# Is my honey authentic ?

# Perspective of QSI Bremen as private and independent expert laboratory on honey authenticity testing



#### Martin Linkogel

State Certified Food Chemist

QSI Bremen, Germany A TENTAMUS COMPANY



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## **QSI Bremen, Germany at a glance**



FOUNDED IN 1954

SINCE 2013 MEMBER OF THE TENTAMUS GROUP

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



AUTHENTICITY TESTING OF HONEY, TEA, COFFEE CANNABIS, PHARMA/GMP





- > 50,000 HONEY SAMPLES PER YEAR
- > 60 YEARS EXPERIENCE IN HONEY TESTING

FULL MEMBER OF THE NMR BRUKER CONSORTIUM SINCE 2014



## What is the business of QSI Bremen?

- QSI is an independent, ISO17025 accredited quality control laboratory and offers a large portfolio of analytical testmethods to customers worldwide
- QSI experts give independent scientific advise and we support our customers with a risk-based and cost-effective quality control strategy and with the scientific interpretation and legal assessment of test results
- QSI developed many inhouse test methods where Norm methods are missing and was for example significantly involved in the dynamic evolution of the NMR Bruker Honey Profiling<sup>™</sup> database since 2014, providing honey expertise to Bruker in weekly discussions in the Bruker consortium and QSI contributed more than 10,000 references to the NMR Bruker database (including generating metadata: microscopic pollen analysis and sensory, IRMS, LC-IRMS, trade analysis, sugar profile, oligosaccharides, foreign enzymes etc.)
- QSI experts actively participate in national and international working groups (DIN, FEEDM, JRC HarmHoney, ISO, USP) for the harmonization of methods



## What is not the business of QSI Bremen?

- QSI is no authority lab and not performing any official controls as long as authorities do not contract QSI for such testing
- QSI is not responsible for method harmonization between honey labs, nevertheless we are highly interested and proactive in this process to facilitate a fair and smooth honey trade and support our customers
- QSI is no academic research institute and not aiming to just publish expert knowledge, methods or results in peer-reviewed journals, but QSI is also engaged in certain R&D projects (FEI projects, academia, diploma and doctoral theses) and continuosly improves inhouse methods with focus on routine quality control (NMR, HRMS etc.), QSI aims to reduce false-positive results, to increase sensitivity to detect foreign sugars in honey and to understand the impact of feeding versus adulteration and the natural variety of honey
- QSI is not responsible for the testing scope and quality control strategy or the testing intentions of our customers (producers, sellers, buyers, traders etc.) and is not involved in their business decisions



# Modes of Economically Motivated Adulteration (EMA)



Ultrafiltration / Diatomaceous earth to remove pollen and origin information



SPMF Meeting Bordeaux Feb 2024

Grey zone: Moisture reduction, harvesting unripe honey

Heat treatment (to inactivate foreign amylases)

## Bee feeding: simple foreign sugars

- Bee feeding is common and legal under Good Beekeeping Practices in winter-time, between the harvests and if the bees starve (drought, bad weather conditions), also in organic honey production
- Feeding syrups are typically simple, not sophisticated, not purified, for example different Levudex<sup>®</sup> syrups (South America), Apifonda<sup>®</sup> Candy (Europe) or own production of beekeepers (often sucrose, beet or cane)
- For easy use of feeding syrups and not to harm the bee's stomach, liquid syrups should be used that do not contain crystals of glucose or sucrose (ideally High Fructose syrups, HFCS)
- In Europe and Asia mainly C3 sugars are used from beet, rice, wheat etc., but also C4 sugars from cane and maize/corn
- In Americas mainly C4 sugars are used from cane and maize/corn, but possibly also C3 sugars from manioc/tapioka etc.



## Adulteration: sophisticated foreign sugars

• Often same sugars like for bee feeding, but the syrups are mostly purified:

A) Purified starch-based syrups: C3 sugars from rice, wheat etc.
 Often < 0.1% oligos, opposed to oligo levels of up to 35% in simple feeding syrups</li>

**B)** Purified sucrose-based syrups: mainly beet sugar (C3 plant) As worst case: inverted syrup, contains only fructose and glucose, no sucrose/oligos

- C4 sugars are not used for adulteration, purified or not, cane or corn syrup (C4 plants) would fail the IRMS > 7.0% and even > 1% LC-IRMS
- Sophisticated C3 syrups are constantly optimized for profit, detection limits of laboratories are evaluated via gradual test mixtures of syrups in honey ("race with the labs"), known or published markers are removed: NMR markers like mannose and DHA, HRMS markers like DFA, SM-B, SM-R, oligos and/or polysaccharides etc.
- HMF is removed (HMF-Decreaser), enzymes are added (Diastase-Plus) to cover syrup and heat damage and to comply with legislation, colorants are added etc.



# Overview foreign sugars (feeding and adulteration)



### Bee enzymes Diastase alpha-Amylase (EC 3.2.1.1) Starch depletion Saccharase / Invertase (EC 3.2.1.20) Sucrose depletion Glucose Oxidase (EC 1.1.3.4) ➡ Glucose depletion, $H_2O_2$ , gluconic acid built (prevents honey from fermentation, ", peroxide activity") further enzymes (Katalase, acidic Phosphatase)



## Overview methods honey authenticity testing (in order of appearance)

- Fellenberg test (1911), Fiehe's test (1936), (foreign dextrins, inverted sugar)
- Pollen Analysis (1978, Louveaux et al., Melissopalynology)
- **13C-EA-IRMS** (C4 sugars; AOAC method 998.12, SCIRA) (1998; White et al.)
- 13C-EA-LC-IRMS (C4/C3 sugars) (2008)
- Beta-Fructofuranosidase (foreign enzyme) (2008)
- Beta-gamma-Amylases (2008)
- Honey-foreign Oligosaccharides and Polysaccharides (HPAEC-PAD) (ca. 2008)
- Thermostable alpha-Amylases (2009)
- Caramel color E150d (2010)
- SM-R (Specific Marker Rice) (2013)
- TM-R (Trace Marker Rice, Arsenic) (2013)
- SM-B (Specific Marker Beet / starch-based syrups from plant roots (manioc)) (2013)
- Famyp (Foreign alpha-amylase profiling) (2015)
- 1H-NMR Bruker Honey Profiling (Nuclear Magnetic Resonance Spectometry,) (2015)
- HRMS / LC-MS/MS (High Resolution Mass Spectrometry) (after 2015)
- Psicose (ca. 2018)



# False-positive results – QSI perspective

- IRMS (harmonized AOAC method 998.12, C4 sugars):
  - Manuka honey (possibly reaction MGO/protein during storage or other reasons)
  - Pine honey (wax wool with very negative isotopic values)
  - Honeys from Africa with high sediment (would require filtration, not allowed)

#### LC-IRMS (using Elflein&Raezke Apiodology 2008 benchmark criteria):

- Small feeding remains from simple C4 sugar syrups (cane, corn) starting from about 1% C4 sugar, depending on syrup and honey
- Honeys with sorghum honeydew (naturally introduced C4 sugars)
- Pine, Bracatinga and other honeydew honeys naturally containing oligosaccharides
- Lavender and acacia honeys, fraction of disaccharides failing (Difference max.)
- Fermented honeys (yeasts contamination impacting protein value, more positive)
- Many other honeys / blends showing high natural variation of minor sugars, fail also due to measurement uncertainty of the method (especially trisaccharides)

### Oligosaccharides and Polysaccharides (HPAEC-PAD):

- Bracatinga honey (Brazil)
- Small feeding remains from simple starch-based syrups detectable (corn, rice, wheat etc.) starting from about 3% level in honey, depending on syrup



# False-positive results – QSI perspective

- NMR Bruker Honey Profiling<sup>™</sup>
  - In certain rare honeys not well reflected in the database (> 28,500 honeys, thereof > 2,000 honeydew honeys), foreign sugar markers might be <u>positive</u> <u>but not related with foreign sugars</u>, but due to the special botanical source (Examples: Lavender honey until Bruker database version 3.1, Jan 2023, honeys with high sucrose or low proline, Aroeira Brazil, Acacium mangium Vietnam, etc.)
  - Certain honeydew honeys with small natural levels of mannose and DHA (e.g. Eucalyptus, Jujube (Ziziphus)), QSI NMR expert assessment recommended
  - Certain fresh or unripe honeys (markers turanose, proline, e.g. rape honey)
  - Honeys with remains of simple feeding syrups (depending on syrup and honey)

## HRMS / LC-MS/MS (markers):

- Honey from Mexico, Cuba, Argentina and Uruguay failing marker SM-B (3-Methoxytyramin), presumably natural plant origin of marker
- Can be very sensitive: if markers are known, starts detecting from 1% to 5%, so that honey with small feeding remains of simple feeding sugars might already fail
- Potentially natural occurrence of other HRMS markers (like SM-B, depending on lab and database of authentic honeys)
- If mannose is used as marker (not by QSI), certain honeydew honeys naturally contain mannose, also DHA needs to be considered in combination to exclude false positives



# False-positive results – QSI perspective

## • Beta-gamma-Amylases:

- Avocado, Pine, Quillaya and Metcalfa honeys, Sorghum honey, other honeys containing honeydew from Metcalfa (also East Europe, Americas) naturally show activities >> 5 U/kg (decision limit)
- Simple starch-based feeding syrup might show high activity if produced by amylases so that already 1% feeding sugar might cause a positive result

#### Beta-Fructofuranosidase:

- Simple sucrose-based feeding syrup (beet, cane) might show high activity if produced by beta-FF so that already 1% feeding sugar remains (technically unavoidable) might cause a positive result (LOQ = 20 U/kg)
- Rotten fruits, feeding yeasts (Brewer's yeast) or inadequate bee feeding supplements produced from Saccharomyces cerevisae can contaminate honey

#### Psicose:

- Chestnut, Jujube and Monte (Argentina) high natural content 0.3% and more (decision limit max. 0.10%)
- Fiehe's test:

- Honeydew honey can be positive, not suitable



# False-negative results – QSI perspective

- IRMS (harmonized AOAC method 998.12, C4 sugars):
  - C3 sugars cannot be detected due to similar isotopic values to honey,
    C3 sugars thus not in the scope of the method (syrups from beet, wheat, rice etc.)

#### LC-IRMS (using Elflein&Raezke Apiodology 2008 benchmark criteria):

 C3 sugars cannot be well detected due to similar isotopic values to honey, but LC-IRMS shows some sensitivity in some cases as the different sugars are separated giving insight and more sensitivity than IRMS, but depending on honey and C3 syrup, levels up to 100% in honey could pass the benchmark criteria, if no oligos are contained in the syrup

### NMR Bruker Honey Profiling<sup>™</sup>

Sophisticated syrups, especially if do not containing markers (like for example mannose/DHA) but only fructose and glucose: not so sensitively detected: up to ca. 20% to 40% syrup might not be detected in Bruker evaluation, even not via unique dilution effects in the NMR (targeted and untargeted), QSI NMR expert assessment recommended for high-risk countries and additional tests like HRMS (further info in QSI+Bruker Webinar, November 2023, watch on demand possible:

https://www.bruker.com/en/news-and-events/webinars/2023/bee-or-not-to-be-is-your-honey-authentic.html)

- Small foreign sugar levels below about 5% to 10% from feeding are mostly not yet detected against the Bruker database



# False-negative results – QSI perspective

## HRMS / LC-MS/MS (markers)

- Depending on HRMS marker database (each lab has own database) and the markers contained in a particular syrup, significant foreign sugar levels might pass (up to 100%)
- Syrups are optimized and purified ("race with labs"), databases require regular updates to cover new syrups (e.g. Mannose/DHA removed, but also other markers)
- Due to lack of method harmonization, different thresholds and markers are applied and a sample could pass in one lab and fail in another lab (below individual threshold)
- Due to +- matrix suppression effects in the HRMS or LC-MS/MS quantification, and a lack of marker references (mostly unknown markers), different sensitivity for different honeys (blossom honey less suppression effects than honeydew honey)
- Might be less sensitive than HPAEC-PAD (Decision limit 0.1%) especially for polysaccharides (DP10-20) due to matrix suppression effects in the HRMS (only about 0.5% threshold in honey for polysaccharides, about 0.05% for oligos)
- If Mannose is tested with 700 mg/kg LOQ (HRMS JRC) false-negatives are possible compared to NMR Bruker (LOQ = 200mg/kg) between 200mg/kg and 700mg/kg level ("From the Hives", if mannose not directly tested by NMR Bruker but only by HRMS)
- Orbitrap HRMS not sensitive enough for SM-B (3-Methoxytyramine), marker not sensitively detected, limit required: 2.4 ppb 3-Methoxytyramin = 5% SM-B at QSI (reference syrup), other labs might have different limit or do not use SM-B as marker



# False-negative results – QSI perspective

## Beta-gamma-Amylases:

- Heat treatment for 7min at 65°C decreases activity to < 5 U/kg (limit authentic) so that after processing method is no longer indicative for foreign sugar syrup

#### • Psicose:

- Many feeding syrups only show a level of close to 0.10% (threshold) in 100% syrup, thus test not sensitive at all to detect such foreign sugar syrup in honey

#### • Oligosaccharides and Polysaccharides (HPAEC-PAD):

- Not present in sucrose-based syrups (beet and cane sugar) and sophisticated, purified starch-based syrups used for adulteration (rice, wheat), 100% syrup passes

#### • Caramel color E150d:

- LC-MS/MS marker levels strongly reduced since method was established in 2010, method likely not sensitive enough anymore for new syrups stained with caramel color E150d
- Different labs use different reference standards for E150d marker calibration, false-negatives possible, even with same E150d marker LOD of 2mg/kg:
  e.g. one lab quantifies 4.0mg/kg (above LOD), other lab same E150d level detected, but due to use of higher concentrated reference material 0.8 mg/kg quantified (below LOD); inhouse method, not published, markers only used by QSI, Intertek, Eurofins Ritterhude
  + authority labs of Czech Republic/Slovakia, other labs might have different results



## Overview EU action "From the Hives" (03/2023)

• The JRC tested a total of 320 import honey samples for foreign sugars markers using 4 modern analytical methods (not accredited at JRC):

IRMS / LC-IRMS (AOAC-method C4 sugars, C4/C3 sugars)
 HRMS (Mannose, DFA, SM-R, Oligosaccharides DP6 to DP9)
 NMR Bruker Honey Profiling <sup>TM</sup> (Mannose confirmation only)
 HPAEC-PAD (Oligosaccharides DP6 to DP9 and Polysaccharides DP10 to DP20)

- A total of 147 samples (46%) failed 1 or more of the 4 tests used by the JRC and were classified as "suspicious" (adulterated)
- The JRC did not specify which tests failed and only partly described the decision criteria for the judgement (see JRC technical report 130227), therefore it finally cannot be answered by QSI, what evidence was obtained from which test for the "suspicious" samples from the different countries



## Origin of suspicious honey consignments "From the Hives"

China 23 66 Ukraine 61 13 27 Argentina 7 Mexico 16 Brazil 10 Turkey 14 Uruquay Most samples failed from undetermined 6 China (90%), United Kingdom Moldova 8 Turkey (93%) and Russia UK (100% also Chinese honeys assumed), New Zealand Cuba but also countries like Zambia Argentina, Mexico, Uruguay and Brasil fail up to 50% Vietnam India Israel For many countries too few samples were Guatemala Georgia tested, might be not representative! Ethiopia Chile 0 10 20 30 40 50 60 70 80 90 100 Number of consignments Non-suspicious







Source DG SANTE report: https://food.ec.europa.eu/safety/eu-agrifood-fraud-network/eu-coordinatedactions/honey-2021-2022\_en

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Source: Ždiniaková, T., Loerchner, C., De Rudder, O., Dimitrova, T., Kaklamanos, G., Breidbach, A., Respaldiza Hidalgo, M.A., Vaz Silva, I.M., Paiano, V., Ulberth, F. and Maquet, A., EU Coordinated action to deter certain fraudulent practices in the honey sector, EUR 31461 EN, Publications Office of the European Union, Luxembourg, 2023, ISBN 978-92-68-01292-5, doi:10.2760/184511, JRC130227

## Results JRC versus QSI (2022 ^)

	IRC	OSI 2022				
Origin	13C-IRMS and 13C-LC-IRMS + HRMS (Mannose 700 mg/kg, Oligos, other markers) + NMR Bruker for confirmation + HPAEC-PAD (Oligosaccharides)	13C-IRMS (AOAC method 998.12) (C4 sugars > 7.0%)	13C-LC-IRMS (C3/C4 sugars)	NMR Bruker + QSI (including mannose 200 mg/kg, many	HRMS (not yet including Oligosaccharides, but	Oligosaccharides (DP5 to DP20) (HPAEC-PAD)
	% suspicious	% adulterated	% positive	% positive	markers SM-B, SM-R etc.) % positive	% positive
Argentina *****	21	0,7	31,4	1,8	4,3	44
Brazil *	44	0,2	3,8	1,5	2,7	11
China	74	0	0,6	31	9,4	2
Cuba **	0	0	0	0	80	0
France ****	not tested	0,5	23,7	8,0	3,5	10
Greece	not tested	2,9	4,3	29	70	60
Guatemala	0	0	11,8	0	0	0
Hungary	not tested	0,2	3,7	17	10	24
India	100	13,3	7,6	35	15	5
Mexico ***	27	0,6	7,8	6,8	33	1
New Zealand	60	5,0	3,7	4,4	0	not tested
Romania	not tested	0,7	3,8	12	4,6	39
Spain	not tested	0,6	0	11,7	0	0
Thailand	not tested	3,3	6,3	21,1	1,6	0
Türkiye	93	3,3	11,9	23,3	<mark>62</mark>	17
United Kingdom	100	0	0	not tested	0	not tested
Ukraine	18	0	1,6	6,4	0,8	2
Uruguay *****	50	1,2	35,4	1,2	1,9	0
Vietnam	50	0,2	16,1	15,0	4,3	0
Total	46	2,5	12,3	20,1	10,7	18,2

\* all positive honeys Oligos: Bracatinga, natural origin of oligos

\*\*\*\* Lavender honey, natural exception for difference max. for disaccharides

\*\* in 100% of Cuban positive samples SM-B > 5% detected (max. 950%), natural origin for Cuba \*\*\* in 100% of Mexican positive samples SM-B > 5% detected (max. 200%), natural origin for Mexico ^ QSI samples from routine tests 2022, submitted by customers, declared origin not verified, might imply also suspect samples, not necessarily representative for the country of origin

\*\*\*\*\* At QSI mostly Sorghum honey naturally causing LC-IRMS Difference max. to fail without detection of C4 sugars Recently also SM-B excluded with natural origin for Argentina and Uruguay

## Conclusion

- Honey authenticity testing and assessment of test results is complex and requires in-depth honey expertise
- Method harmonization and standardization of the assessment of results (limits/thresholds) is required (so far only IRMS (AOAC method 998.12) and privately owned NMR Bruker Honey Profiling<sup>TM</sup> evaluation harmonized)
- False-positives are possible for nearly all techniques applied and need to be considered (honey expert assessment recommended)
- False-negatives are also possible and due to the race of fraudsters with the labs in optimizing sophisticated syrups, databases need to be updated regularly + the sensitivity of the test methods must be increased continuosly
- Feeding is a potential source for positive results not related with EMA, therefore bee feed should be tested if it has a potential impact on the quality and authenticity of honey (Field of tension: technically unavoidable feeding remains introduced by bees vs. overfeeding, no timely stopping)
- Methods should be evaluated in independent ring trials if they are "fit-for-purpose" (for example "HarmHoney project" of EU JRC)



# Thank you for your attention!

Do you have any questions? Get in touch



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Martin Linkogel +49 421 596 607 0 <u>martin.linkogel@tentamus.com</u>



# **Backup Slides**

## Summary NMR Bruker Honey-Profiling<sup>TM</sup> (current database version 3.1)

#### Key advantages

- Harmonized: Bruker protocol and database evaluation (worldwide)
- Comparable results and assessment from different labs using NMR Bruker Honey-Profiling<sup>TM</sup> Problems of previous database version 2.0 (until July 2021) with false-positive markers related to processing and heat treatment were successively eliminated, comparability is continously improved and assured in ring trials between honey labs and independent proficiency testing schemes (Pro-PTS)
- Accredited under ISO 17025 including authenticity and foreign sugar evaluation (Bruker)
- Accepted by most honey traders in most countries around the world, also used by authorities (e.g. Canada) and further routinely used since years by honey packers in the EU and India
- Universal: detects all different kinds of foreign sugars (C3/C4), including yet unknown syrups via detection of "dilution effects" (targeted and non-targeted against large Bruker database)
- **Sensitive:** increasing sensitivity for foreign sugars with each new database version, but also not too sensitive and typically not detecting small, technically unavoidable feeding remains
- **Robust:** NMR is a precise, accurate and robust technique, low variation of results, no confirmation measurements required
- **Transparent:** detailed Bruker NMR report available including illustration of foreign sugar markers, performance of sample also depicting borderline ranges for each marker

#### Potential known risks

- False-positives: in the case of certain rare honeys (e.g. Bracatinga, Aroeira) not reflected in the database yet and unripe honeys (e.g. Litchi, Rape): NMR honey experts are required and the Bruker evaluation needs to be improved (regular database updates), microscopic pollen analysis recommended for final assessment of a honey batch
- False-negatives: Might be less sensitive for certain sophisticated C3 syrups (> 20% syrup could pass)



## Summary HRMS / LC-MS/MS (High Resolution Mass Spectrometry)

#### Key advantages

- Sensitive: Most recent, promising state-of-the-art technique to sensitively detect sophisticated foreign C3 sugars from rice, beet, wheat, manioc, but also C4 sugars from cane or corn etc. Via targeted HRMS markers, useful for application in high-risk countries to detect EMA
- Safe: Identification of selected HRMS markers either by LC-HRMS directly or by LC-MS/MS

#### Potential known risks

- Not comparable: Results and judgement from different labs show significant differences due to different instruments, different methods, different databases with selected targeted markers and experiences of each lab, different database approaches (only foreign sugar database in combination with different markers required to be positive for a judgement vs. sugar and authentic honey database, where - like for Bruker NMR - one marker failing is sufficient for the judgement). This has been shown in several ring trials between honey labs (QSI, Intertek, FoodQS) and within the FEEDM (EU packers, 5 honey labs, especially HRMS trial 04/2022), might cause significant troubles in the honey trade if used as major EMA screening method
- False-positives: due to the high sensitivity, potentially already detecting small, technically unavoidable feeding remains, especially for oligosaccharides and polysaccharides from simple starch based feeding syrups, but also other markers that might naturally occur (e.g. SM-B)
- False-negatives: Might fail with 100% syrup, if syrup markers removed/not known to lab/database
- Not harmonized: No commercial HRMS database available, no official method and protocol available, no threshold limits defined, no markers harmonized yet, just a few marker published (SM-R, DFA etc.) in contrast to hundreds of unpublished markers used by the different labs



# Summary 13C-LC-IRMS (C4/C3 sugars)

Key advantages

- Sensitive, selective and specific: no other analytical method allows for differentiation between C3 and C4 sugars. Adds value to the AOAC method 998.12 which cannot investigate the different sugar fractions in honey and only detects C4 sugars. Enhances sensitivity for C4 sugars and partly also detects C3 sugars at higher levels (depending on syrup and honey composition and isotopic values). Gives useful insight into the sugar composition and foreign sugar origin, only method that can detect Sorghum honeydew (Argentina, Uruguay)
- Harmonized analytical method: to be published by CEN soon and analytical results are well comparable between different labs and numerous proficiency tests available (FIT-PTS, Bipea etc.)

#### Potential known risks

- Not harmonized assessment: different limits and benchmark criteria are used by different labs, no official limits available, causes troubles in the honey trade and frequent false-positives, if benchmark criteria of Apidology 2008 are used (too stringent difference max., oligo criterion not valid, just based on 7 honeydew honey not containing natural oligos, not considering high variation of method, as indicated by JRC and many ring trials)
- Too sensitive for C4 sugars: due to the high sensitivity for C4 sugars, method fails already starting from 1% foreign C4 sugars, mostly from technically unavoidable feeding remains (see JRC 104749 honey final report 2016, chapter 5.1) and not EMA
- False-negatives: up to 100% C3 syrup could be not detected (depending on syrup and honey)
- False-positives: Sorghum honeys are detected false positive (natural C4 sugars colleted by bees), exceptions are further known for fresh citrus, acacia and lavender honey, but not official, expert knowledge of the honey labs required for correct assessment



### Summary HPAEC-PAD (Oligosaccharides and Polysaccharides) Key advantages

- Sensitive: State-of-the-art technique to sensitively detect oligosaccharides (DP5 to 9) and polysaccharides (DP10 to 20) from starch-based syrups (foreign C3 sugars from rice, wheat, manioc as well as C4 sugars from maize/corn) and certain bee feeding beet or cane sugars
- **Robust:** Less matrix suppression compared to HRMS / LC-MS/MS (no differences between blossom and honeydew honey), aqueous dextrin solution can be used for limit assessment

#### Potential known risks

- Not comparable detectors: Results and assessment from different labs potentially show significant differences due to different detectors used: besides PAD (<u>P</u>ulsed <u>A</u>mperometry) also ELSD (<u>E</u>vaporative <u>Light S</u>cattering) is used, less sensitive and less selective for reducing sugars like PAD
- Not comparable scope: Some labs analyze only DP 4 to DP 7, some labs state ">DP 5 investigated", QSI tests for Oligosaccharides DP5 to 9 and Polysaccharides DP10 to 20, possibly resulting in significantly different assessment, depending on the syrups present and the scope investigated
- False-positives: due to the high sensitivity potentially already detecting small, technically unavoidable feeding remains, especially from simple starch based feeding syrups (Argentina, Mexico, but also East and Southeast Europe) but also from Apifonda<sup>®</sup> (mainly sucrose, other sugars and oligosaccharides and polysaccharides at high level), starts detecting from about 3%
- False-negatives: Not sensitive enough for sophisticated syrups with < 0.1% oligosaccharides and polysaccharides
- Not harmonized: no official method and protocol, scope of DP investigated not standardized, some labs quantify, some labs just identify against a corn starch dextrin standard (like QSI) above a 0.1% threshold, no independent proficiency testing or ring trials available

