

Characterization of honey for geographical origin authentication

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INTRODUCTION & AIM

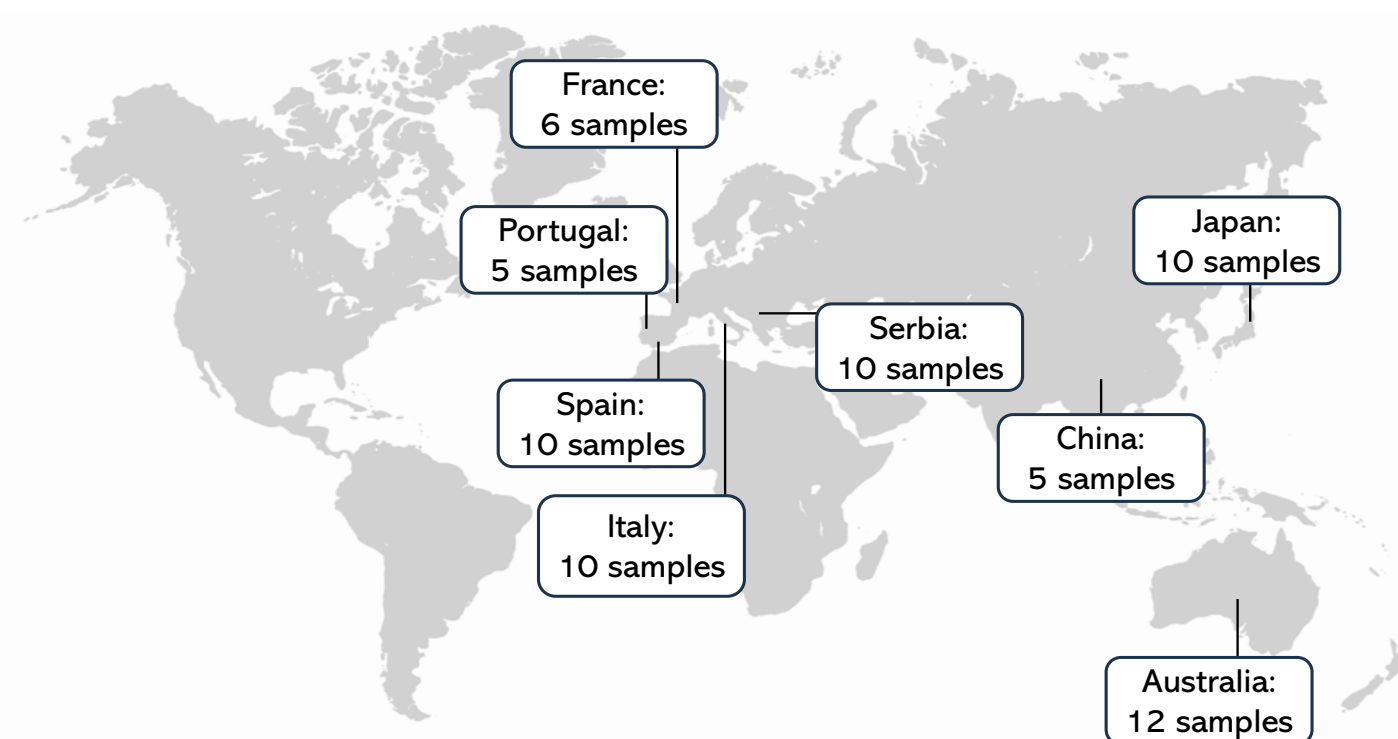
Nowadays, the quality of food products is strongly associated, among other factors, to their geographical origin. This has led to the implementation of quality schemes such as the Protected Designation of Origin (PDO), which enhance the value of the products from specific regions. Given the notable differences in quality and prices of several food products, cases of fraud related to geographical origin mislabelling are a growing concern. In the particular scenario of honey, fraud cases arise from price differences between products of different origins and qualities. For this reason, to ensure its high quality and prevent fraud cases, it is important to recognize the differences in the chemical attributes of honey from different origins.

This work aims to characterize and authenticate honey from 8 countries by determining diverse spectrophotometric indexes, physicochemical properties, and LC-MS/MS polyphenolic profiling

METHOD

HONEY SAMPLES

68 samples from 8 countries



METHODOLOGY

Physicochemical parameters

pH

Conductivity

Brix index by refractometry

Water content by refractometry

Spectrophotometric assays

Antioxidant assays¹

Reducing sugars by 3,5-dinitrosalicylic acid (DNS) assay²

Total Phenolic content by Folin-Ciocalteu (FC)

Total Flavonoid Content

Ferric Reducing Antioxidant Power (FRAP)

LC-MS/MS analyses



Chromatographic separation:

- Agilent 1100 Series
- Kinetex® C18 (100×4.6 mm i.d., 2.6 µm)
- Sol. A: HCOOH 0.1%; Sol. B: ACN
- Flow rate: 0.8 mL·min⁻¹
- Gradient (%B): 0–1 min: 5–10%; 1–4 min: 10–16%; 4–8 min: 16%; 8–8.5 min: 16–25%; 8.5–13.5 min: 25–60%; 13.5–16 min: 60–100%; 16–16.5 min: 100%; 16.5–16.6 min: 100–5%; 16.6–22 min: 5%

MS conditions:

- AB Sciex 4000 QTrap hybrid triple quadrupole/linear ion trap mass spectrometer
- Acquisition mode: Multiple Reaction Monitoring (MRM) targeting 39 compounds
- ESI in negative mode
- N₂ as curtain gas: 10 a.u.
- N₂ as ion source gas 1: 50 a.u.
- N₂ as ion source gas 2: 50 a.u.
- Ionization temperature: 400 °C
- ionspray voltage: 2.5 kV

DATA ANALYSIS



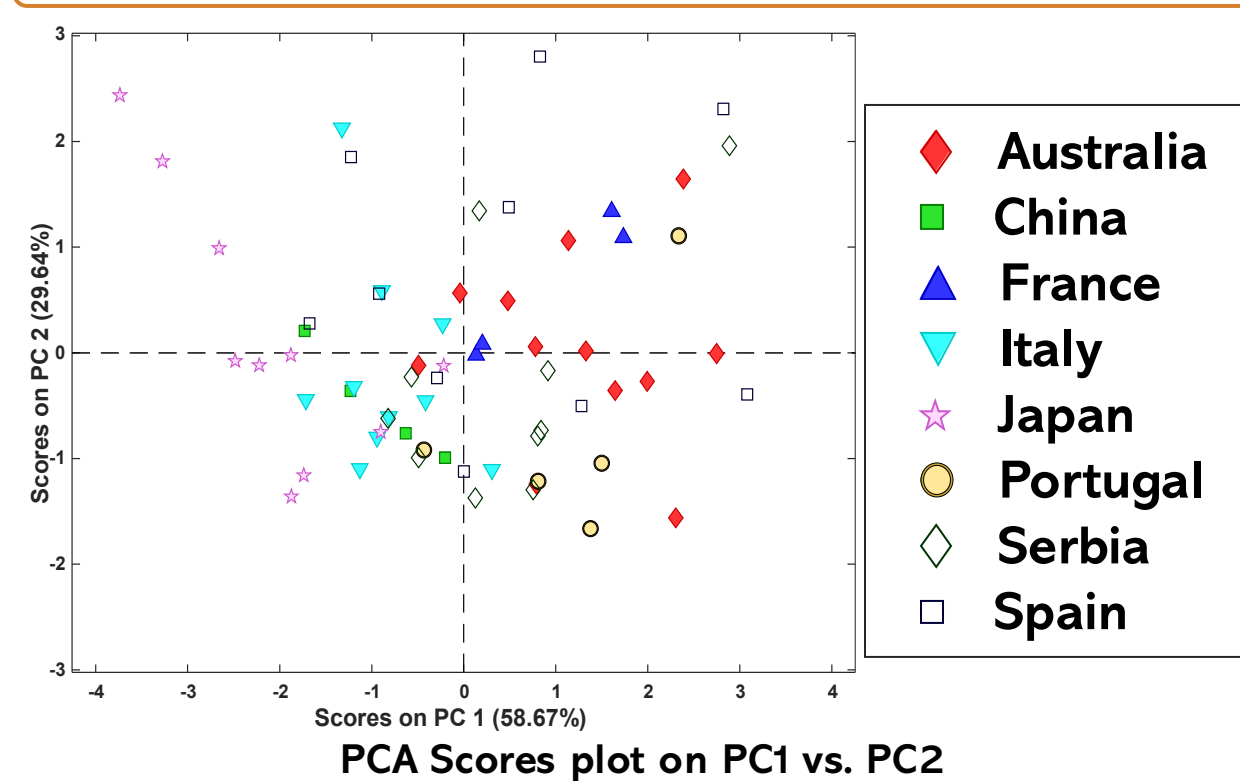
Solo 8.6
(Eigenvector Research)

Principal Component Analysis (PCA)

Partial Least Squares Discriminant Analysis (PLS-DA)

RESULTS & DISCUSSION

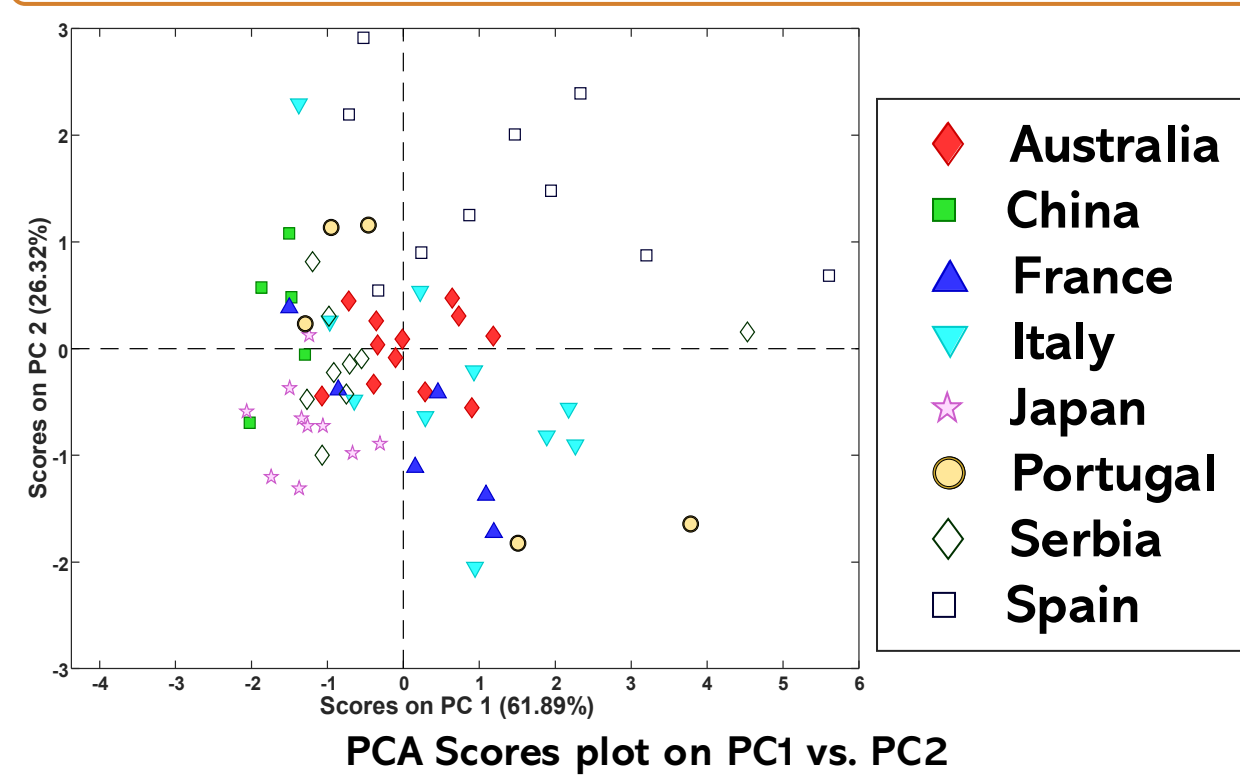
PHYSICOCHEMICAL PROPERTIES



PLS-DA results

Sensitivity (%): 70–100 (excluding Spain)
Specificity (%): 49.2–83.9 (excluding Serbia)
Classification error (%): 18.0–42.7 (excluding Spain)

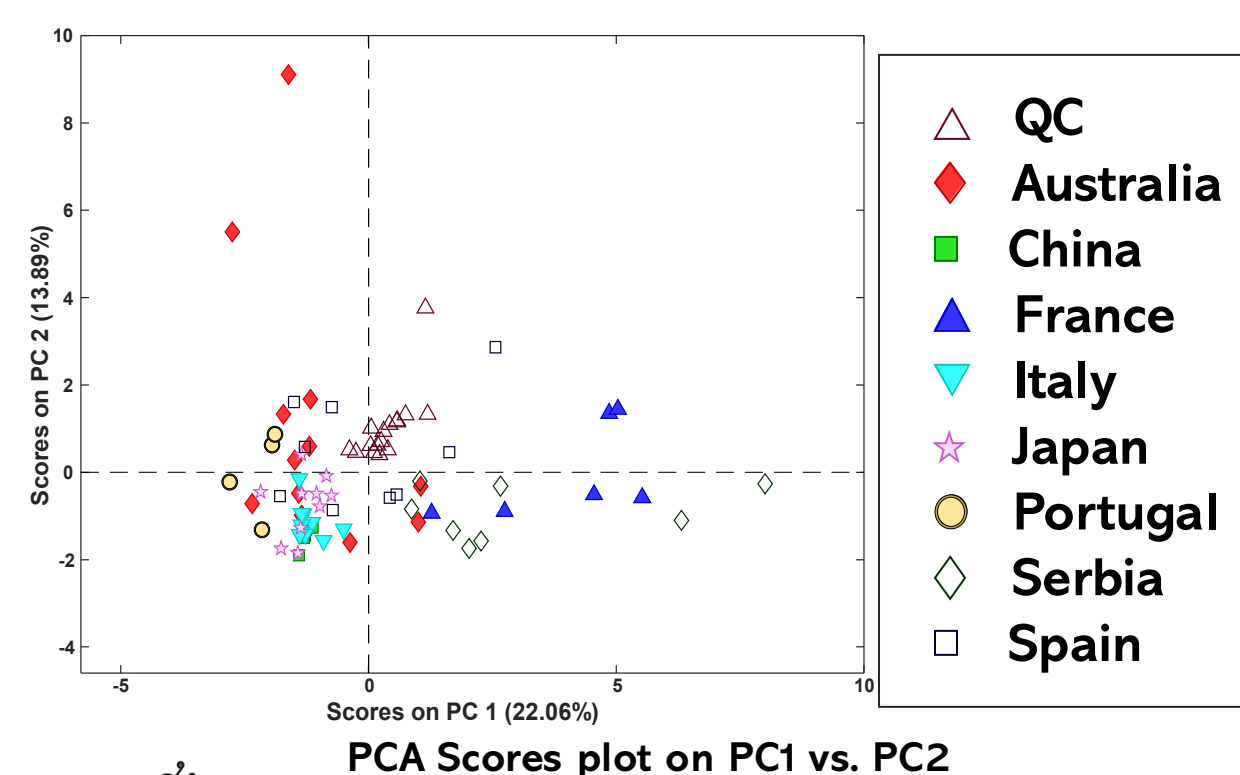
SPECTROPHOTOMETRIC ASSAYS



PLS-DA results

Sensitivity (%): 33.3–100 (excluding Italy and Portugal)
Specificity (%): 65.5–89.5
Classification error (%): 10.3–50.5

LC-MS/MS



PLS-DA results

Sensitivity (%): 66.7–100 (excluding Australia)
Specificity (%): 61.1–100
Classification error (%): 1.7–34.4

Some tendencies can be observed in the exploratory analyses and, in general, good values of classification figures were obtained, considering the complexity of the matrix and the simplicity of the measured parameters

CONCLUSION

A good classification performance was achieved using the measured properties (i.e. physicochemical parameters, spectrophotometric indexes, and LC-MS/MS profile), highlighting the potential of these variables to address honey geographical origin authenticity

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