Honey Authenticity: An Integrated Approach with FlavourSpec® (GC-IMS), Visible Spectroscopy (VIS), and Molecular Biology (digital PCR)

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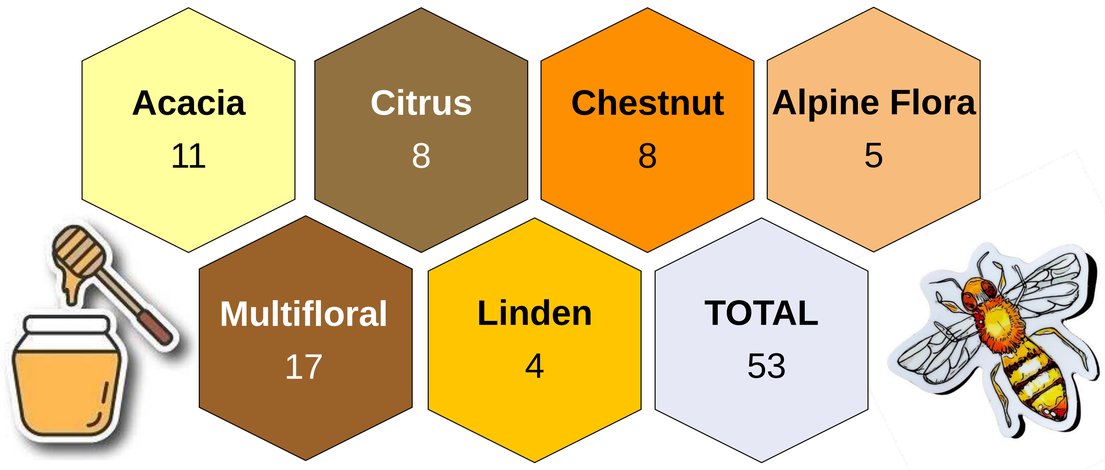
1. Objectives

1. **Verification of Honey Authenticity:** Botanical Origin
2. Comparison and Integration of **Complementary Analytical Techniques**
3. Identification of **Anomalous Samples (Outliers)**
4. **Screening to Support Traditional Analyses** with rapid and effective methods

2. Samples Analyzed



3. Integrated Multi-Instrumental Approach

a — FlavourSpec® (GC-IMS)

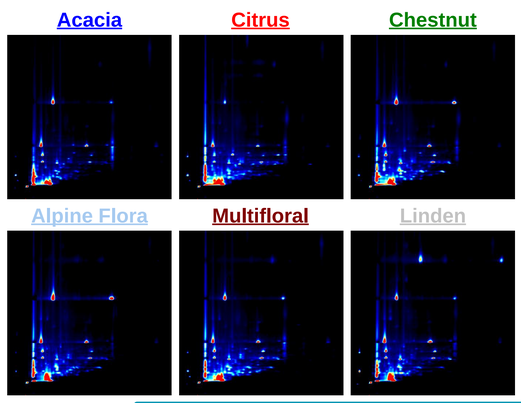
Chemical Fingerprinting of Volatile Compounds

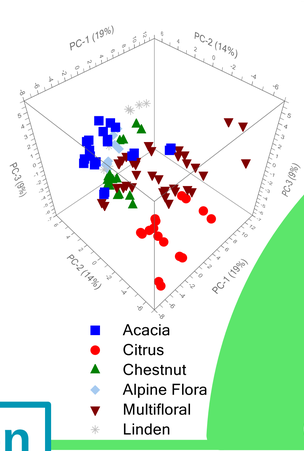
Materials and Methods

- **(2.00 ± 0.02) g** of raw honey in a sealed 20 mL vial, heated to **40°C for 8 minutes**
- Injection of V=0.5 mL of **static headspace**
- Analysis time: **36 minutes per vial**

Results

- Background noise elimination (**deNoise** + **Percentile** Modules, GAS Dortmund)
- Unique pseudo-images for each honey





b — VIS Diffuse Reflectance Spectroscopy

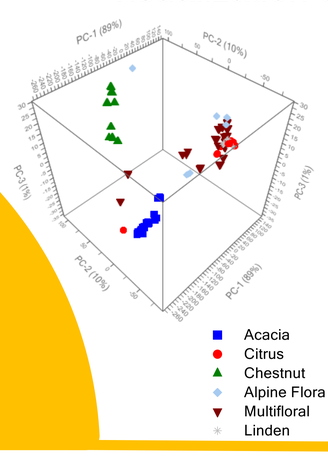
Chemical Fingerprinting of Raw Honey Pigments

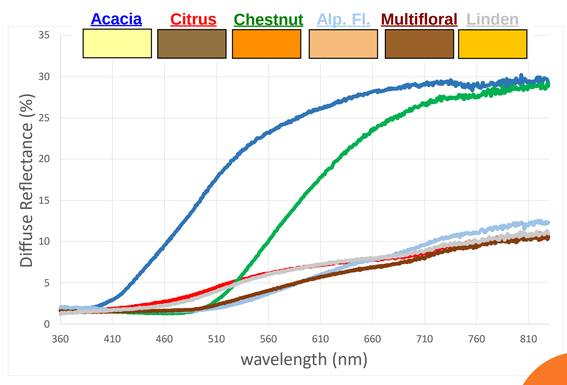
Materials and Methods

- Honey previously dissolved in a **water bath**, placed in a disposable plastic cuvette
- Spectral range: 360–830 nm, in **integrating sphere**
- Analysis time: **~ 5 seconds per cuvette**

Results

- Measurement of **diffuse reflectance % vs. nm**
- **Color analysis:** parameterization and visualization of raw honey (colorimetric spaces)





c — VIS Transmission Spectroscopy

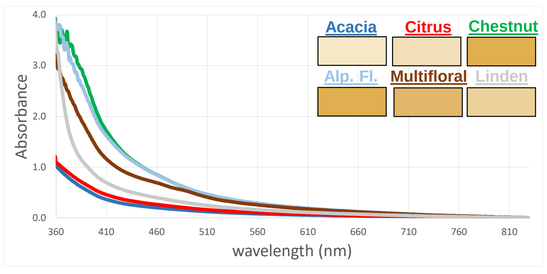
Chemical Fingerprinting of Honey Pigments Diluted in Water

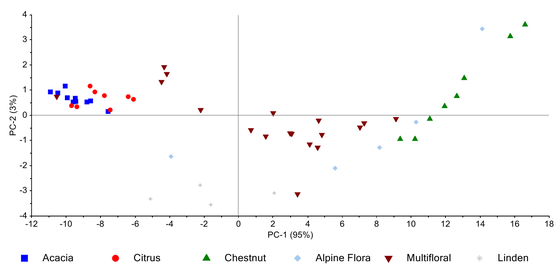
Materials and Methods

- 2 g of honey + 2 g of milliQ H_2O, **dissolved in a water bath**, placed in a disposable plastic cuvette
- Spectral range: 360–830 nm, in **transmission**
- Analysis time: **~ 5 seconds per cuvette**

Results

- Measurement of **absorbance vs. nm**
- **Color analysis:** shades of orange/brown of aqueous honey extracts (colorimetric spaces)





d — Digital PCR

Quantification of Plant DNA

Materials and Methods

- **Workflow**: DNA extraction; Amplification.
- **14 molecular markers** specific to certain plant species typical of honey: 2 plastidial + 12 nuclear.

Results

- The number of *housekeeping* gene copies is correlated to the amount of pollen in the analyzed honeys.
- In acacia honey, the percentage of species-specific DNA meets the standards.