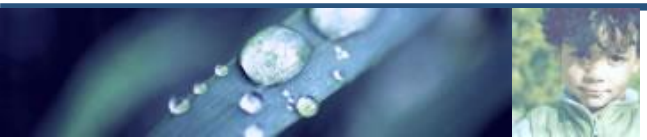
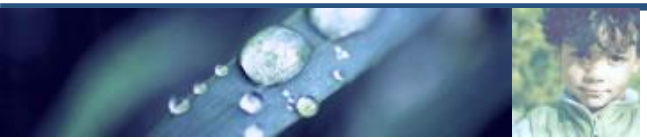


Food authenticity testing: a challenge with solutions

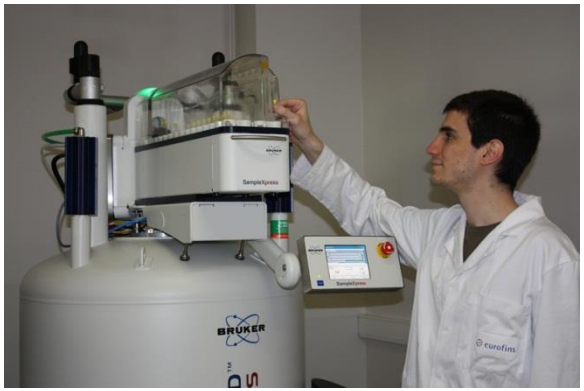
Dr. Eric Jamin
Eurofins Authenticity Competence Center, Nantes (France)

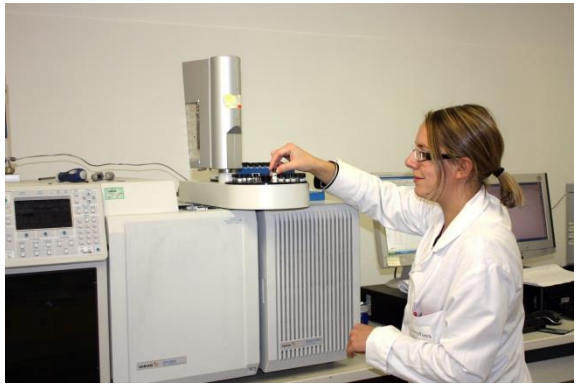


eurofins

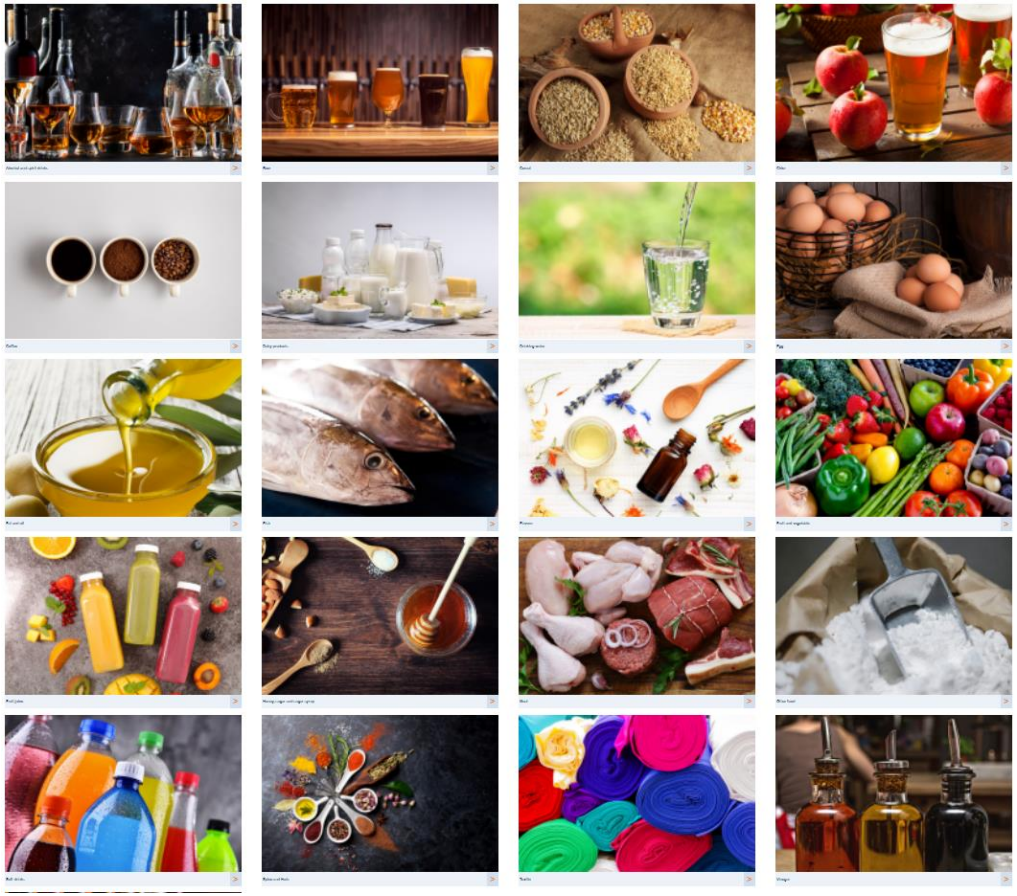
Authenticity competence centre:
Authenticity testing pioneers since 1987

Nantes, France





Authenticity Testing portfolio



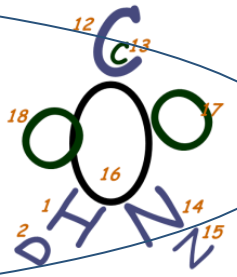
Frequent requests

- Sugar addition
- Water addition
- Botanical origin
- Geographical origin
- Fruit content
- Undeclared additives
- Labelling check
- Naturality (high value compounds)
- Purity (high value compounds)
- Production process

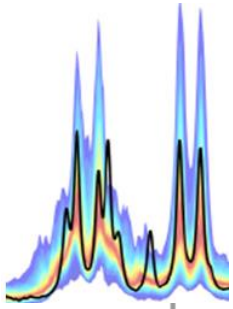
All food ingredients are potentially at risk
Learn more about currents issues, trends, etc. in our International Food Integrity Bulletin

The authenticity testing toolbox

- **Stable isotopes**
  - Molecules origin, natural products geo origin

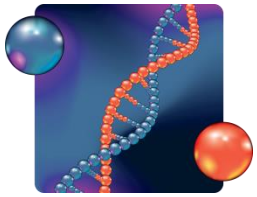


- **Profiling methods**
  - Whole matrices fingerprint, non-targeted approach



- **Chemical composition methods (e.g. chromatography)**
  - Identification & quantification of defined compounds

- **Molecular Biology**
  - Identification of animal species, plant varieties

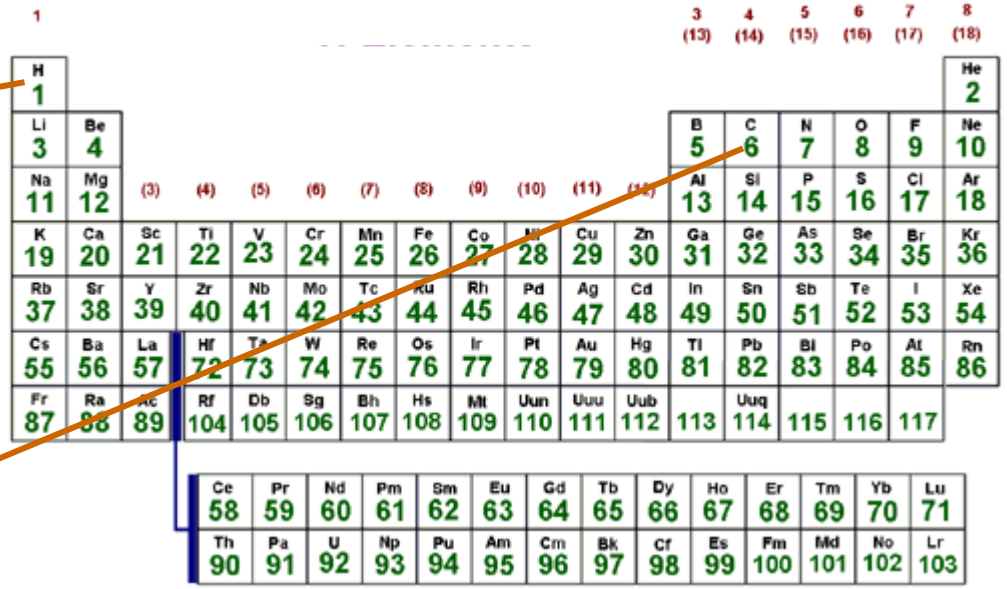


Stable isotope analyses

Stable isotope approach

$^{1}_{1}H$ / $^{2}_{1}H$

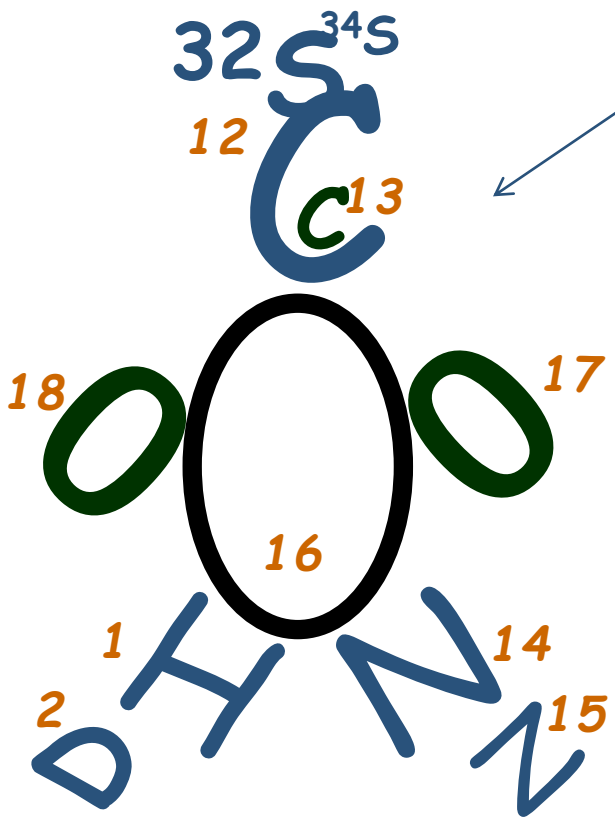
$^{12}_{6}C$ / $^{13}_{6}C$



Isotopes are different forms of a single element - the same number of protons, but differing numbers of neutrons.

Stable isotopes in natural abundance

Natural products are composed of 5 main elements C, H, O, N and S



These elements are naturally present in several isotopic forms (same atomic number, different weights),

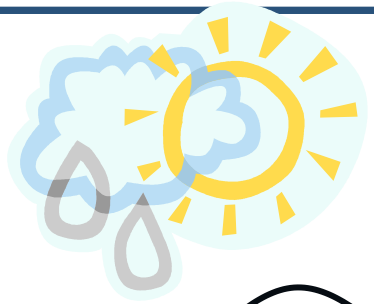
Isotopes distribution is influenced by natural phenomena and human processes:

- **Precursors (natural / synthetic)**
- **Metabolism (botanical origin, regime)**
- **Environment (geographical origin)**

Isotopic fractionation due to metabolism & physiology: example of plant primary metabolism

eurofins

Where does the sugar in a plant come from :



Carbon dioxide

CO_2

(-8 ‰)

C_3

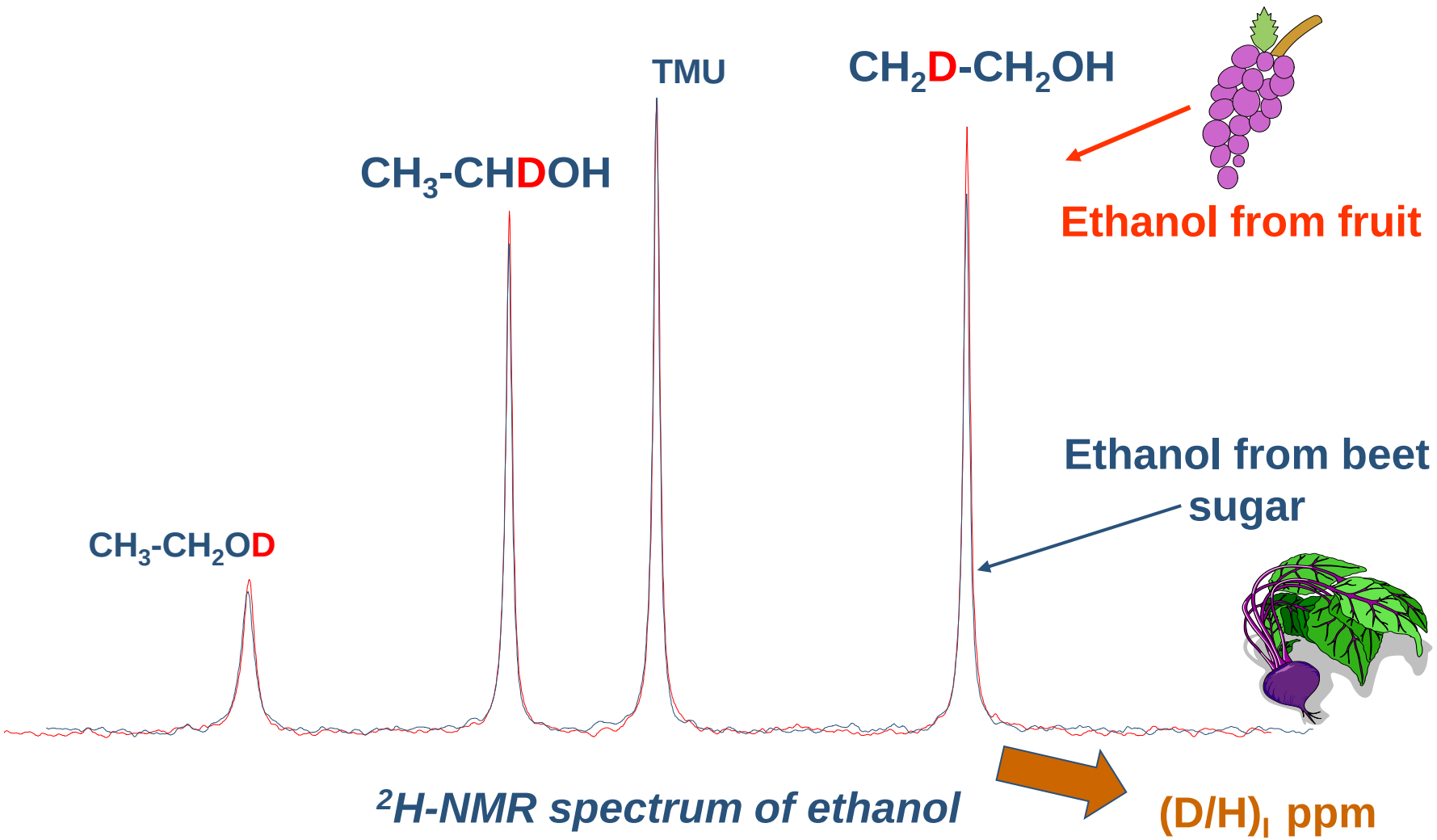
most plants

values around -25‰

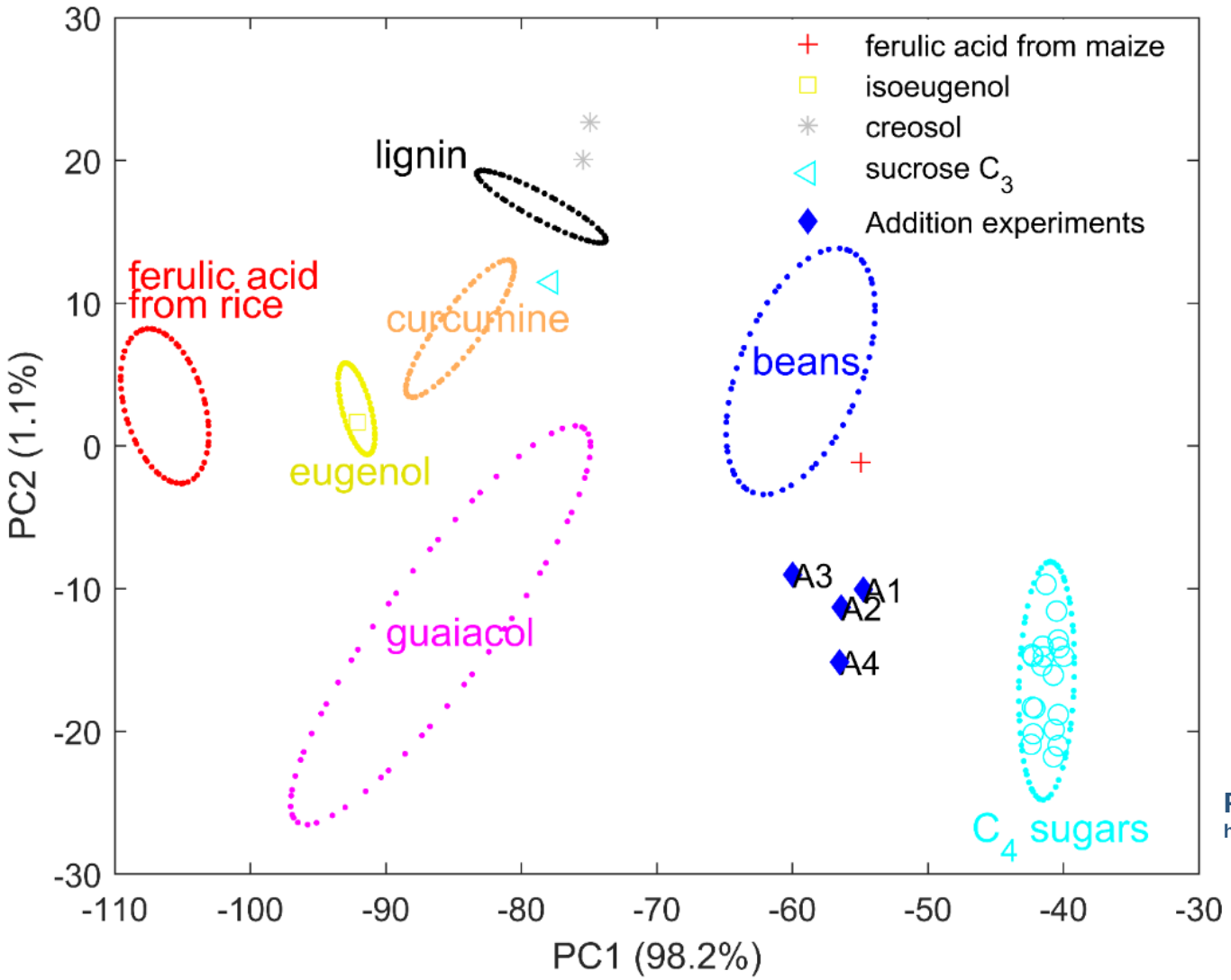
C_4

Gramineae family e.g. sugarcane, maize, values around -10‰

SNIF-NMR® of ethanol: detection of beet sugar



Vanillin authentication by SNIF-NMR



Food Control 130 (2021) 108345
https://doi.org/10.1016/j.foodcont.2021.108345

Examples of adulterations tracked by isotope testing

- Undeclared addition of cheaper sugar / ethanol
- Undeclared addition of water (wine or direct juice)
- Undeclared addition of artificial organic acids (citric, malic, tartaric) to fruit products
- Undeclared addition of artificial vitamin C in fruit products
- Undeclared addition of artificial flavour compounds (e.g. vanillin)
- Geographical origin mislabelling

Isotopic methods recognition: international standards

Method (year)	Product	Fraction	Technique	Isotope ratios
AOAC Official method 995.17 (1995)	fruit juice	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
AOAC Official method 998.12 (1998)	honey	honey & proteins	IRMS	$^{13}C/^{12}C$
AOAC Official method 2000.19 (2000)	maple syrup	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
AOAC Official method 2004.01 (2004)	fruit juice & maple syrup	Ethanol (from fermentation)	IRMS	$^{13}C/^{12}C$
AOAC Official method 2006.05 (2006)	Vanillin	Vanillin	SNIF-NMR	$(D/H)_i$
CEN ENV 12140 (1996)	fruit juice	Sugars	IRMS	$^{13}C/^{12}C$
CEN ENV 12141 (1996)	fruit juice	water	IRMS	$^{18}O/^{16}O$
CEN ENV13070 (1998)	fruit juice	pulp	IRMS	$^{13}C/^{12}C$
OIV-MA-AS311-05, OIV-OENO 426-2011 (2011)	wine & spirits	Ethanol	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
OIV-MA-AS312-06, OIV/OENO 381/2009 (2009)	wine & spirits	Ethanol	IRMS	$^{13}C/^{12}C$
OIV-MA-AS2-12, OIV/OENO 353/2009 (2009)	wine & spirits	Water	IRMS	$^{18}O/^{16}O$
CEN, EN 16466-1:2012 ; OIV-OENO 527-2015 (2015)	vinegar	Acetic acid	SNIF-NMR	$(D/H)_{CH3}$
CEN, EN 16466-2:2012 ; OIV-OENO 510-2013 (2013)	vinegar	Acetic acid	IRMS	$^{13}C/^{12}C$
CEN, EN 16466-3:2012 ; OIV-OENO 511-2013 (2013)	vinegar	Water	IRMS	$^{18}O/^{16}O$
OIV-MA-AS314-03 ; OIV-OENO 512-2014 (2014)	sparkling wine	CO2	IRMS	$^{13}C/^{12}C$

AOAC = Association of Official Analytical Chemists (USA)
CEN = European Committee for Standardization (EU)
OIV = International Organisation of Vine and Wine

Developed & collaboratively validated by Eurofins

Honey: 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)

Recent EU OLAF-JRC study: 46% of honeys imported into EU were found suspect

- **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**

Optimal offer
covering the main potential fraud risks

- **NMR profiling (AA0SG)**
  - ➢ **broad authenticity and integrity screening**

- **Stable isotope testing via EA-IRMS (PAJM)**
  - ➢ **detecting some sugars with an optimum detection limit**

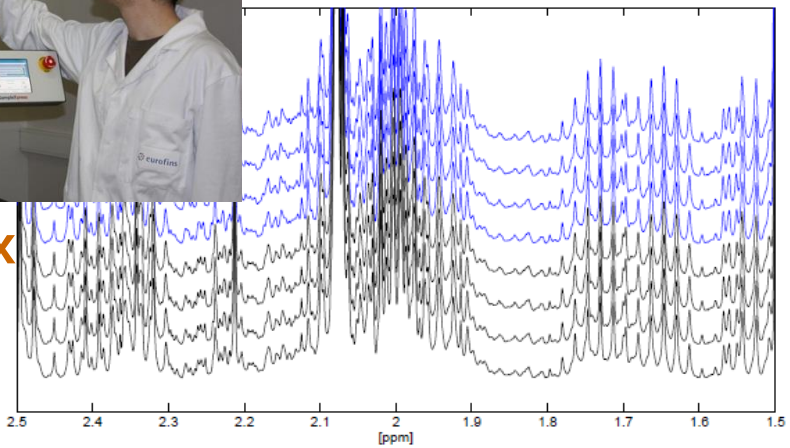
- **LC-HRMS (THP01)**
  - ➢ **detecting other sugars with an optimum detection limit**

- **Pollen/sensorial/conductivity analysis (PTH03)**
  - ➢ **confirming botanical origin**

NMR profiling
High Resolution ^{1}H NMR

eurofins

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

Lab 1
Lab 2

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

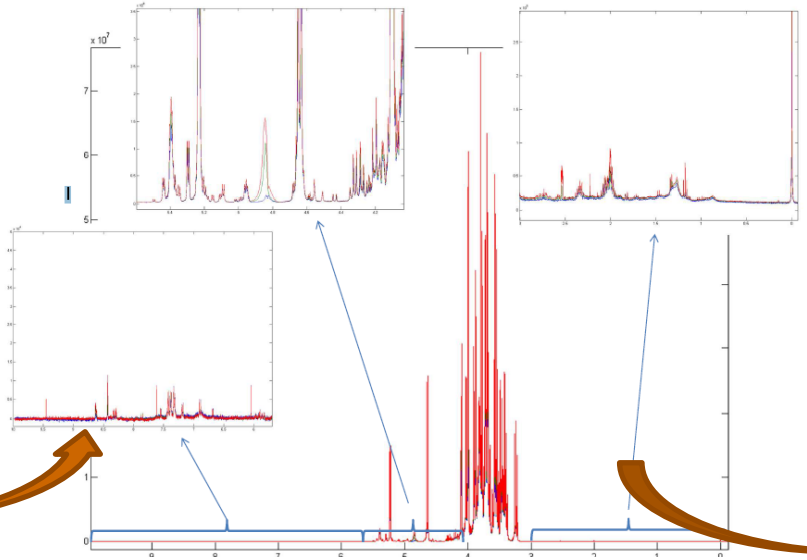
Marc Spiteri [a], Eric Jamin [a,*], Freddy Thomas [a], Agathe Rebours [a], Michèle Lees [a], Karyne M. Rogers [b], Douglas N. Rutledge [c]

[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

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

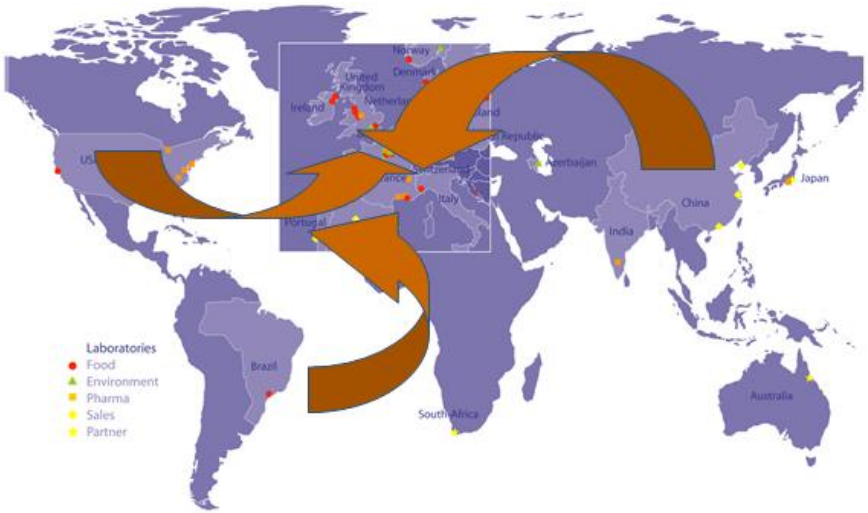
- Allows to quantify key-quality parameters
- Allows to confirm botanical origins of mono-floral honey

Alternative to conventional methods

NMR profiling
Building a worldwide authentic honey database

Reference samples : > 30000
>130 different botanical « families »

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



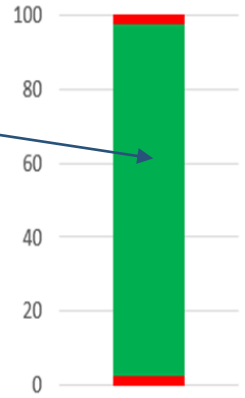
In spite of the diversity of all sources, which allows to differentiate them, honey spectra also possess common spectral characteristics

Our criteria for detecting sugar addition are very conservative and robust across all botanical / geographical origin, thus avoiding false positives

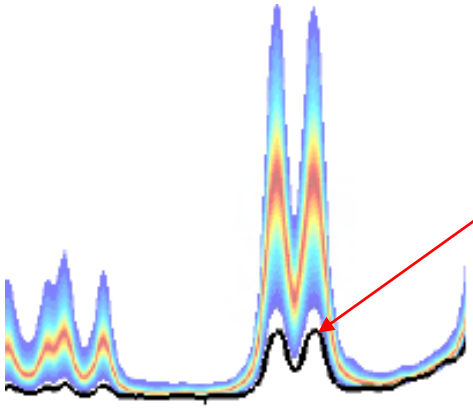
NMR profiling
Detection of added sugars (both C3 & C4)

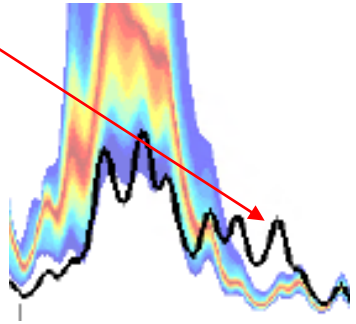
eurofins

Interpretation limits based on 95% quantiles of reference honey populations



Adulterated honeys





Indirect detection:
Dilution effects

Direct detection:
Markers of sugar syrup addition

17

NMR profiling
Quantitative parameters
1) Sugars & HMF

- **Glucose, Fructose, Glucose + Fructose, sucrose**
- ➢ **Comparison with directive 2001/110/EC**

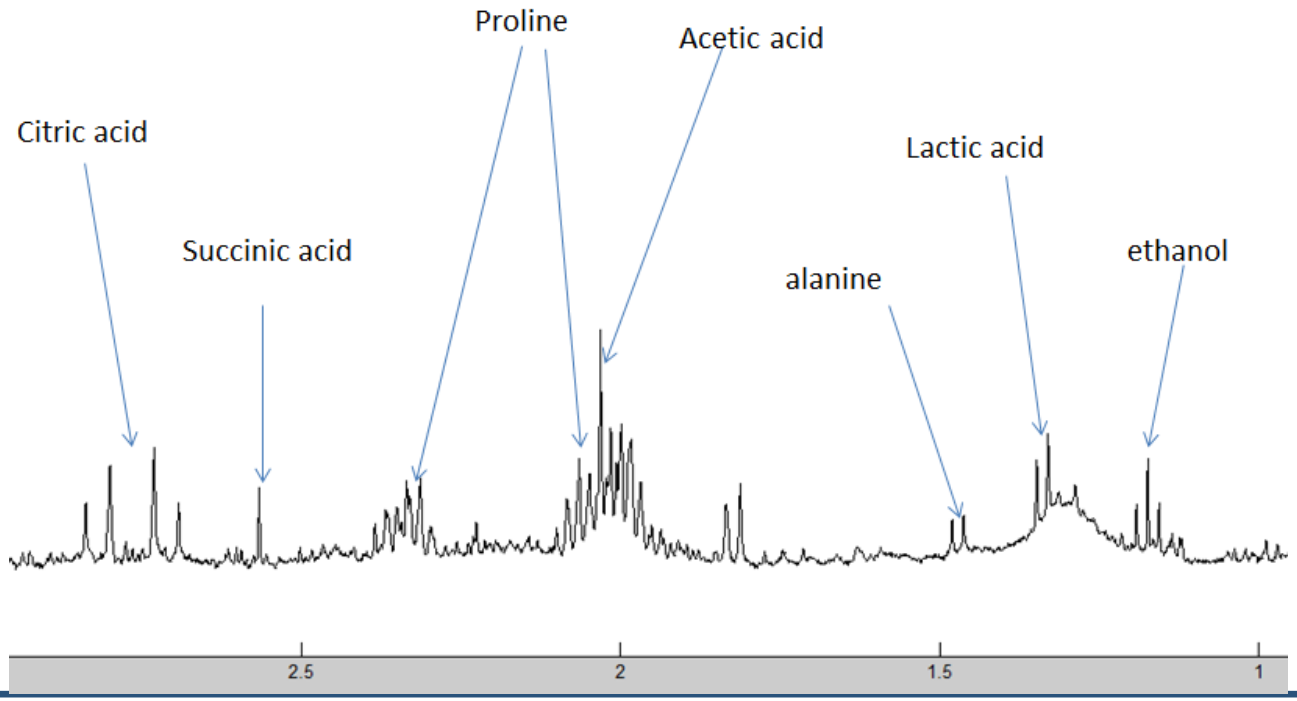
- **Fructose / Glucose, turanose**
- ➢ **Comparison with litterature & databases**

AA0SG	AA	1H-NMR profiling of honey (sugars, HMF, sugar add)	Method : Internal, NMR	
(a)	Fructose		41.5 (± 3.1) g/100 g	
(a)	Glucose		32.0 (± 2.6) g/100 g	
(a)	Fructose / Glucose		1.30 (± 0.10)	
(a)	Glucose+Fructose		73.5 (± 4.7) g/100 g	
(a)	Sucrose		0.8 (± 0.5) g/100 g	<= 5 (general ; Dir. 2001/110/CE)
(a)	5-HMF		10 (± 6) mg/kg	<= 40 (general ; Dir. 2001/110/CE)
(a)	Turanose		1.42 (± 0.19) g/100 g	

>= 60 | Council Directive 2001/110/EC | General | >= 45 Honeydew honey

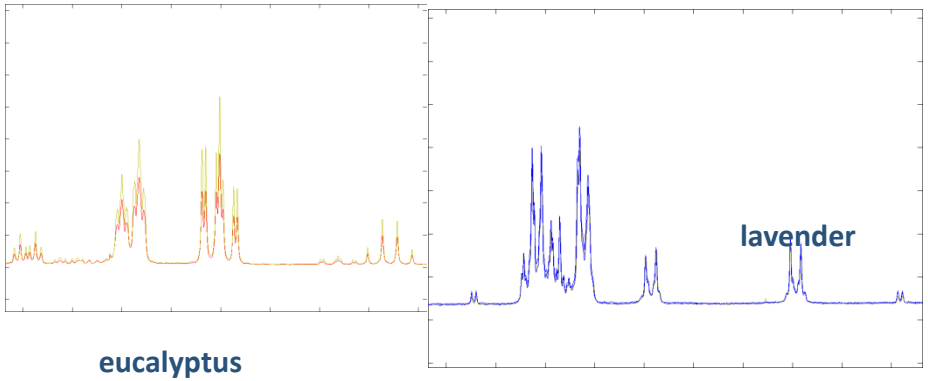
NMR profiling
Quantitative parameters
2) Others

- citric acid
- fermentation markers (acetic, succinic, and/or lactic acid, ethanol)



NMR profiling
3) Confirmation of the botanical origin

For monofloral honeys → Specific markers
For botanical species



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

NMR profiling
3) Confirmation of the botanical / geographical origin, e.g. Manuka honey

eurofins

Food Chemistry 217 (2017) 766–772

Contents lists available at ScienceDirect

Food Chemistry

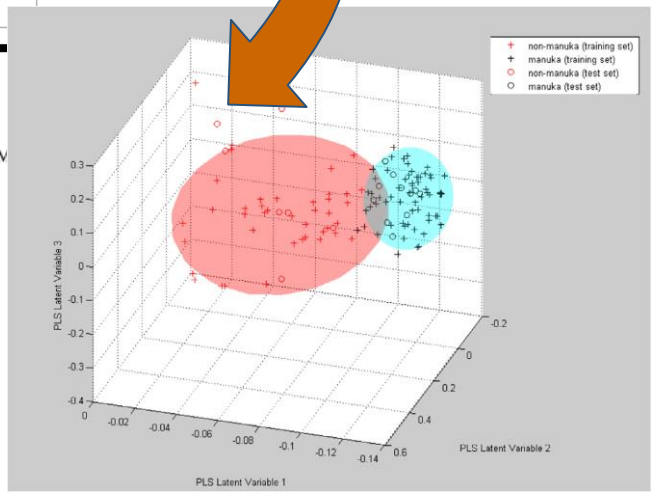
journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

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

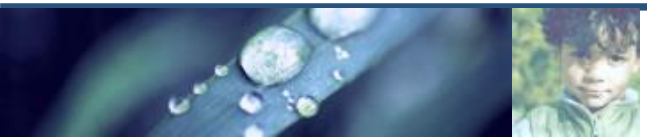
Marc Spiteri [a], Karyne M. Rogers [b], Eric Jamin [a], Freddy Thomas [a,*], Sophie Guyader [a], Michèle Lees [a], Douglas N. Rutledge [c]

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



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**



Food authenticity testing: a challenge with solutions

Dr. Eric Jamin
Eurofins Authenticity Competence Center, Nantes (France)