OP-225 [Beekeeping Technology and Quality]
Detection of Economically Motivated Adulteration in Honey Using Untargeted Liquid Chromatography-Mass Spectrometry and Chemometrics

Jule Hansen, Christof Kunert
Eurofins Food Integrity Control Services GmbH, Germany

The addition of sugar syrups in honey are currently detected by approaches based on stable isotopes and the specific detection of targeted marker compunds or foreign enzymes. Recently, we have developed a new data processing strategy that allows us to transfer the advantages of the robust method Proton Nuclear Magnetic Resonance (1H-NMR) to the highly sensitive method untargeted Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) [1]. The detection of foreign sugars requires a sufficient sensitivity, and we can show that the distinction between pure and economically motivated adulteration in honey is possible. The classification accuracies range from 98 to 100%, while the accuracy of the quantitative syrup content estimation using regression models depends on the respective syrup. For rice and beet syrup, the deviations from the true proportion were in the lower percentage range, while for high-fructose corn syrup they were much higher, in some cases exceeding 20% [2]. The method we present here shows great promise for the reliable and sensitive detection of syrups in honey, making it suitable for routine laboratory applications.

[1] Hansen, J.; Kunert, C.; Münstermann, H.; Raezke, K.-P.; Seifert, S. Application of Untargeted Liquid Chromatography-Mass Spectrometry to Routine Analysis of Food Using Three-Dimensional Bucketing and Machine Learning. Sci. Rep. 2024, 14, 16594.

[2] Hansen, J.; Kunert, C.; Raezke, K.-P.; Seifert, S. Detection of Sugar Syrups in Honey Using Untargeted Liquid Chromatography–Mass Spectrometry and Chemometrics. Metabolites 2024, 14, 633.

Detection of Economically Motivated Adulteration in Honey Using untargeted LC-HRMS and Chemometrics

Jule Hansen

Eurofins Food Integrity Control Services GmbH

Untargted LC-HRMS and Chemometrics

- **L**iquid **C**hromatography **H**igh **R**esolution **M**ass **S**pectrometry
  - Detect and identify components of honey
- Chemical fingerprint
  - All signals
  - Difficult to evaluate manually
- Chemometrics
  - Which ones are similar?
  - Which ones stand out?
- ✓ Authentication of honey

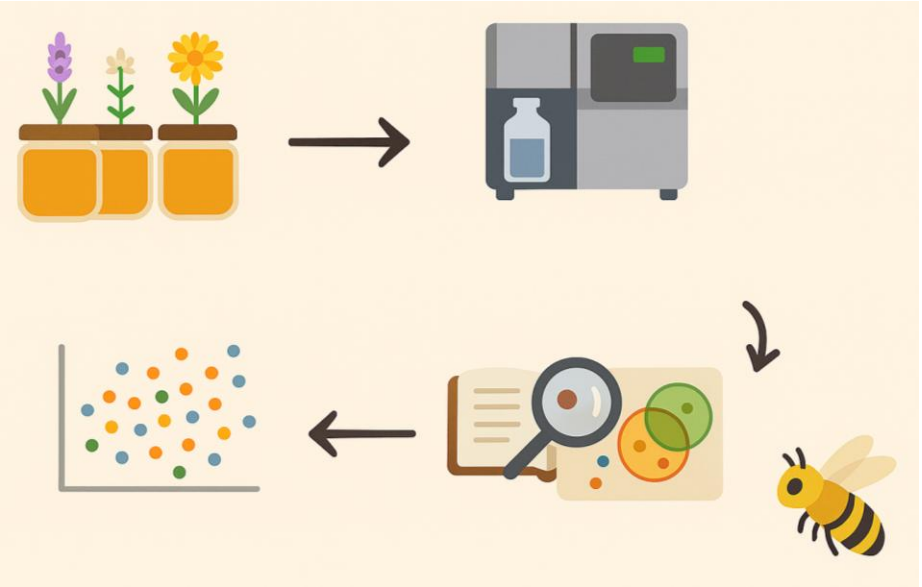


Illustration generated with AI (Microsoft Copilot)

Targed versus Untargeted LC-HRMS

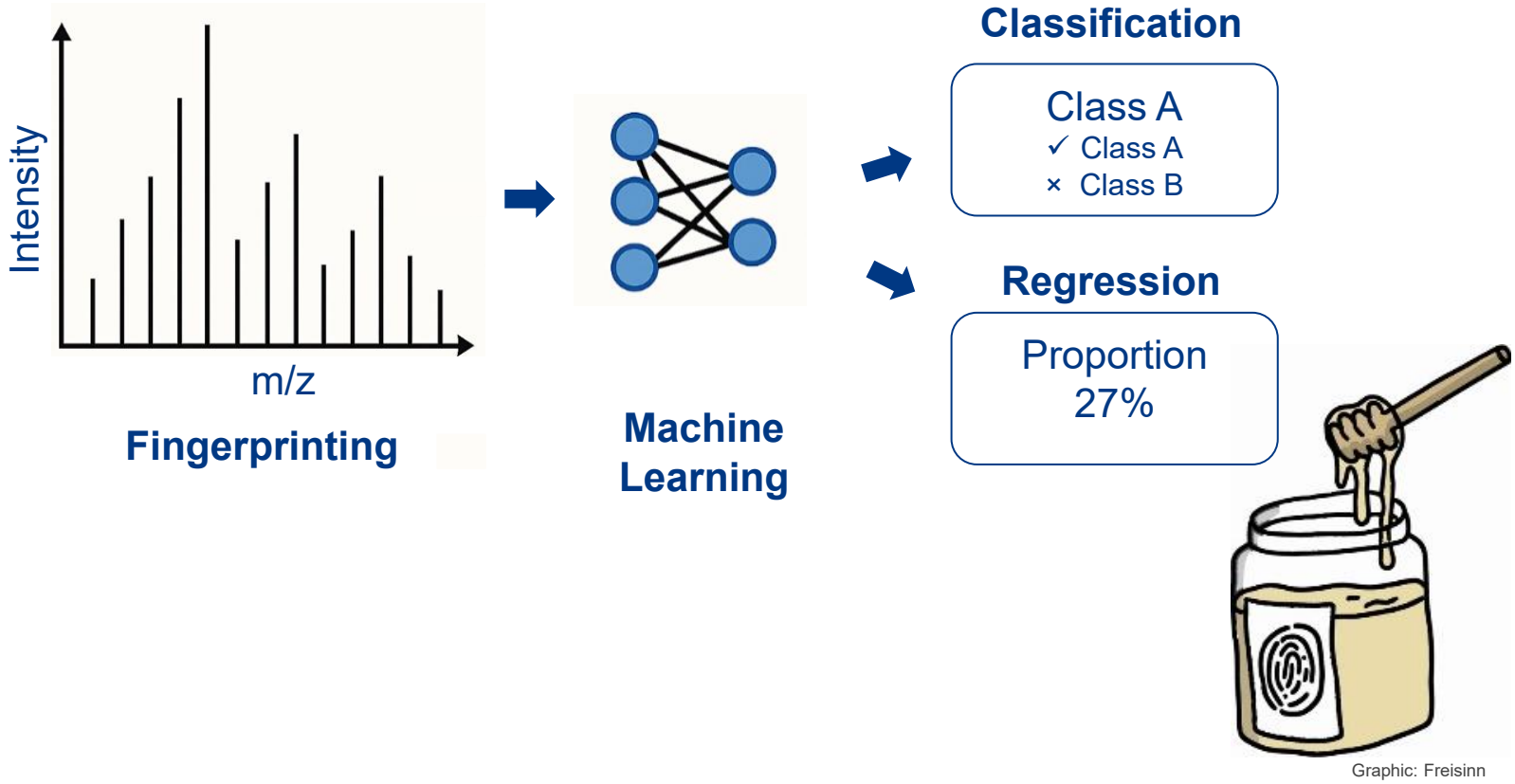
- Targeted Analysis
  - Focused search
  - Fast, sensitive and precise
  - Find specific known marker compounds

- Untargeted Analysis
  - Explores all detectable compounds
  - Potential reveal of unknowns or fraud
  - Reduced risk of false-positives

Illustration generated with AI (Microsoft Copilot)

Chemometrics and Machine Learning

- Mathematics and statistics → understand complex chemical data
- Machine Learning: Type of AI where computers learn from data

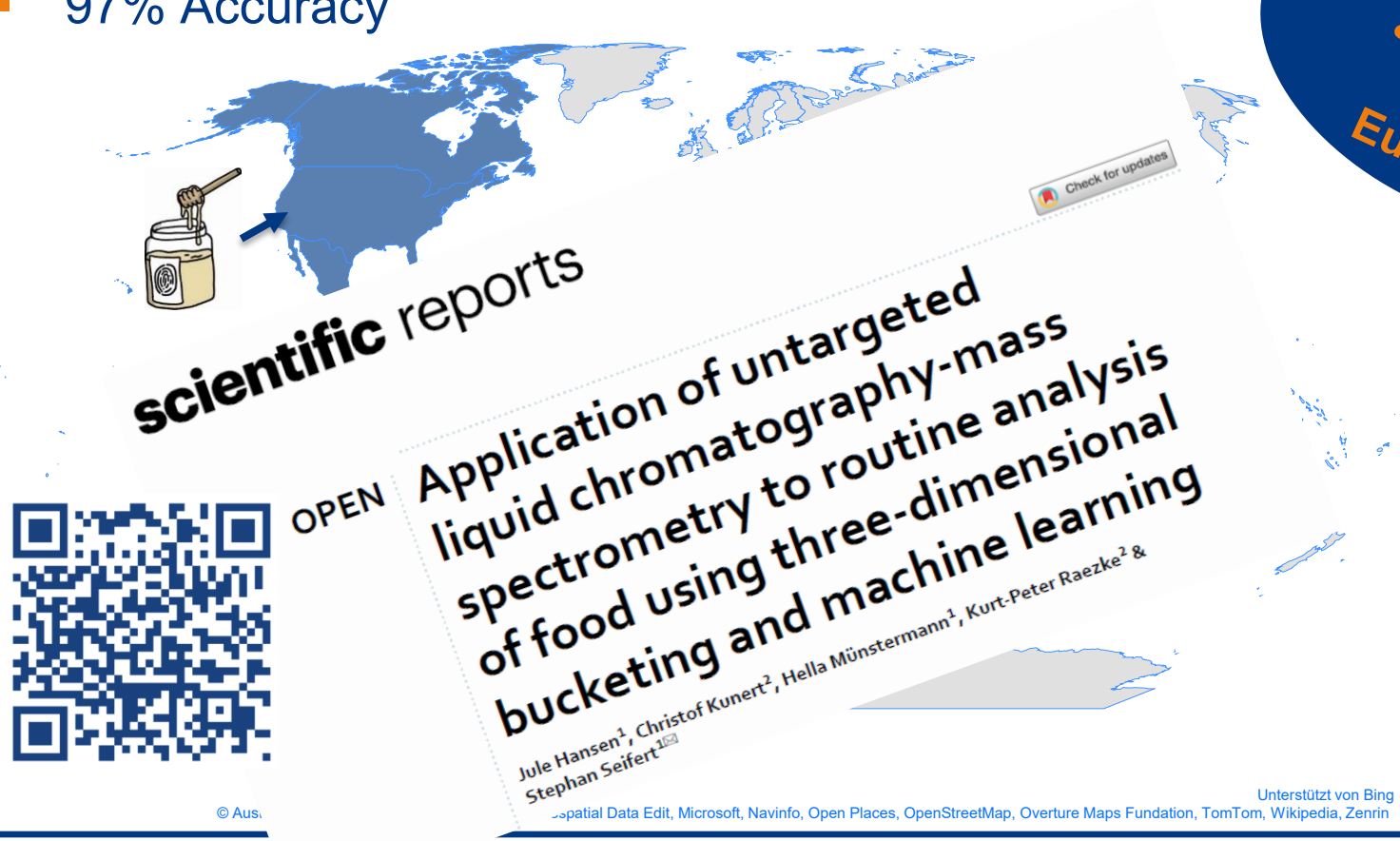


Graphic: Freisinn

Which country does the honey come from?

- Classification model: geographical origin
- Based on 15700 honey samples → continuous growth
- 97% Accuracy

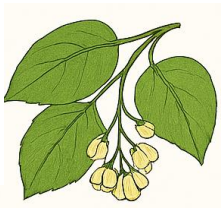
Exclusively available at Eurofins



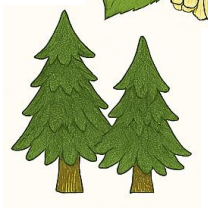
What type of honey is it?

- Classification model: botanical origin
- Based on 2500 honey samples → continuous growth
- 91% Accuracy

Exclusively available from Eurofins


Linden

Pepper


Forest

Canola

Orange Blossom

Acacia

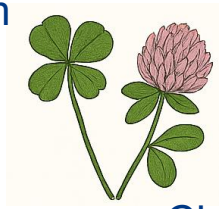
Buckwheat

Wildflower

Sunflower

Lavender

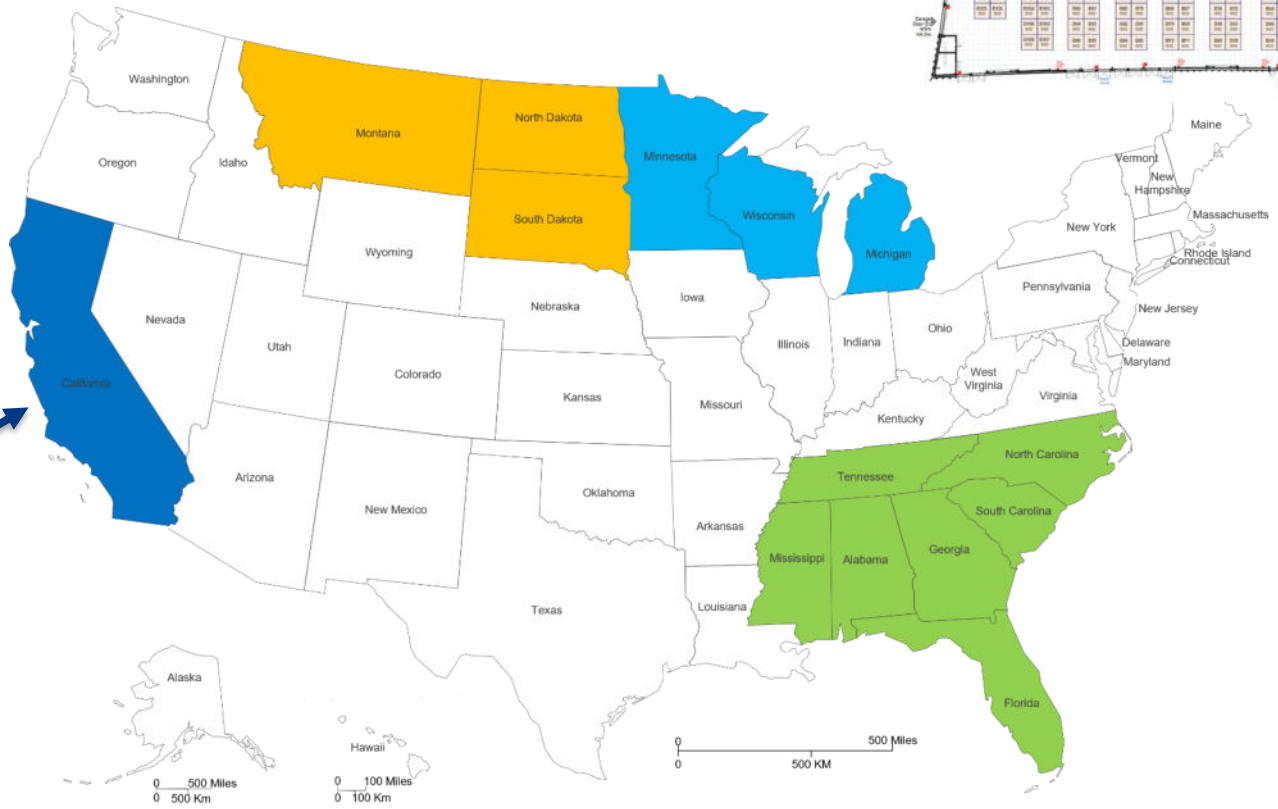
Mustard


Clover

Sage

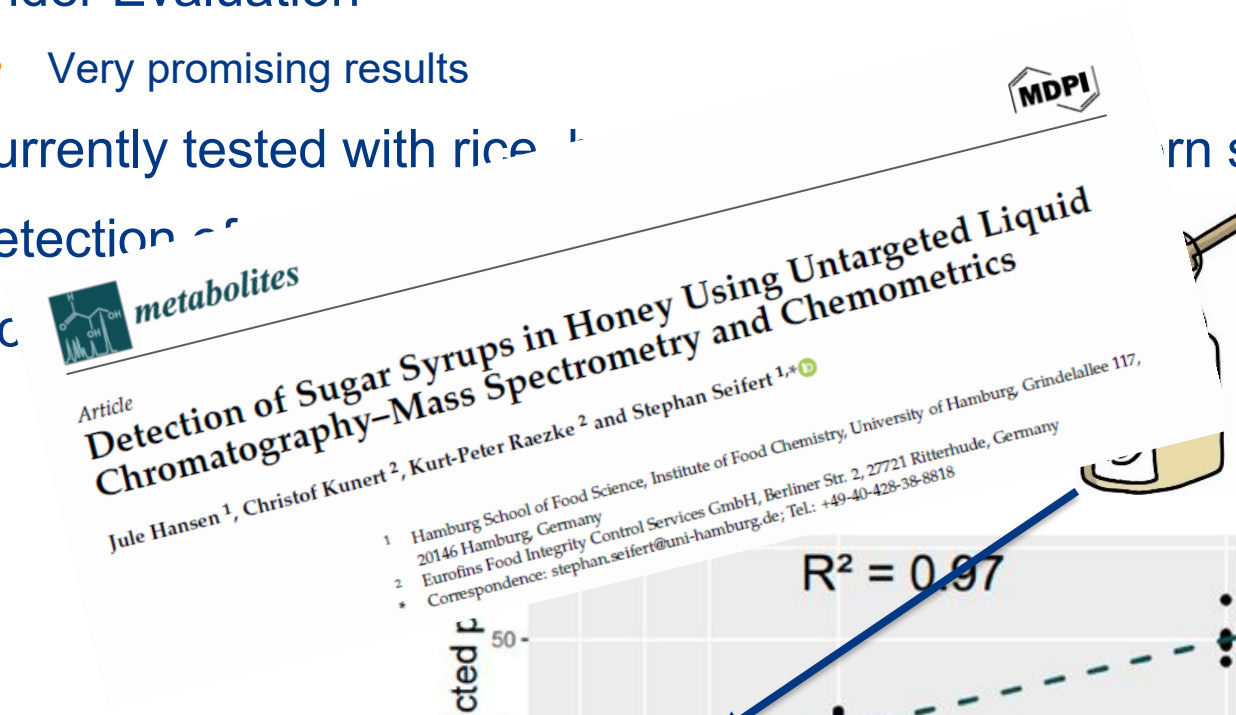
Which region does the honey come from?

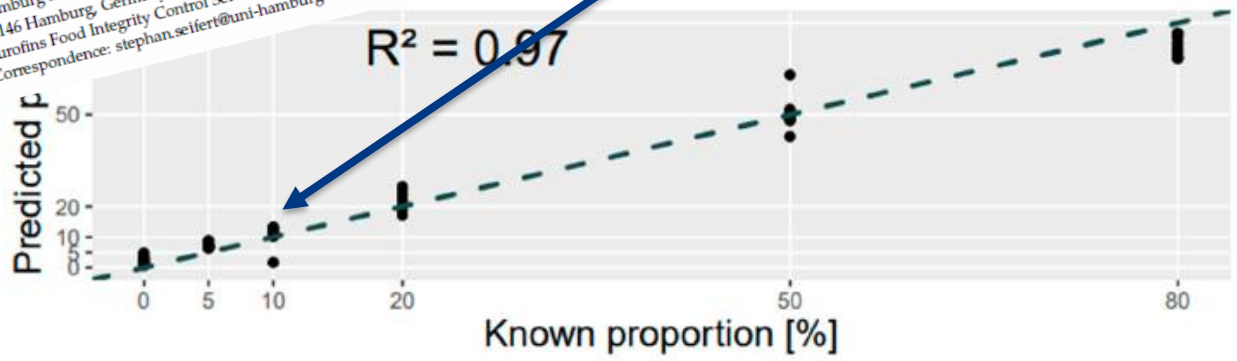
- Adaptable to specific authentication issues
- Open to specific inquiries



How much syrup is in the honey?

- Regression approach
- Under Evaluation
  - Very promising results
- Currently tested with rice [illegible] rn syrup
- Detection [illegible]
- Inc [illegible]





Summary: LC-HRMS Fingerprinting

- With one LC-HRMS measurement, we can determine:

✓ Country of origin

✓ Botanical origin

✓ Regional origin

✓ Syrup detection

✓ Syrup quantification

→ USA

→ Orange Blossom

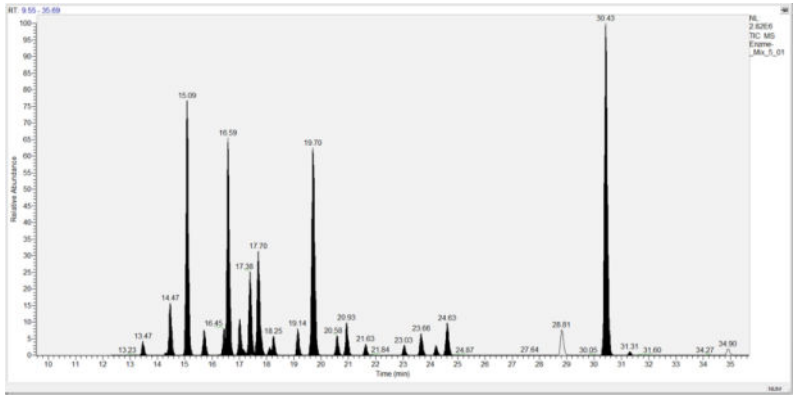
→ California

→ Addition of beet syrup detected

→ Quantification: 10%

Newest method developments

Foreign and honey native enzymes detection in honey by Proteomics



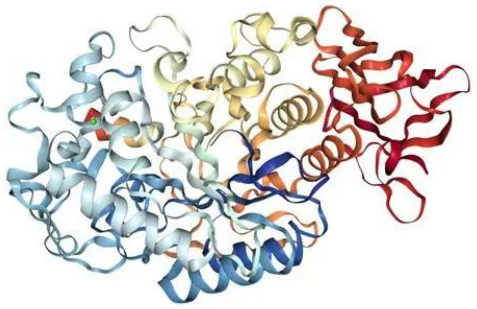
New Testing Recommendation for adulteration detection in honey

LC-HRMS – LC-IRMS – NMR - Proteomics

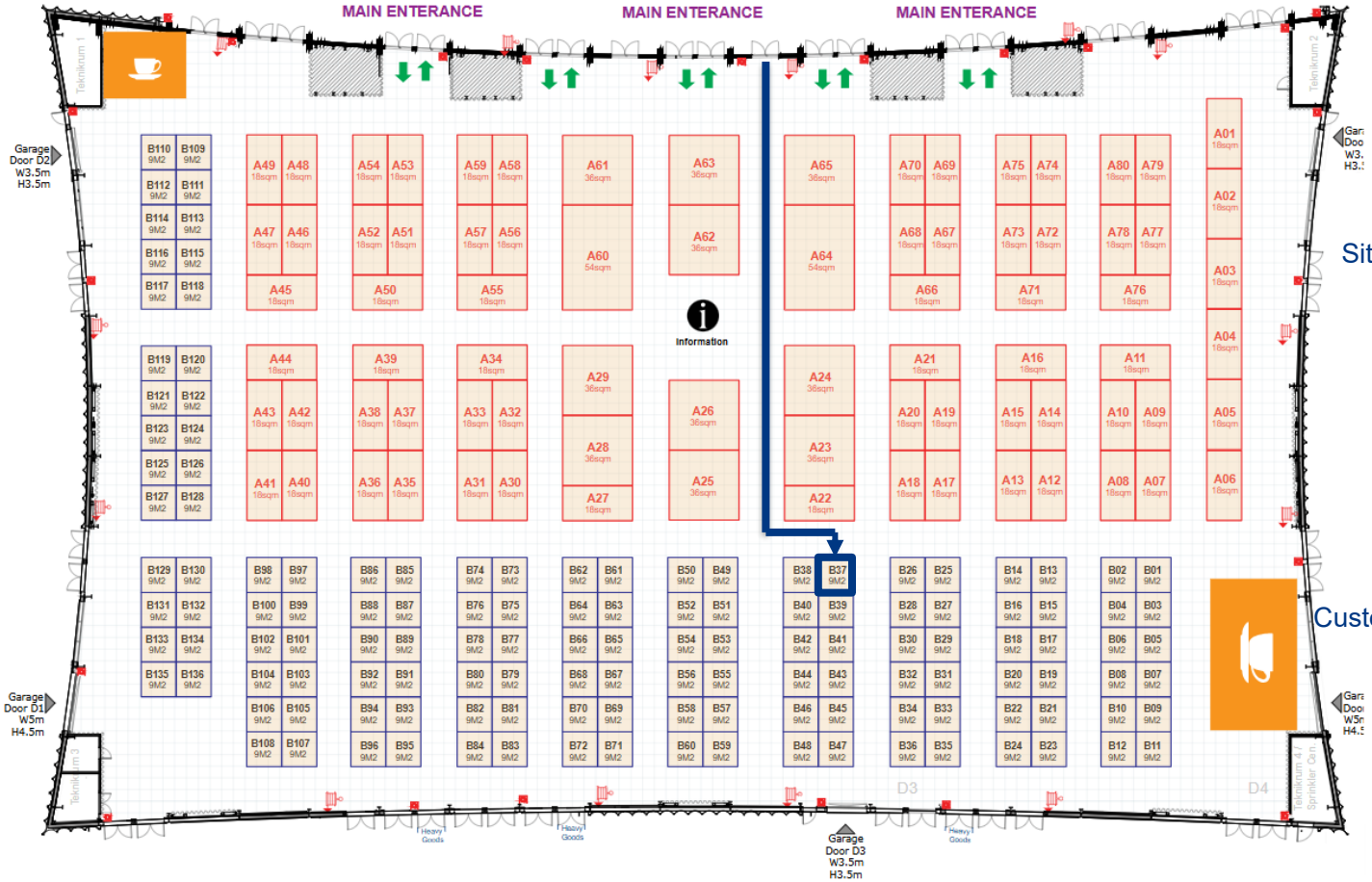
Detection of foreign and honey native enzymes using proteomics

- Added enzymes like amylase and invertase can mimic natural honey properties or speed up processing
- Foreign enzymes are used for sugar syrup production
- Proteomics is like fingerprinting proteins
  - Larger molecules than in metabolomics (small molecules like sugars, acids, and other chemical compounds)
  - Proteomics helps detecting illegal processing or enzyme addition (directly or indirectly through sugar syrups)
  - Metabolomics helps detect sugar adulteration or mislabeling of origin
- Mass spectrometry as “protein scanner”

For more information please visit us at Booth 37



You can find us here: B37



Dr Christof Kunert
Site Manager / Authenticity Testing

Renata Domsky
Customer Service and Administration

Elena Schnelker
Account Manager