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QUANTIFICATION OF BEESWAX ADULTERANTS VIA FT-IR AND ^{1}H-NMR SPECTROSCOPY

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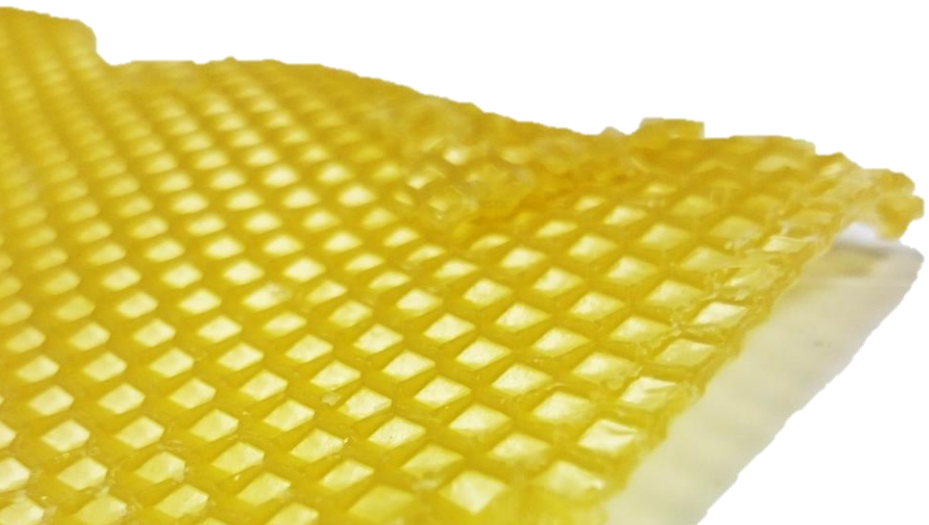
BEESWAX ADULTERATION AND TESTING - CURRENT STATUS

THE ISSUE

- Unintended and intended adulterations of beeswax observed
  - Paraffins, stearic acid, or tallow
- Impact on health of bee population
  - Stearic acid harm bees (Reybroeck, 2018)
- Adulterated comb foundations may impact composition of honey (Svečnjak *et al.*, 2019)



THE REGULATIONS & METHODS

- Ph. Eur. 9.0

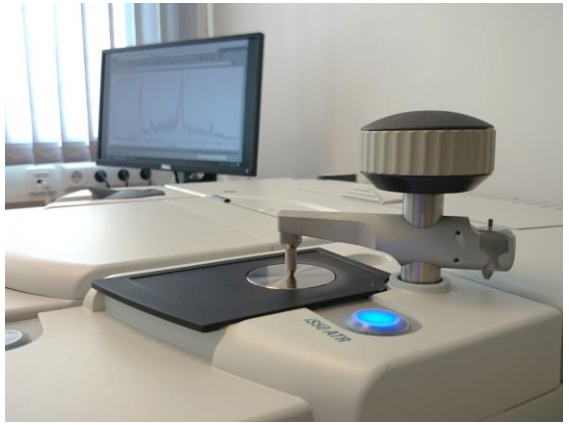
  Commission Regulation (EU) No. 231/2012

  RAL-GZ 041 (Quality Assurance for Candles)
  - Melting point, acid value, saponification value, ester value, total hydrocarbon content […]
- Further testing methods (e.g. GC)
- Most methods demand time, space, and capacity

	Paraffin	Stearic acid	Tallow
	54–56 °C		
Density	10	3	20
Melting temperature	30	30	40
Acid value	10	2	10
Saponification value	10	3	15
Ester value	5	5	10
Ratio number	10	15	10

adapted from Bernal *et al.*, 2005; unit % of adulteration

THE PROJECT



Techniques used

- FT-IR-ATR spectroscopy
  - Feasibility already demonstrated (e.g. Maia *et al*., 2013; Svečnjak *et al*., 2015)
  - Straight-forward approach
- ^{1}H-NMR spectroscopy
- (GC-FID)

Samples

- Beeswaxes: Collaboration partner, routine samples
- Adulterants: Paraffins, stearic acid, palmitic acid, tallow, (carnauba wax)

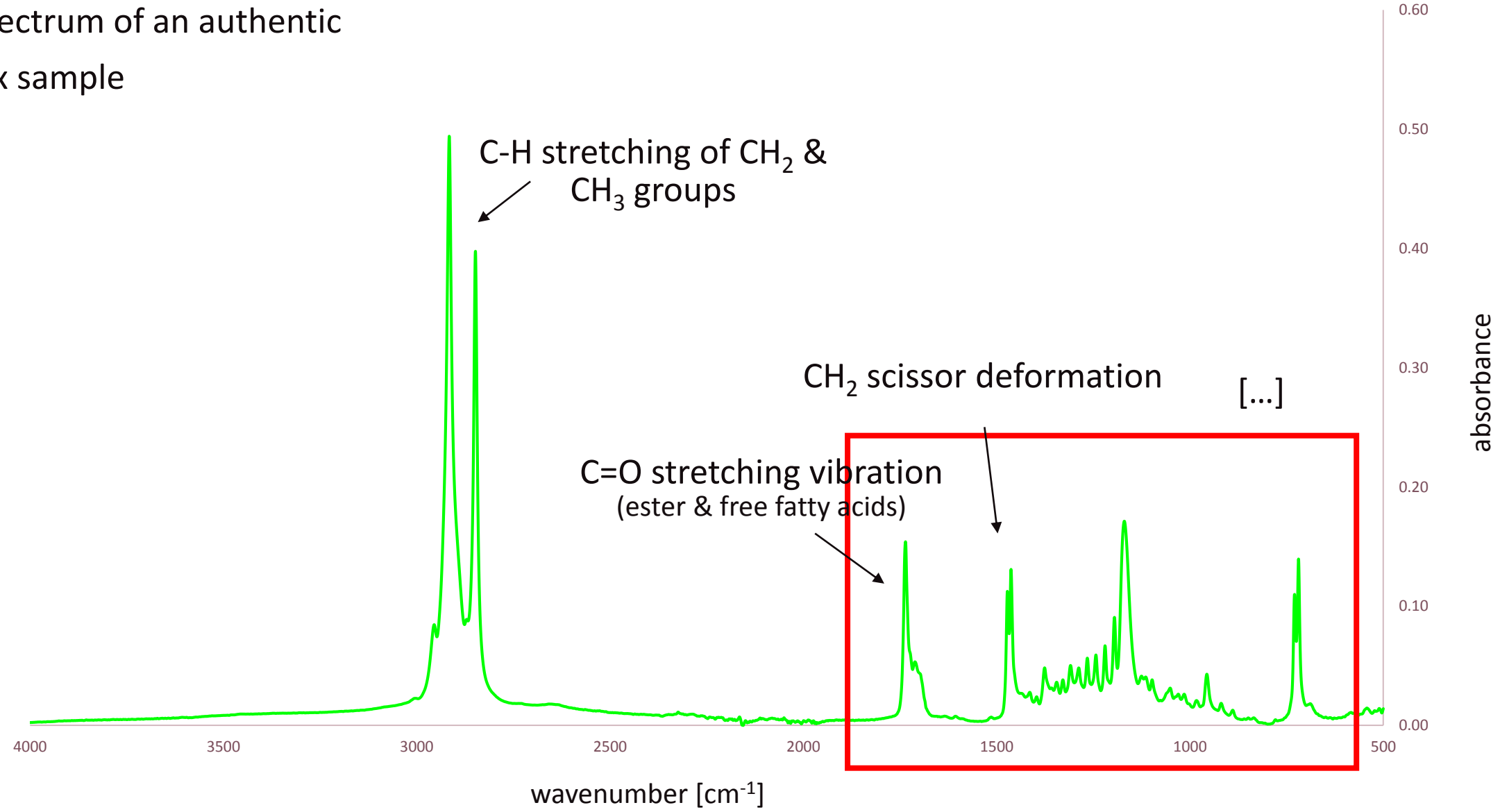
Statistics

- Partial least squares (PLS) regression models

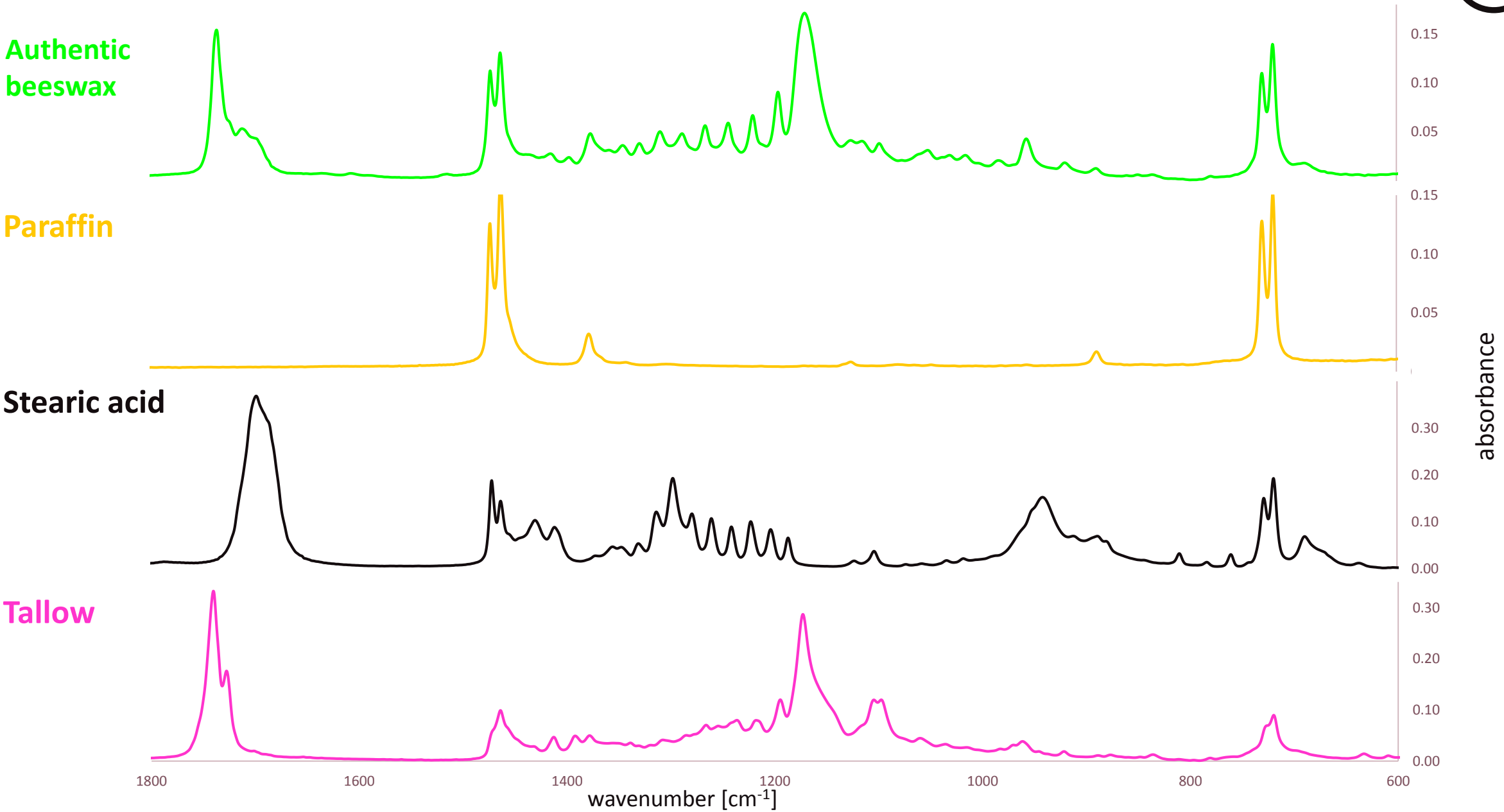
Validation and accreditation

QUANTIFICATION OF COMMON BEESWAX ADULTERANTS VIA FT-IR

FT-IR spectrum of an authentic beeswax sample



SPECTRAL DATA OF AUTHENTIC BEESWAX AND ADULTERANTS (FT-IR)



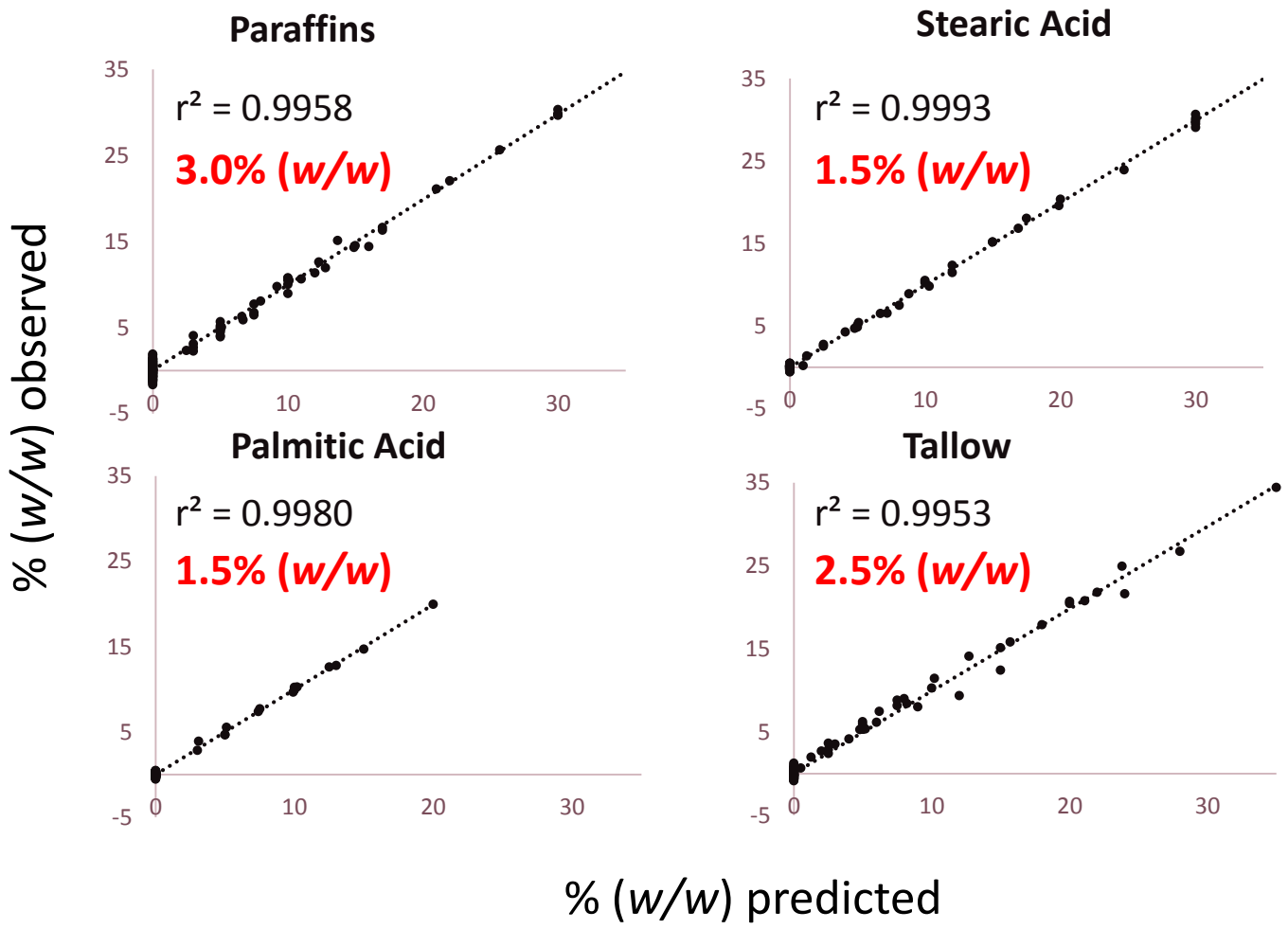
SET-UP OF PARTIAL LEAST SQUARES (PLS) REGRESSION MODELS (FT-IR)

Training-set samples

	# (Version 1)
Authentic beeswaxes	~ 50
Paraffins (2 MPs)	44
Stearic acid	23
Palmitic acid	15
Tallow	35

- GC-FID-checked, additional analyses conducted
- High diversity (origin, processing)
- 2-fold adulterated samples integrated into statistical models

  (stereo-detection possible)

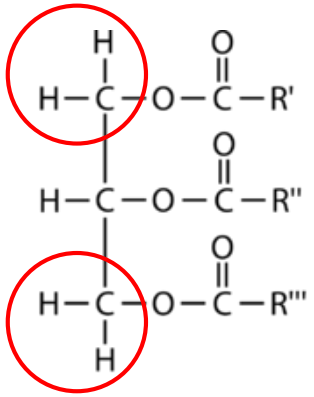


Linearity plots calculated via leave-one-out cross validation

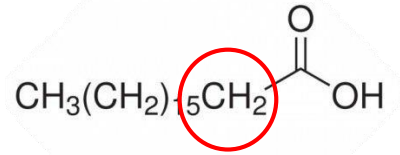
ADULTERANT-ASSOCIATED SIGNALS IN THE ^{1}H-NMR SPECTRUM

Beeswax + 5% tallow (*w/w*)

Signal distribution of authentic beeswaxes (n=51)

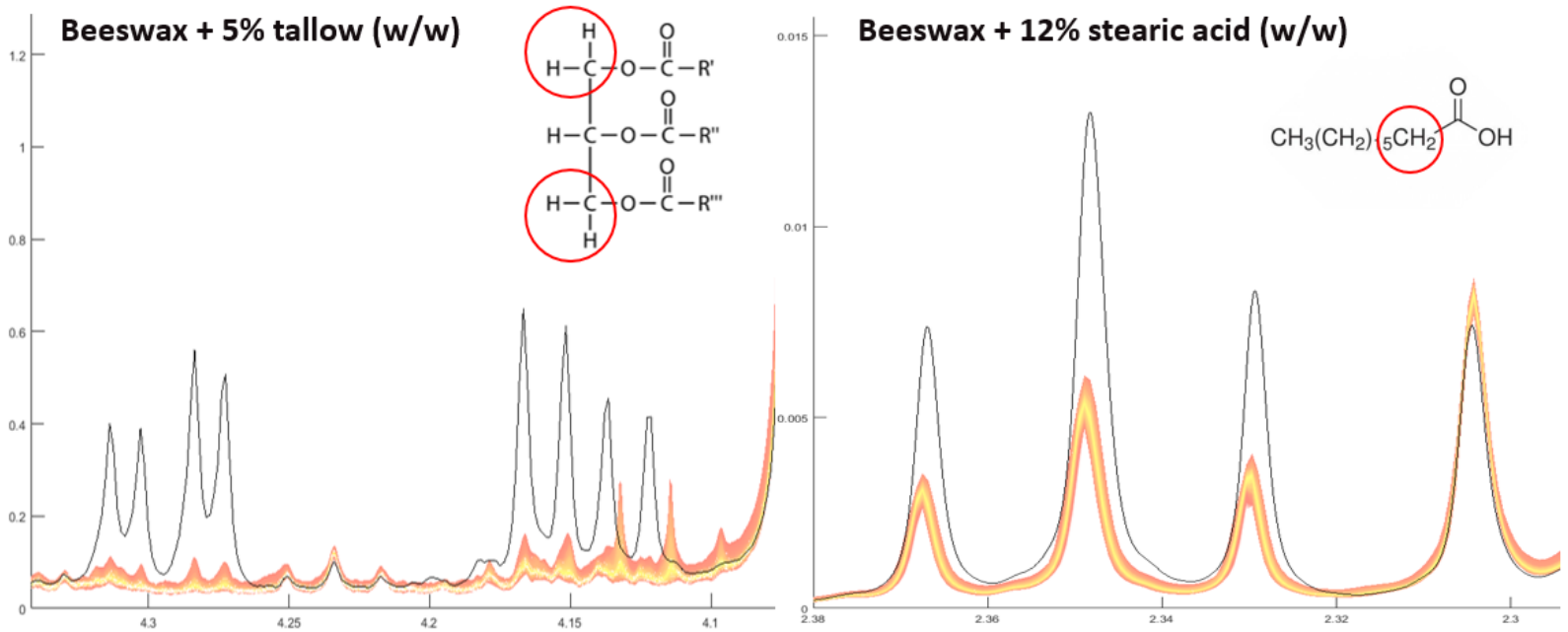


Beeswax + 12% stearic acid (*w/w*)



QUANTIFICATION OF ADULTERANTS VIA ^{1}H-NMR - SUMMARY

- Spectral data successfully used for set-up of PLS regression models (paraffin, fatty acids, tallow)
- No stereo-detection of stearic and palmitic acid possible
- Tallow (triacylglycerides) observable in low concentrations
- Quantification limits similar to FT-IR results
- Validation finished



FUTURE PROJECT: ^{1}H-NMR FOR DETERMINATION OF GEOGRAPHICAL ORIGIN

Background

- ^{1}H-NMR spectral data used to confirm the geographical origin of honey

- Preliminary results:

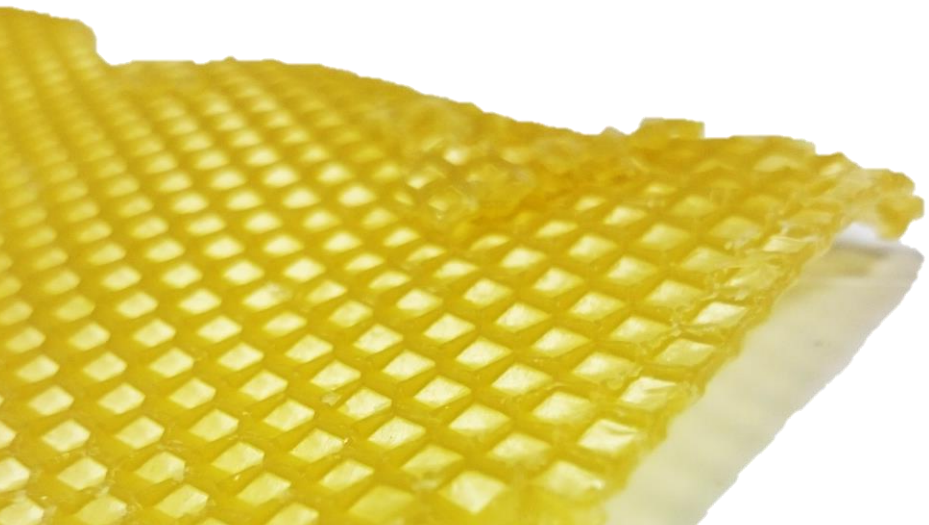
(linear discriminant analysis (LDA))

- Extension of database and confirmation of preliminary results necessary and in progress

FINAL REMARKS

- Both FT-IR and ^{1}H-NMR allow the set-up of PLS regression models for quantification of common beeswax adulterants
- Sensitivity is at least comparable to those obtained by classical wet lab methods
- Accreditation in March 2019 by German accreditation body (DAkkS)

- Official regulations and limits for alternative methods needed



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THANK YOU FOR YOUR ATTENTION

IN CASE OF QUESTIONS, FEEL FREE TO ASK

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