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Liquid Chromatography-Mass Spectrometry Fingerprinting To Authenticate Honey Origin

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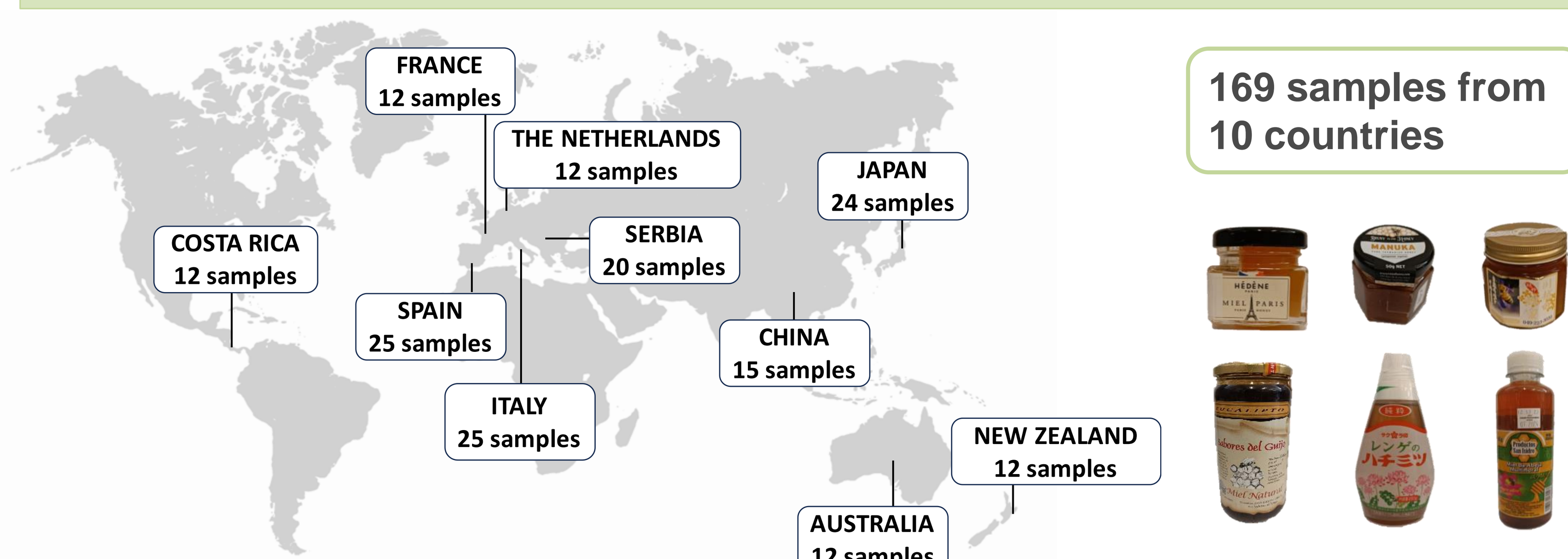
INTRODUCTION & AIM

Honey is a natural food sweetener made by bees (*Apis mellifera*) that contains an important number of bioactive substances, such as polyphenols, which provide health benefits to humans, being therefore highly appreciated by society. These special characteristics, together with the great variability of products due to its worldwide production, have placed honey as one of the products most susceptible to manipulation for illicit purposes, with adulteration with sugars or botanical and geographical origin mislabelling being the most common fraudulent practices. For that reason, the development of feasible analytical methodologies to assess honey authenticity is required.

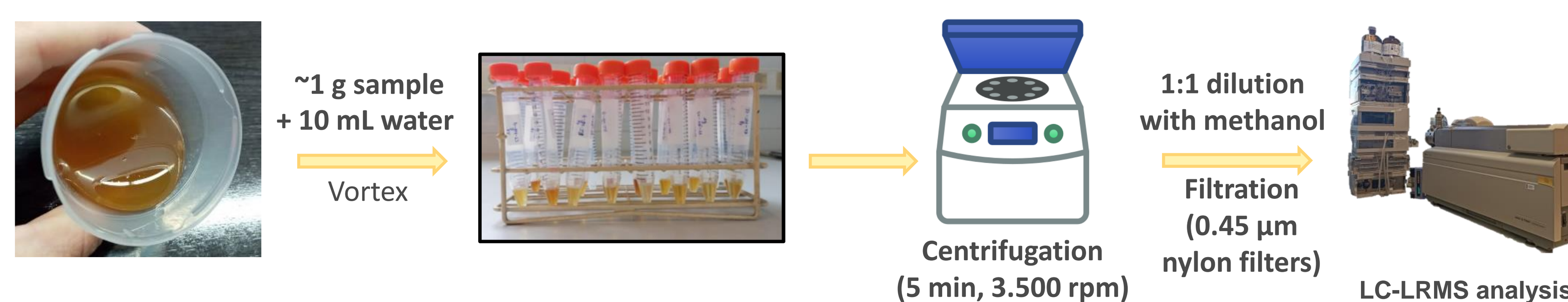
In this work, a liquid chromatography coupled with mass spectrometry (LC-MS) fingerprinting methodology employing a hybrid triple–quadrupole/linear ion trap mass analyser in negative ESI mode was evaluated to assess honey geographical origin.

METHOD

HONEY SAMPLES



SAMPLE TREATMENT



Quality Control (QC): Blended sample with 50 µL of each honey extract.
Analyzed every 10 samples to control the reproducibility and robustness of the methodolgy

LC-LRMS CONDITIONS

Chromatographic Separation:

- **Instrument:** Agilent 1100 Series HPLC
- **Column:** Kinetex® C18 (100 × 4.6 mm I.D., 2.6 µm partially porous particle).
- **Mobile Phase:** (A) HCOOH 0.1%; (B) CH3CN
- **Flow-rate:** 400 µL/min
- **Injection volume:** 5 µL
- **Gradient program:** 0-5 min 3% B; 5-13 min 3-95% B; 13-15 min 95% B; 15-15.5 min 95-3% B; 15.5-20 min 3% B

Mass Spectrometry:

- **Instrument:** AB Sciex 4000 QTrap
- **Ionization:** ESI on negative mode
- **Acquisition:** Full scan (*m/z* 100-550)
- **ESI parameters:**
  - **Curtain gas Flow-rate (N_2):** 10 a.u.
  - **Ion source gas 1 and 2 (N_2):** 50 a.u.
  - **Spray voltage:** -2500 V
  - **Source temperature:** 400 ºC
  - **Declustering potential:** -80 V

DATA TREATMENT

Data matrix building:

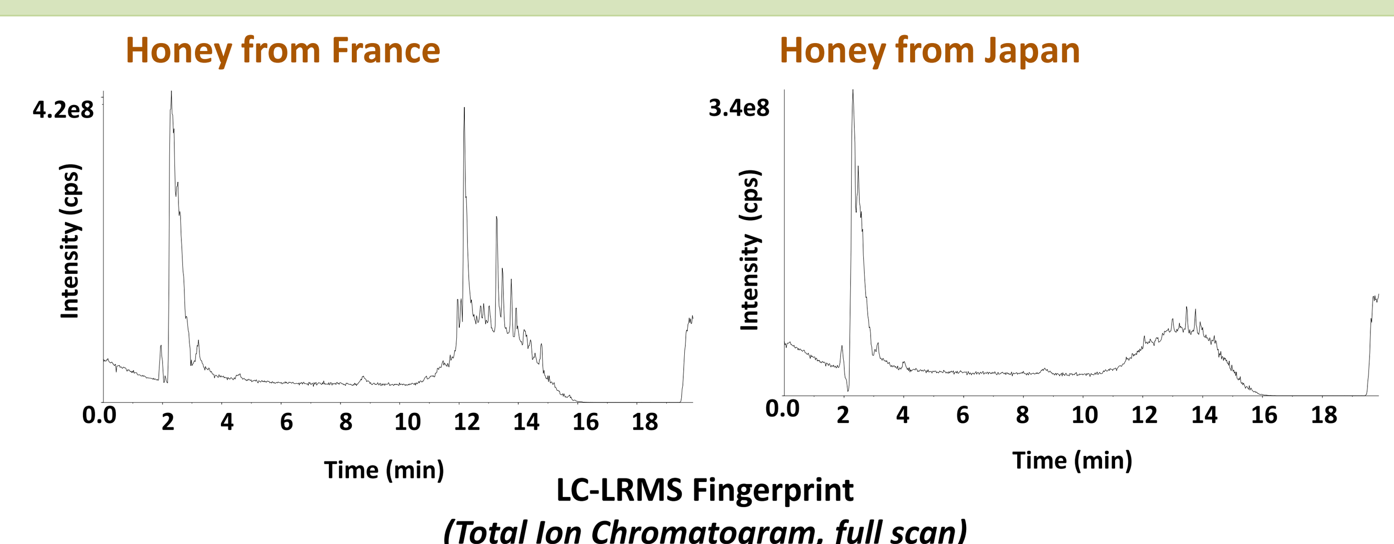
- **MSConvert software:** chromatographic raw data conversion into mzXML output format
- **mzMine 3 software:** to obtain data matrices

Chemometric data analysis:

- **SOLO 8.6 chemometric software:**
  - **Principal Component Analysis** (PCA)
  - **Partial Least Squares-Discriminant Analysis** (PLS-DA)

RESULTS & DISCUSSION

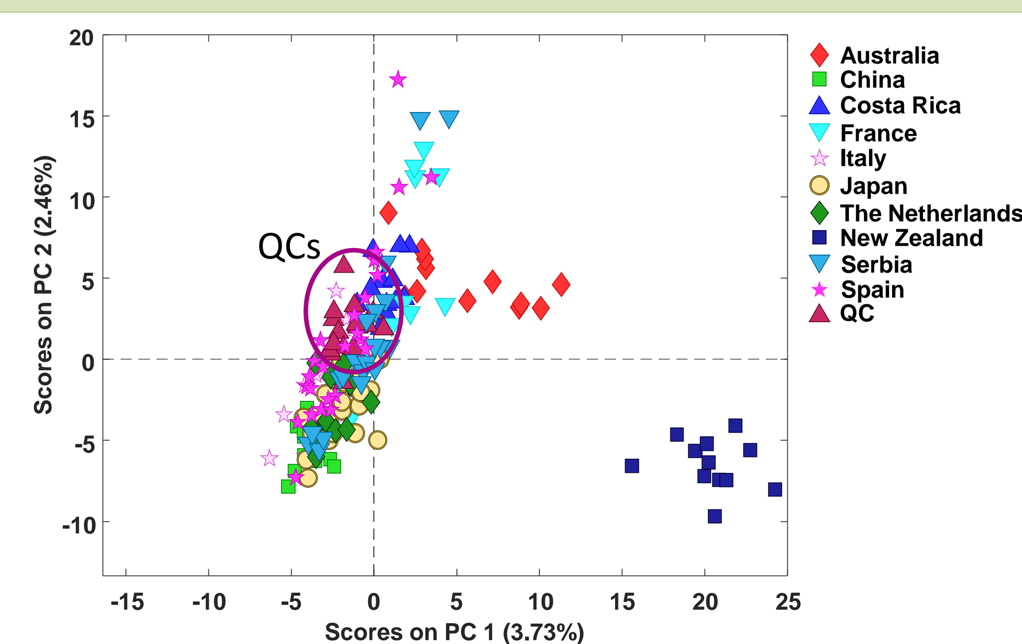
HONEY LC-LRMS FINGERPRINTS



LC-LRMS Fingerprint
(Total Ion Chromatogram, full scan)

Important differences in the LC-LRMS fingerprints were observed, suggesting that these fingerprints may be good sample chemical descriptors to assess honey authentication according to their geographical production origin.

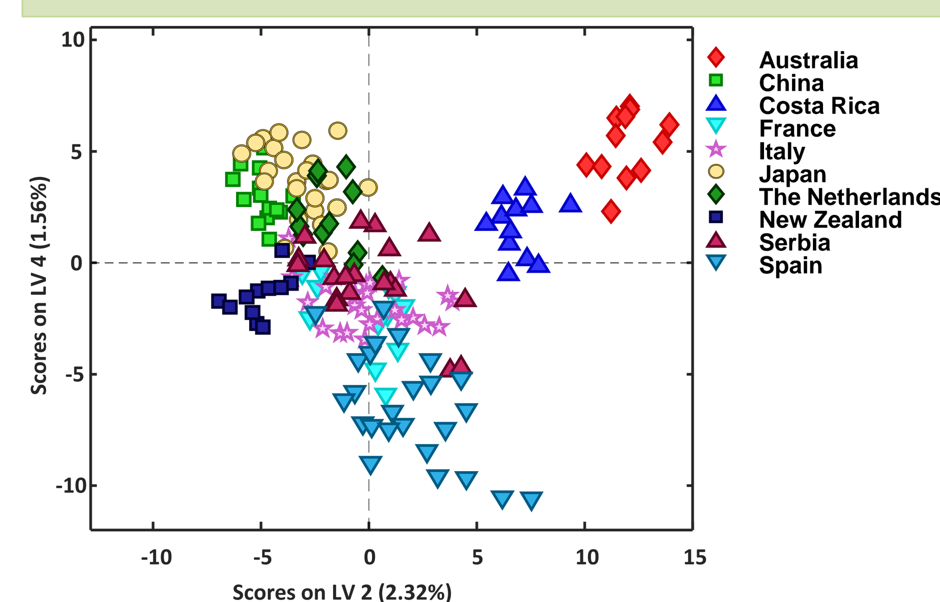
EXPLORATORY PCA



QCs are well grouped. Thus, the proposed methodology is both reproducible and robust.

PCA scores plot of PC1 vs. PC2

CLASSIFICATION OF HONEY SAMPLES BY GEOGRAPHICAL PRODUCTION ORIGIN BY MULTICLASS PLS-DA AND BY CLASSIFICATION DECISION TREE



PLS-DA scores plot of LV2 vs LV4 (4 LVs to build the model)

Multiclass PLS-DA results:

	Calibration	Cross-validation
Sensitivity (%)	90-100	46.7-100
Specificity (%)	74.3-100	80.6-100
Classification error (%)	0-12.8	0-29.6

PLS-DA Prediction results when applying a Classification Decision Tree:

	Calibration	Cross-validation	Prediction
Sensitivity (%)	100	78.6-100	25-100
Specificity (%)	98.6-100	92.6-100	78.9-100
Classification error (%)	0-0.7	0-14.9	0-38.9

CONCLUSION

The proposed methodology, based on LC-LRMS fingerprinting, demonstrated to be both reproducible and robust. Additionally, the fingerprints obtained were good chemical sample descriptors for the classification based on geographical origin, obtaining acceptable values of classification error considering the high number of samples and classes analyzed.

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