The COLOSS BEEBOOK: Standard methods for *Apis mellifera* beeswax research

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The COLOSS BEEBOOK
Volume III, Part 1

- Standard methods for *Apis mellifera* product research (*Editors: Vincent Dietemann, Peter Neumann, Norman Carreck and James D Ellis*):
  - Standard methods for *Apis mellifera* propolis research (2016)
  - Standard methods for *Apis mellifera* brood as human food (2016)
  - Standard methods for *Apis mellifera* royal jelly research (2017)
  - Standard methods for *Apis mellifera* beeswax research (2019)

- Volume III, Part 1I: honey, bee venom, pollen / bee bread
  - plan to be published in 2020

BEEBOOK Beeswax chapter: Standard methods for *A.mellifera* beeswax research

- Long lasting project:
  - 2016 - 2019
- Summary on the chapter:
  - comprehensive - 108 pages, 65 figures, 23 tables
  - 10 (7) sections covering different aspects of beeswax research
  - the best practice approach and detailed protocol steps for all methods described - reproducibility of experiments and results
  - 13 contributors from 8 countries (Croatia, Germany, Italy, Portugal, Poland, Switzerland, Turkey, USA)
- Methods presented:
  - Recipe-like description of protocols
  - List of equipment, materials and reagents required
  - Advantages and disadvantages

Chapter sections:

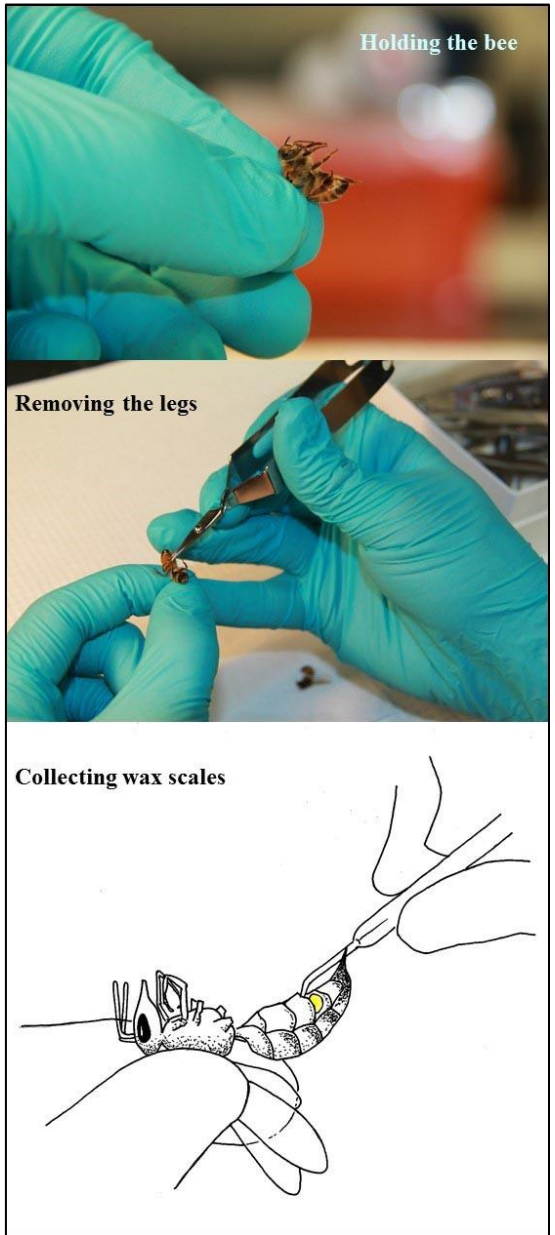
1. Introduction
2. Beeswax sampling, processing and storage
3. Standard methods for research on wax gland cells and production of wax scales
4. Methods for investigating honeycomb cell properties and comb construction
5. Standard methods for beeswax chemical characterization
6. Standard methods for beeswax authenticity and quality control
7. Standard methods for detection of pathogens in beeswax
8. Overview of other methods and perspectives
9. Acknowledgements
10. References

Beeswax sampling, processing and storage

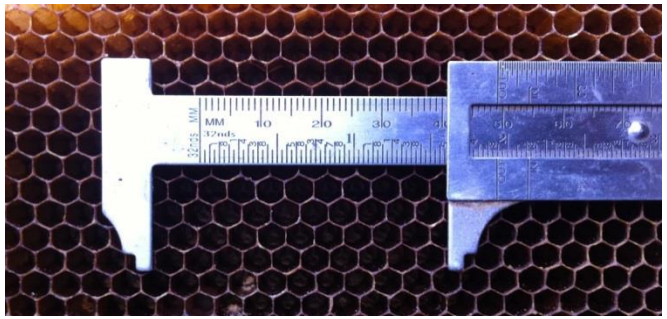
- Sampling - strongly depended on the aim of the study / method
- Collection of different types of beeswax specimens:
  - Wax scales (from individual bees, fallen)
  - Comb wax (wild-built combs from the hive)
  - Market beeswax (comb foundations, wax blocks)
  - Specific types of beeswax samples:
    - old comb, wax (hive) debris - detection of pathogens
    - wax caps, wax in honey - IRMS analysis
- Beeswax processing - melting
- Beeswax storage

Standard methods for research on wax gland cells and production of wax scales

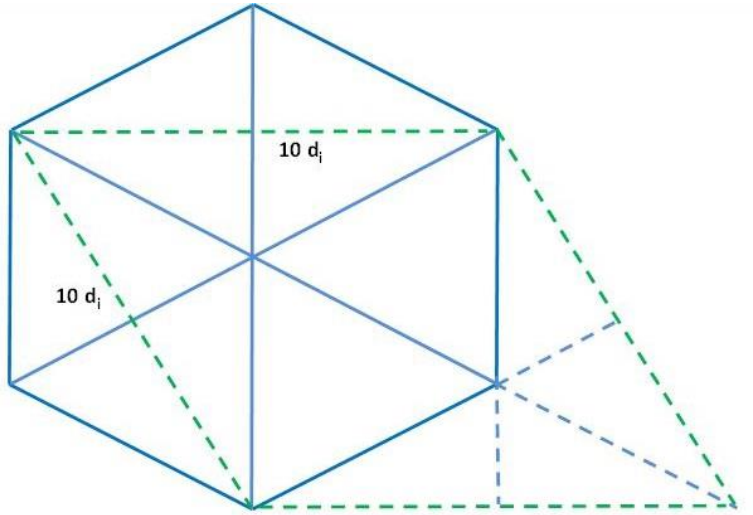
- Overview on wax scales production
- Collection of wax scales
  - Collecting wax scales from individual bees
    - Selecting and anesthetizing individual bees
    - Collecting wax scales from the wax mirror
  - Collecting fallen wax scales from cages
    - Modified plastic cup cages
    - Specially designed cages
- Wax scale measurements
  - Recording scale size
  - Measuring scale mass



Methods for investigating honeycomb cell properties and comb construction



- The two-dimensional structure of the hexagonal cell
- Linear measurements along cell diameters
  - Sampling the comb using linear measurements along cell diameters
  - Measurements and analyses conducted on photographs
  - Addressing the regularity of the cell
  - Investigating interspecific and intraspecific cell size variability
  - Interpreting historical data on cell size



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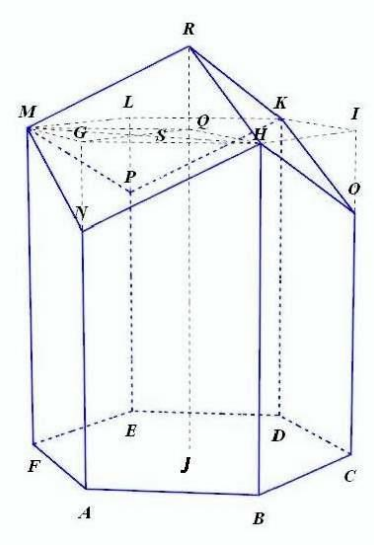
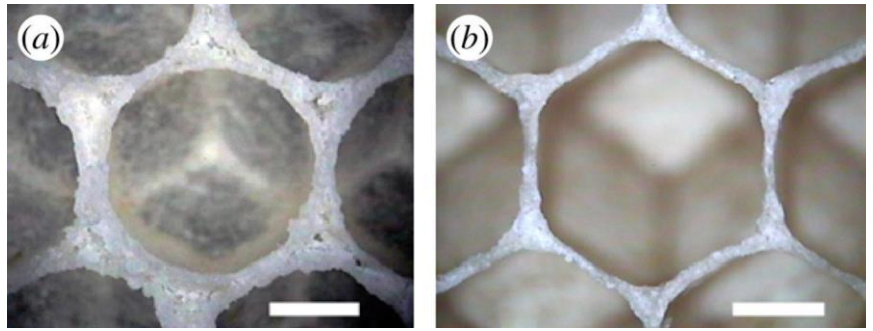
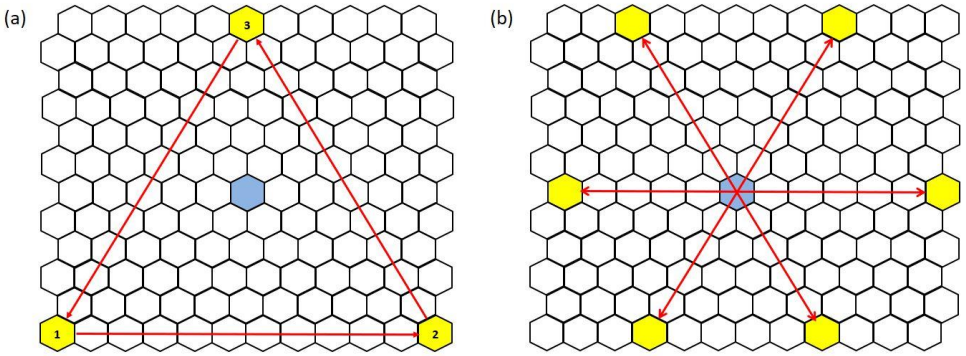


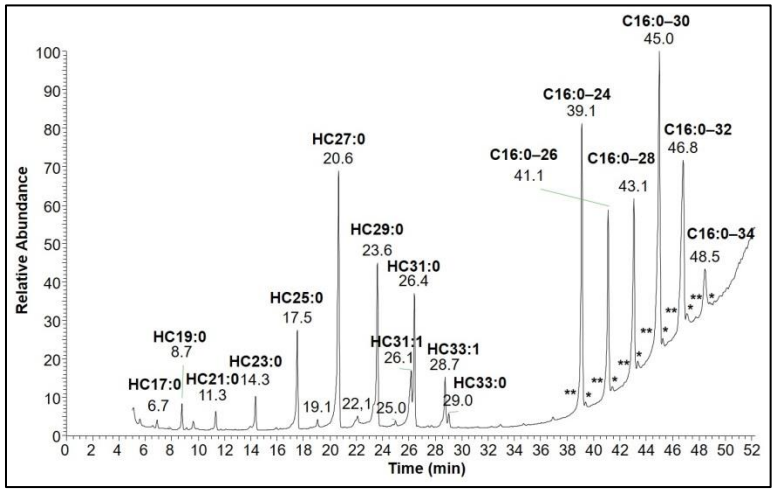
- The three-dimensional structure of the comb
  - Measuring the depth and estimating the internal volume of the cell
  - Investigating the bottom of the cells
  - Estimating the width and the external volume of the comb
  - Estimating the capacity of the comb
- Comb construction research
  - Investigating comb construction and its growth pattern
  - Investigating timing and type of comb construction

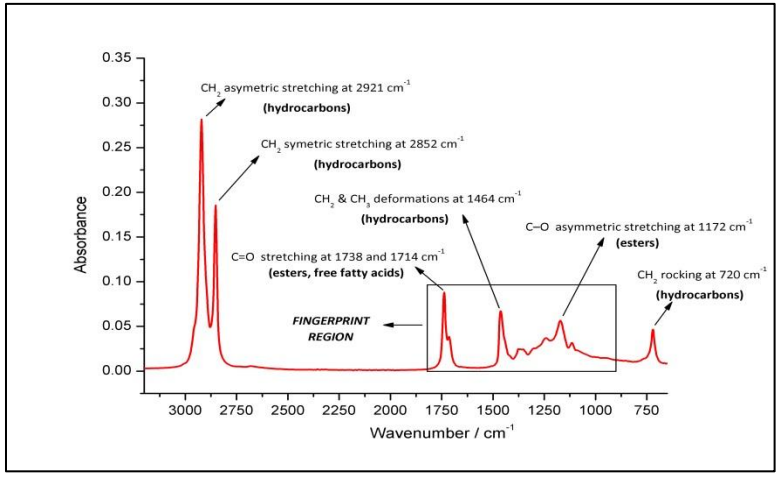




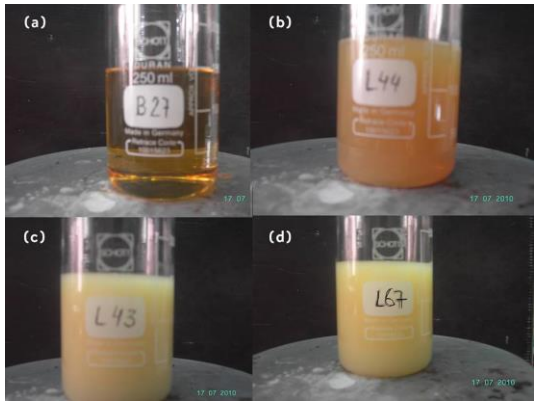
Standard methods for beeswax chemical characterization

- Chemistry of beeswax - overview on major constituents
- Gas chromatography-mass spectrometry (GC-MS) and other GC-coupled techniques
  - Determination of beeswax hydrocarbons by GC-MS
    - qualitative and quantitative analysis
  - Simultaneous analysis of monoesters and hydrocarbons by GC-MS and GC-FID
  - Simultaneous analysis of fatty acids and fatty alcohols by GC-MS and GC-FID
- Infrared (IR) spectroscopy (FTIR-ATR)
- Determination of ash content and mineral composition
- Sampling and analysing beeswax for hydrogen isotope ratios – IRMS (wax caps, beeswax in honey)

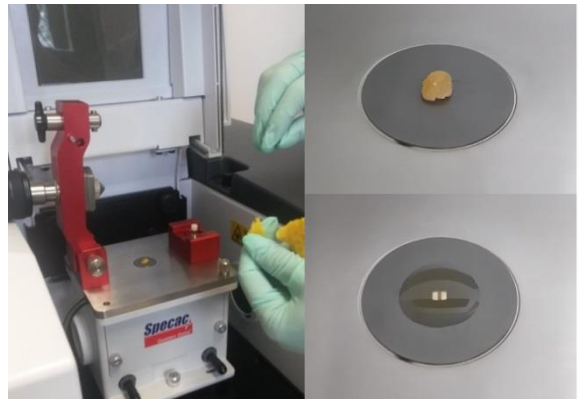


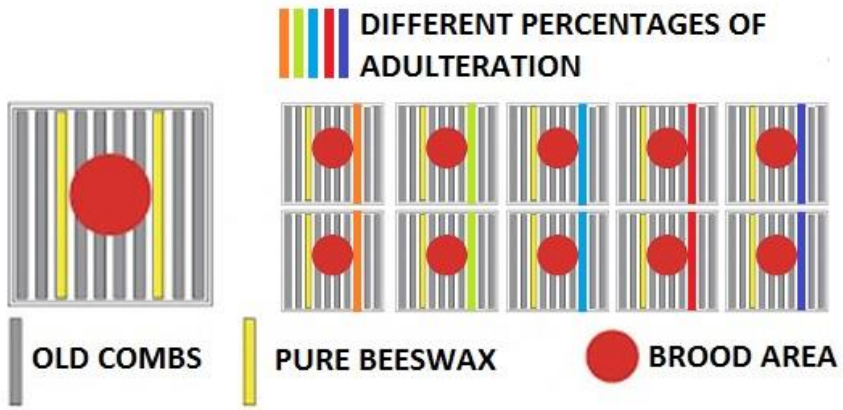


Standard methods for beeswax authenticity and quality control - *adulteration*



- Legislation and quality control of beeswax
- Standard methods for beeswax adulteration detection
  - Beeswax authentication by classical analytical methods
    - drop point, melting point, acid, saponification and ester value, iodine number, peroxide value, detection of carnauba wax, detection of ceresin, paraffin and other waxes, detection of glycerol and other polyols
    - Sensory analysis of beeswax
  - Adulteration detection by GC-MS technique
  - Adulteration detection by FTIR-ATR spectroscopy
  - Investigating brood survival on adulterated comb foundations (bioassay)





Standard methods for beeswax authenticity and quality control - *pesticide residues*

- Standard methods for detection of pesticide residues in beeswax
  - Residues in beeswax - overview
  - Trace-level determination of pesticides in beeswax by LC-MS
    - Protocols for extraction and purification (OCLLE protocol, SPE protocols,, modified QuEChERS protocol)
    - LC-MS detection
  - Chromatographic multiresidue analysis by GC-MS
    - Protocols for extraction and purification (DE protocol, SPE protocol, QuEChERS protocol)
    - GC-MS detection
  - Other methods for pesticides detection in beeswax

Standard methods for detection of pathogens in beeswax

- Early detection of American foulbrood by beeswax analysis
  - Determination of *Paenibacillus larvae* spores in beeswax
  - Isolation of *P. larvae* spores from beeswax and wax (hive) debris
  - PCR protocols
- Detection of other pathogens / pests in beeswax and hive debris
  - European foulbrood - detection of *Melissococcus plutonius*
  - *Ascosphaera apis*
  - *Nosema* spp.
  - Small hive beetle (*Aethina tumida*)
    - Protocol for determination of *A. tumida* in wax (hive) debris in the context of monitoring programs

Thank You for Attention

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