Honey authenticity seminar, London 13 11 2019

HONEY:
NMR Authenticity testing

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Context

- **Honey is one of the « top 10 » products regarding the risk of food fraud, according to an EU report dated 8/10/2013:**

Draft report on the food crisis, fraud in the food chain and the control thereof 2013/2091 (INI)

- **Fewer bee hive survival & lower yields worldwide, but yet global production increase, with large differences in price**
- **The main fraud encountered in honey is sugar addition**
- **Other frauds may concern mislabelling of the botanical and/or geographic origin**

➢ **This slide show aims to describe the state-of-the-art and Eurofins analytical offer, including recent developments**

Our vision of honey market needs

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Analyses must help to enforce regulations & protect brands but:

- Need for a high number of parameters
- Often limited time, and
- Always a limited budget

- Targeted analysis efficiency is limited, as fraudsters are always pushing limits

Need to select the most efficient tests & Include untargeted screening

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Optimal offer covering the main potential fraud risks

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- **NMR profiling**
  - ➢ broad authenticity and integrity screening
- **Stable isotope testing via EA-IRMS**
  - ➢ detecting C4 sugars with an optimum detection limit
- **Pollen/sensorial/conductivity analysis**
  - ➢ confirming botanical/geographical origin and quality

NEW

- **LC-HRMS**
  - ➢ detecting some sugars with an optimum detection limit

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Honey Expert laboratories

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**Our expert laboratories are ISO 17025 accredited*
for all above mentioned honey tests**

Eurofins Authenticity competence centre (Nantes, France)
** accredited by COFRAC under registration n° 1-0287, details on www.cofrac.fr*

Eurofins Food Integrity Control Service GmbH (Bremen, Germany)
** accredited by DAkkS under registration n° D-PL-20965-01-00, details on www.dakks.de*

NMR profiling
High Resolution ^{1}H NMR

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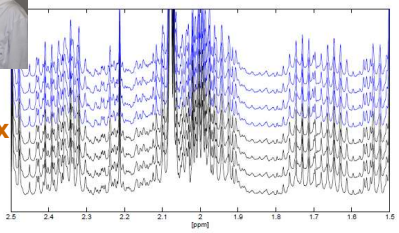
Observation domain:
from ppm to %

Key Strengths:
Broad observation of the whole matrix
High Reproducibility
Discrimination power



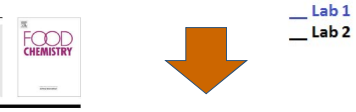
Eurofins initial publication

Food Chemistry 189 (2015) 60–66
Contents lists available at ScienceDirect
Food Chemistry
journal homepage: www.elsevier.com/locate/foodchem

Fast and global authenticity screening of honey using ^{1}H-NMR profiling

Marc Spiteri [a], Eric Jamin [a,*], Freddy Thomas [a], Agathe Rebours [a], Michèle Lees [a], Karyne M. Rogers [b], Douglas N. Rutledge [c]

[a] Eurofins Analytics France, Rue Pierre Adolphe Bobierre, B.P. 42301, F-44323 Nantes Cedex 3, France
[b] National Isotope Center, GNS Science, 30 Gracefield Road, Seaview, P O Box 31 312, Lower Hutt 5040, New Zealand
[c] AgroParisTech, UMR1145 Ingénierie Procédés Aliments, 16, rue Claude Bernard, 75005 Paris, France

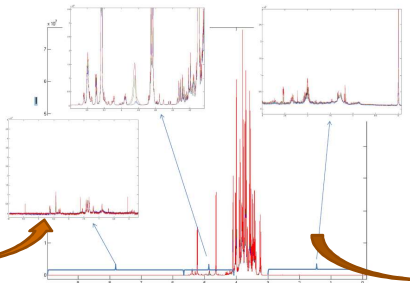


Since 2018:
SOP aligned with Bruker Honey consortium

NMR profiling
Benefits of a non-targeted method

- The ^{1}H-NMR spectrum is obtained on the whole matrix

Unique spectral fingerprint



- Detects markers and spectral effects of adulteration

Authenticity proof

- Allows to quantify key-quality parameters
- Allows to confirm botanical origins of mono-floral honey

Alternative to conventional methods

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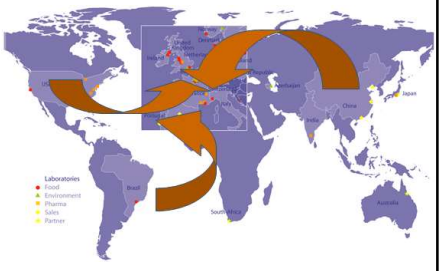
NMR profiling
Building a worldwide authentic honey database

Reference samples : > 10000
>100 different botanical « families »

Collected from local producers
In more than 65 countries worldwide
over more than 15 years



The inclusion of samples in the statistical models requires:

- Availability of metadata (botanical & geographical origin)
- Compliance of other methods results
- Coherence with other samples of the same kind (95% confidence intervals)

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Analytical Method lifecycle: a continuous improvement process within flexible accreditation

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Market need

Method re-validation

Method improvement:

Scope, Efficiency, etc.

Project opening:
state of the art
Technologies
Databases
New Ideas?

Method development:

Go / No Go

Method initial validation

PRO-PTS: Proficiency Testing for NMR PROfiling methods

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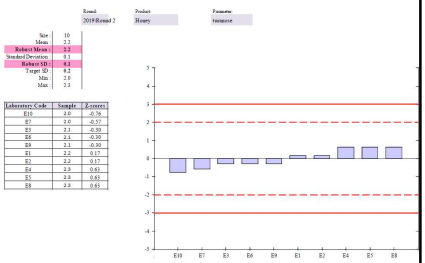
- **METHODS CONCERNED**
  - Wine NMR profiling
  - Juice NMR profiling
  - Honey NMR profiling
  - Coffee NMR profiling
  - Spices NMR profiling
  - Oils NMR profiling

Since 2017: 3 times a year
Ca 20 participants (10 for honey)
Each lab is free to use its internal SOP

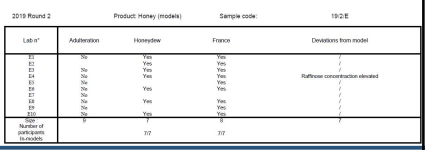


- **STATISTICAL ANALYSIS**
  - Calculation of z-scores using robust statistics

According to the ISO/IUPAC/AOAC International Harmonised Protocol for Proficiency Testing of analytical laboratories.

- Includes non targeted analysis / data interpretation

Cheks global agreement between laboratories



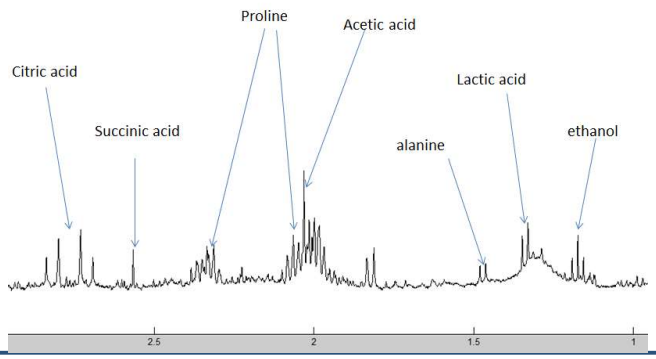
NMR profiling
Quantitative parameters
1) Sugars & 5-HMF

- **Glucose + Fructose, sucrose, 5-HMF**
  - **Comparison with directive 2001/110/EC**
- **Fructose / Glucose, Turanose**
  - **Comparison with litterature & databases**

AA0SG	AA	1H-NMR profiling of honey (sugars, HMF, sugar add)	Method : Internal, NMR	
(a)	Fructose		35.5 (± 2.8) g/100 g	
(a)	Glucose		31.0 (± 2.6) g/100 g	
(a)	Fructose / Glucose		1.15 (± 0.10)	
(a)	Glucose+Fructose		66.5 (± 4.3) g/100 g	>= 60 (blossom honey - miel de fleurs) >= 45 (Honeydew honey - miel de miellat) (Dir. 2001/110/CE)
(a)	Sucrose		<0.5 g/100 g	<= 5 (general ; Dir. 2001/110/CE)
(a)	5-HMF		22 (± 7) mg/kg	<= 40 (general ; Dir. 2001/110/CE)
(a)	Turanose		1.42 (± 0.19) g/100 g	

NMR profiling
Quantitative parameters
2) Others

- **citric acid**
- **fermentation markers (acetic, succinic & lactic acid, ethanol)**
- **Amino-acids**



NMR profiling
3) Detection of added sugars (both C3 & C4)

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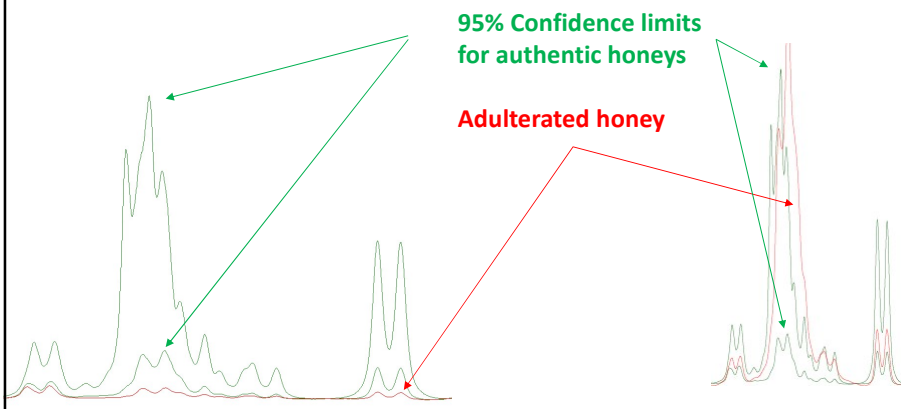

Indirect detection:
Dilution effects

Direct detection:
Markers of sugar syrup addition

NMR profiling
3) Detection of added sugars (both C3 & C4)

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- **Analytical criteria for detecting sugar addition include:**
  - Concentration thresholds for typical honey compounds
  - Concentration thresholds for sugar syrup markers
  - Thresholds & Ratios between specific signals (ca 40 parameters)
  - Quantile plot for selected spectral windows

- **Reporting:**
  - "The NMR profiling test"…
- …"did not reveal any peculiarities in this sample."
- or "shows an untypical spectrum, and points to an addition of exogenous sugar."

- **Interpretation harmonisation with Bruker consortium**
  - Information sharing
  - Case studies discussions

NMR profiling
4) Confirmation of the botanical origin

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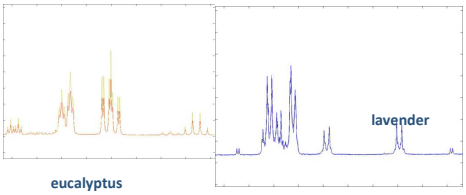
For monofloral honeys → Specific markers For botanical species



→ Complementary to pollen analysis (independant from potential pollen manipulation)

NMR profiling
3) Confirmation of the botanical origin
Manuka honey

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Food Chemistry 217 (2017) 766–772

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Combination of 1H NMR and chemometrics to discriminate manuka honey from other floral honey types from Oceania

Marc Spiteri [a], Karyne M. Rogers [b], Eric Jamin [a], Freddy Thomas [a,*], Sophie Guyader [a], Michèle Lees [a], Douglas N. Rutledge [c]

[a] Eurofins Analytics France, Rue Pierre Adolphe Bobierre, B.P. 42301, F-44323 NANTES Cedex 3, France
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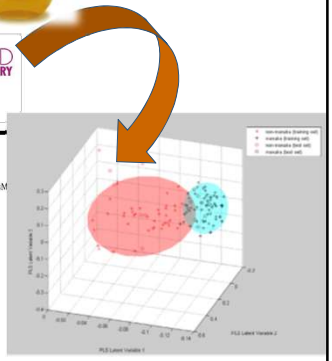

High value => fraud risk
Production << world consumption

- **Specific model to differenciate pure Manuka from non-100% manuka honey**
- **Specific test AA06C controls non peroxide activity**
- **High occurrence of non-compliances**

Stable isotope testing via EA-IRMS
Carbon 13: AOAC 998.12 official method

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Honey

Proteins extraction

Proteins = internal reference

Honey = mainly sugars

Difference of ^{13}C deviations

Detection and « quantification » of C4 plant (cane, maize) sugar
Limitation: does not detect C3 plants sugar (from wheat, rice, beet, etc.)

Stable isotope testing via EA-IRMS
Carbon 13: LC-IRMS

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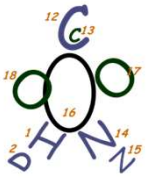

Honey

LC separation of sugars

Glucose

Fructose

Sucrose

Difference of ^{13}C deviations

Enhanced Detection of C4 plant (cane, maize) sugar
Limitation: does not detect C3 plants sugar (from wheat, rice, beet, etc.)

Stable isotope testing via EA-IRMS
Geographical origin check by multi-isotope analysis

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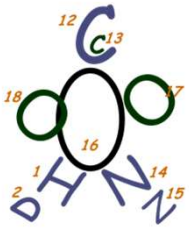
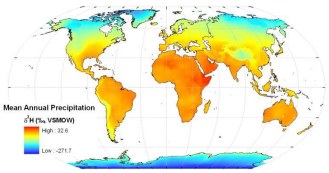
Isotopic fingerprint



Place of production



- Package PAA4W (^{13}C, ^{15}N, ^{2}H), based on European database
- Test A4010 (^{87}Sr) for local origins based on geology data

Complementary to pollen analysis (independant from potential pollen manipulation)

LC-HRMS
Advantages of High Resolution MS

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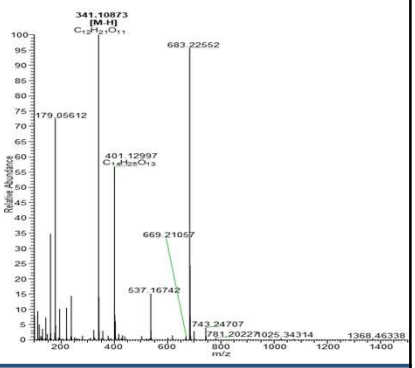
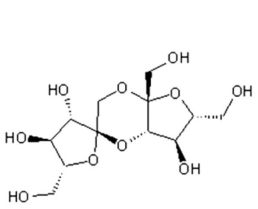
- Capable of separating mass fragments at the fifth decimal place (exact mass) where MS/MS instrumentation was limited to single-digit mass unit.
- high-resolution MS narrows down the possible chemical formulas of a unknown compound

Element	Nominal Mass	Accurate Mass
^{12}C	12	12.00000000
^{1}H	1	1.007825037
^{16}O	16	15.99491464
^{14}N	14	14.003074008

- Example: Sucrose Mass
  342.1 → *274 possible chemical formulas*
  342.11621 → *1 possible chemical formula*
  $C_{12}H_{22}O_{11}$

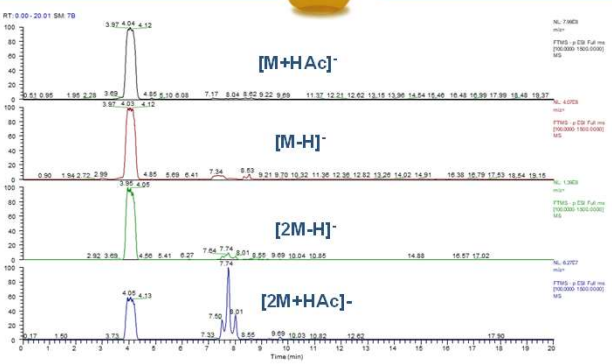


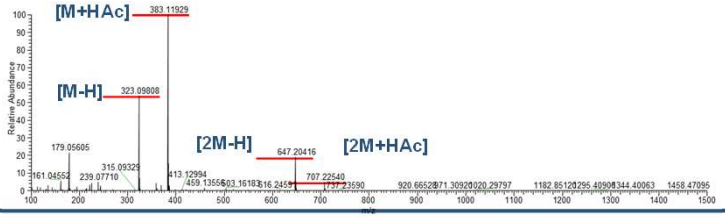
LC-HRMS
Specific Marker example : Difructose Anhydride



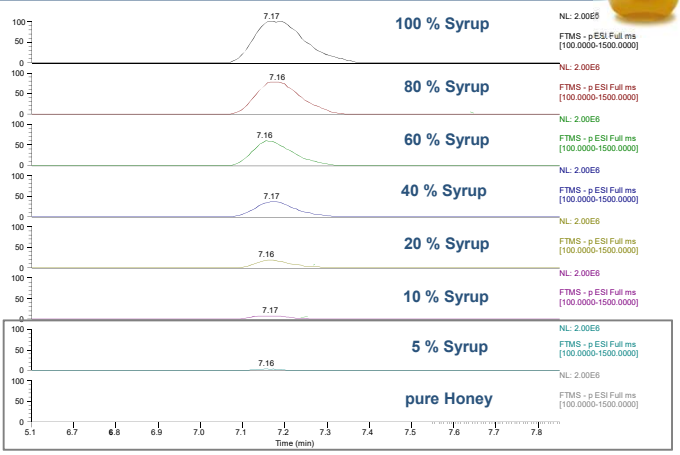
Tricyclic disaccharides formed during caramelization (14 known Isomers)

HFCS and Inverted Syrup



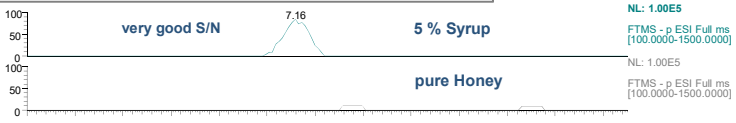


Limit of Detection for Syrup Additions



- **Detection of 5% Syrup Addition**

Scaling by 20x



Case study of multi-methods approach: Corsica honeydew honey

- **Microscopic analysis**
  (pollen, non soluble elements)
  +Sensorial analysis → In agreement with the product description
- **C13-IRMS:** → No C4 sugar addition
- **NMR:**
  very high citric acid content
  (1327mg/kg) → Adulteration with sugar suspected
- **LC-HRMS:**
  sugar syrup addition detected → Adulteration with sugar confirmed

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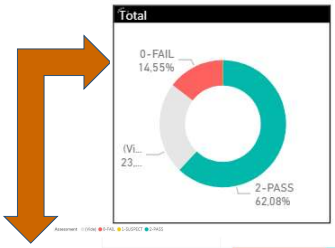
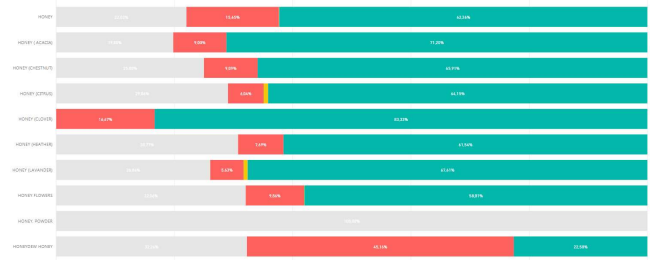
Non compliance statistics (all tests) (From 2013 to 2019)



All honey tests



Disclaimer :

Non compliance rates presented here are based on samples sent by customers and analyzed in Eurofins Analytics France. They may not reflect accurately the market situation in each country / segment

But they can be used as qualitative indications for major risks for each ingredient supply on a worldwide basis.

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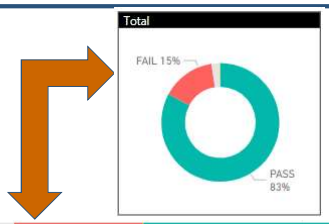
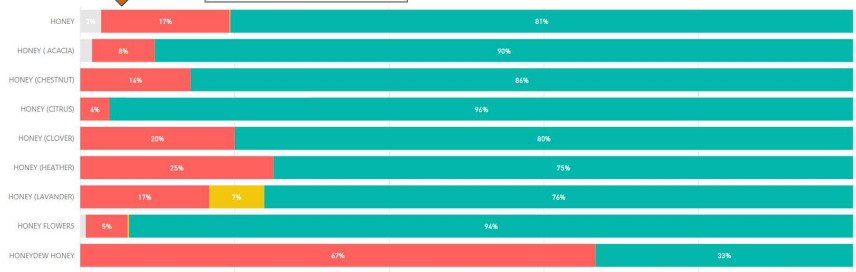


NMR profiling only



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Thank you for your attention !

Dr. Eric Jamin

Authenticity Competence Centre Manager

Eurofins Analytics France (Nantes)