and micro-elements, organic acids, flavonoids, and other polyphenols, that contribute to a greater or lesser extent on its organoleptic and physicochemical properties.

The great diversity of botanical varieties and countries of production has given rise to products with disparity in quality and prices, also increasing fraudulent practices. In this line, developing methods capable of characterizing honey and authenticating and certifying not only its botanical variety but also its geographical origin is essential in order to avoid distrust in society or economic losses in the beekeeping sector. In this sense, non-targeted chromatographic approaches are gaining relevance to address food authentication issues. These fingerprinting approaches pursue to register as many chemical instrumental features from the analyzed samples as possible (chromatographic, spectroscopic, etc.) without the requirement of knowing the identity of the known/unknown metabolites responsible for those responses, thus obtaining feasible and cheaper methodologies not requiring the use of chemical standards for metabolite identification.

The aim of the present contribution is to evaluate the potential of HPLC-UV fingerprints to assess honey geographical production region, and to detect and quantify honey adulterations based on blended-adulterated honeys produced in two different countries.

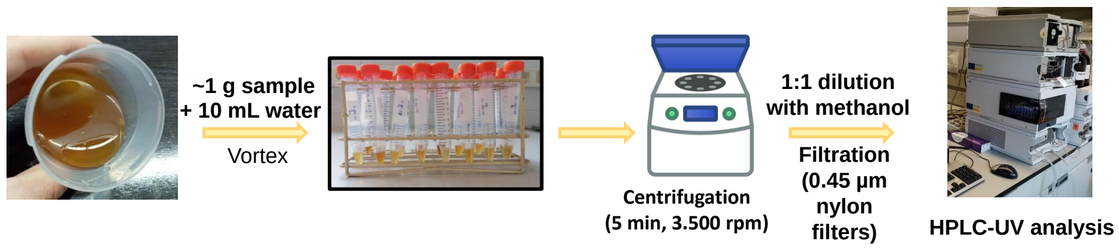
METHOD

ANALYZED SAMPLES



157 Honey samples

SAMPLE TREATMENT



INSTRUMENTATION

Instrument: Agilent 1100 Series HPLC

Column: Kinetex C18 (10 cm × 4.6 mm, 2.6 µm)

Mobile phase:

A. Water with 0.1% formic acid

B. Acetonitrile

Flow-rate: 400 µL·min^{-1}

Gradient:

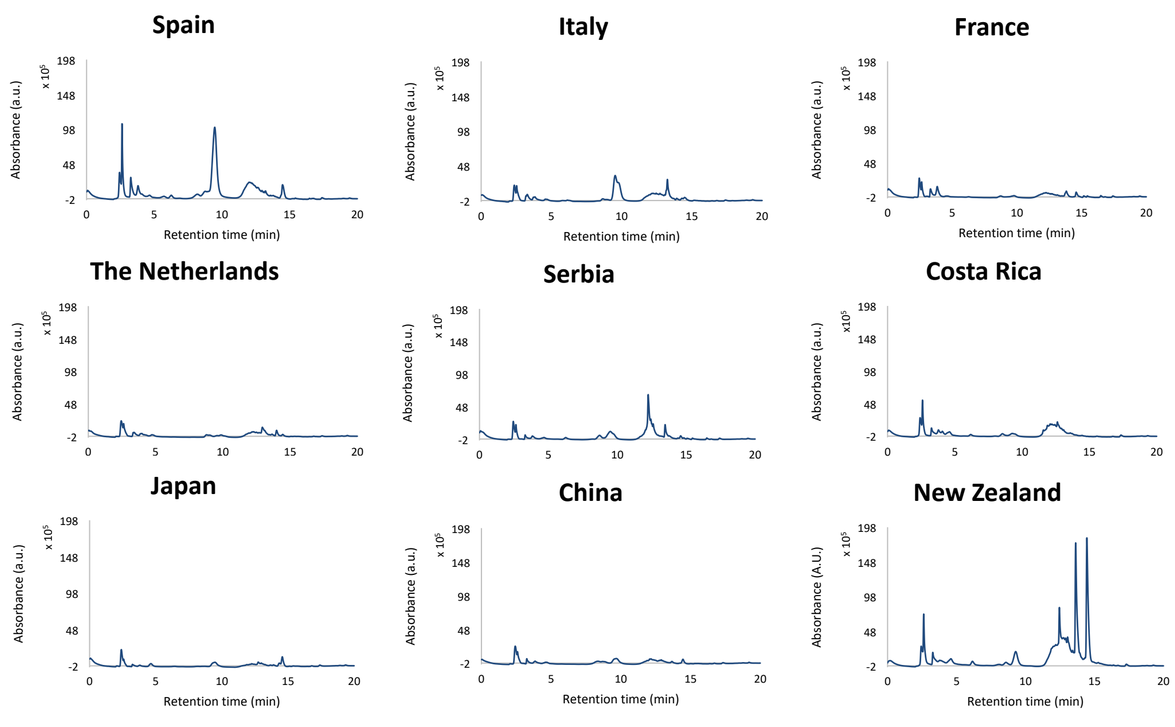
Time [min]	Solvent B [%]	Elution mode
0-5	3	Isocratic
5-13	3-95	Lineal
13-15	95	Isocratic
15-15.5	95-3	Lineal
15.5-20	3	Isocratic

UV acquisition: 280 nm

Injection volume: 5 µL

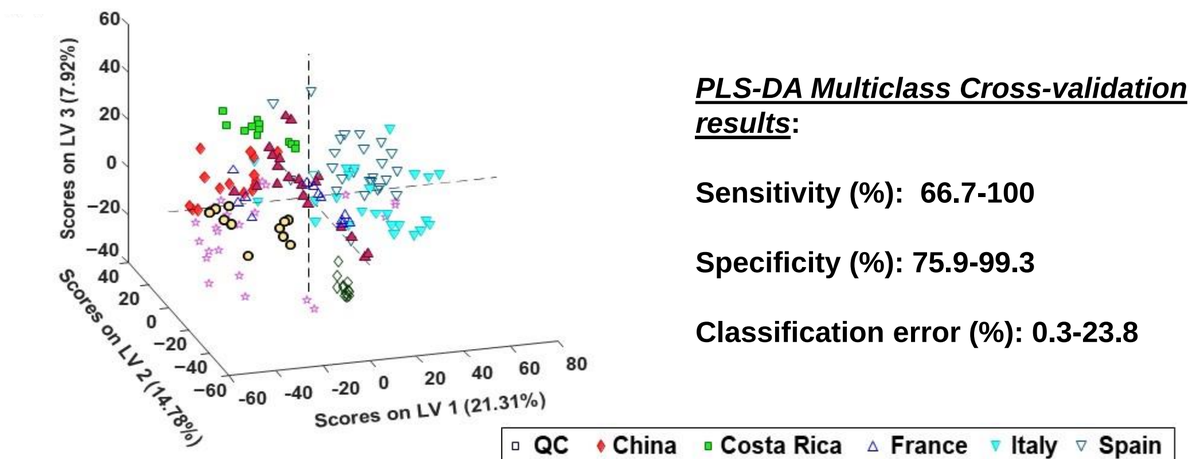
RESULTS & DISCUSSION

Non-targeted HPLC-UV Fingerprints



CLASSIFICATION OF HONEY SAMPLES BY PARTIAL LEAST SQUARES-DISCRIMINANT ANALYSIS (PLS-DA)

PLS-DA score plot of LV1 vs. LV2 vs. LV3 (with 6 LVs)



***PLS-DA Multiclass Cross-validation results*:**

Sensitivity (%): 66.7-100

Specificity (%): 75.9-99.3

Classification error (%): 0.3-23.8

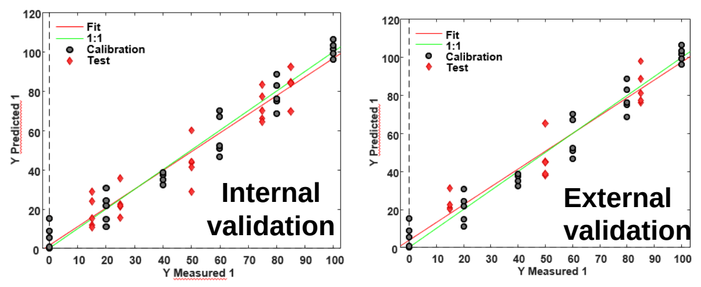
***PLS-DA Cross-validation results by using a Classification Decision Tree*:**

Sensitivity (%): 87.5-100

Specificity (%): 78.6-99.3

Classification error (%): 0-17

DETECTION AND QUANTITATION OF HONEY FRAUDS BASED ON BLENDED-ADULTERATED HONEYS FROM TWO DIFFERENT COUNTRIES BY PARTIAL LEAST SQUARES (PLS) REGRESSION



PLS data results:

Calibration errors: 4.7-9.7%

Cross-validation errors: 9.4-13.9%

Prediction errors: 8.5-15.9%

Spanish Eucalyptus adulterated with Italian Eucalyptus Honey

CONCLUSION

HPLC-UV fingerprints were excellent sample chemical descriptors to assess honey geographical origin, specially when PLS-DA with a Classification Decision Tree was employed. Good prediction errors were also obtained in the detection and quantitation of adulterations.

ACKNOWLEDGEMENTS

This research was funded by the project PID2023-147160OB-C22, financed by the Agencia Estatal de Investigación. The authors also want to acknowledge the Agency for Administration of University and Research Grants (Generalitat de Catalunya, Spain) under the project 2021SGR-00365, and María de Maetzu Unit of Excellence (Research Institute of Nutrition and Food Safety, INSA-UB, University of Barcelona), Grant CEX2021-001234-M, funded by MCIN/AEI/10.13039/501100011033