"Keep Honey Fraud at Bay"
Honey Authenticity Testing by 1H-NMR

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Honey-Profiling overview

Honey-Profiling is an application module of the **FoodScreener platform**

- Standardized procedures for sample preparation and measurement to ensure data reproducibility and the usage of common data analysis tools.

- Sample measurement and data analysis are fully automatized

- Sample preparation requires 2,5 mg of honey diluted by 1 to 4 in water followed by pH adjustment

Sugar syrups in honey

- According to EU-Directive of 2001 and Codex Alimentarius, honey should be exempt of foreign sugars.

- Foreign sugars can be:

    - a deliberate addition to honey in order to stretch the product.

    - a consequence of **bee feeding** with sugar syrups.

- Feeding bees with industrial sugar syrups: ≈ 40 times less expensive than feeding with honey.

- Sugar syrups need to be handled with care and be given outside of production periods.

- Famille Michaud, the first reseller of honey in France tests all the honeys they buy with their NMR instrument. **They find sugar syrups in 10% of the French honeys tested.**
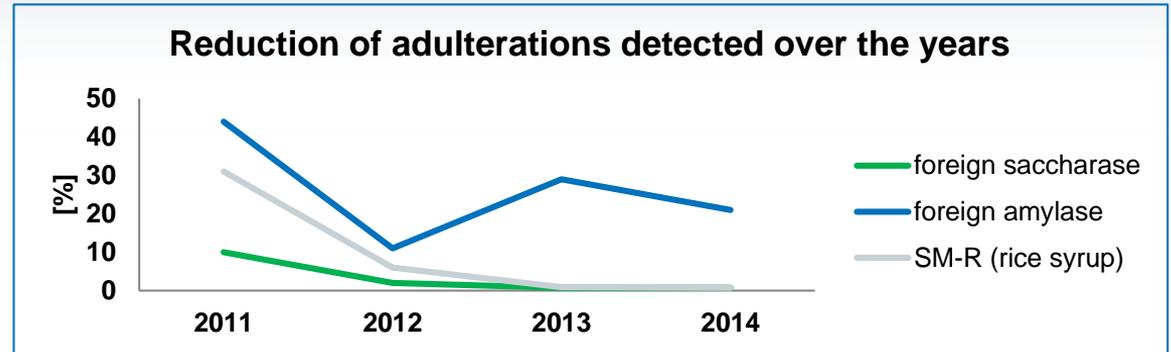
https://www.francetvinfo.fr/replay-jt/france-2/20-heures/video-les-abeilles-nourries-au-sirop-de-betterave_3521387.html

Detection of Sugar Syrups
with specific methods

BRUKER

- **Foreign enzymes**
  - ○ Particular enzyme

- **Single Markers**
  - ○ Rice syrup
  - ○ Beet syrup

- **AOAC EA-IRMS**
  - ○ C4-type sugars

➢ **You need to know what you are looking for**

Reduction of adulterations detected over the years

— foreign saccharase
— foreign amylase
— SM-R (rice syrup)

With the courtesy of QSI GmbH

- Detection of **25%** of adulterated honeys in Honey-Profiling Database

- 13C EA-IRMS only: acceptance of approximately 3/4 adulterated honeys.
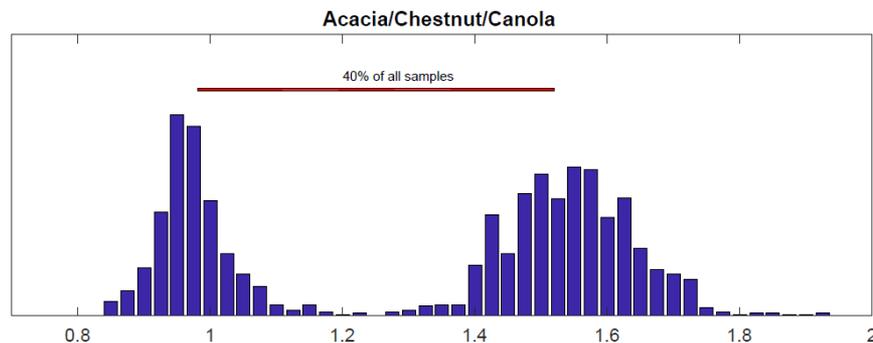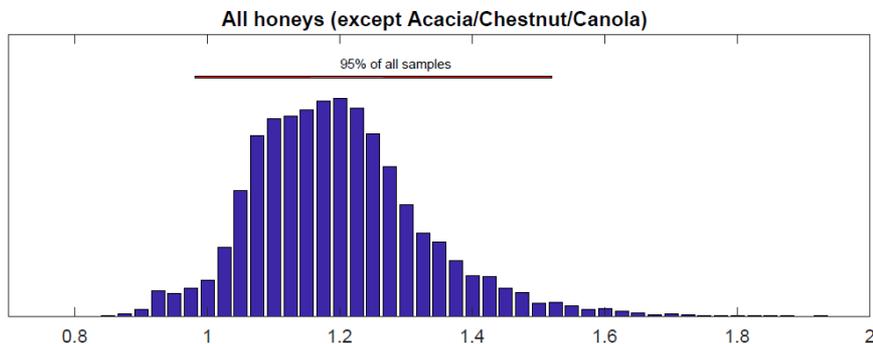
➢ **Tests are easy to deceive**

Detection of Sugar Syrups
with NMR Honey-Profiling

- **Quantitative analysis** of many markers: up to 60 markers per sample

- The values are compared to the reference thresholds

- Reference thresholds have been determined and validated thanks to a large database of authentic and known adulterated honeys from various countries and botanical varieties:

  - 16100 authentic samples / 1900 known adulterated samples

  - > 50 countries & 100 botanical varieties.

  - Monofloral & polyfloral honeys from a single country source.

  - Blends of honeys from different countries.

  - Industrial honeys (or "baker honey")

Why using a database ?
Validation of markers and related thresholds

All honeys (except Acacia/Chestnut/Canola)

95% of all samples

0.8 1 1.2 1.4 1.6 1.8 2

Acacia/Chestnut/Canola

40% of all samples

0.8 1 1.2 1.4 1.6 1.8 2

Example of Fructose/Glucose ratio

- Independently of the analytical technique used (NMR, MS, HPLC-UV…) and of the parameter measured (unknown compound or identified molecule like e.g. mannose):
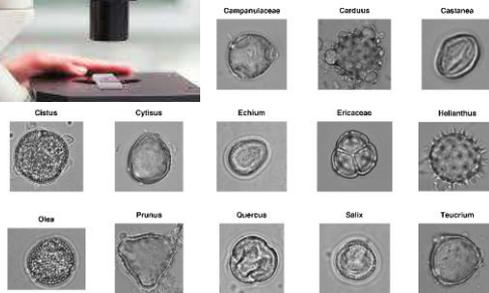
  To define what is "normal" it is mandatory to have a set of representative samples, to make sure markers chosen are not naturally present in a specific type of honey.

  ➢ **AVOID THE RISK OF FALSE POSITIVE**

- That is even more important when looking to low concentrated compounds (like e.g. secondary metabolites of plants)

- In NMR Honey-Profiling, the markers are all in the spectral regions related to sugars, which is less sensitive to origin.

False declaration of origin

- Hiding real country of origin or variety.

- Intend: Avoid higher tariff rules, avoid further testing, reach higher market value

- Transhipment = sending the honey to another country where it is relabeled before being exported.

- **Pollen grains manipulation** to deceive detection by pollen analysis:

  Filtration of endogeneous pollen grains and addition of pollen grains from another country

Floral source (e.g. Manuka)

Geo. origin (e.g. Transhipment)

Riddle of how 1,700 tons of manuka honey are made... but 10,000 are sold

Detection of false declaration of origin
Pollen analysis

- Identification and counting pollen grains by microscopy

- Requires a trained expert and is time consuming

➢ Not working on pollen-filtered honeys.

➢ Not able to differenciate the real pollens from exogeneous pollen grains.

Detection of false declaration of origin
Marker compounds

- Compounds commonly used as markers can often be synthetically produced and added into the honey.

- E.g. DHA and MGO are cheap chemicals

- Non-compliance of marker compounds can be used to detect a fraud

- However, **compliance of 1 or few markers are not sufficient to prove authenticity**

Compound	Value	Unit	LOQ	Reference Range		Flag
3-phenyllactic acid	682	mg/kg	300	388	2300	🟢
dihydroxyacetone (DHA)	378	mg/kg	20	235	3513	🟢
methylglyoxal (MGO)	243	mg/kg	30	110	1486	🟢

Riddle of how 1,700 tons of manuka honey are made... but 10,000 are sold
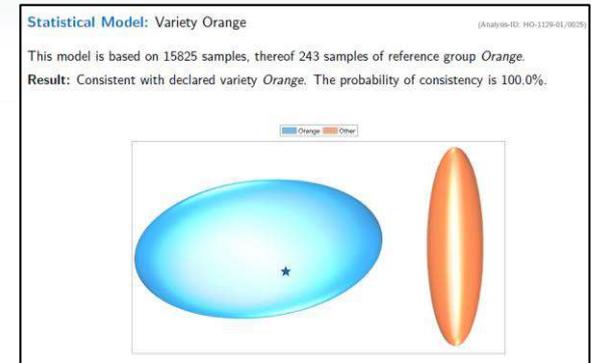
23 Aug, 2016 1:30pm   ⏱ 3 minutes to read

Research by the main honey producers' organisation in New Zealand has revealed that 1,700 tonnes of manuka are produced there each year. Photo / Brett Phibbs

Detection of false declaration of origin
NMR Honey-Profiling

- Based on the **complete chemical composition of the honey** observed by 1H-NMR

- Statistical analysis of the NMR spectrum: untargeted buckets/variables.

- Differentiation of the supposed/declared origin or variety with all other ones present in the Database (50 countries / 100 varieties).

- Statistical models have been validated by Monte Carlo cross validation (Criteria: TP > 98%)

  ➤ *Very difficult/ impossible to deceive*

  ➤ *No need for an expert and fast*

  ➤ *Applicable to pollen-filtered honey*

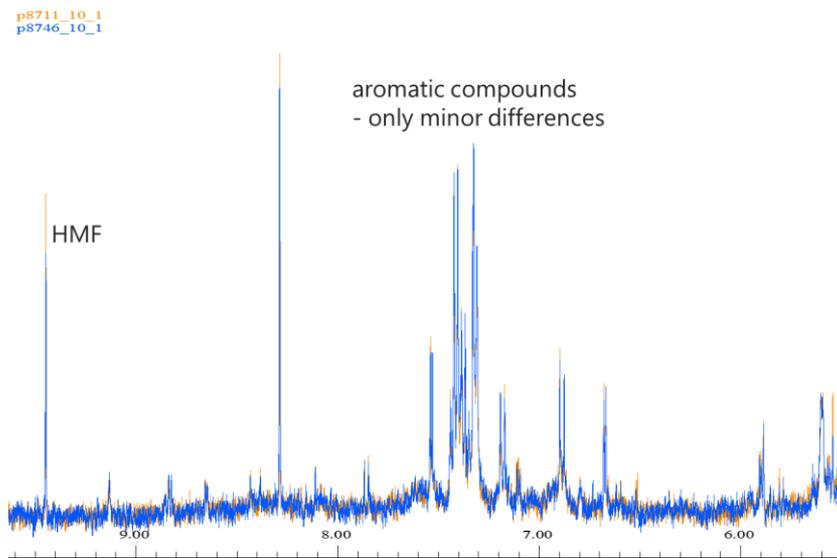Statistical Model: Variety Orange   (Analysis-ID: HO-1129-01/0025)

This model is based on 15825 samples, thereof 243 samples of reference group *Orange*.

Result: Consistent with declared variety *Orange*. The probability of consistency is 100.0%.

Verification of Labeling of Origin
on pollen-filtered honey

^{1}H-Spectra of USA/ARG. (40:60) Honey: raw vs filtered
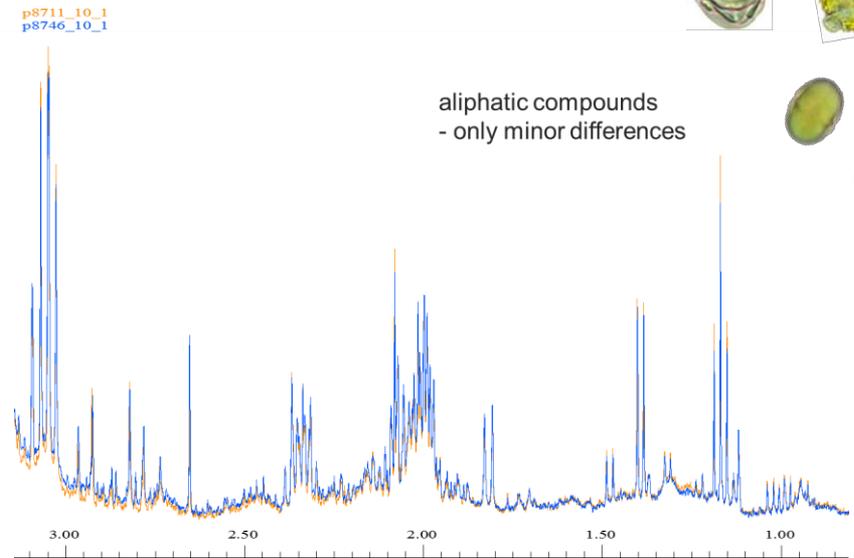
Verification of origin possible even on pollen-filtered honey with NMR !

Additional information with NMR
Honey-Profiling

- **Detection of atypical samples**
  - Statistical comparison of the NMR profile with "normal" profiles from the same floral source
  - Potential to detect new frauds at early stage.

- **Honey constituent's quantification**
  - Sugars, organic acids, amino acids, freshness and quality criteria
  - Absolute quantification
  - Comparison to reference values (obtained by our Database samples)

- **Regulated parameters in EU-directive / Codex Alimentarius**
  - HMF, glucose+fructose, sucrose
  - Absolute quantification
  - Conclusion according to directive.

Why using NMR and Honey-Profiling

- An **all-in-one method**, comprehensive authenticity test:
  - Detection of sugar syrups
  - Verification of labelling/declaration of origin (country and botanical source)
  - Detection of atypical samples
  - Quality control (composition, freshness, regulated parameters)

- **deeply validated method**, relying on a huge Database of 18000 well-characterized samples

- Several labs (including Bruker BAS lab) accredited **ISO17025** for Honey-Profiling

- no NMR expertise required. Easy to operate, fully automated, no regular instrument cleaning

- Fast: 20 – 25 min / sample

- Difficult to deceive

- Wide and ww network of partners and users

NMR Honey-Profiling network

- A **worldwide network** of partners and customers.

- Honey-Profiling is a **centralized database** to which states offical laboratories can contribute. The goal is to have a database of authentic material covering all its natural variations and to define **common/harmonized purity criteria**

- Possibility to develop new models according to country-specific needs **much quicker**, and with **better performance**.

    i.e to characterize one country it is necessary to have samples from other countries to know what are the differences that should be looked for.

- **Round tables** have been kicked-off to discuss the NMR purity criteria defined amongst honey experts

If you want to learn more, just stop at our **booth B58** ! We are happy to answer all your questions ☺