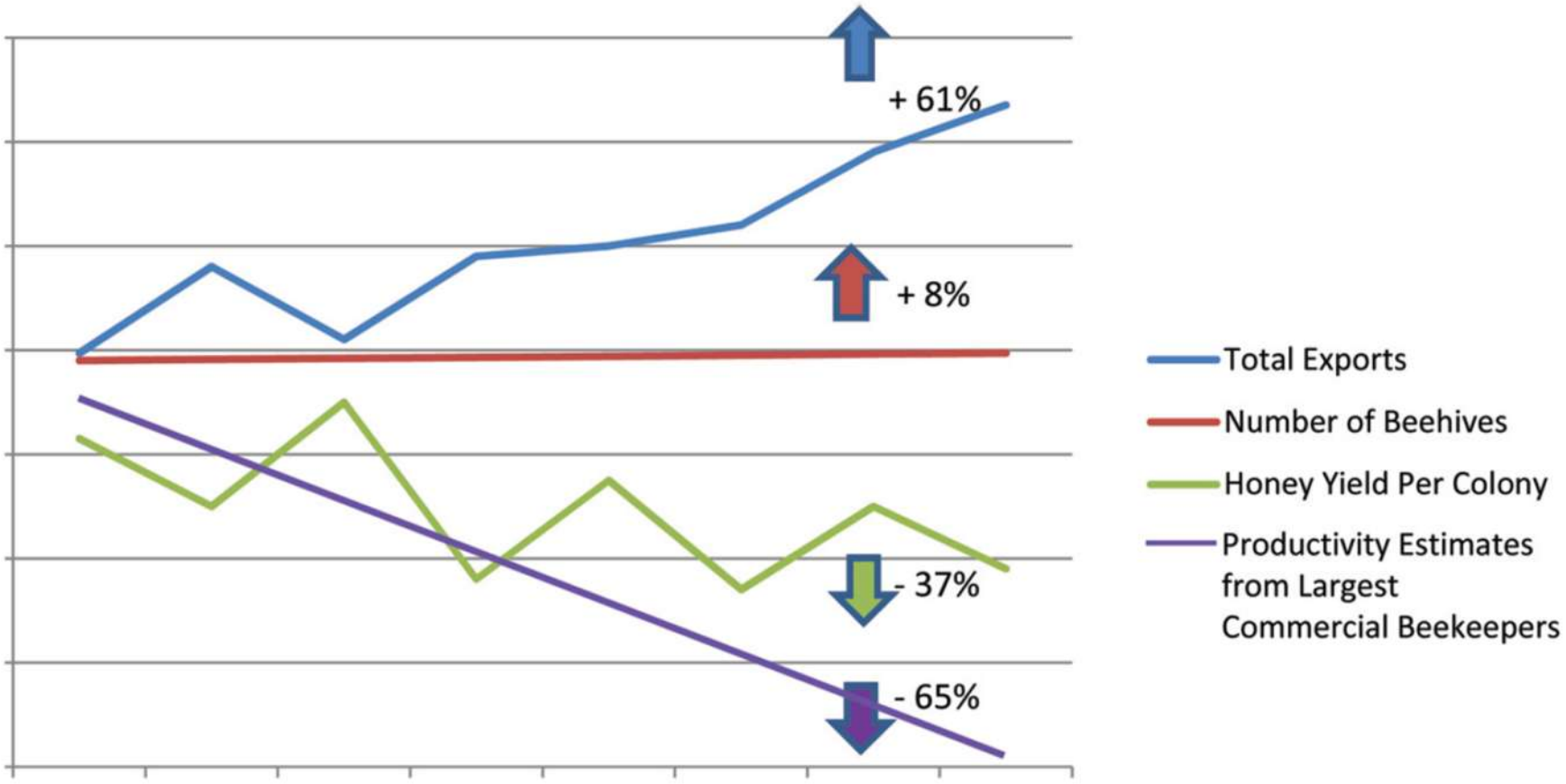


Identification of Syrup Markers using High Resolution Mass Spectrometry (LC-HRMS)

Tobias Wiezorek

Tentamus Locations - Honey

Why do we need to be concerned?



R. Phipps, American Bee Journal, September 2019

LC-High Resolution Mass Spectrometry

Yet another method to detect adulteration?

Aren‘t there enough methods already?

„Designer“ Syrup Offer on Alibaba 1

Rice Syrup for production of honey. Passes SMR, TMR, TLC, C3, C4, C13 Tests.

Fructosa jarabe de arroz para la miel (BS SMR TMR TLC C3 C4 C13 prueba pasar F55 F48)

$500.00 - $750.00

Pedido mín.: 1 Tonelada métrica

„Designer“ Syrup Offer on Alibaba 2

Honey Blend Syrup Pass the C3 C4 C14 TMR SMR Oligosaccharids

FOB **Reference** Price: Get Latest Price

$0.35 - $0.43 / Pieces | 1 Ton/Tons (Min. Order)

Contact Supplier

Leave Messages

Payment: More

Shipping: Alibaba.com Ocean Shipping Service from China to U.S

Get shipping quote

„Honey Blend Syrup Pass the C3 C4 C14 TMR SMR Oligosaccharids“

Probably referring to ^{13}C-Isotopes (C_4/C_3 sugars)

LC-High Resolution Mass Spectrometry

Yet another method to detect adulteration?
Aren‘t there enough already?

YES!

LC-HRMS needed
as it is able to detect adulterations that otherwise remain undiscovered.

Targeted vs. Non-targeted

TARGETED ANALYSIS

Looks at specific molecular masses and fragments (single substances, e.g. residue analysis, structure elucidation)

Scans an entire mass range (high resolution finger-printing of com-pound mixture)

NON-TARGETED ANALYSIS

Foreign Enzymes
13C-Isotopes

NMR
HRMS

Two different approaches

Illustration by
Carmen.Diaz.Amigo@ Focos-Food.com

NMR vs. LC-HRMS

NMR

- Profiling of Sugars
- Profiling of other major compounds
- Low sensitivity (conc. range mg/kg)

Sensitivity

LC-HRMS

- Profiling of minor compounds
- High sensitivity (conc. range µg/kg)

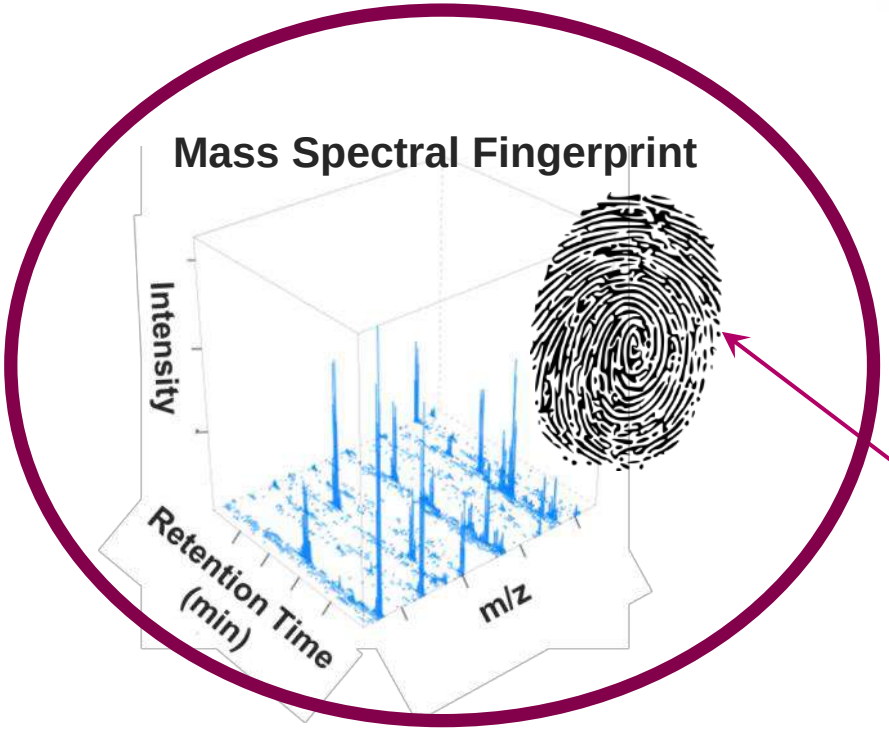
→ **Complementary Methods as different concentration ranges are targeted.**

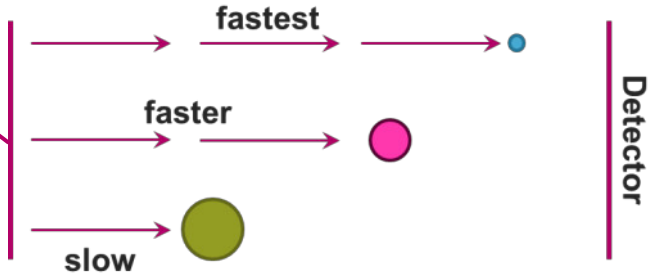
How it works 1 – non-targeted Analysis

Sample preparation

LC-HRMS

Accurate Mass with ToF

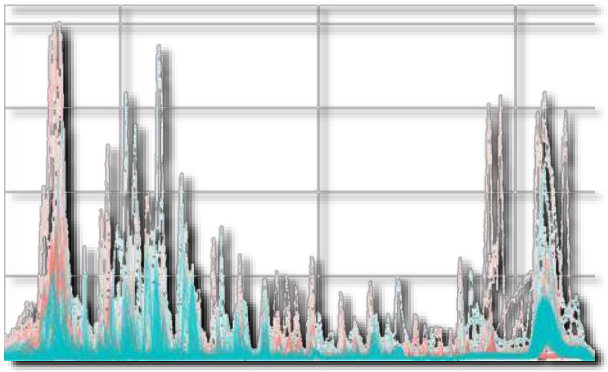




ToF = Time of Flight
→ very precise measurement of molecular masses
Bigger molecules slower than smaller molecules

How it works 2 – Data Evaluation

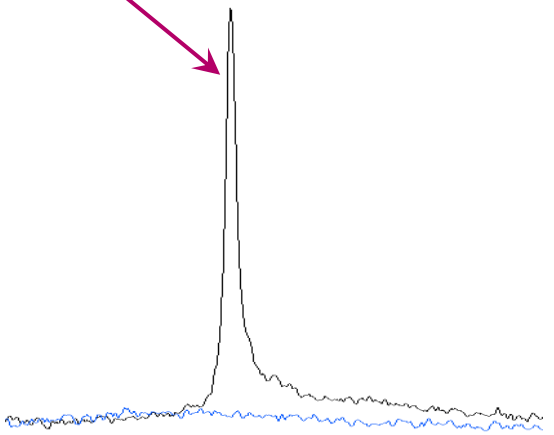
Fingerprint of authentic Honeys



Comparison

Statistics

Multiple Syrup Marker



Authentic Honey (blue, no peak)

Fingerprint of Syrups

A dataset of proven adulterated (e.g. with NMR, Isotopes,…) samples will be checked using the newly found LC-HRMS-markers.

How it works 2 – Data Evaluation

Syrup Marker

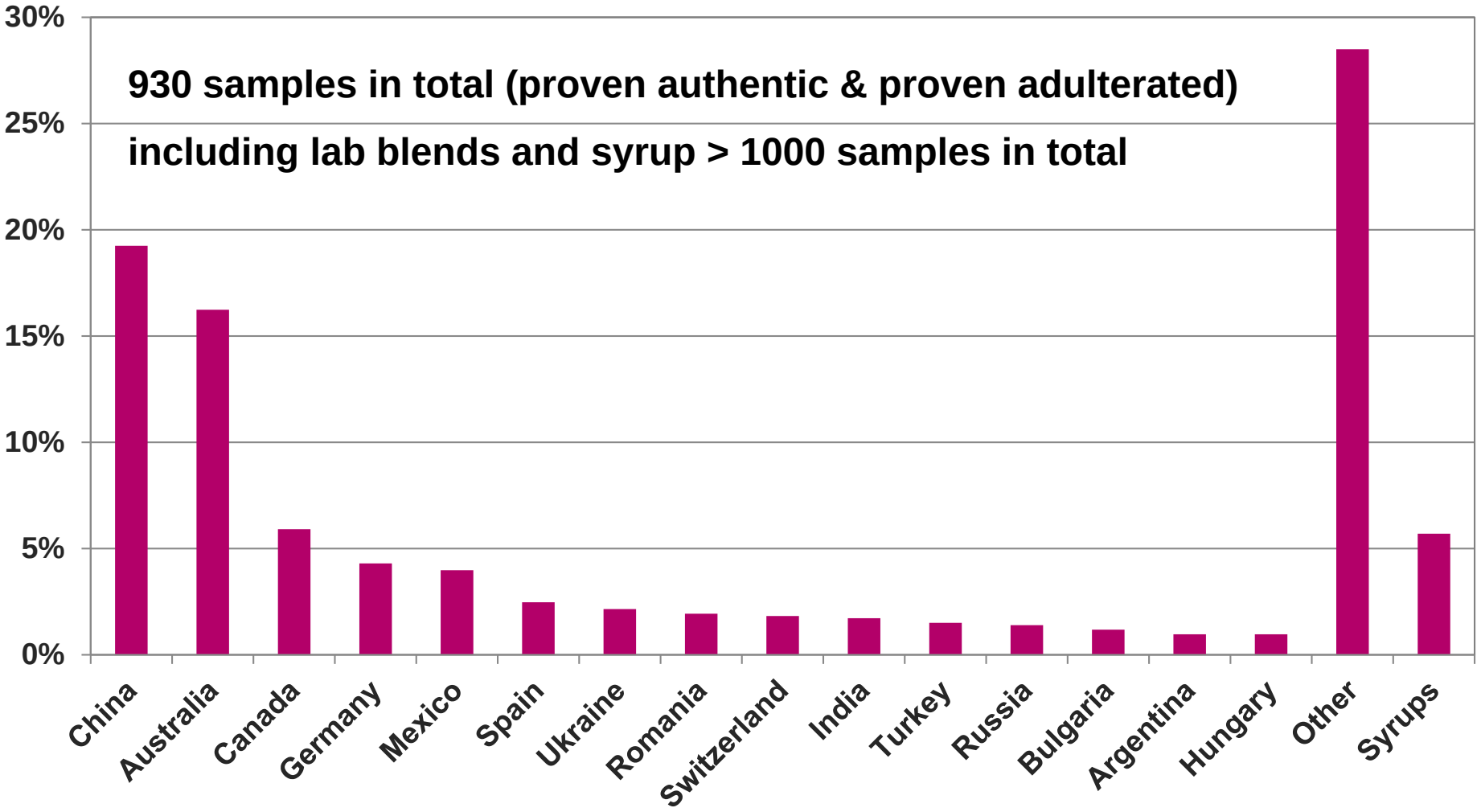
Nature created a vast number of substances!

It is absolutely indispensable to have a set of authentic honeys to exclude natural occurrence or to define natural concentration ranges of newly found markers.

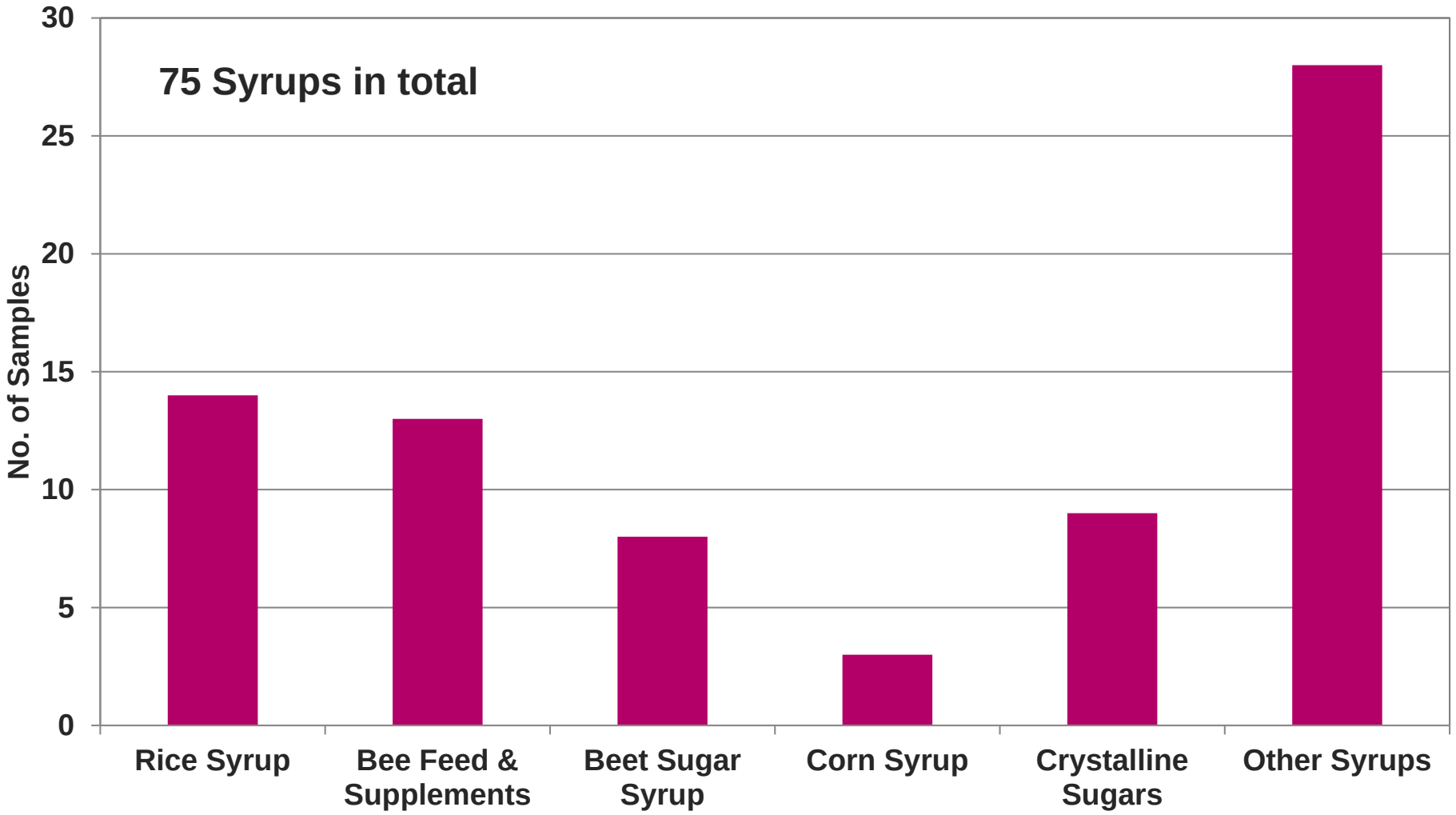
Occurrence of a set of markers that <u>does not naturally occur</u> in honey is sufficient to prove addition of syrup.

checked using the newly found LC-HRMS-markers.

Origins of HRMS-Database Samples



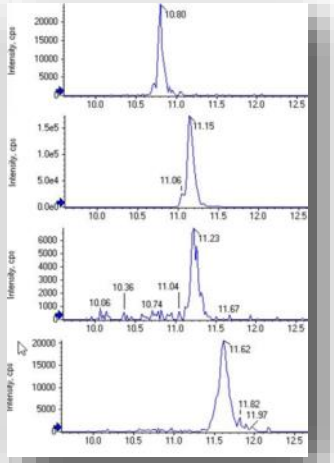
Syrups in HRMS-Database



Results non-targeted LC-HRMS

- More than 1000 samples analyzed
  - Occurrence of a single marker is enough if that marker could not be found in authentic honeys
- Far over 100 syrup markers found

LC-HRMS is very sensitive. It is nearly impossible to prevent interferences e.g. through sample prep in a routine environment.



→ Know your Background

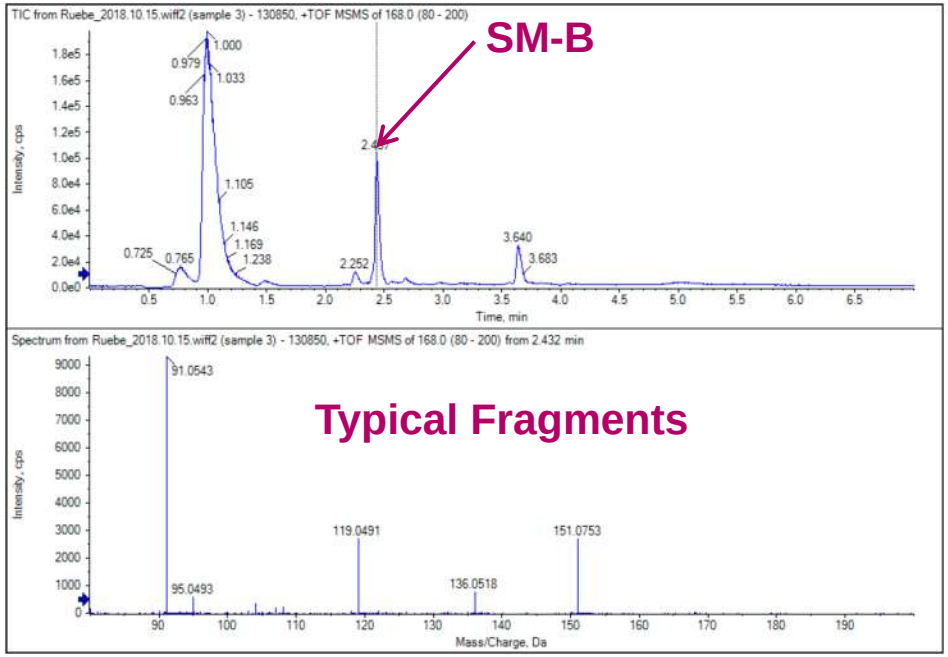
- Interferences
  - 12 from plastic containers
  - 33 from syringe filters
  - 10 from cosmetic products (e.g. hand lotions)

Further capabilities of HRMS

<u>Identification of Substances</u> by analysis of accurate mass

Molecular mass of SM-B (Specific Marker Beet Sugar) has been long known

So far it was unknown what substance is hiding behind SM-B

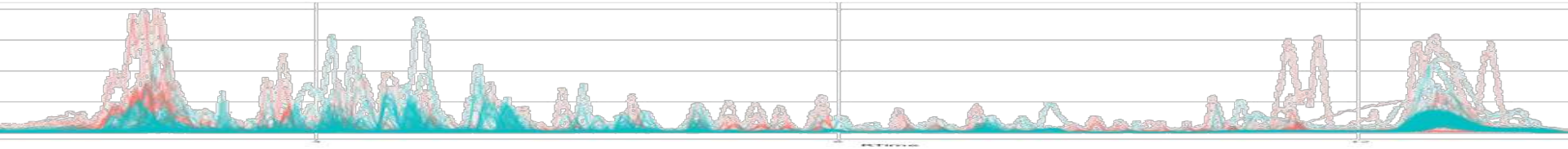


Molecular structure unknown

In close cooperation with foodQS

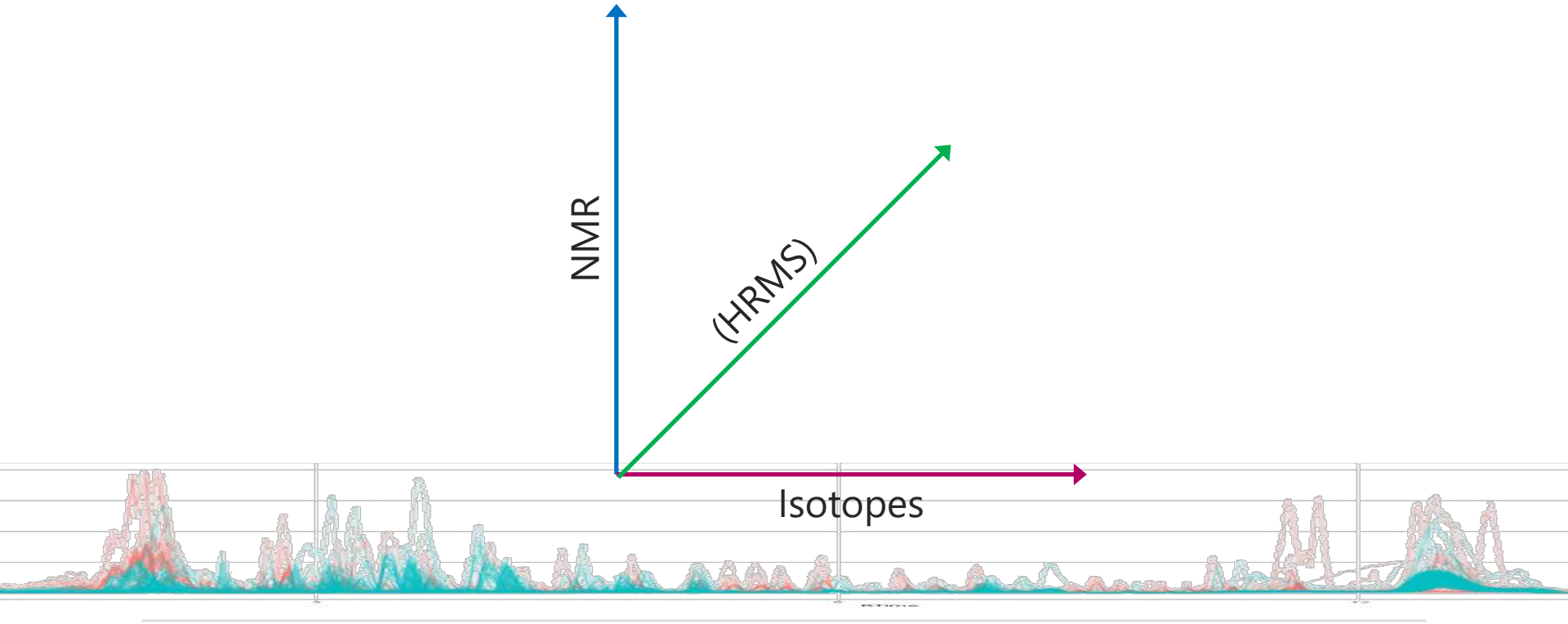
Outlook

- Extending dataset for syrup and honey
- Further statistical evaluation
- Further identification of new marker masses for
  - Adulteration
  - Botanical Origins
  - Processing
- Preparation of further blends of honey and syrup to better discriminate feeding and adulteration
- Transfer of markers from LC-HRMS to Triple Quad for routine analysis



Strategy for your adulteration test

- Orthogonal testing gives highest certainty
- Screening techniques makes adulteration very difficult but also allows differenciation between feeding and commercial adulteration
- Screening techniques demand a database

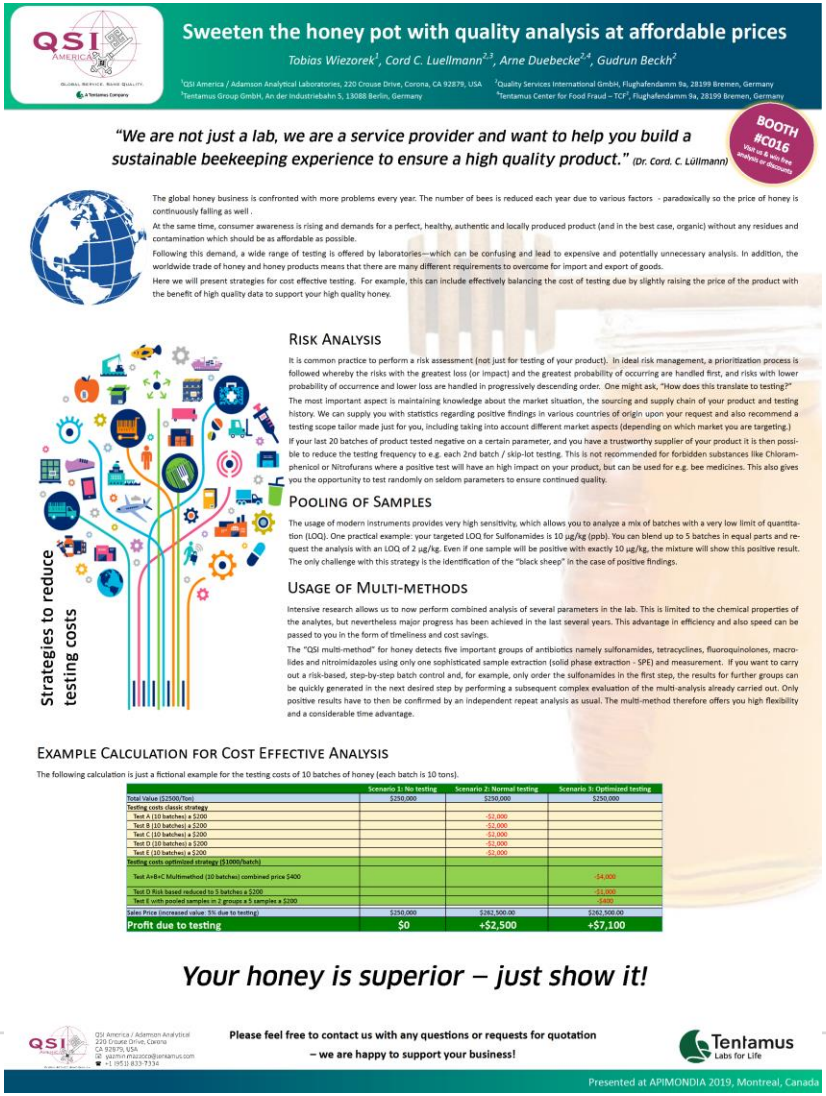


Recommendation

Poster P.16.380 - "*Sweeten the honey pot with quality analysis at affordable prices*"

Visit us at Booth C016

See also GenuHoney Booth C131.



Sweeten the honey pot with quality analysis at affordable prices

Tobias Wiezorek[1], Cord C. Luellmann[2,3], Arne Duebecke[2,4], Gudrun Beckh[2]

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[3]Tentamus Group GmbH, An der Industriebahn 5, 13088 Berlin, Germany [4]Tentamus Center for Food Fraud – TCF², Flughafendamm 9a, 28199 Bremen, Germany

"We are not just a lab, we are a service provider and want to help you build a sustainable beekeeping experience to ensure a high quality product." (Dr. Cord. C. Lüllmann)

BOOTH #C016 Visit us & win free analysis or discounts

The global honey business is confronted with more problems every year. The number of bees is reduced each year due to various factors - paradoxically so the price of honey is continuously falling as well .

At the same time, consumer awareness is rising and demands for a perfect, healthy, authentic and locally produced product (and in the best case, organic) without any residues and contamination which should be as affordable as possible.

Following this demand, a wide range of testing is offered by laboratories—which can be confusing and lead to expensive and potentially unnecessary analysis. In addition, the worldwide trade of honey and honey products means that there are many different requirements to overcome for import and export of goods.

Here we will present strategies for cost effective testing. For example, this can include effectively balancing the cost of testing due by slightly raising the price of the product with the benefit of high quality data to support your high quality honey.

Strategies to reduce testing costs

Risk Analysis

It is common practice to perform a risk assessment (not just for testing of your product). In ideal risk management, a prioritization process is followed whereby the risks with the greatest loss (or impact) and the greatest probability of occurring are handled first, and risks with lower probability of occurrence and lower loss are handled in progressively descending order. One might ask, "How does this translate to testing?"

The most important aspect is maintaining knowledge about the market situation, the sourcing and supply chain of your product and testing history. We can supply you with statistics regarding positive findings in various countries of origin upon your request and also recommend a testing scope tailor made just for you, including taking into account different market aspects (depending on which market you are targeting.)

If your last 20 batches of product tested negative on a certain parameter, and you have a trustworthy supplier of your product it is then possible to reduce the testing frequency to e.g. each 2nd batch / skip-lot testing. This is not recommended for forbidden substances like Chloramphenicol or Nitrofurans where a positive test will have an high impact on your product, but can be used for e.g. bee medicines. This also gives you the opportunity to test randomly on seldom parameters to ensure continued quality.

Pooling of Samples

The usage of modern instruments provides very high sensitivity, which allows you to analyze a mix of batches with a very low limit of quantitation (LOQ). One practical example: your targeted LOQ for Sulfonamides is 10 µg/kg (ppb). You can blend up to 5 batches in equal parts and request the analysis with an LOQ of 2 µg/kg. Even if one sample will be positive with exactly 10 µg/kg, the mixture will show this positive result. The only challenge with this strategy is the identification of the "black sheep" in the case of positive findings.

Usage of Multi-methods

Intensive research allows us to now perform combined analysis of several parameters in the lab. This is limited to the chemical properties of the analytes, but nevertheless major progress has been achieved in the last several years. This advantage in efficiency and also speed can be passed to you in the form of timeliness and cost savings.

The "QSI multi-method" for honey detects five important groups of antibiotics namely sulfonamides, tetracyclines, fluoroquinolones, macrolides and nitroimidazoles using only one sophisticated sample extraction (solid phase extraction - SPE) and measurement. If you want to carry out a risk-based, step-by-step batch control and, for example, only order the sulfonamides in the first step, the results for further groups can be quickly generated in the next desired step by performing a subsequent complex evaluation of the multi-analysis already carried out. Only positive results have to then be confirmed by an independent repeat analysis as usual. The multi-method therefore offers you high flexibility and a considerable time advantage.

Example Calculation for Cost Effective Analysis

The following calculation is just a fictional example for the testing costs of 10 batches of honey (each batch is 10 tons).

	Scenario 1: No testing	Scenario 2: Normal testing	Scenario 3: Optimized testing
Total Value ($2500/Ton)	$250,000	$250,000	$250,000
Testing costs classic strategy			
Test A (10 batches) a $200		-$2,000	
Test B (10 batches) a $200		-$2,000	
Test C (10 batches) a $200		-$2,000	
Test D (10 batches) a $200		-$2,000	
Test E (10 batches) a $200		-$2,000	
Testing costs optimized strategy ($1000/batch)			
Test A+B+C Multimethod (10 batches) combined price $400			-$4,000
Test D Risk based reduced to 5 batches a $200			-$1,000
Test E with pooled samples in 2 groups a 5 samples a $200			-$400
Sales Price (increased value 3% due to testing)	$250,000	$263,500.00	$263,500.00
Profit due to testing	**$0**	**+$2,500**	**+$7,100**

Your honey is superior – just show it!

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Please feel free to contact us with any questions or requests for quotation – we are happy to support your business!

Tentamus Labs for Life

Presented at APIMONDIA 2019, Montreal, Canada

Thank You!

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