

The European Commission's science and knowledge service

Joint Research Centre



State of play of the actions in view of better honey authentication and new developments

JRC-Directorate F:
Health, Consumers and Reference Materials
Alain MAQUET

CDG Animal Products – Beekeeping sector, Bruxelles, BE– 17/05/2019

➤ Recommendations

- ✓ Harmonization of analytical methods
- ✓ Biobank of honeys, sugar syrups and bee feeding products
- ✓ European honey reference database
- ✓ Validation of emerging analytical methods

Presented at the CDG Animal Products – Beekeeping sector on 21/11/2017.

JRC TECHNICAL REPORTS

Scientific support to the implementation of a Coordinated Control Plan with a view to establishing the prevalence of fraudulent practices in the marketing of honey

Results of honey authenticity testing by liquid chromatography-isotope ratio mass spectrometry

Administrative Arrangement

N° SANTE/2015/E3/JRC/SI2.706828

Eric Aries, Julien Burton, Luis Carrasco,
Olivier De Rudder, Alain Maquet

2016

Technical Round Table on Honey Authentication

JRC-Geel, Belgium

25 January 2018

Meeting Report

March 2018



➤ Conclusions

- ✓ Critical review of the current definition of identity and purity criteria of honey.
- ✓ Acceptance / rejection criteria for authenticating honey.
- ✓ Appropriate analysis of the vulnerability of the honey supply chain and improved traceability system.

Technical Round Table on Honey Authentication

JRC-Geel, Belgium

25 January 2018

Meeting Report

March 2018



➤ Conclusions

- ✓ Screening methods to economise testing.
- ✓ Analytical methods to detect emerging fraud cases should be developed and already existing methods should be validated.
- ✓ A mechanism for providing quality assurance tools should be established.
- ✓ Chemical and biological characteristics of genuine honeys (including blends), bee feeding products, and products from inappropriate practices should be generated and stored in a publicly available database.

➤ Conclusions

- ✓ Harmonisation/standardisation of the definition of food fraud is necessary;
- ✓ Collaboration within and among Member States and with European Commission needs improvement;
- ✓ Leverage the power of data analytics to fight food fraud;
- ✓ 'Authentomics' can only become reality if appropriate databases are available;
- ✓ Centres of competence should share the workload of detecting fraud in the food chain;
- ✓ Supply of harmonised methods of analysis has to keep pace with technological progress.

First technical meeting of the Knowledge Centre for Food
Fraud and Quality
JRC, Geel, Belgium
4-5 December 2018

Meeting Report

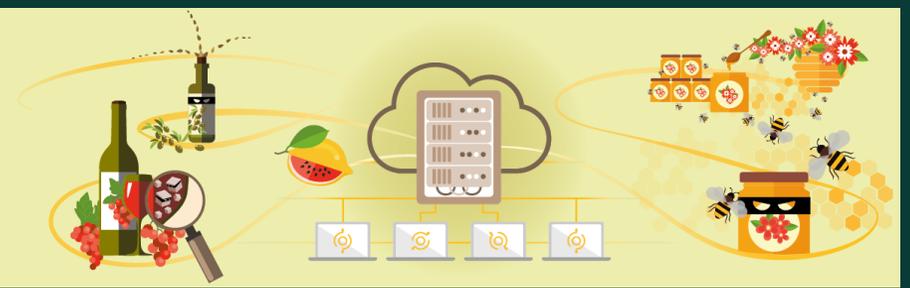


February 2019

1

Ongoing Actions

Knowledge Centre
for Food Fraud
and Quality



➤ Monthly JRC Food Fraud Reporter.

THE  TIMES

Supermarkets stung by funny honey adulterated with sugar and E numbers

Jonathan Leake, Science Editor
March 31 2019, 10:01am,
The Sunday Times

Food and drink Retail
European Union



Tests on supermarket honey suggest some versions may contain foreign sugars, colourings or flavourings
GETTY

The Food Standards Agency (FSA) has issued a warning about alleged adulteration of honey after tests suggested many popular supermarket brands may be impure.

The tests suggest some supermarket versions may contain foreign sugars, colourings or flavourings.

Search ▾ International edition

The Guardian

Rosie Taylor
Wed 30 Jan 2019 2:48 GMT

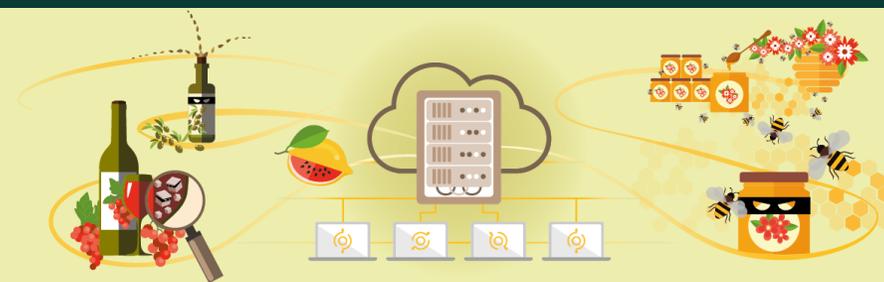
New Zealand brings first 'fake mānuka honey' prosecution

Company is accused of adding synthetic chemicals, including one used in tanning lotion, to honey

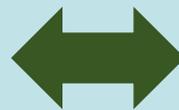


Ongoing Actions

Knowledge Centre
for Food Fraud
and Quality



➤ Food Authenticity Knowledge Base.



CEN/TC 275 N 1992

[CEN/TC 275](#)
Food analysis - Horizontal methods
E-mail of Secretary: carola.seiler@din.de
Secretariat: DIN

FACG Method Priorization June 2018

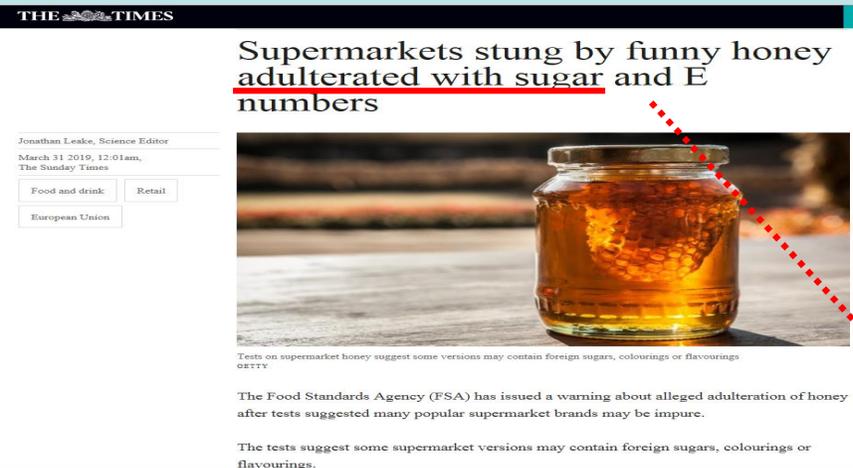
Date of document	2018-08-17
Expected action	Next Meeting
Due Date	2018-10-11

Background

The Food Authenticity Coordination Group (FACG) had the task to search for methods which can be used for analysing authenticity of foods and further needs.

Ongoing Actions

➤ Food Authenticity Knowledge Base.



Search results

Home Browse Dashboard

Login

Search Options

CN Code

Enter only the first four digits

Food Category

Honey And Royal Jelly ▼

Analytical Technique

Any Technique ▼

Level of use

Any Level ▼

Type of fraud

- Dilution
- Substitution
- Concealment/masking

Search Results (11)

Showing results 1 to 10

[Entry #41](#)

Food Category: Honey and royal jelly, Honey

Description of Issue: Addition of undeclared C4 plant-derived sugars

Type of Fraud: Substitution

Issue: Product composition

Level of use: Official

[Entry #57](#)

Food Category: Honey and royal jelly, Honey

Description of Issue: Adulteration of honey by addition of sugars

Type of Fraud: Substitution

Issue: Product composition

Level of use: Used routinely by industry

Ongoing Actions

Entry #226

Food Integrity Issue

Food Category:	Honey and royal jelly
Commodity Detail:	Honey
CN Code:	0409 00 00
Description:	Adulteration of honey by addition of sugar syrups. Determination of honey sugars (trehalose, glucose, fructose, isomaltose and sucrose) by applying HPLC coupled to a Pulsed Amperometric Detector (PAD). This method has been expanded and is used for the official control of sugar profile in honey samples (fructose, glucose, sucrose, turanose, maltose, melezitose, erlose, isomaltose, raffinose and trehalose). It is a more sensitive technique compared to a HPLC coupled to a refractive Index Detector (RI) or GC coupled to a Flame Ionization Detector (FID). The addition of fructose or industrial glucose could change the fructose/glucose ratio, which has to be 1 - 1.2 in pure honey. Maltose is usually present in honey in low quantities (30 mg.g ⁻¹) and is suggested as a marker of natural honey. Higher amounts of maltose concentration may indicate adulteration of honey by sugar syrup or starch hydrolysate.
Type of Fraud:	Substitution
Issue:	Product composition
Importance:	Often
Location in Supply Chain:	Agriculture producer, Food manufacturer, Trader

Analytical Strategy

Method Status

Method Use

Data + References

Method Use

Sample Preparation:	Each honey sample is diluted with ultra-pure water, mixed well and filtered.
Further Information on the Method:	Determination of fructose, glucose, sucrose, trehalose, isomaltose, turanose, melezitose, raffinose, maltose and erlose by HPLC-PAD.
Rules for Interpretation:	The addition of fructose or industrial glucose could change the fructose/glucose ratio, which has to be 1 - 1.2 in pure honey. Maltose is usually present in honey in low quantities (30 mg.g ⁻¹) and is suggested as a marker of natural honey. Higher amounts of maltose concentration may indicate adulteration of honey by sugar syrup or starch hydrolysate.
Compendial Approach / Trade Recommended Methods (e.g. OIV, AIJN, IOC, IDF, etc.):	Harmonised method of the International Honey Commission (IHC 2009)
Compendial Approach / Trade Recommended Methods Links:	International Honey Commission
Data Processing (multivariate):	
Pre-processing:	Not applicable.
Multivariate Mode:	Not applicable.
Complexity:	None of the above



Welcome on the Homepage of the International Honey Commission (IHC).



The International Honey Commission (IHC) was formed in 1990 in order to create a new world honey standard. All modern routine honey analysis methods were collaboratively tested and compiled as "Harmonised methods of the European Honey Commission" published in Apidologie, extra issue, 1-59, 1997. Based on these methods the Codex Alimentarius Standard and the EU Honey Directive were revised.

Next, the major European unifloral honeys were characterised in a special issue of Apidologie (2004), allowing the international trade with these honeys.

In 2009 the "Harmonised methods" were updated.

Presently the IHC is chaired by Gudrun Beckh, Germany, co-chairs are Teresa Sancho, Spain and Ligia Bicudo de Almeida Muradian, Brazil.

Current Objectives of the IHC

The specific current objectives of the IHC are

- work out better and new analysis methods of honey and the other bee products
- inform members of the group on current aspects of quality and control of honey and other bee products
- work out standards for other bee products besides honey
- work out quality criteria for specific honeydew honeys

Do you like to join?

Yes! you may find our membership form - please fill in the personal data and your skills. If you need more space, please feel free to use it - it is a word document, so you are able to change. We are looking forward to hearing from you!

[Reports and Conferences](#)

[Publications and Standards](#)

Ongoing Actions:

Inter-laboratory comparison exercise on LC-IRMS* – Preliminary results



- ILC was organised to assess the performance of the method.
- 15 laboratories registered and 14 submitted results.
- 8 samples (6 honeys, 2 standard maltotriose) dispatched to measure the $\delta^{13}\text{C}$ values.
- 5 different techniques were applied: LC-IRMS based on Elflein method, LC-IRMS based on Cabañeros method, LiquidFace-IRMS, IC-IRMS and LC-TC-IRMS.

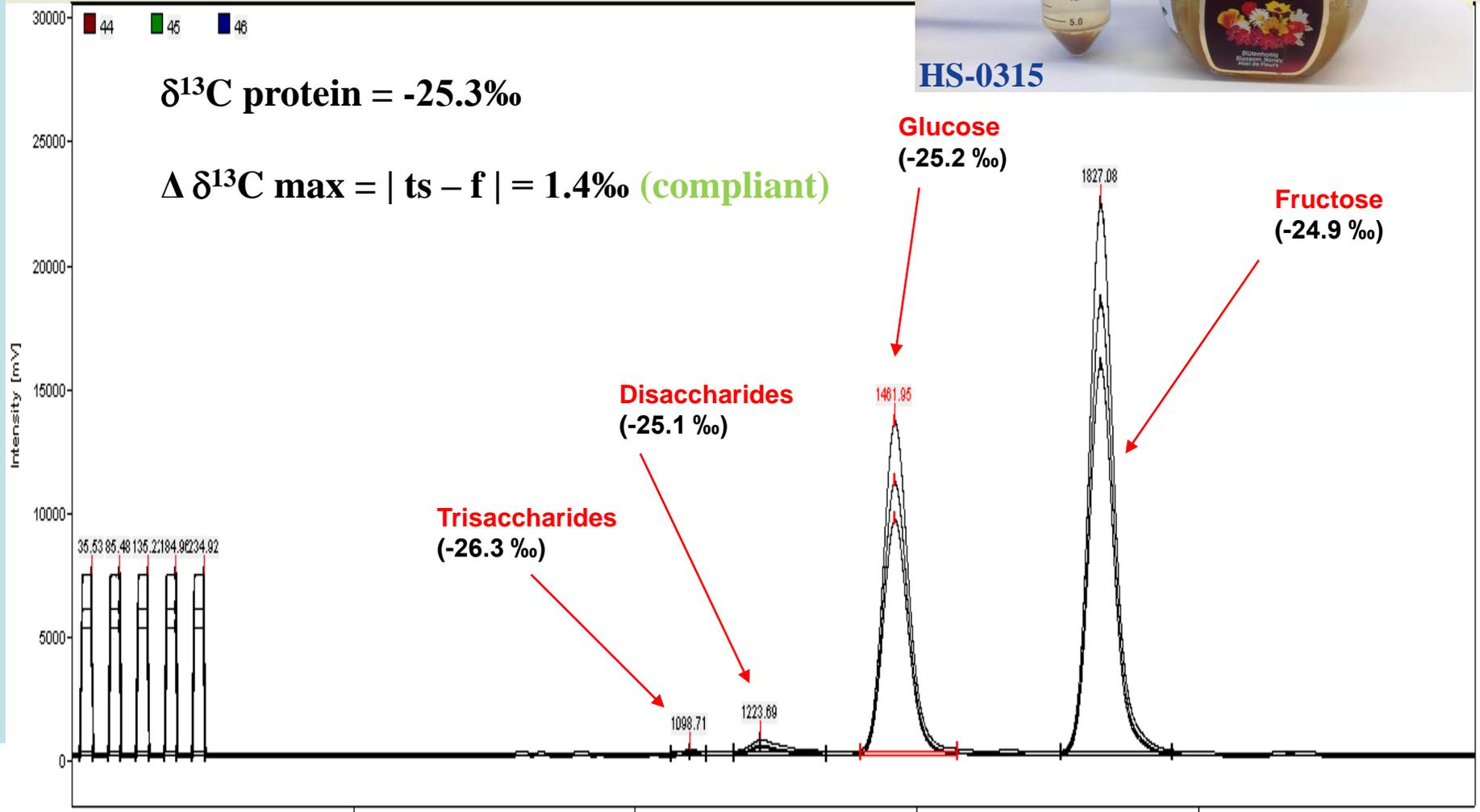
* Liquid chromatography linked to Isotope Ratio Masse Spectrometry; IC-IRMS: Ion chromatography IRMS; LC-TC-IRMS: LC thermal conversion IRMS.

Ongoing Actions: LC-IRMS

Compliant polyfloral honey
using EA/LC-IRMS



File Name: C:\Thermo\Isodat NT\Global\User\LC IsoLink Dionex Interface\Results\ACQ-Results\Acquisition-0664.dxf

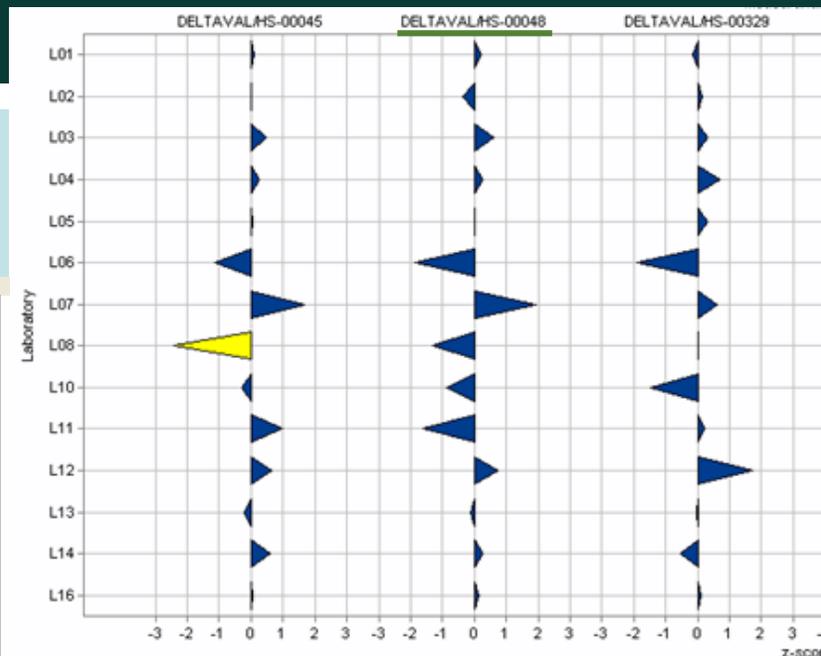
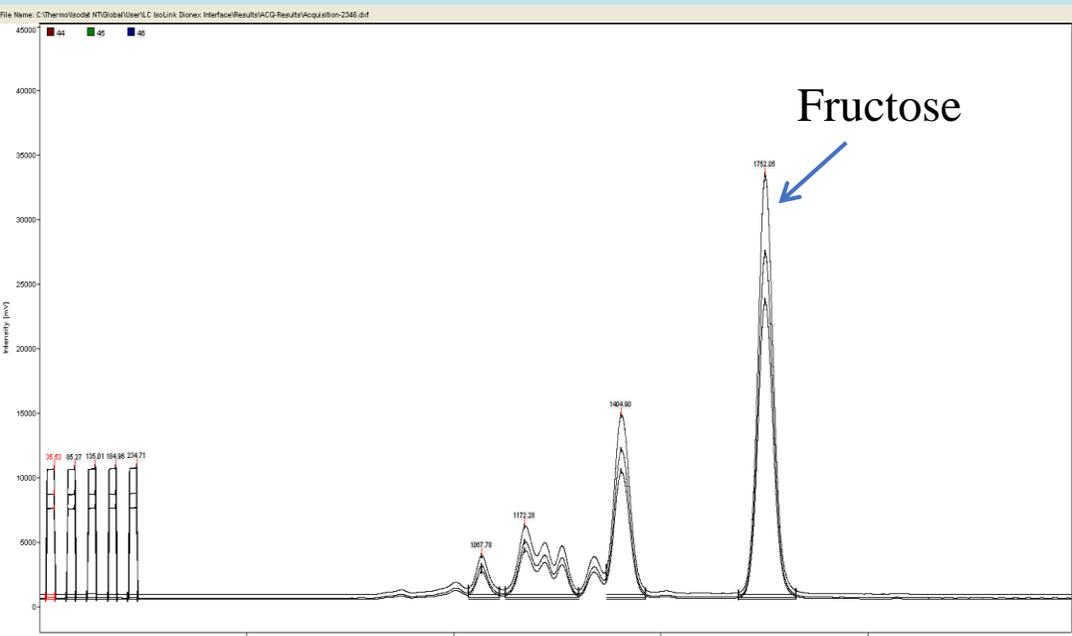


HS-0315

Ongoing Actions: ILC on LC-IRMS – Preliminary results

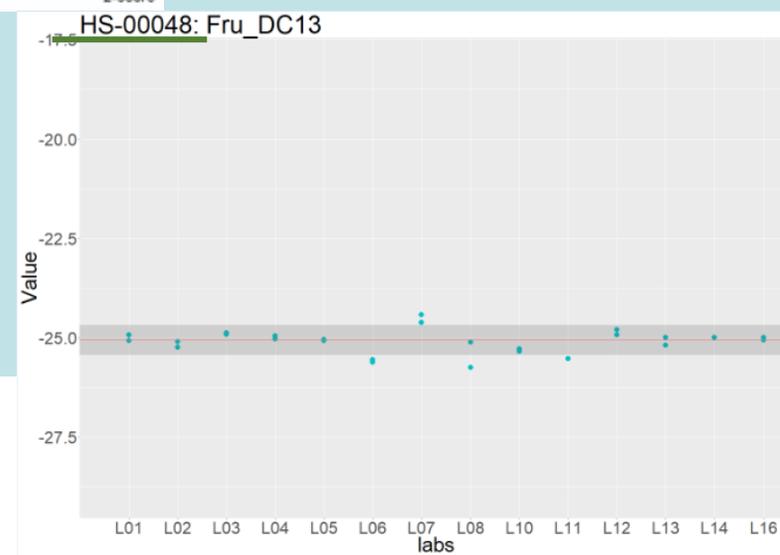


HS-00048

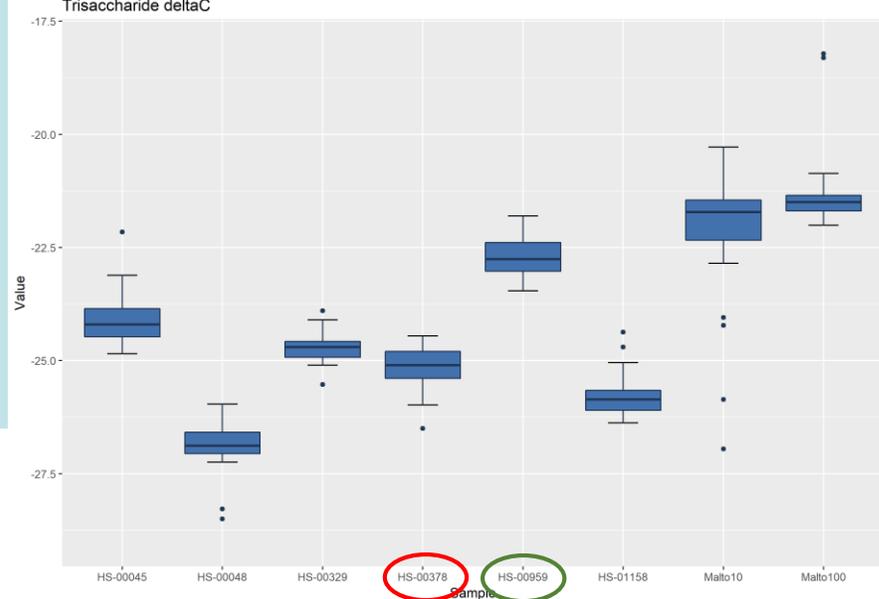
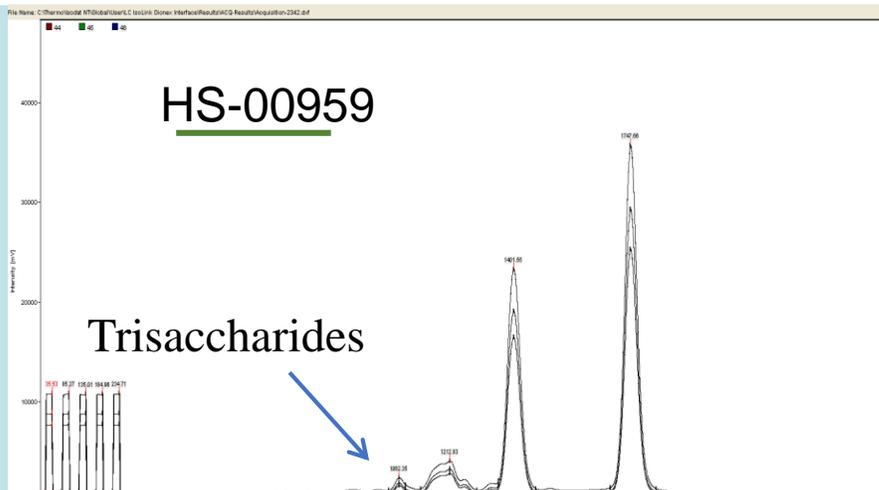


Z-scores

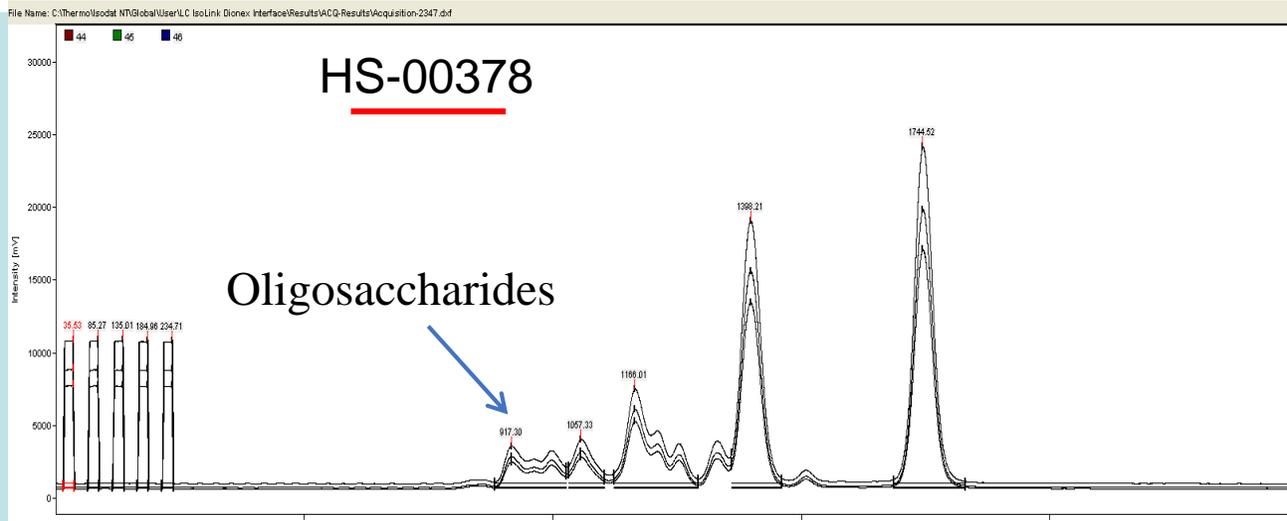
$\delta^{13}\text{C}$ values



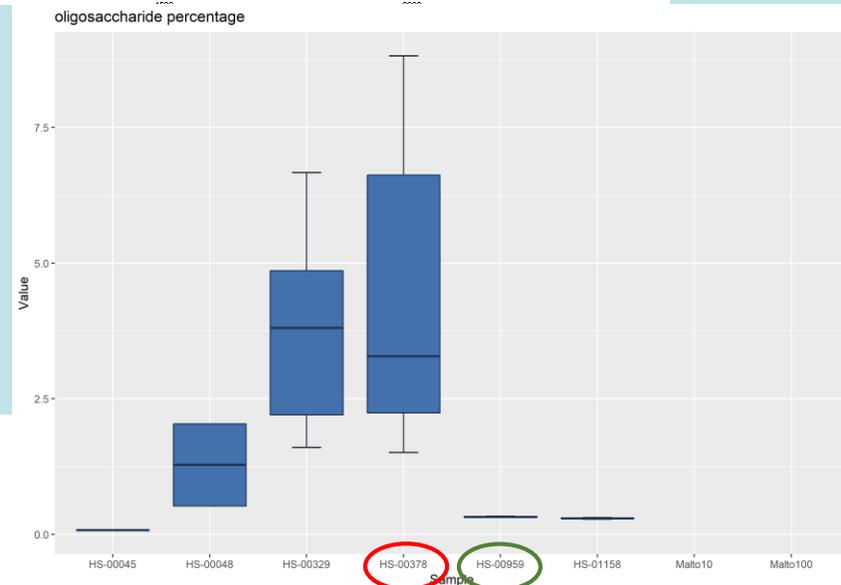
Ongoing Actions: ILC on LC-IRMS – Preliminary results



$\delta^{13}\text{C}$ values



% peak area oligosaccharides



Ongoing Actions: ILC on LC-IRMS – Preliminary results



- Laboratories performed generally well.
- Few outliers detected.
- Low relative reproducibility for $\delta^{13}\text{C}$ values (F/G/Di-/Tri-) among labs.
- High variation in estimating the % peak area of oligosaccharides.
- Method fit-for-purpose except IC-IRMS which needs more investigations.
- **Next:** Establish an harmonized approach (peak integration & threshold levels); then draft a Standard Operating Procedure.
- **Next:** Evaluate decision criteria for identifying adulteration with new ILC.

Ongoing Actions: Metabolomics approach by LC - High Resolution Mass Spectrometry



- Technology providing a comprehensive metabolome coverage (both semi-and non-volatiles can be analysed in one run).
- High sensitivity, resolution and mass accuracy.
- Combinations of targeted (profiling) and untargeted (fingerprinting) approaches.
- Identification of unknown markers / adulterants and metabolites.

Metabolomics approach by LC - High Resolution Mass Spectrometry



➤ Profiling of oligo- & polysaccharides

- ✓ Analysis of sugar syrups, bee feeding products, honey samples, and “authentic” honey samples.
- ✓ Quantification of mono-, di-, oligosaccharides & markers (mannose, AFGP*, DFA, etc.)
- ✓ Establishment of quantitative threshold levels to differentiate between adulterated and non-adulterated honey samples.

* AFGP: 2-acetylfuran-3-glucopyranoside; DFA: difructose anhydrides.

Metabolomics approach by LC - High Resolution Mass Spectrometry



- **Untargeted metabolomics (fingerprint)**
- Possibility to identify unknown markers and adulterants and to re-analyse data in case of new syrups entering the market.
- Establishment of classification models to differentiate between adulterated and non-adulterated honey samples.
- Establishment of classification models to differentiate honey samples based on their geographical origin and floral composition.

Metabolomics approach by LC - High Resolution Mass Spectrometry

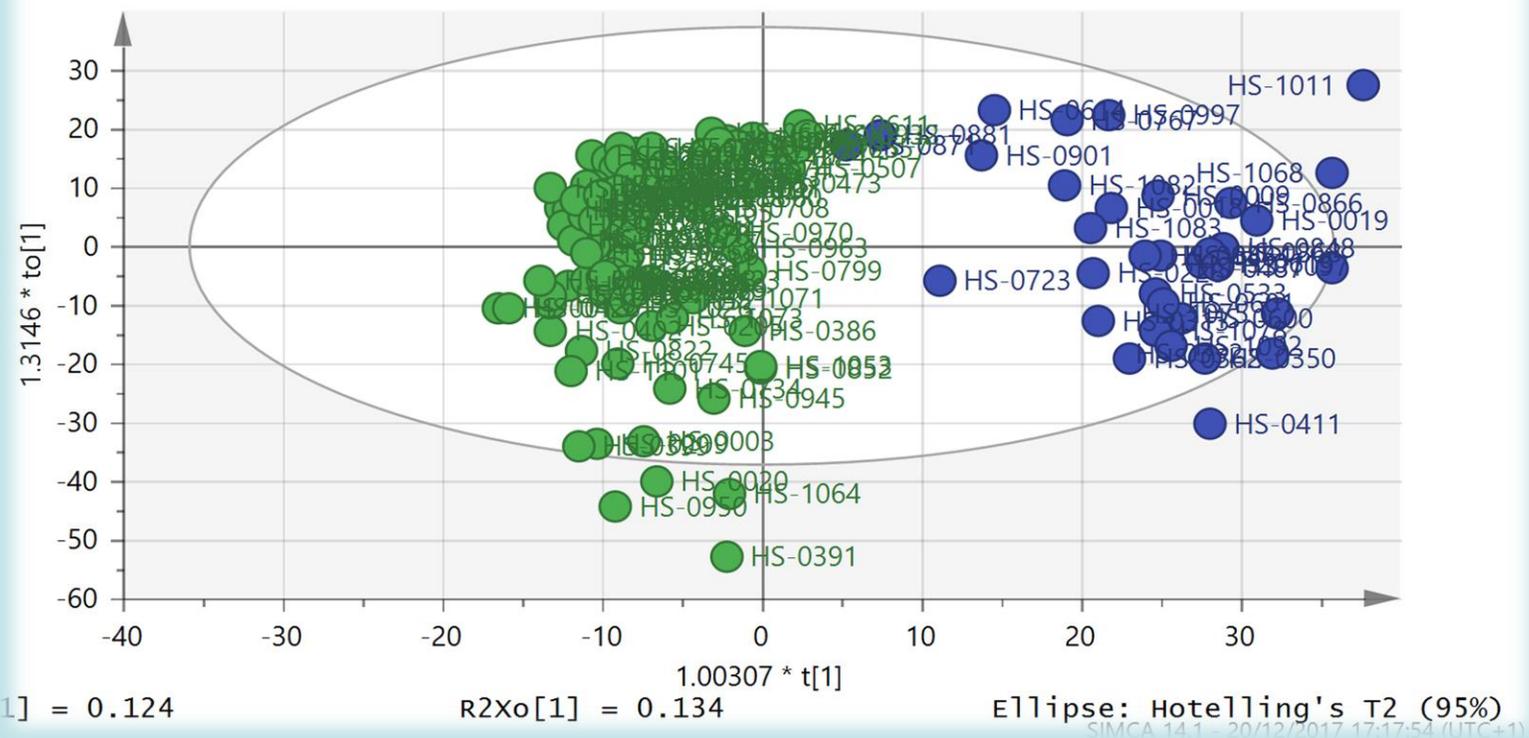


Tutorial_RAWdata3_CV30_diff.M3 (OPLS-DA), >200

Scaled proportionally to R2X

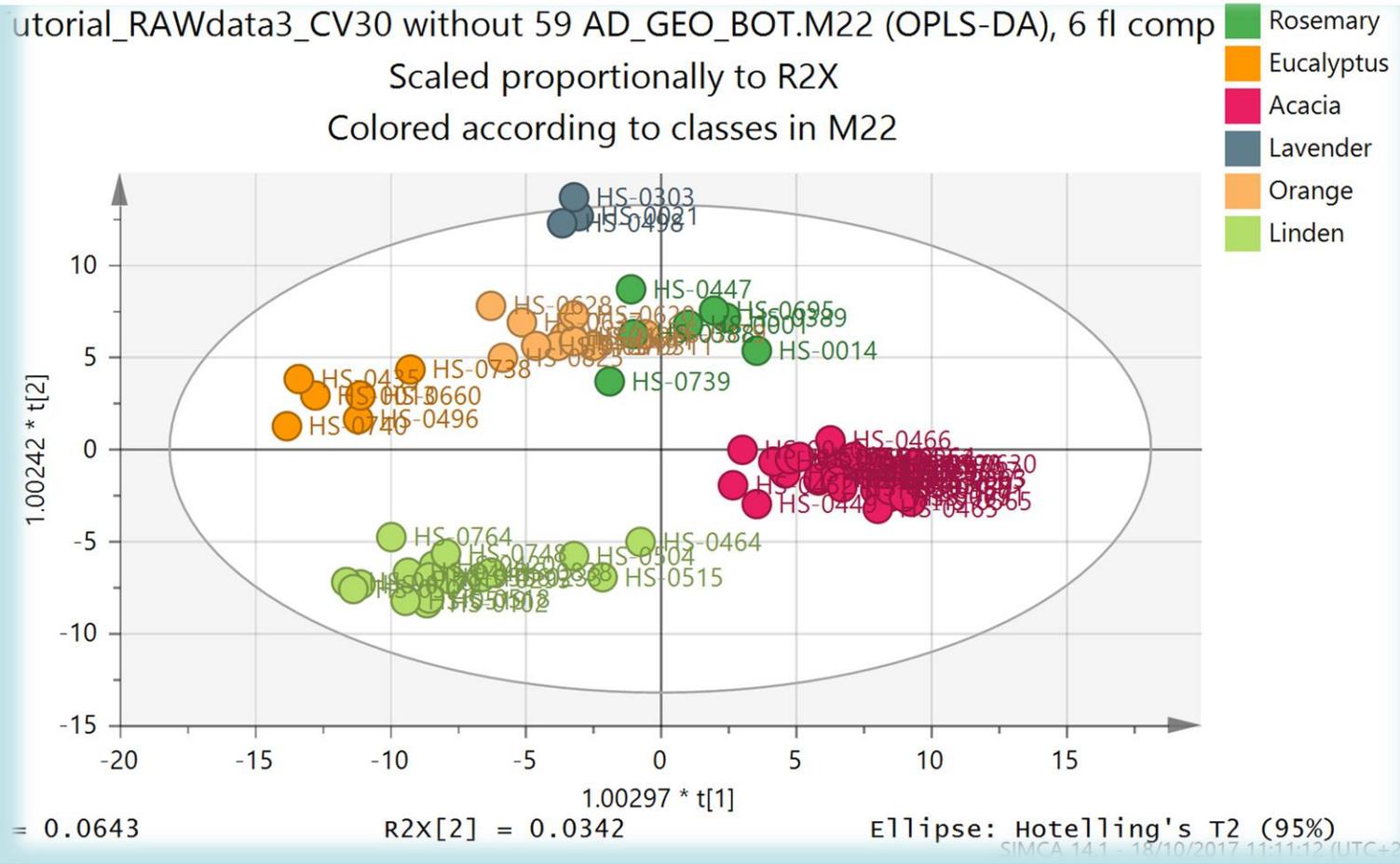
Colored according to classes in M3

no
Yes



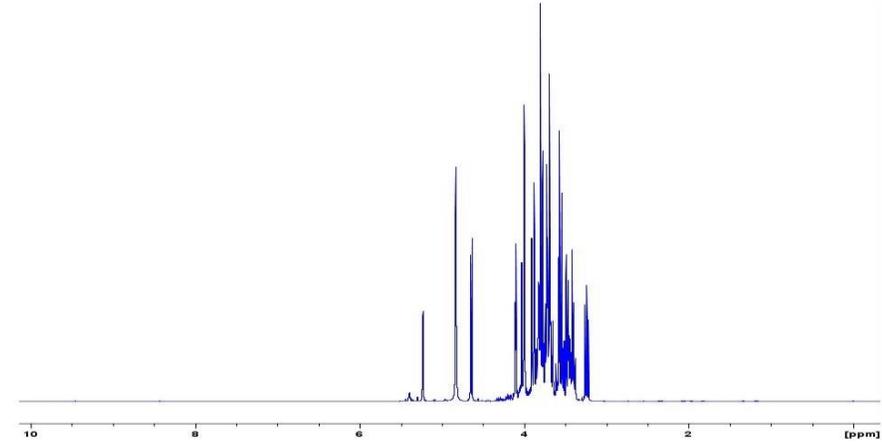
217 honey samples with a detectable polysaccharide profile
R2=0.9, Q2=0.897

Metabolomics approach by LC - High Resolution Mass Spectrometry



**From 286 honey samples including only monofloral varieties with (n>3), removing polyfloral and unknown – Total: 80 samples
 R2=0.917, Q2=0.588**

Ongoing Actions: Metabolomics approach by Proton Nuclear Magnetic Resonance (^1H -NMR)



- **Comparison**
- **Evaluation**
- **Result**

Ongoing Actions: Sugar and oligo-polysaccharide profilings by HPAE-PAD*



- High sensitivity.
- Specific for sugars and maltodextrins.
- Low cost.
- High sample throughput.
- Straightforward technique for official control purposes.

* High-Performance. Anion-Exchange Chromatography with Pulsed Amperometric Detection – Chromatographie d'échange d'anions à haut pH couplé à la détection par ampérométrie pulsée.

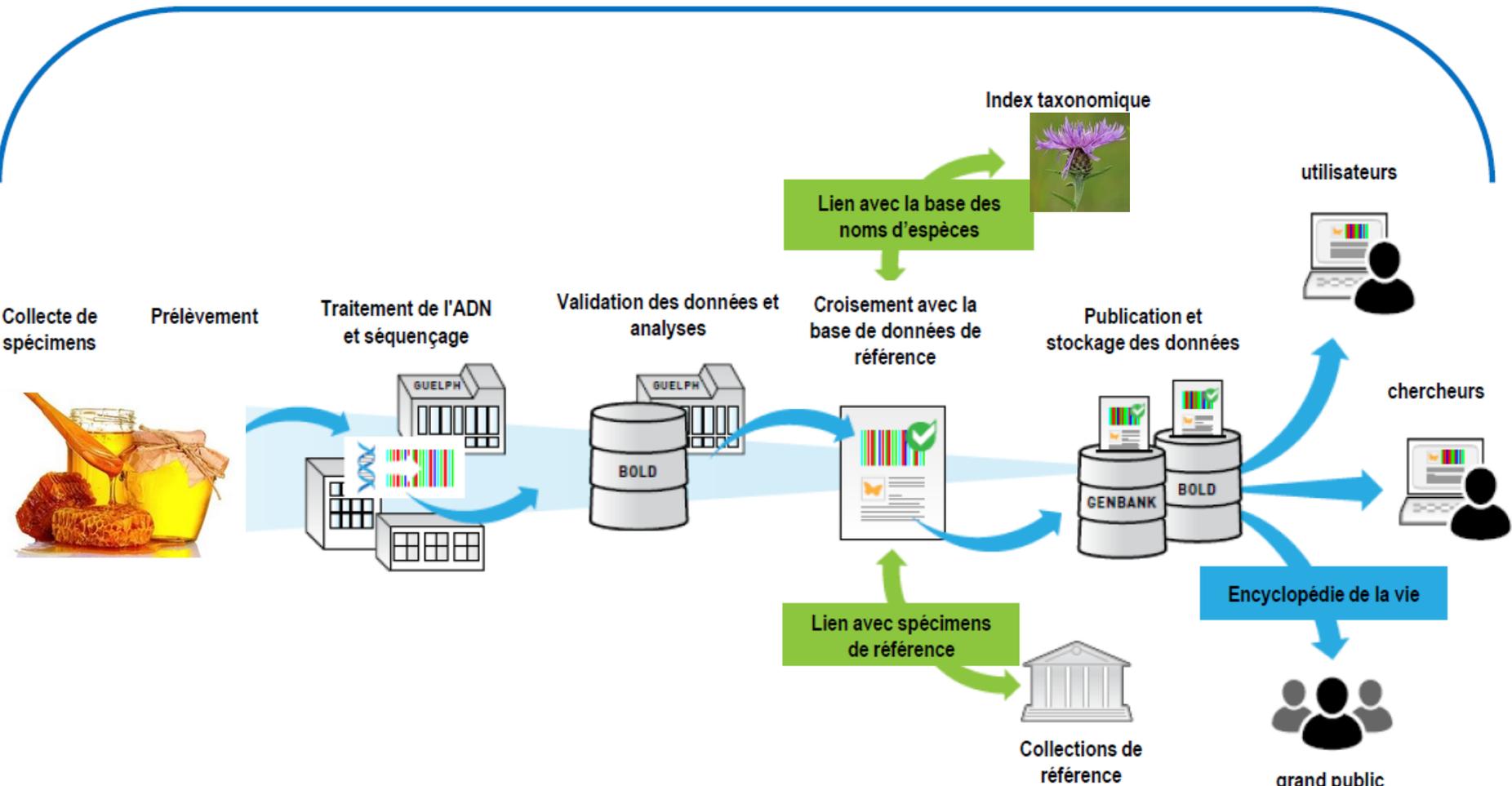
Sugar and oligo-polysaccharide profilings by HPAE-PAD



- Quantification of main carbohydrates (Trehalose, Arabinose, Glucose, Fructose, Melibiose, Isomaltose, Maltulose, Sucrose, Gentiobiose, Turanose, Melezitose/Palatinose, Raffinose, Stachyose, Kestose, Maltose, Erllose, Panose, Nystose and Maltotriose).
- Quantification of maltodextrins from DP4 to DP10.
- Establishment of threshold levels for authentication of honey.
- Identification of adulteration based on ratios of sugars & presence of oligo- and polysaccharides.

Ongoing Actions: DNA Metabarcoding

The « barcoding pipeline »



Bold = barcode of life data

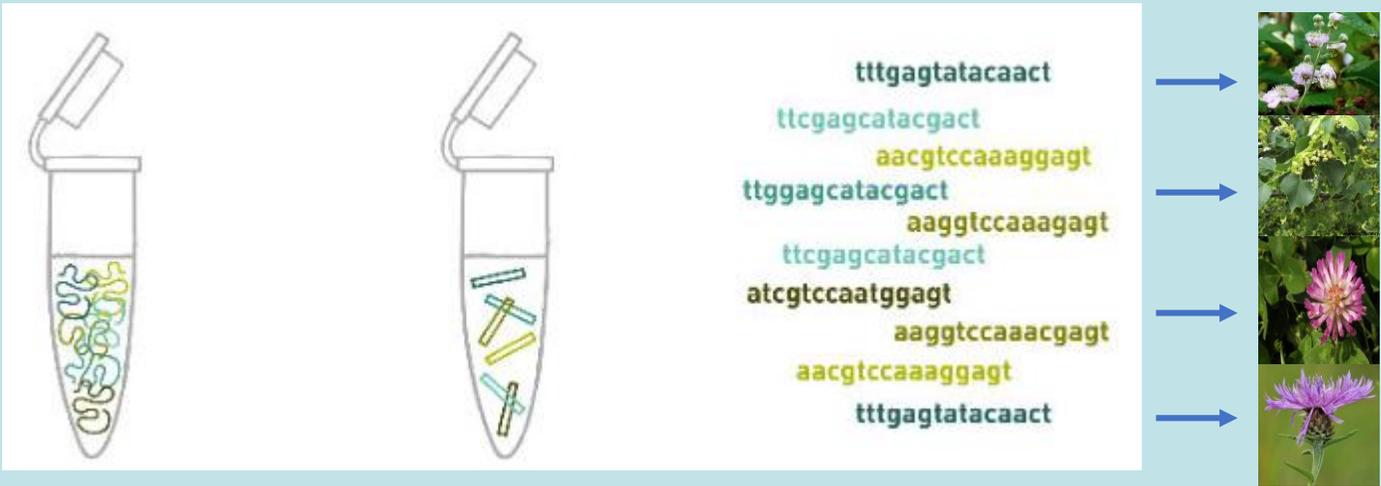
Source: DREAL, FR

Ongoing Actions: DNA Metabarcoding

❖ Identification of botanical origin of honeys by metabarcoding using next-generation sequencing



Source: Bruker



Source: AllGenetics

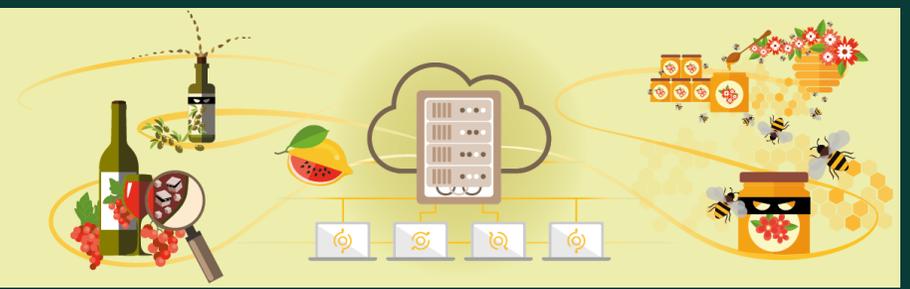
Ongoing Actions: DNA Metabarcoding

➤ Remarks

- ✓ DNA metabarcoding has the advantage over melissopalynology in that it does not require a high level of taxonomic expertise, a greater sample size can be screened and it provides greater resolution for some plant families.
- ✗ However, it does not provide a quantitative approach and pollen present in low levels are less likely to be detected.

Outlook

Knowledge Centre for Food Fraud and Quality



- Release of the Food Authenticity Knowledge Base.
- Operate a 2nd Inter-laboratory comparison exercise on LC-IRMS and publish a Standard Operating Procedure.
- Collect authentic honeys, bee feeding products, syrups and full traceable blends.
- Continue testing the metabolomics approach (profiling and fingerprinting) by Nuclear Magnetic Resonance, LC-HRMS and HPAE-PAD, and the metabarcoding.
- Technical meeting with competent authorities of the Member States on data sharing and design of compositional databases.

THANKS



Any questions?

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