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

<table>
<thead>
<tr>
<th></th>
<th>Paraffin</th>
<th>Stearic acid</th>
<th>Tallow</th>
</tr>
</thead>
<tbody>
<tr>
<td>54–56 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>10</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Melting temp.</td>
<td>30</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Acid value</td>
<td>10</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Saponification</td>
<td>10</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Ester value</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ratio number</td>
<td>10</td>
<td>15</td>
<td>10</td>
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</tbody>
</table>

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

- C-H stretching of CH$_2$ & CH$_3$ groups
- CH$_2$ scissor deformation
- C=O stretching vibration (ester & free fatty acids)

wavenumber [cm$^{-1}$]

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

- Authentic beeswax
- Paraffin
- Stearic acid
- Tallow
SET-UP OF PARTIAL LEAST SQUARES (PLS) REGRESSION MODELS (FT-IR)

Training-set samples

<table>
<thead>
<tr>
<th></th>
<th># (Version 1)</th>
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<tbody>
<tr>
<td>Authentic beeswaxes</td>
<td>~ 50</td>
</tr>
<tr>
<td>Paraffins (2 MPs)</td>
<td>44</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>23</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>15</td>
</tr>
<tr>
<td>Tallow</td>
<td>35</td>
</tr>
</tbody>
</table>

• 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:

  ![Confusion Matrix Diagram]

  (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
THANK YOU FOR YOUR ATTENTION

IN CASE OF QUESTIONS, FEEL FREE TO ASK

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