Optimization of FTIR-ATR spectroscopy for beeswax adulteration detection

Lidija Svečnjak, Ivan Mihaljević

University of Zagreb Faculty of Agriculture,

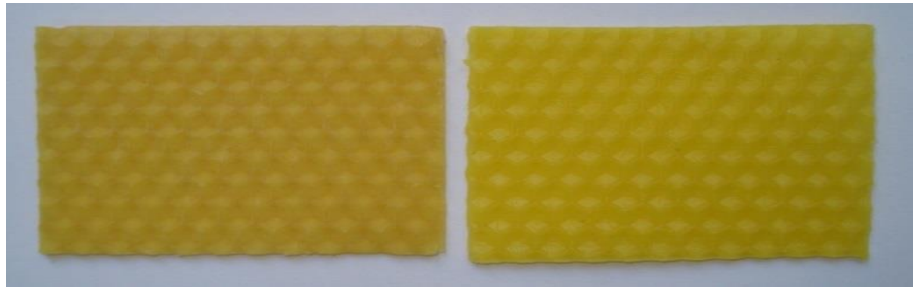
Laboratory for bee products analysis and bee biology,

Zagreb, Croatia

Beeswax adulteration issue

- long-present and **growing problem**
- still no internationally defined quality criteria and analytical methods for routine beeswax authenticity control - apiculture sector

- current analytical methods proposed by ***IHC:***
  - beeswax composition criteria for routine testing based
    - 10 classical **physico-chemical parameters** determined in accordance with EP and DGF
    - temperature > 100 °C - anomalous values
- alternative analytical methods:
  - gas chromatography - mass spectrometry (GC/MS analysis)
  - sensory analysis

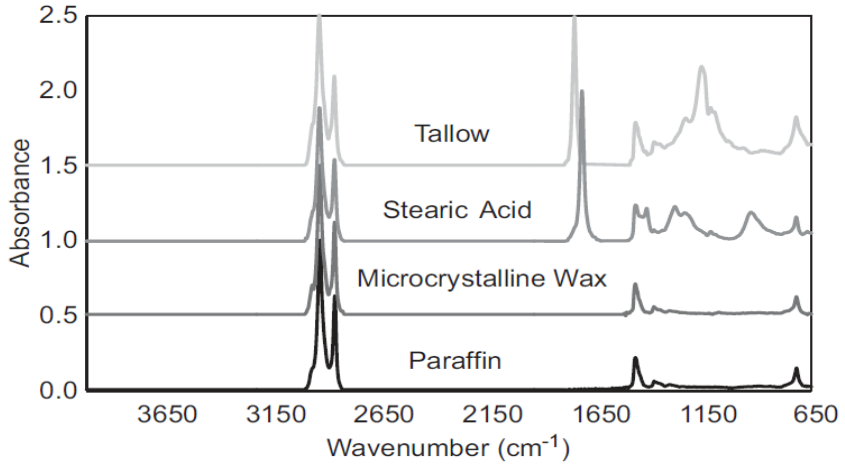


Genuine comb foundation

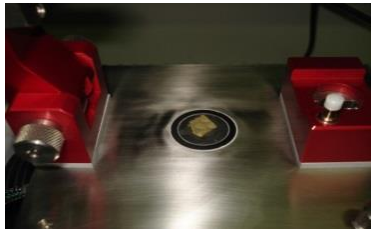
Adulterated comb foundation (paraffin)

FTIR-ATR spectroscopy and beeswax adulteration detection

- The most recently developed analytical tool
- ***Maia et al. (2013)*** - feasibility study revealed good detection limits (≤ 5%) for:
  - paraffin
  - microcrystalline wax
  - tallow
  - stearic acid



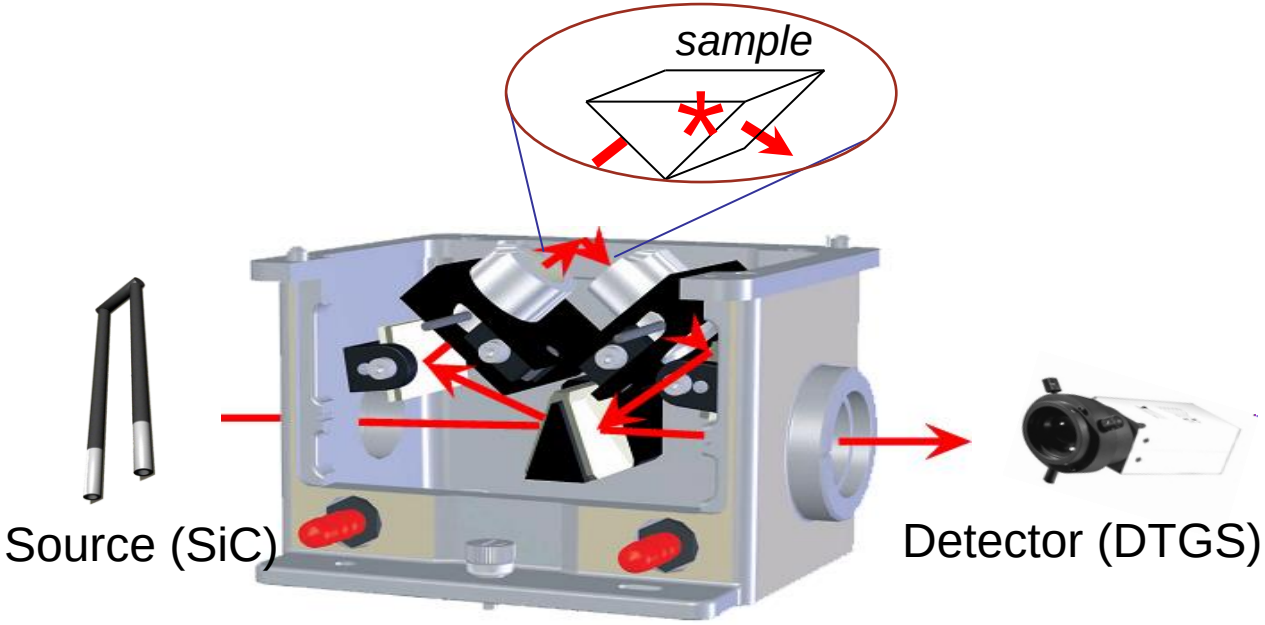
- ***Svečnjak et al. (2015)*** - established a procedure that enables rapid beeswax adulteration detection - research focused on bringing the method to the level of applicability for beeswax routine quality control
- ***Svečnjak et al. (2019) - BEEBOOK -*** optimization in terms of both qualitative and quantitative analytical procedure

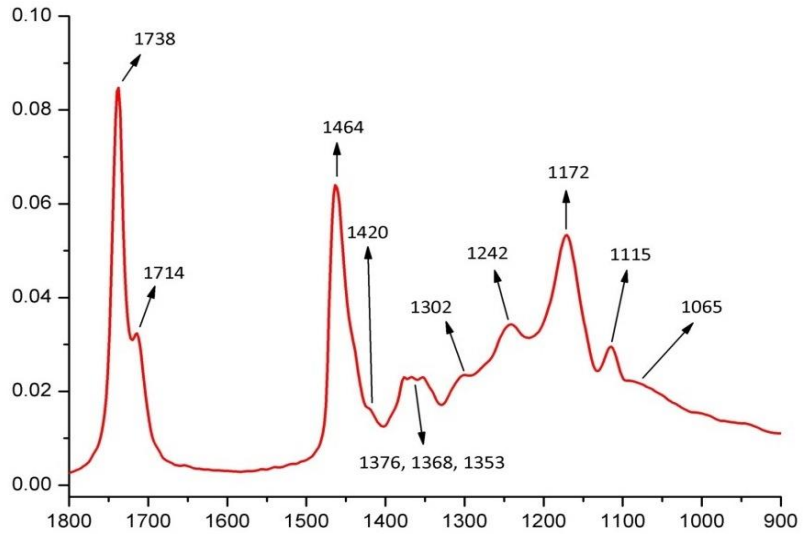


Comb foundation sample prepared for the acquisition of its IR spectrum

Advantages of FTIR-ATR spectroscopy

- Rapid and cheap
- Reagent-free method
- Non-destructive
- Easy-to-use
- Requires small amount of a sample (~ 0.03 g)
- Information on the total chemical composition of a sample - chemical fingerprint
- Suitable for detection of all adulterant types
- Reliable - accuracy, precision, detection limits (<3%)



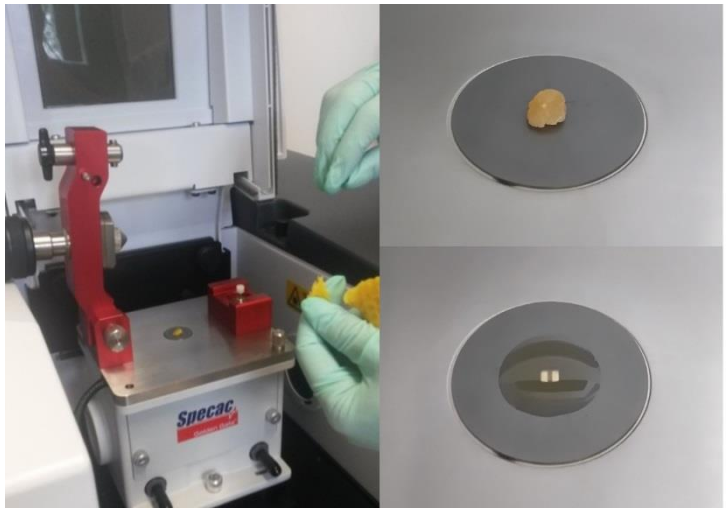


IR spectrum (chemical fingerprint)

Calibration procedure

Generating IR spectral database of the reference samples

- Preparation of in-house reference standards (RS):
  - sampling of the reference specimens
    - genuine beeswax (wild-built combs from the hives)
    - adulterants - paraffin, tallow, stearic acid, carnauba wax, etc.
  - preparation of adulterant - beeswax mixtures (ABM)
    - containing different proportions of adulterants (10, 20, 30… 100 %)
      - precise weighting (w/w)
      - melting and homogenisation (3 hours at 90 °C)
      - re-solidification, storage
  - acquisition of IR spectra of prepared RS
    - samples in liquid form - heating of ATR plate at 75 °C
    - spectral range: 4000 - 400 cm^{-1} (mid-IR region)
    - resolution: 4 cm^{-1}; 64 scans



Calibration procedure

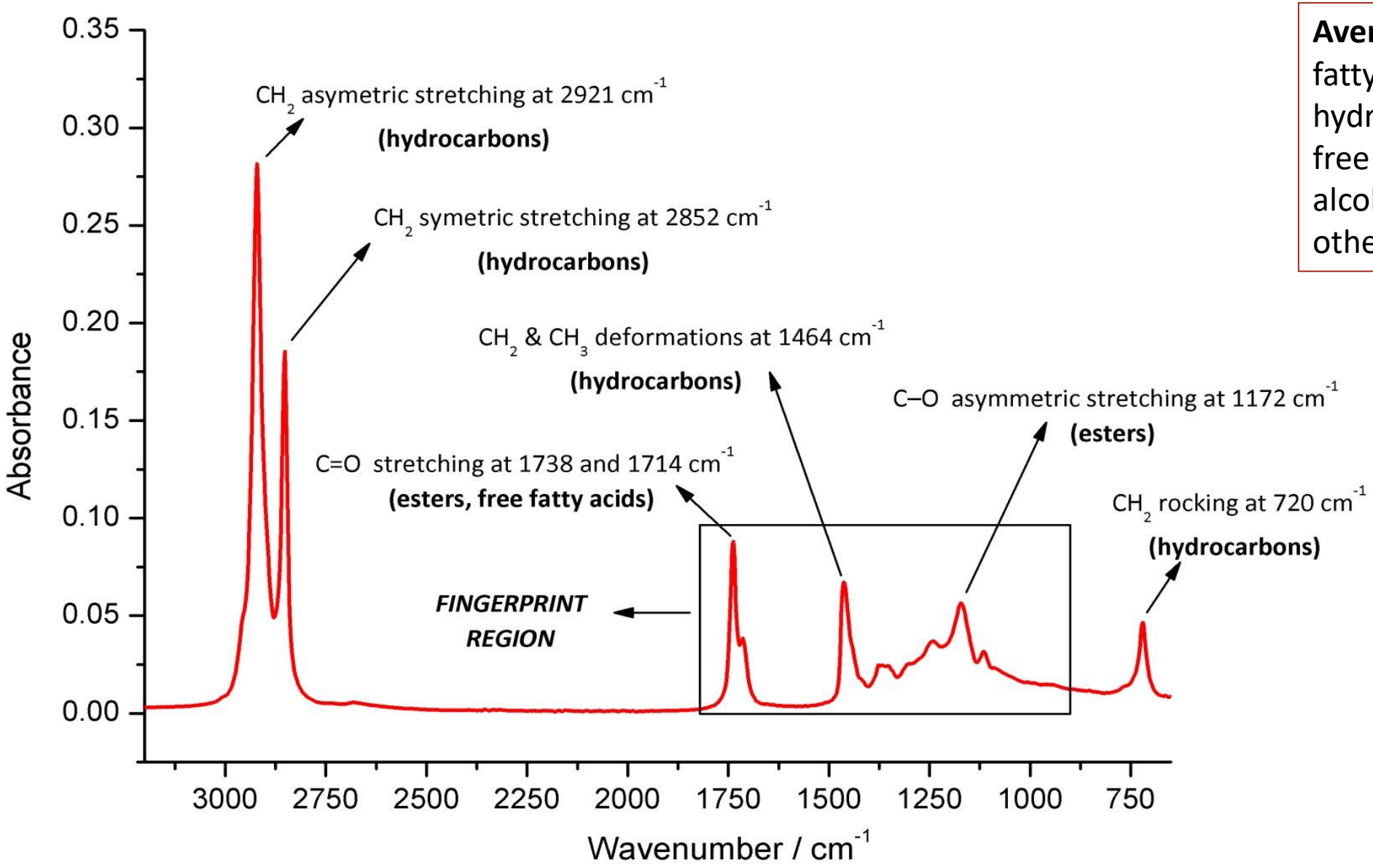
Detection of adulterants using IR spectral data

- Visual exploration of spectral features - beeswax, adulterants, ABM
  - identification of adulterant - specific spectral region(s) of interest indicative for adulteration detection

- Construction of a calibration curve using the spectral data of RS
  - Estimation of the coefficient of correlation (R)
  - Selection of spectral region (peak areas) showing the best correlation effects
    - $R \geq 0.998$
  - Generating a calibration curve based on the best correlation results
  - Statistical (linear regression) analysis - prediction strength (R^2) and error (SE)
    - $R^2 \geq 0.997$, $SE < 0.05\%$

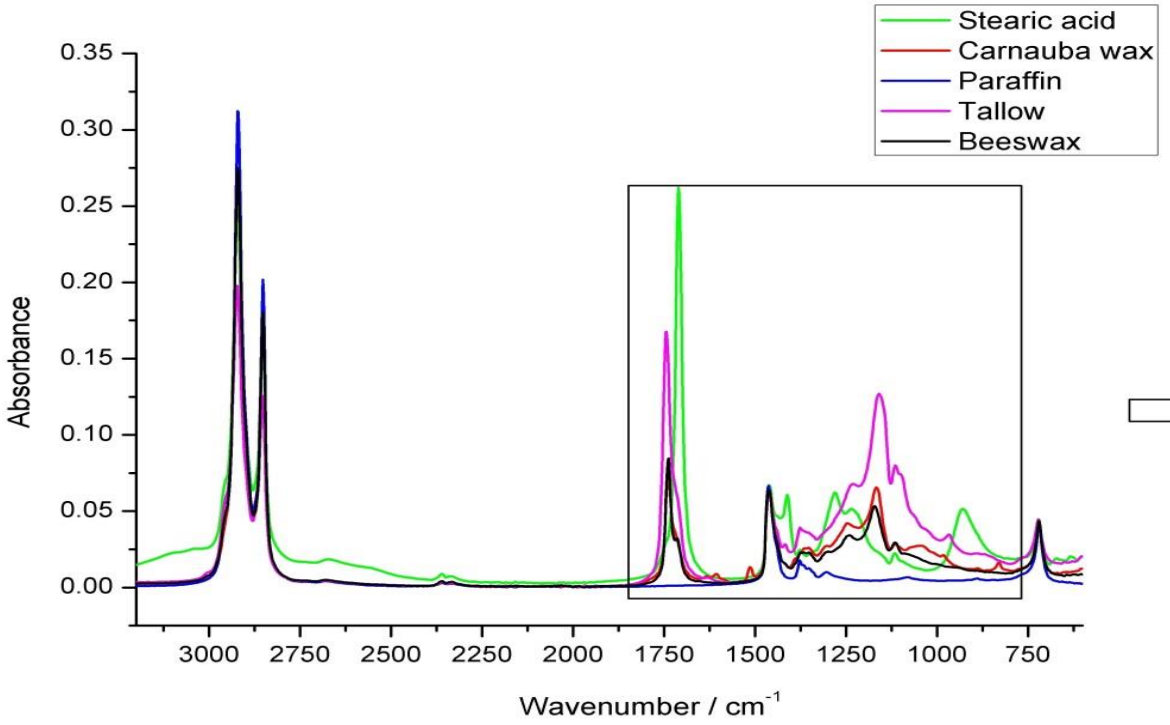
- Detection / quantification of adulterants in test samples (unknown chemical background) - market beeswax samples (comb foundations, wax blocks)

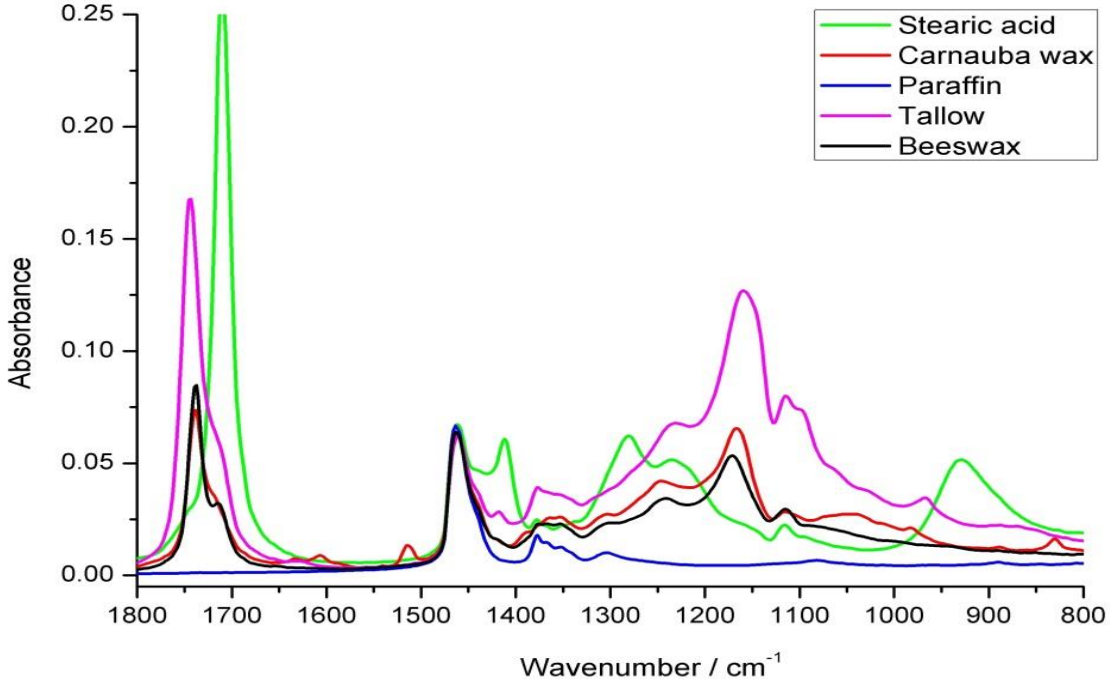
Average beeswax composition:
fatty acid esters (~67%)
hydrocarbons (~14%)
free fatty acids (~13%)
alcohols (~1%)
other components (~5%)



Characteristic FTIR-ATR spectrum of *A. mellifera* beeswax with assignation of underlying molecular vibrations (absorption bands)

Comparative spectral features: beeswax vs. selected adulterants





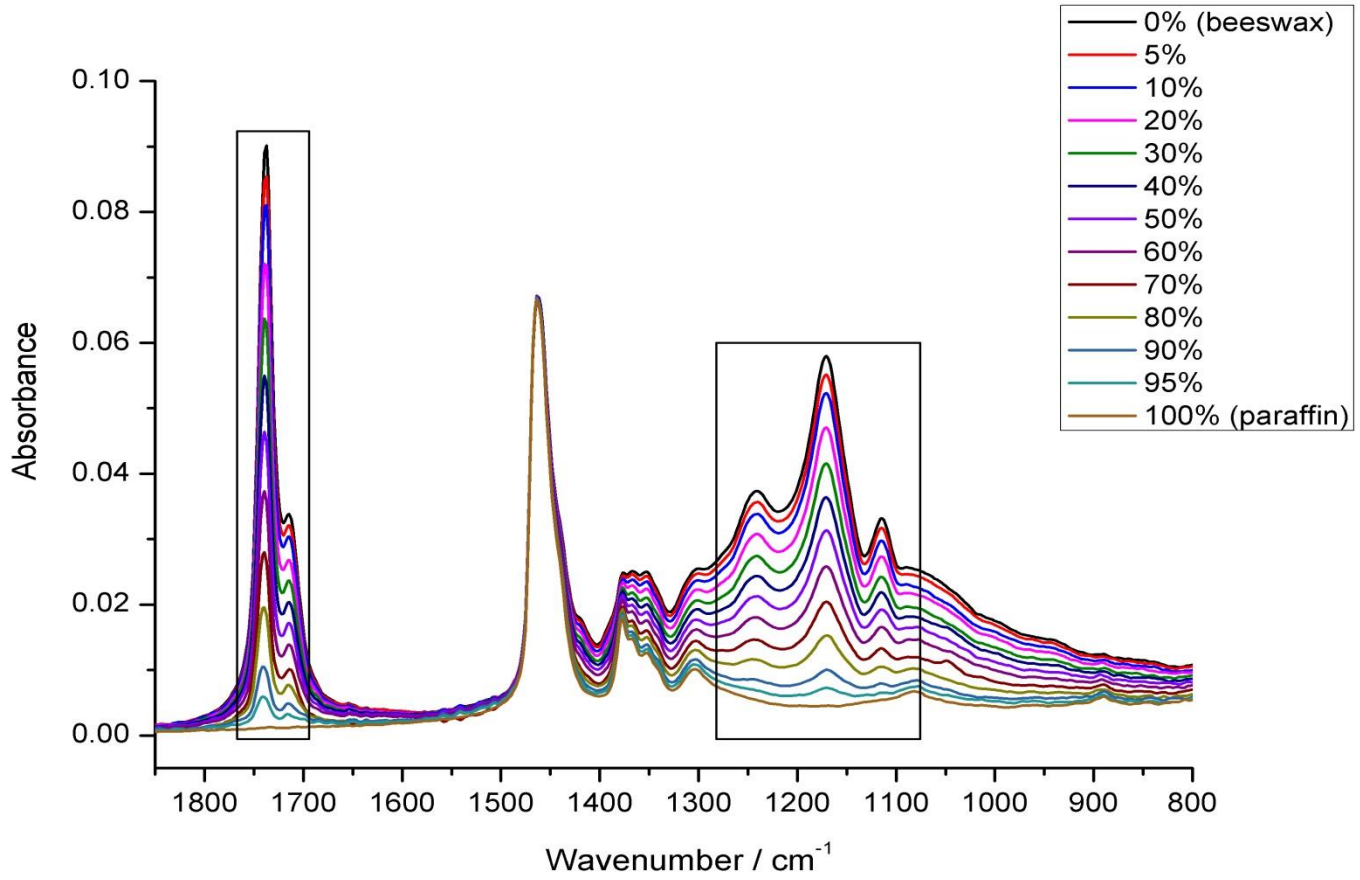
Paraffin wax

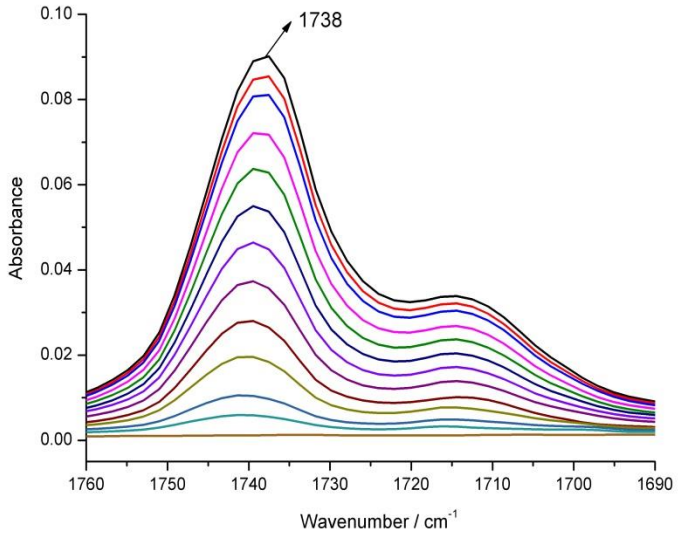
Stearic acid

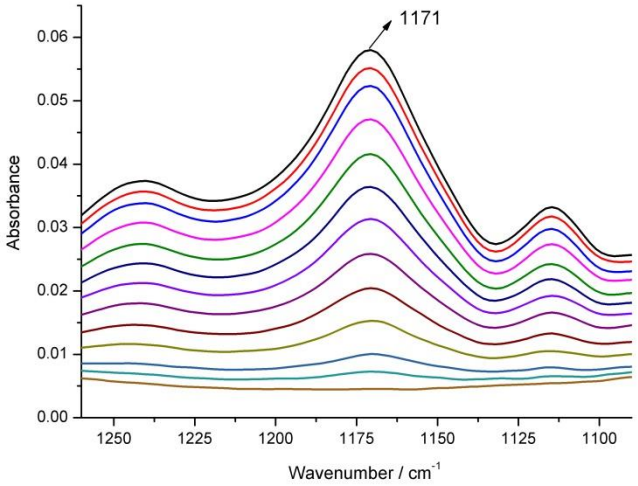
Beef tallow

Carnauba wax

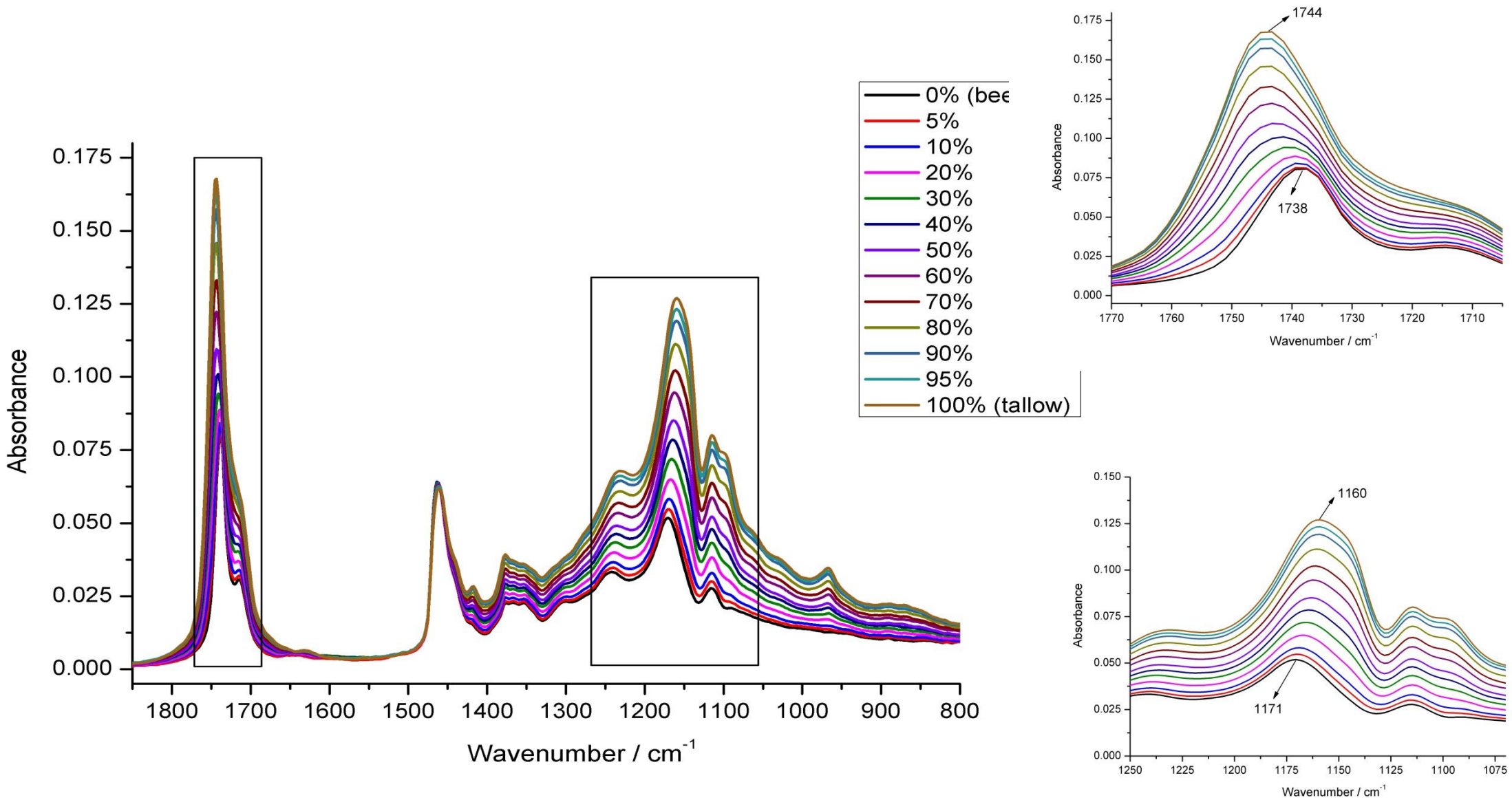
Paraffin - beeswax mixtures



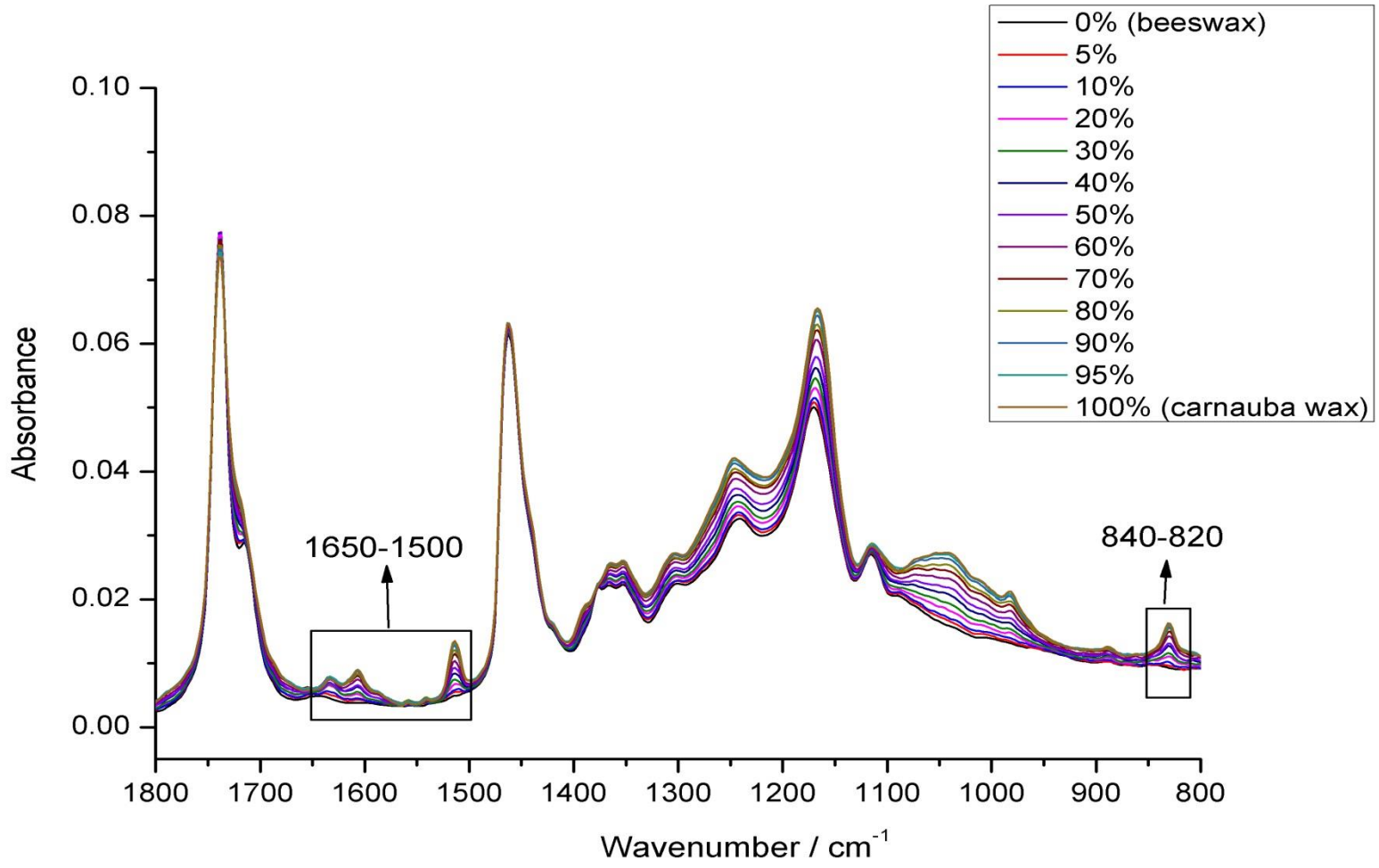


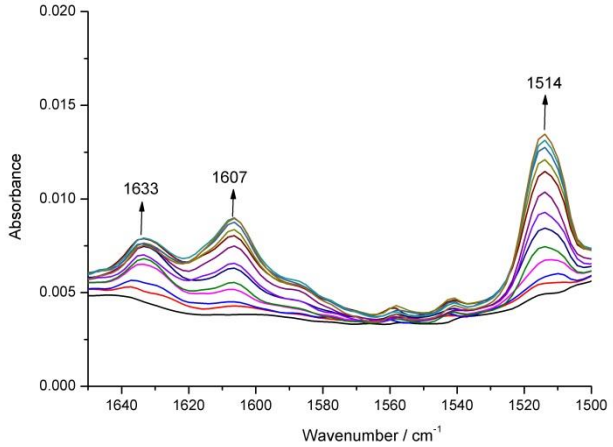


Tallow - beeswax mixtures

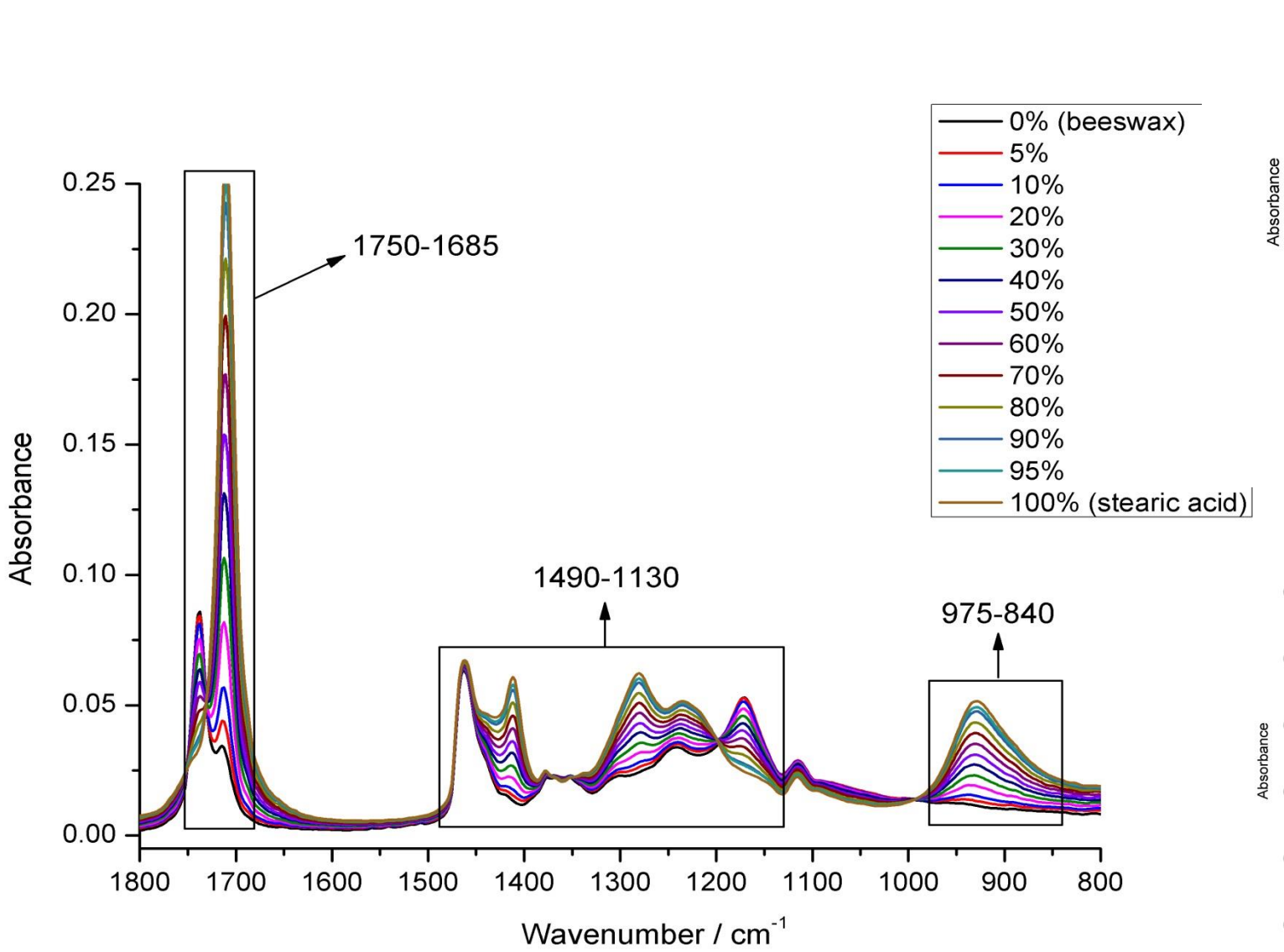


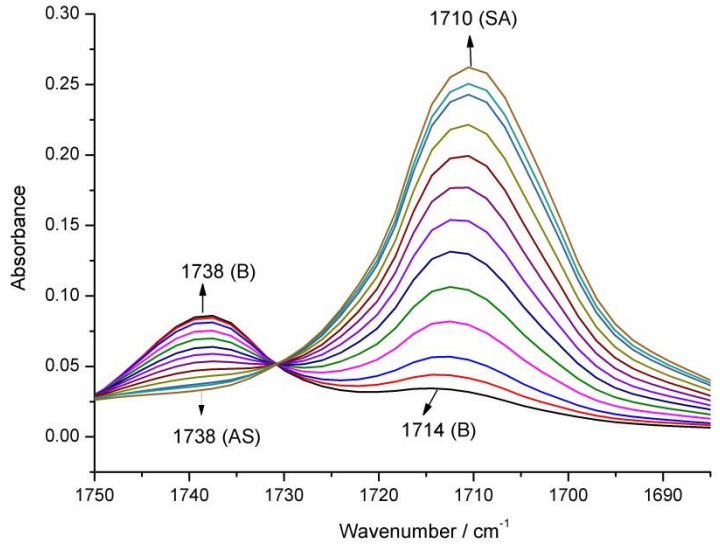
Carnauba wax - beeswax mixtures

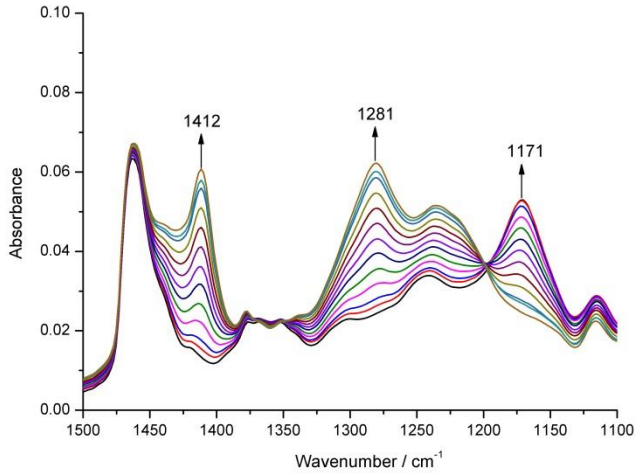




Stearic acid - beeswax mixtures

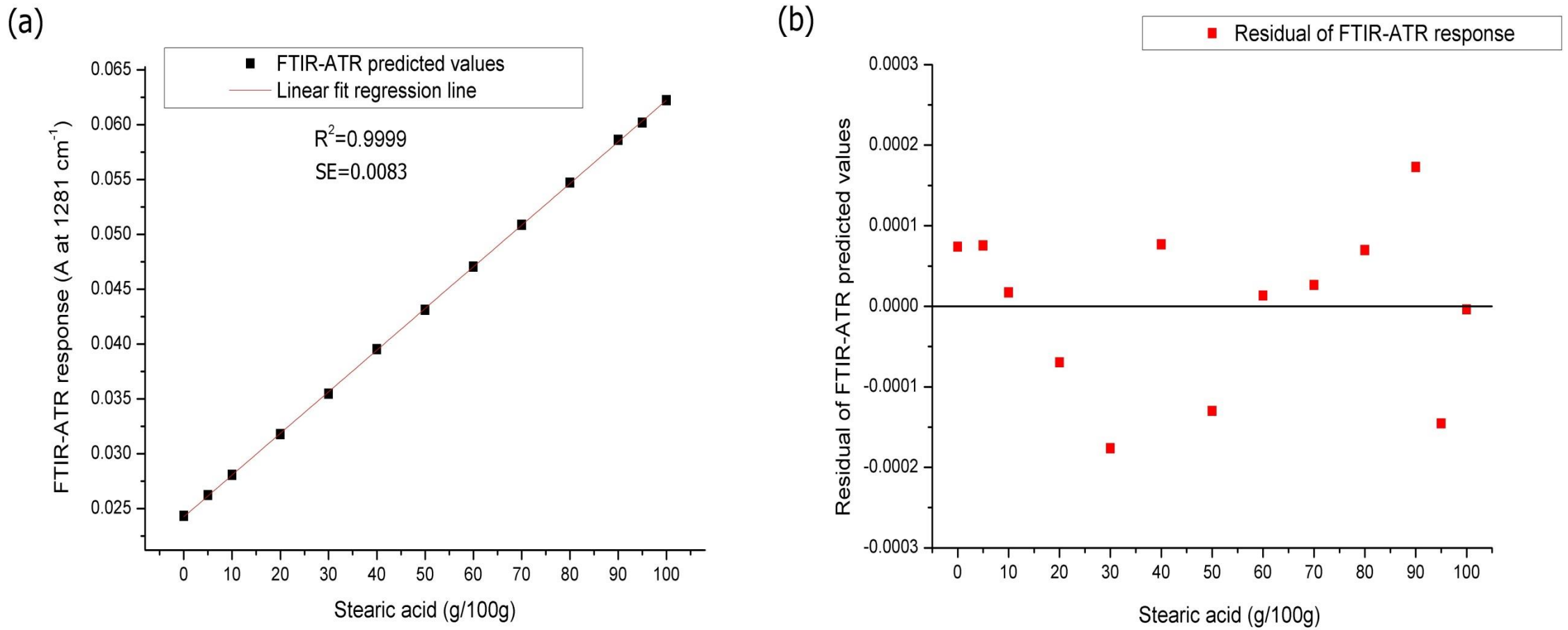






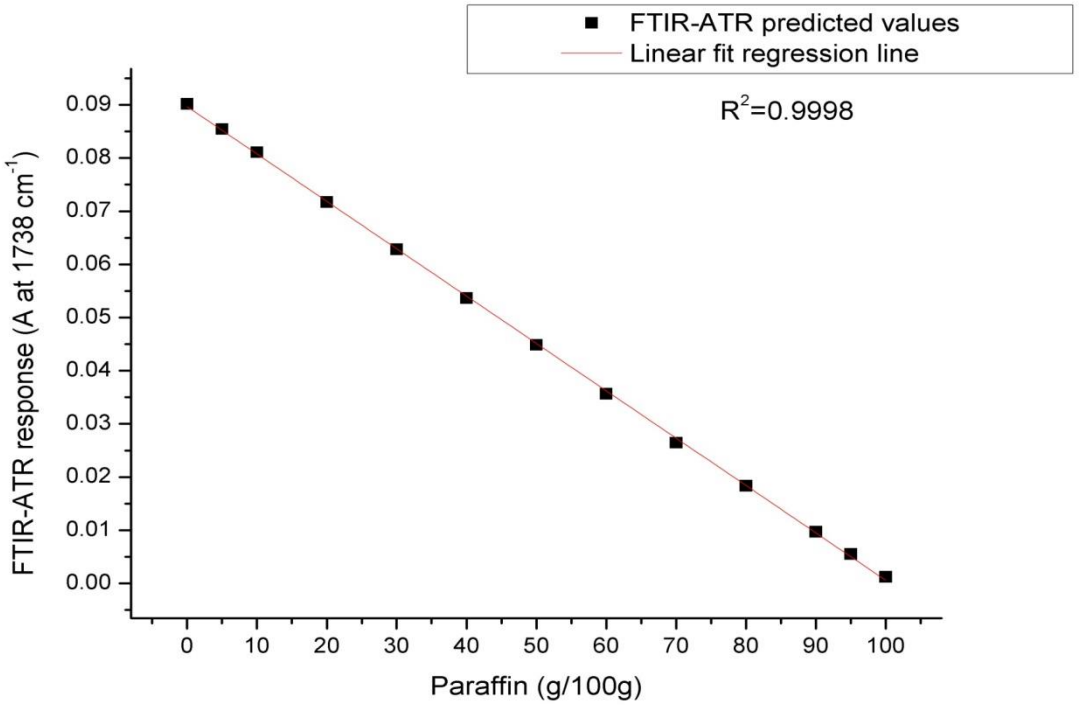
Reference standard (adulterant-beeswax)	**Reference peak (cm^{-1})**	**Peak area (cm^{-1})**	**Predominant beeswax compound associated to reference peak**	**Correlation coefficient (R)**
Paraffin	1738	1750-1727	monoesters	***0.9999****
	1171	1195-1147	esters of aliphatic acids	***0.9998*** **
Tallow	1738	1753-1724	monoesters	0.9969
	1171	1195-1148	esters of aliphatic acids	***0.9995****
Stearic acid	1738	1747-1730	monoesters	0.9971
	1710	1721-1707	free fatty acids	0.9982
	1412	1423-1400	esters (shoulder)	0.9989
	1281	1308-1253	free fatty acids	**0*.9996****
	929	978-880	none	0.9983
Carnauba wax	1633	1638-1628	none	0.2609
	1607	1610-1603	none	0.9859
	1514	1523-1506	none	***0.9995****
	830	740-820	none	0.9889

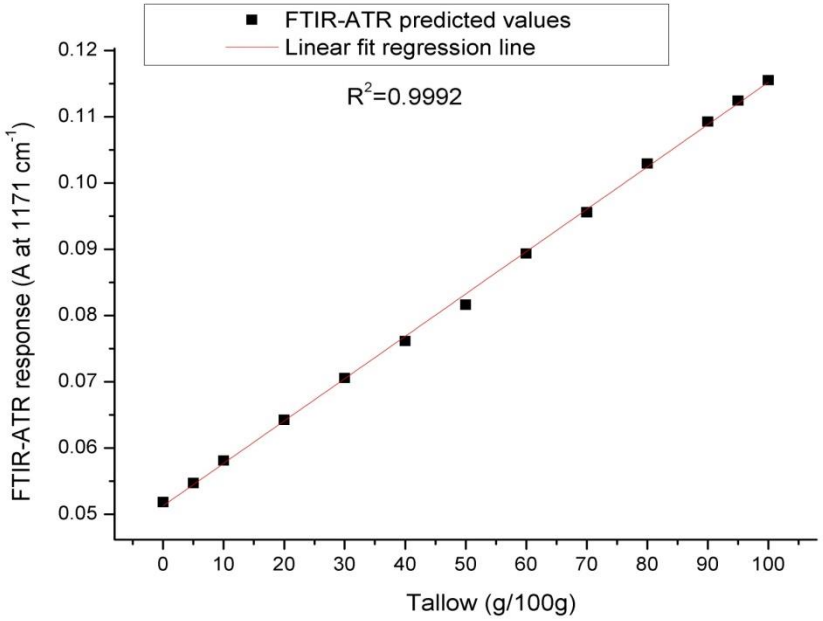
Determination of a correlation coefficient conducted on the target peaks of indicative spectral regions of four different adulterant - beeswax mixtures

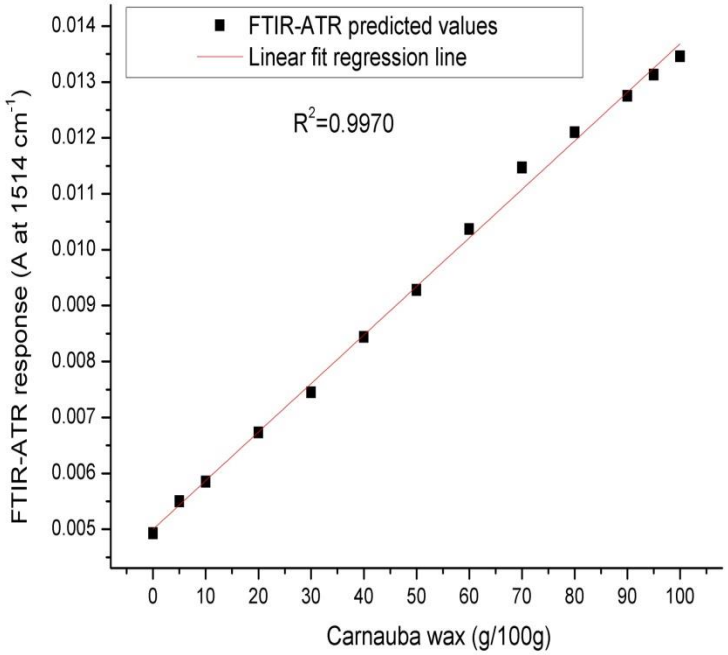


(a) Prediction performance parameters of the calibration curve constructed for determination of the stearic acid share in beeswax: a scatter plot of instrument response data (FTIR-ATR predicted values) vs. real stearic acid share values;
(b) residuals of FTIR-ATR prediction

Prediction performance parameters of the calibration curve constructed for determination of paraffin, beef tallow and carnauba wax

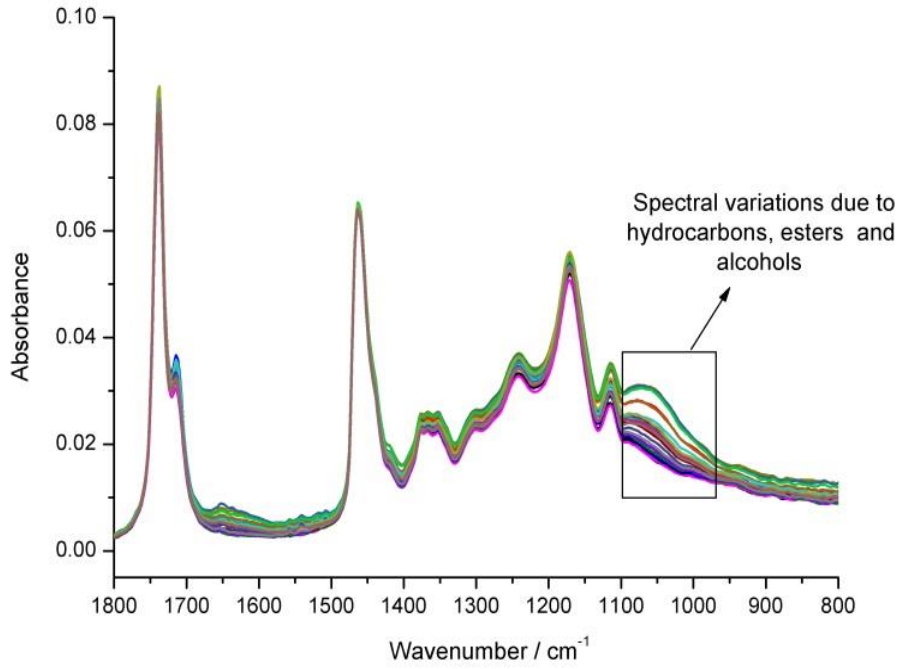


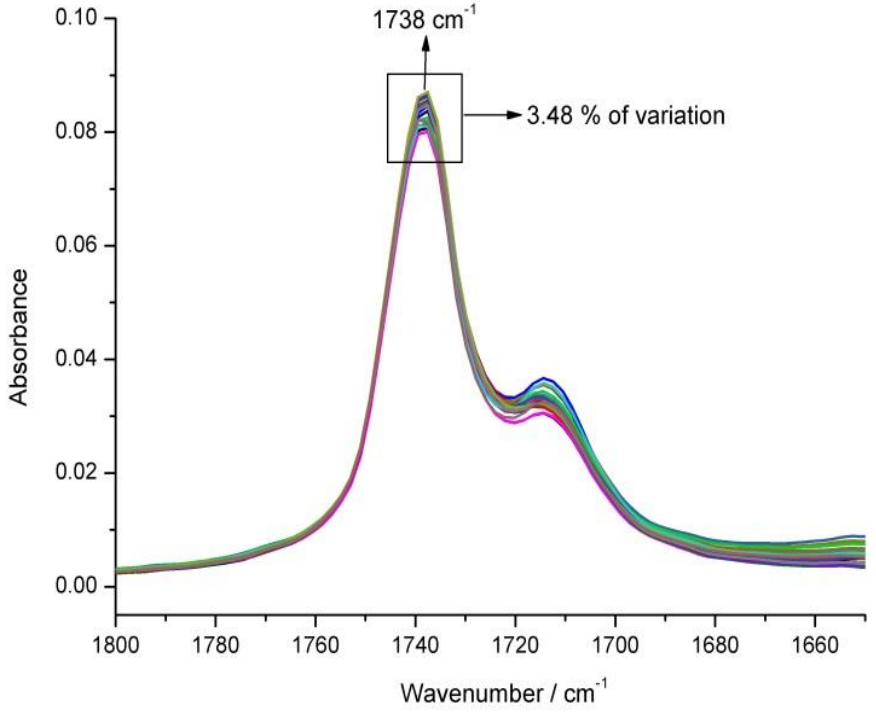




Final interpretation of results

- Complete result should include an estimate on (along with $R^2 \geq 0.997$, SE< 0.05%):
  - Measurement uncertainty arising from the sample
    - minor spectral variations identified in different genuine beeswax samples (3.5% var.)
  - Instrument related measurement uncertainty
    - repeatability (<0.5%)





Thank You for Attention