



Introduction

Honey quality is evaluated, according to the *Codex Alimentarius*, by determining a number of physicochemical parameters (moisture, electrical conductivity, pH, free acidity, colour, sugar content and hydroxymethylfurfural content, diastase and invertase activities, etc.) using AOAC official methods, which are costly, long, tedious, and consume considerable amounts of solvents and reagents. To overcome these drawbacks, vibrational spectroscopy and chemometrics are proposed. Rapid and non destructive vibrational spectroscopic techniques allow to obtain information without performing any special sample preparation, and determine various physicochemical parameters in a single run. The objective of the present work is to develop a **novel rapid method based on FT-Raman spectroscopy and chemometrics to determine honey physicochemical parameters.**

Honey physicochemical parameters

□ **Moisture:** Water content is related with honey spoilage due to fermentation. Monofloral honey classification. Honey and honeydew discrimination. Official method: IRAM 15931 – Honey moisture content by refractrometry.

□ **Electrical conductivity** depends on the honey mineral content. Honey electrical conductivity is 0.06-2.17 mS/cm. Monofloral honey classification. Honey and honeydew discrimination. Official method: IRAM 15945.

□ **pH:** Honey pH values are 3.5 - 5.5 are due to organic acids responsible for honey flavour and microbiologic stability. Official method: IRAM 15938.

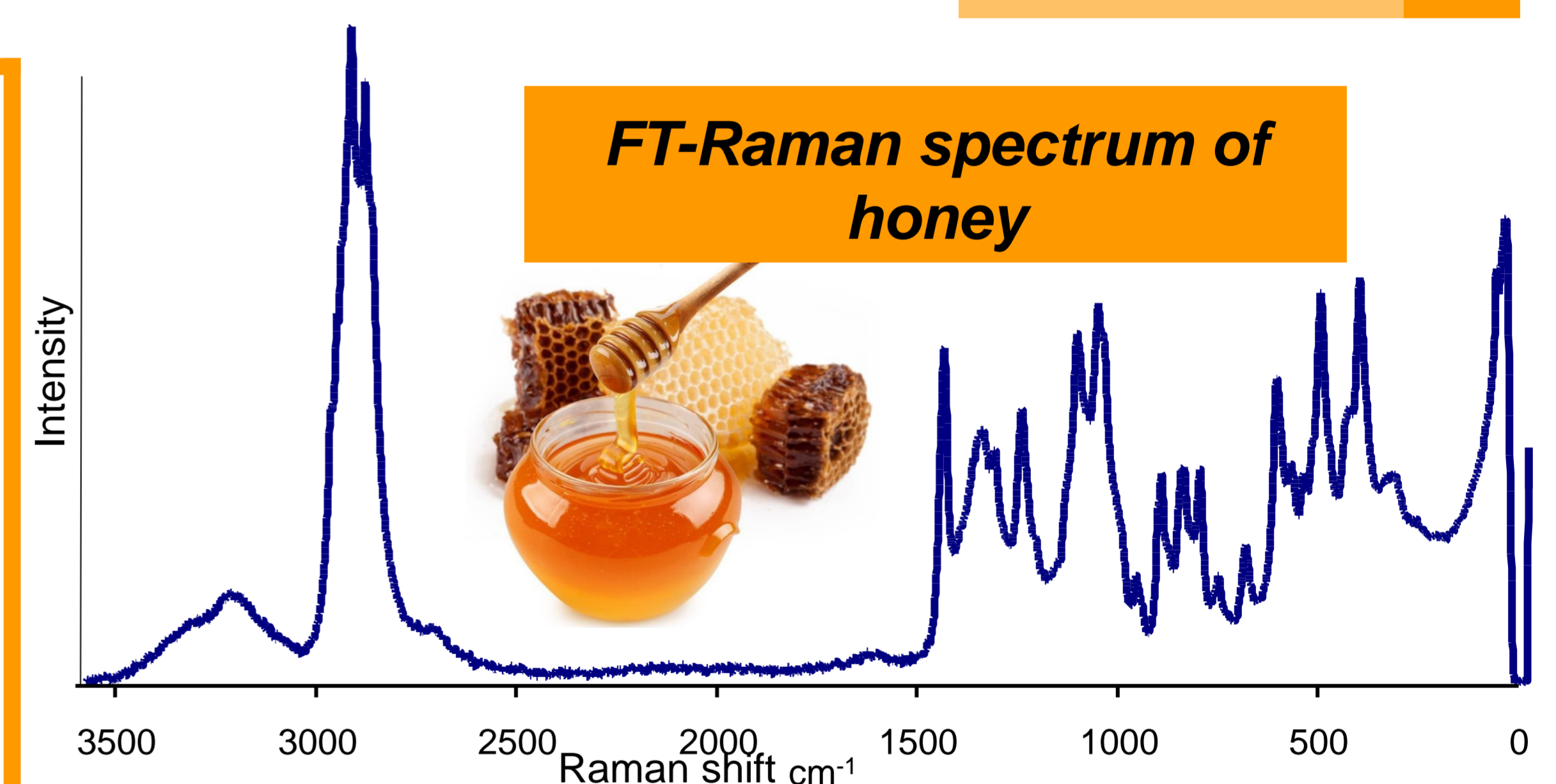
□ **Free acidity:** Gluconic acid is the major acid in honey. Monofloral honey classification. Official method: IRAM 15933.

□ **Colour:** Honey colour goes from white to different amber tones. Monofloral honey classification. Official method: IRAM 15941-2 – Honey Pfund colour.

□ **Sugars** are the major honey constituents, being about 95% of honey dry weight. Fructose (F) and glucose (G) contents and F/G ratio for monofloral honey classification. Sugar profile for honey and honeydew discrimination. Official method: IRAM 15946 – Honey sugar composition by HPLC.

Optimization of FT-Raman experimental conditions

Sample temperature (-80°C, -18°C, 4°C, RT, 40°C)	→	40°C	Optimised parameters
Laser power (600 mW, 700 mW, 800 mW)	→	800 mW	
Sample position on the Z axis (100, 105, 110, 120, 140)	→	105	
Resolution (4 cm ⁻¹ , 8 cm ⁻¹)	→	4 cm⁻¹	
Number of scans (8, 16, 32, 64, 128)	→	128 (~ 4 min)	



FT-Raman equipment: Vertex 70 – RAM II Bruker FT-Raman spectrometer, equipped with a Nd:YAG laser with an output at 1,064 nm (9,398.5 cm⁻¹), and a liquid-nitrogen cooled Ge detector. The measurement accessory is pre-aligned, only the Z-axis of the scattered light is adjusted to set the sample in the appropriate position regarding the local point. Samples are placed in classical glass tubes of an internal diameter of 12 mm and a length of 75 mm, which are introduced into a dedicated sample Al holder developed at the CRA-W to assure repeatable position of the sample in front of the laser beam. The sample holder is placed in the sample compartment.

Multivariate data analysis: Partial Least Squares regression

PLS regression model	Y range	Preprocessing	PLS comp	R-cal	R-val	RMSEP	% R-RMSEP
Fructose (g/100g honey)	30 - 43	full MSC + centering	5	0.811	0.786	0.938	3.1 - 2.1
Glucose (g/100g honey)	22 - 39	full MSC + centering	5	0.793	0.767	1.586	7.2 - 4.1
F/G ratio	1.0 - 1.7	full MSC + centering	6	0.787	0.750	0.061	6.3 - 3.6
F+G	55 - 78	full MSC + centering	5	0.848	0.827	1.793	3.3 - 2.3
Turanose (g/100g honey)	2.0 - 3.5	full MSC + centering	5	0.793	0.764	0.349	17 - 10
Maltose (g/100g honey)	2.4 - 6.1	full MSC + centering	6	0.839	0.803	0.600	25 - 10
Sacarose (g/100g honey)	1.5 - 3.7	full MSC + centering	8	0.907	0.647	0.390	26 - 10
Erllose (g/100g honey)	1.7 - 3.1	full MSC + centering	5	0.804	0.756	0.417	25 - 13
Moisture (%)	14 - 22	full MSC + centering	6	0.843	0.814	0.722	5.0 - 3.3
Free acidity (meq/kg honey)	11 - 25	full MSC + centering	4	0.769	0.752	5.7	23 - 7.9
Colour (mm Pfund)	45 - 121	full MSC + autoscaling	2	0.881	0.879	10.5	23 - 8.7
Electrical conductivity (microS/cm)	359 - 990	full MSC + centering	6	0.860	0.827	92.4	26 - 9.3

Samples: 313 honeys (2013 & 2014)
X matrix: FT-Raman spectra (3734 variables)
Y matrix: Physicochemical parameters
3-fold Cross-validation

Multivariate data analysis:

Software: The Unscrambler 9.1 (Camo Process AS, 1986-2004)

- FT-Raman spectra of honey contain information related **honey physicochemical parameters.**
- PLS models for **Fructose, F/G ratio** and **F+G** perform **better** than classical analytical methods.
- PLS models for **Glucose** and **Moisture** present **satisfactory** %R-RMSEP.
- PLS models for di- and tri-saccharides, free acidity, colour and electrical conductivity only present acceptable % R-RMSEP in the upper range of the calibration curve → More samples and further modelling is required.