



Melissopalynology – not only a tool for the determination of the botanical and geographical origin of honey and a short excursion into the NMR analysis of honey produced by *Apis dorsata*

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Quality Services International GmbH

Madhu Duniya, 6 - 10 November 2023, Ho Chi Minh City

Quality Services International (QSI Bremen): Profile



FOUNDED IN 1954

SINCE 2013 MEMBER OF
THE TENTAMUS GROUP

170 STAFF
(FOOD) CHEMISTS,
BIOLOGISTS, PHARMACISTS
AND OTHER EMPLOYEES



LAB WITH FOCUS ON
AUTHENTICITY AND
ANALYTICAL TESTING
FOR HONEY,
PHARMA/GMP, TEA,
COFFEE & CANNABIS



ISO 17025 ACCREDITED
GMP CERTIFIED



HUB SERVICES via
QSI AMERICA, QSI MEXICO,
QSI INDIA, QSI VIETNAM



QSI - A LAB FOR LIFE



QSI Bremen: Main Lab Equipment

CHROMATOGRAPHY

- LC-MS/MS 7500, 5500+, 5500 (Sciex), 6495C (Agilent)
- LC-HRMS 6600, X500R (Sciex), Orbitrap Exploris 240 (Thermo)
- GC-MS/MS 7010B (Agilent)
- HPLC-DAD/FLD/ELSD
/RID, HPAEC-PAD,...(Thermo, Shimadzu, Agilent)

ISOTOPE RATIO ANALYSIS

- LC-IRMS LC IsoLink™ II (Thermo)
- EA-IRMS CM-CRDS (Picarro Inc)

NMR

- AVANCE NEO FOODSCREENER SYSTEMS (400 MHz/Bruker)

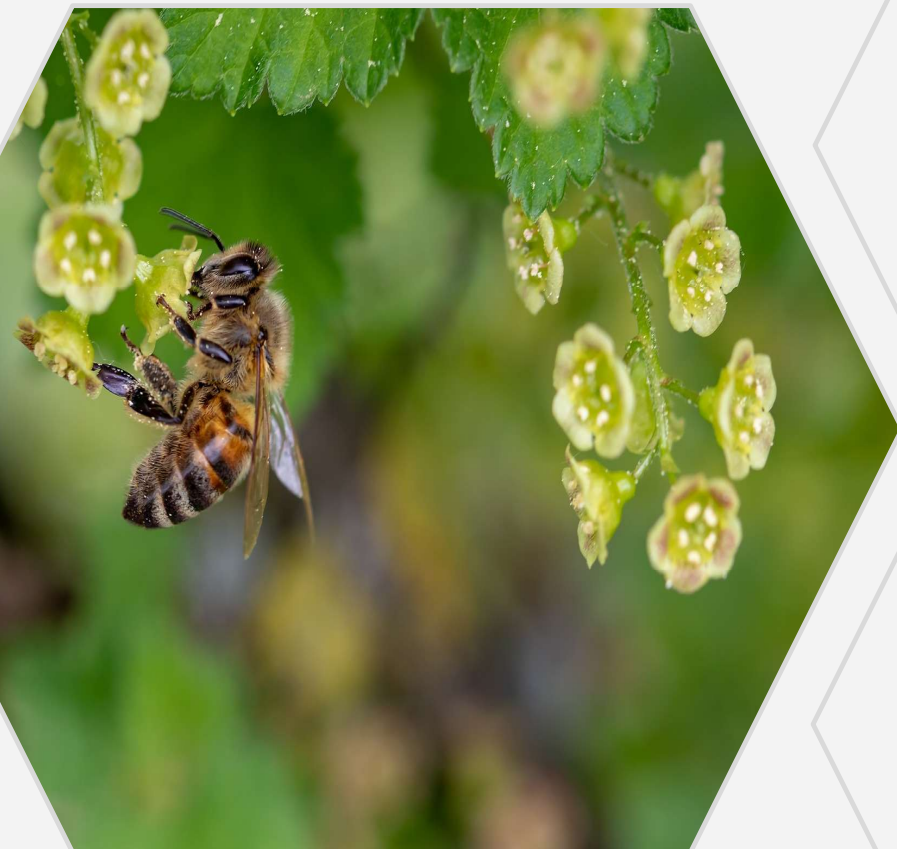
FURTHER IMPORTANT EQUIPMENT:

- Colorimetric/
enzymatic robotics Gallery™ Discrete Analyzers (Thermo)
- Microscope Ni-U/DIC (for Melissopalynology/Nikon)



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QSI BREMEN ANALYSES FOR HONEY



QSI tests for Europe and US, Canada

Quality Parameters

- Melissopalynology:
Pollen analysis (incl. sensory, electrical conductivity)
- + Trade Analysis (Diastase, free acid, HMF)
- + Sugar spectrum
 - Saccharase
 - Ethanol
 - Color, etc.

Residues, Contaminants

- Pesticides incl. Varroacides (Amitraz, Coumaphos, Neonicotinoids, Glyphosate etc.)
- Antibiotics (Sulfonamides, Tetracyclines, CAP etc.)
- Pyrrolizidine alkaloids (PA)
- Heavy metals (lead etc.)
 - GMO, Microbiology



Authenticity / Quality

- NMR Bruker Honey Profiling™
- HRMS (Orbitrap)/LC-MS/MS
- LC-IRMS (incl. AOAC 998.12)
 - Oligosaccharides
 - Foreign Enzymes (incl. Famyp, beta-Fructofuranosidase, beta-gamma Amylases, thermostable alpha-Amylases)
- Caramel color E150d
- SM-R, SM-B, TM-R, Psicose, Mannose etc.



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Beginning of melissopalynology in Europe



Up to now, numerous publications from various countries worldwide regarding

- geographical origin (typical pollen spectra for certain regions)
- pollen atlases
- monofloral honeys (minimum percentages)
- honeydew honey

2022: start of ISO/AWI 13667- specifying requirements and test methods for analysis the pollens in honey.

2014/2016: *presentations on honey atypical particles in the honey sediment*

2004: Von der Ohe, Persano Oddo, Piana, Morlot, Martin: Harmonized methods of melissopalynology

2002: DIN10760:2002-05 Examination of honey - determination of the relative pollen frequency

1998: *Rußmann: Yeasts and glycerol in Blossom honeys - Detection of fermentation or stopped fermentation*

1995: *Kerkvliet, Shrestha, Tuladhar, Manandhar: Microscopic detection of adulteration of honey with cane sugar and cane sugar products*

1978: Louveaux, Maurizio, Vorwohl: methodes of melissopalnyology

1895: Pfister: Trying a microscopy of the honey

Microscopic picture

pollen

- geographical origin
 - typical pollen combination, presence of „marker pollen“
- botanical origin
 - different classes: under-, over- and normal represented

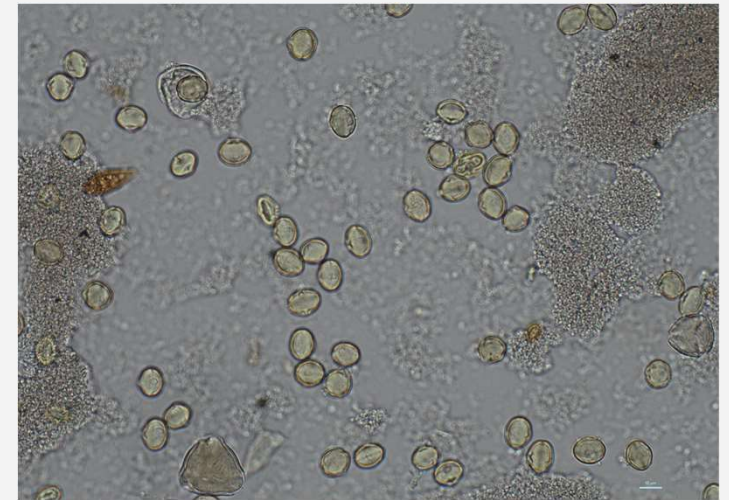


sediment

pollen

Verification of the declaration (botanical and geographical origin):

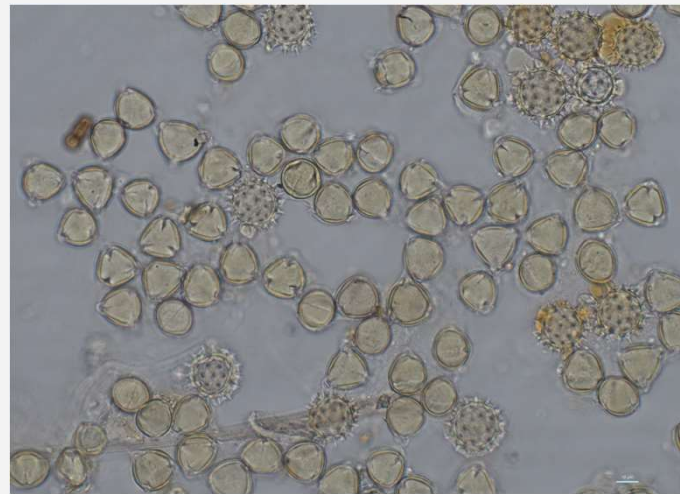
- botanical origin (in connection with chemical-physical parameters and sensory characteristics)
 - due to various factors (e.g. blossom structure) a different number of pollen gets into the honey → different pollen density for each botanical source



Chestnut Honey: over-represented



Citrus Honey: under-represented



Litchi Honey: normal-represented

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sediment

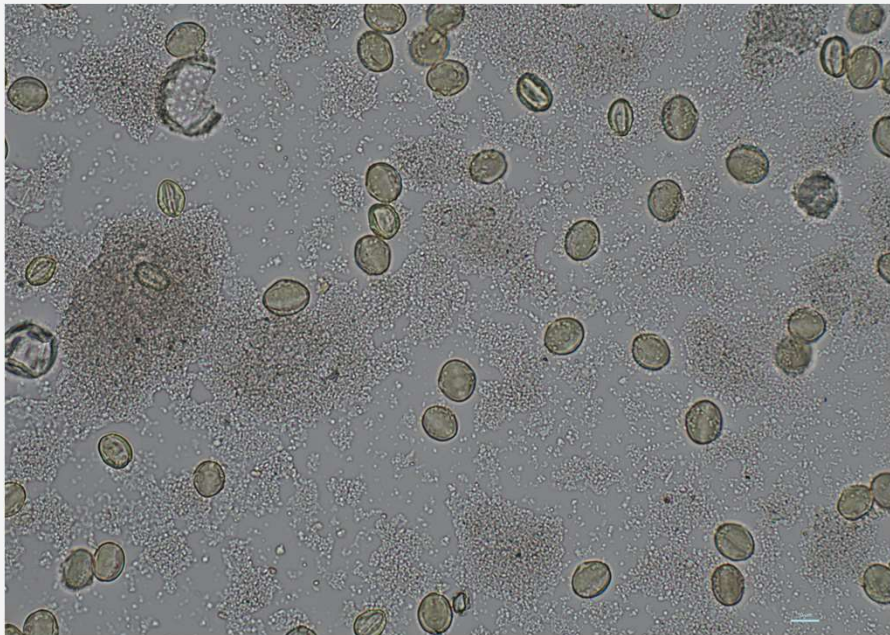
- honeydew elements
 - indicators for honeydew
- other sediment components
 - typical for honey (e.g. bee hairs)
 - yeasts
 - atypical for honey (starch, atypical particles, filter particles)

- ✓ Verification of the declaration
- ✓ Indication of good beekeeping practice or of possible filtration

Sediment: honeydew elements

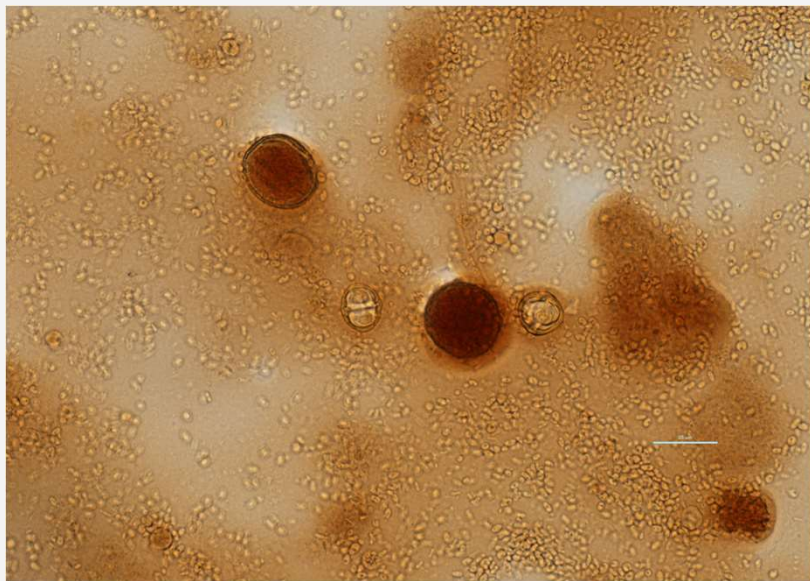
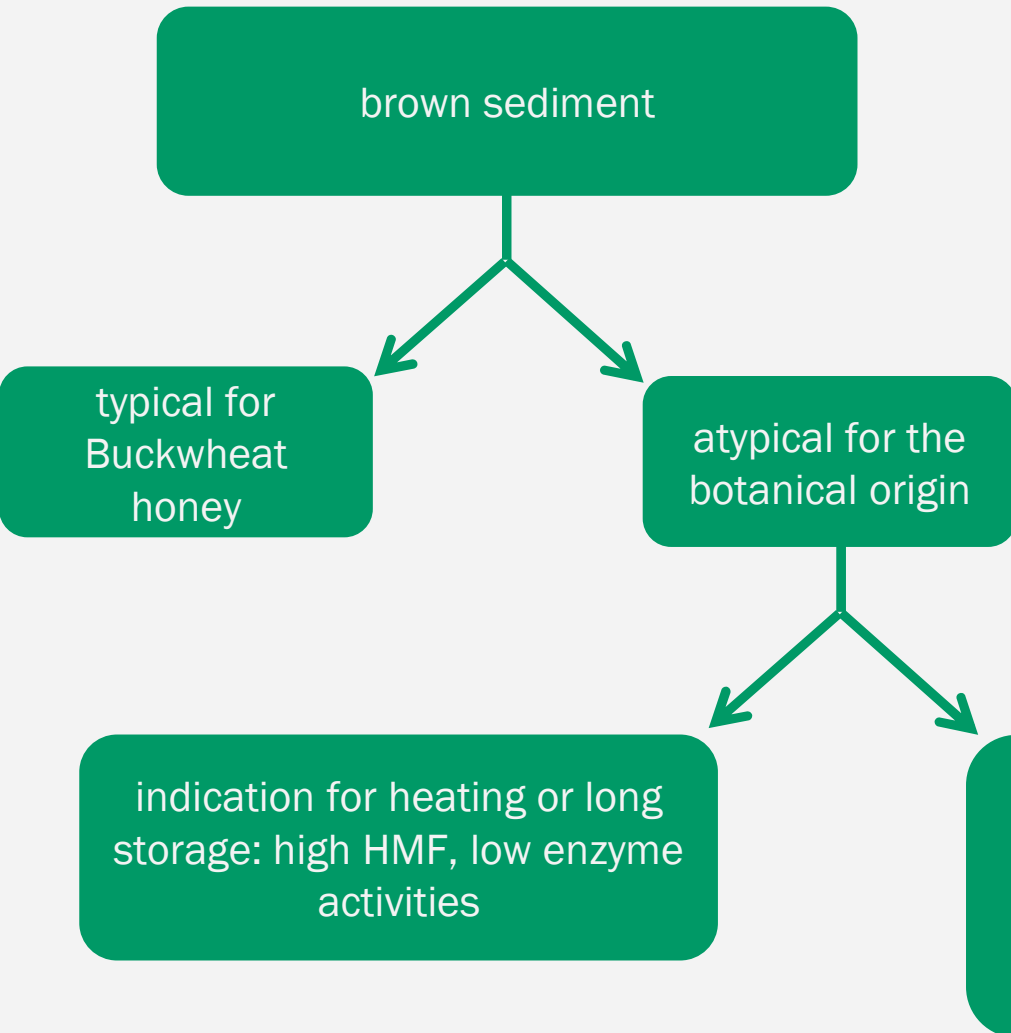


Pine honey from Greece (magnification: 400x):
wax wool and fungi elements



Chestnut honey from Franc (magnification: 400x):
crystalline matter

Sediment: suspicious

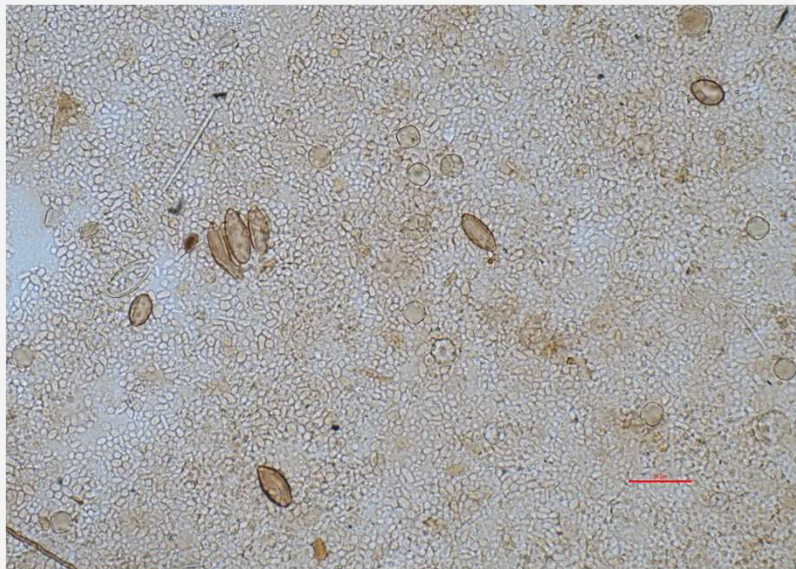


Example: Philippines (magnification 400x): brown sediment and brown pollen grains -> HMF: 1698 mg/kg (EU Honey Directive 110/2001/EC: max. 80 mg/kg for tropical origins)

Sediment: yeasts

classification according to Rußmann (1998):

/10 g honey	
< 10.000	very low
10.000 – 100.000	low
100.000 – 500.00	medium
500.000 – 1.000.000	many
> 1.000.000	extremely high



Parameters for a fermentation or a stopped fermentation:

- increased yeast content (> 500 000/10 g honey)
- glycerol > 300 mg/kg
- deviating sensory

Exception: honeydew honey

sample from the Philippines (magnification 400x):

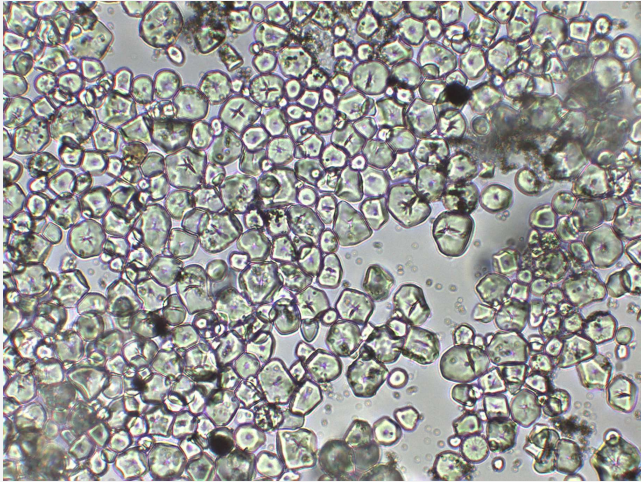
ETOH: 898,5 mg/kg

Yeast: extremely increased

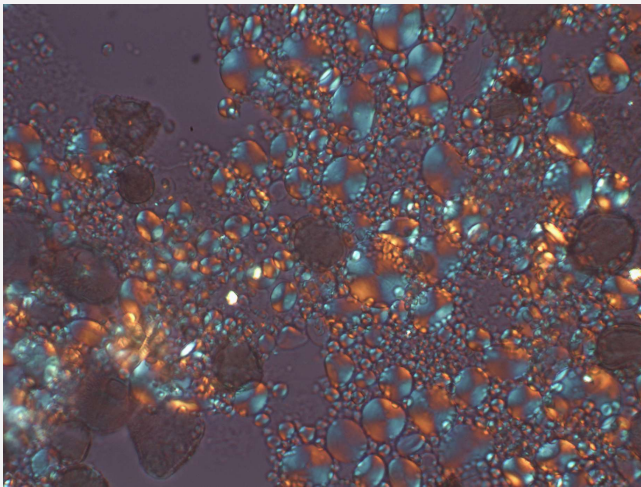
Consistency: very liquid (high water content)

Sensory: fermented

Sediment: starch



Mexico: magnification 600x



Germany: magnification 600x (with polarization filter)

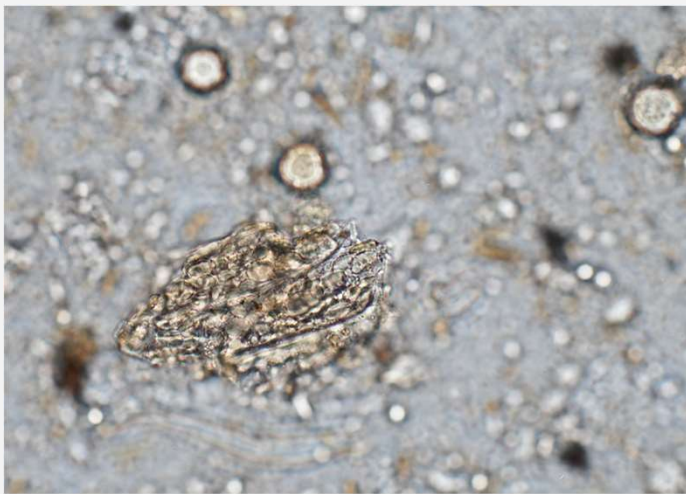
Occurrence: normally < 1 %

How do starch grains get into the honey?

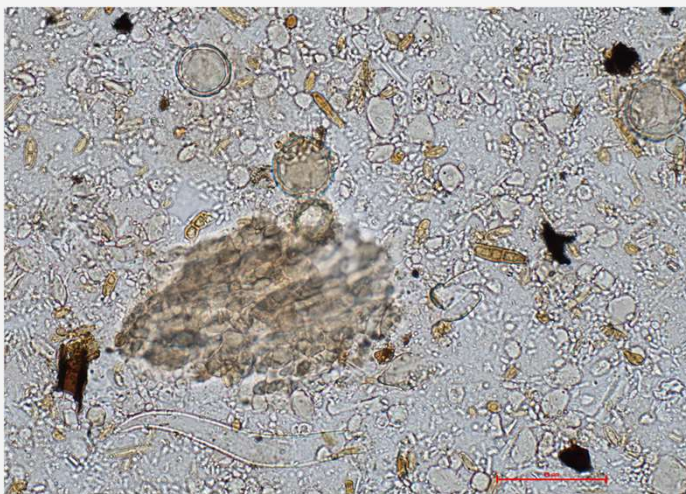
- adulteration with starch-based honeys
- use of bee drugs (starch as carrier material)
- feeding of bees
- starch entry by the bees

Assessment: honeys with contents > 30 % (based on pollen content) do not comply with the EU Honey Directive 110/2001/EC

Sediment: particles of bee feeding



Vietnam (magnification 400x)

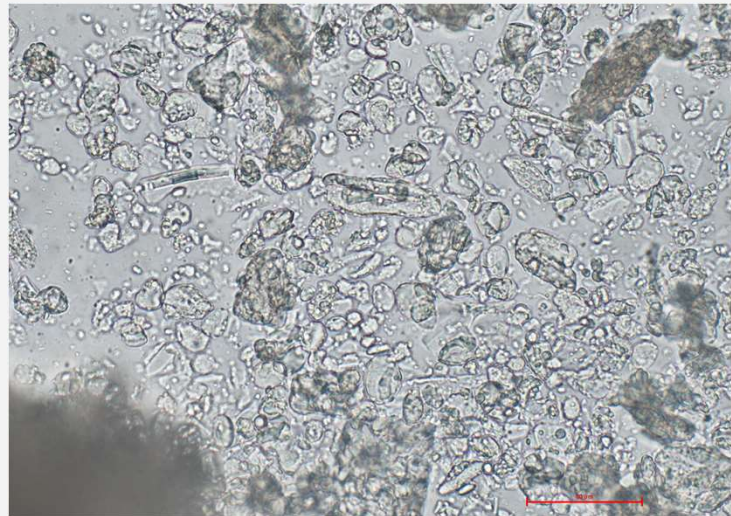


Presence of particles of bee feeding in the honey sediment due to extreme feeding.

In Vietnam soybean flour is often used as protein source for the bees.

Soybean is classified as allergen according to EU legislation (allergen labeling).

- ELISA/PCR-testing



Reference: organic soybean flour from the supermarket (magnification: 400x)

Sediment: honey atypical particles



blend of China and Central America (magnification: 400x)

Since 2012 observation of strange particles in certain honeys (mainly from China or blends with China):

- brown or white particles
- occurrence in different sizes (small – big)
- without any surface structure, but appearance is very characteristic

sensory: not typical for honey (malty or metallic aroma)

An adulteration could be detected later by specific methods.

- Comparisons with reference material showed that it could be soy protein.

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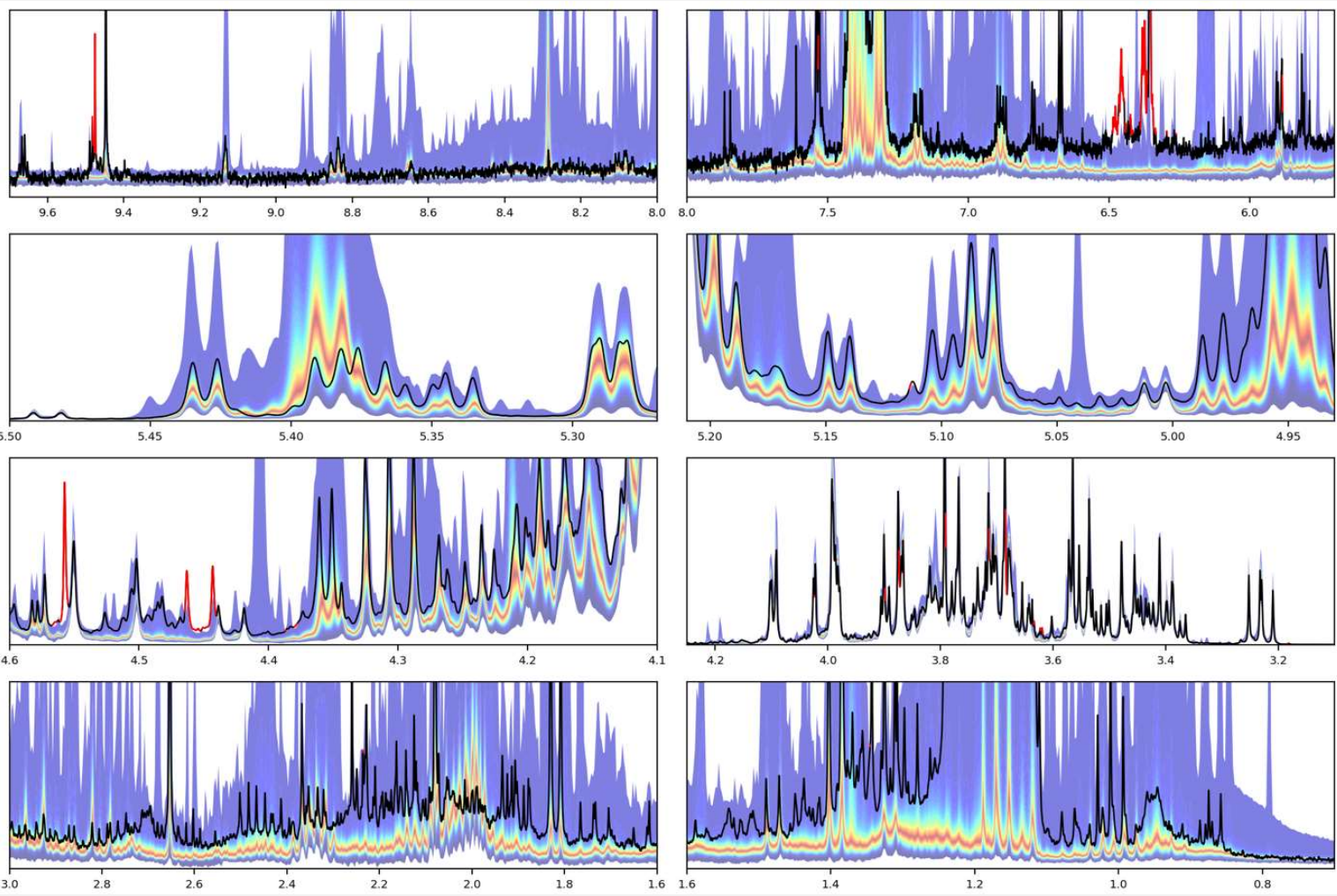
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- ✓ Relevant for honeydew honey
- ✓ valuable additional information on e.g. good beekeeping practice

NMR spectrum of Apis Dorsata honey



- Aliphatic area 0-3 ppm (org. and amino acids, ethanol)
- Sugar area 3-6 ppm
- Aromatic area 6-10 ppm (org. and amino acids, HMF)
- The bot. and geog. origin can be observed in the aliphatic and aromatic sections

Spectrum (black) in comparison with Bruker Honey-Profiling database (coloured background --> orange represent an average honey)

- sugars are similar
 - differences in the acid composition
- own database for Apis Dorsata honey has to be built up



THANKS

Do you have any questions?
Get in touch

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