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# JRC's contribution to fight food fraud


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**Joint Research Centre**
the European Commission's
in-house science service


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# Food fraud

- Prevention of fraud in the agri-food chain and promotion of authentic products is a major element to ensure the commercial success of European high-value agri-food products on the internal and international markets.
- Fraudulent activities such as mis-description of food or extension of genuine products with cheaper substitutes will negatively impact on consumer's trust and the competitiveness and profitability of honest producers in the EU.

# Food fraud

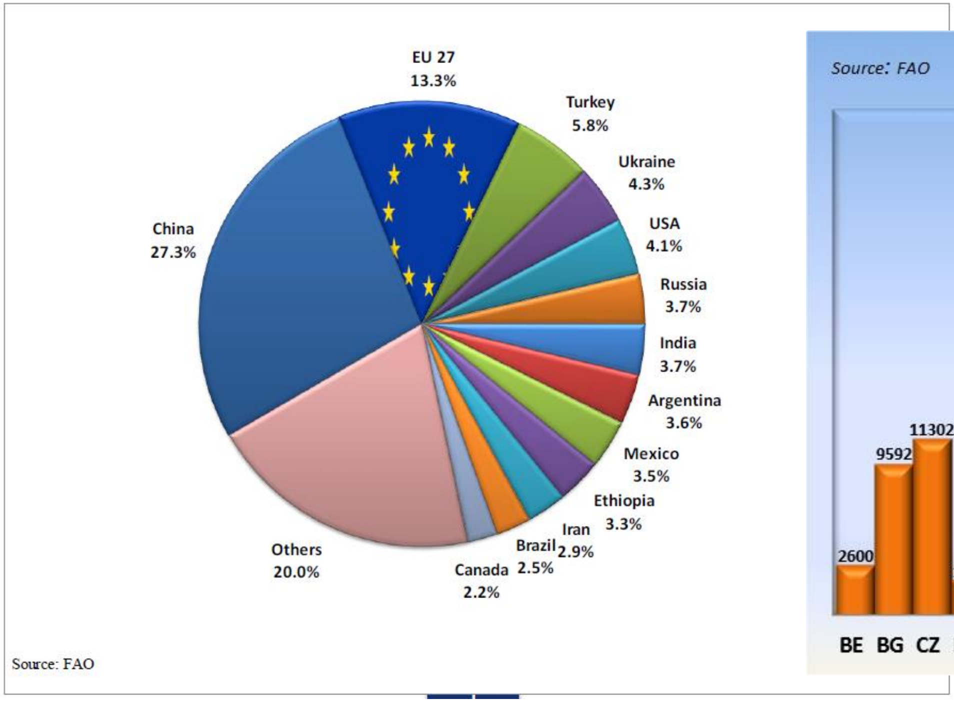
- **In this respect, DG SANTE** (Directorate-General for Health and Food Safety) **is organizing coordinated control plans across the EU Member States to detect fraud in fish, honey and wine markets.**

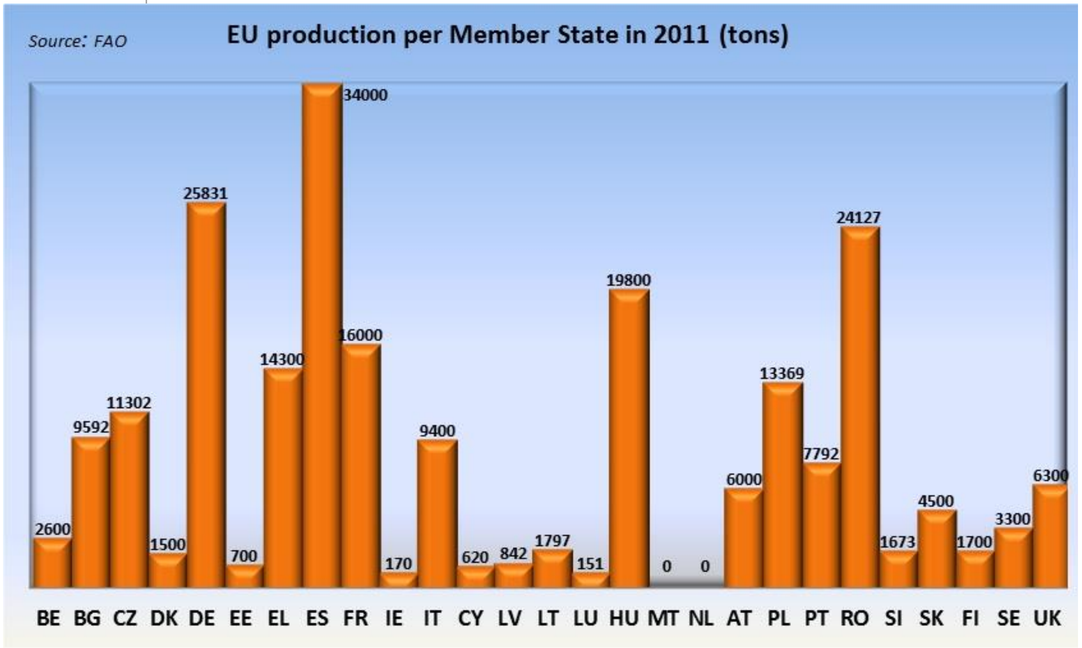
- **A Food Fraud Network has been established to improve the capability of competent authorities to:**

  - detect and prevent violations of food chain rules, also across borders and in potential cases of “food fraud”.

  - collect the information which is needed (in accordance with applicable national rules) to further refer a case to investigation/ prosecution.

# Honey in Europe




**The European Union is the world's second honey producer, and plays an important part in the trade of agriculture products.**

# EU COORDINATED CONTROL PLAN ON HONEY (DG SANTE, DG JRC, Member States)

- All Member States + CH and NO participated
- 2237 samples tested
- Non compliances:

| Non-compliance | Physico-chemical parameters | Botanical source | Geographical origin | Sugar | Other labelling | **Total** |
|---|---|---|---|---|---|---|
| % non-compliant samples | 2% | 7% | 2% | 6% | 2% | **19%** |

- *Suspicions of non-compliances:*

| Nature of the suspicion | Pollen content and declared geographical origin | Adulteration with sugar | **Total** |
|---|---|---|---|
| % suspect samples among the remaining samples | 2% | 11% | **13%** |

***""The results concerning adulteration with sugar are only preliminary and further investigations were conducted on 1200 samples that will be tested with advanced laboratory methods.""***

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# Composition of authentic honey

*Fructose ~ 31 - 49%*

*Glucose ~ 23 - 41%*

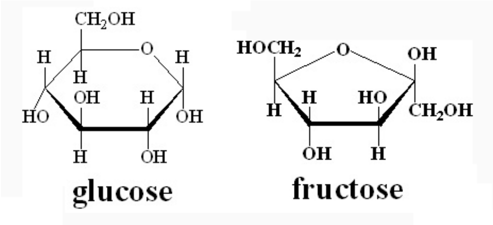
*Sucrose ~ 0.2 - 10%*

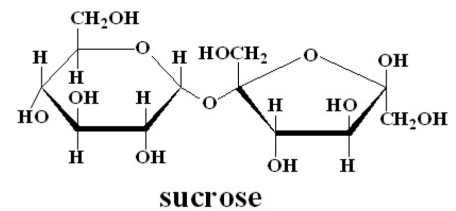
*Water ~ 18%*

*Other ingredients up to 6%*

➢ Oligosaccharides 3-5%

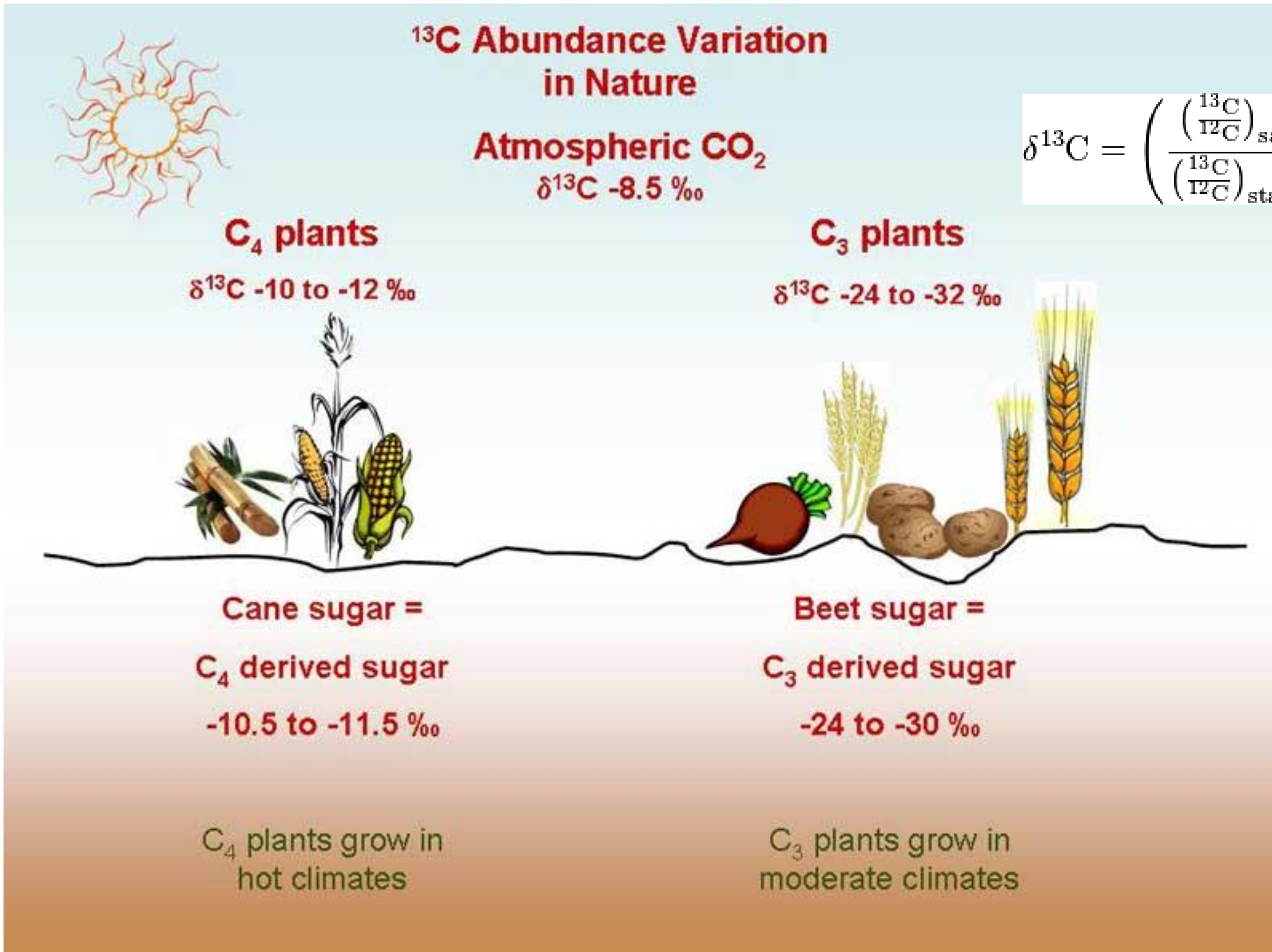
- tri-saccharides (melezitose, raffinose, erlose, etc.)
- traces of tetra-saccharides and penta-saccharides



# IRMS concept

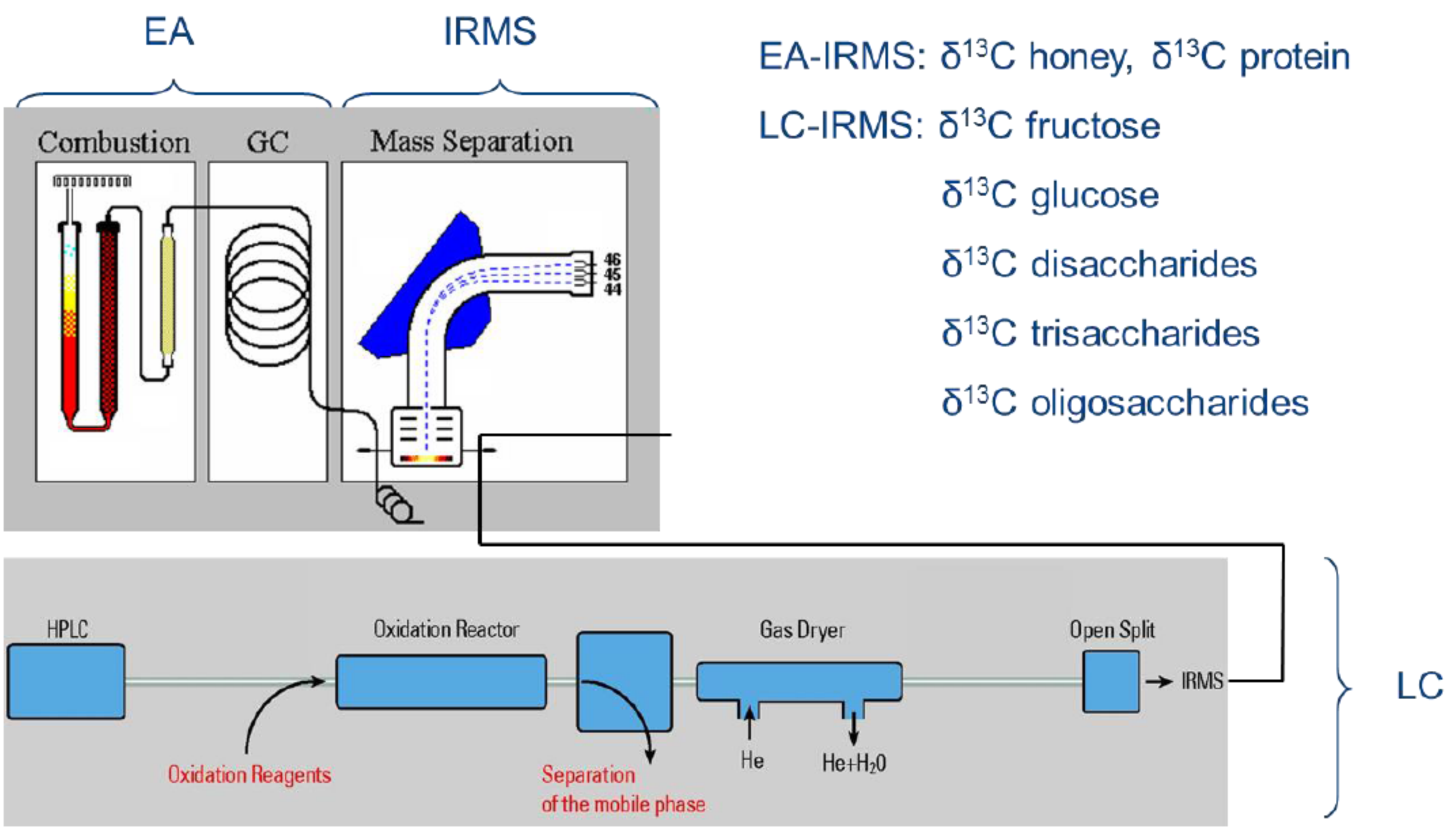
$$\delta^{13}C = \left( \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) * 1000\ ‰$$



# IRMS concept

## LC-IRMS: δ13C-EA/LC-IRMS

**δ13C EA/LC-IRMS**




EA-IRMS: δ13C honey, δ13C protein

LC-IRMS: δ13C fructose

δ13C glucose

δ13C disaccharides

δ13C trisaccharides

δ13C oligosaccharides

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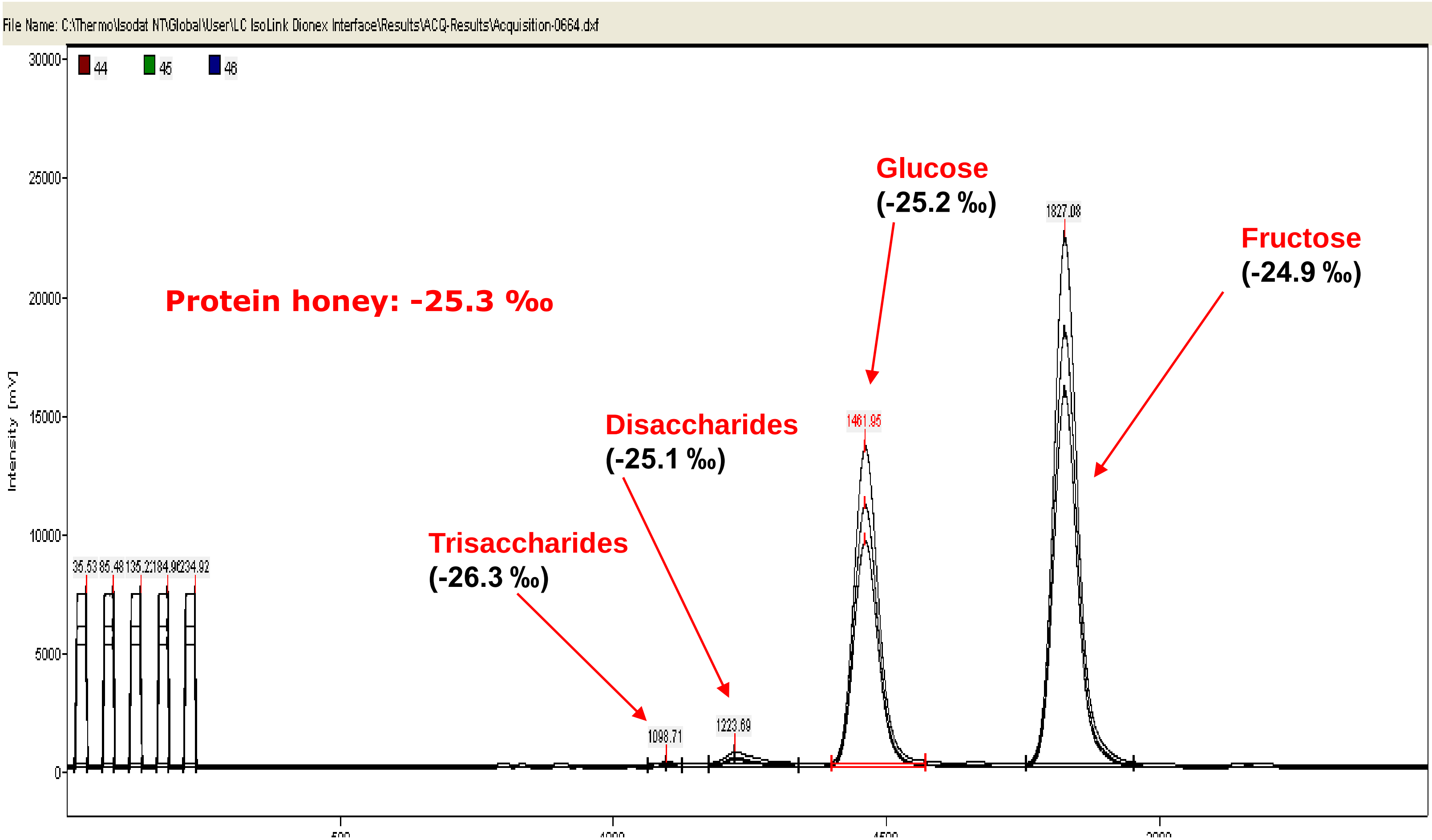
# Authentic honey (C3 plants)

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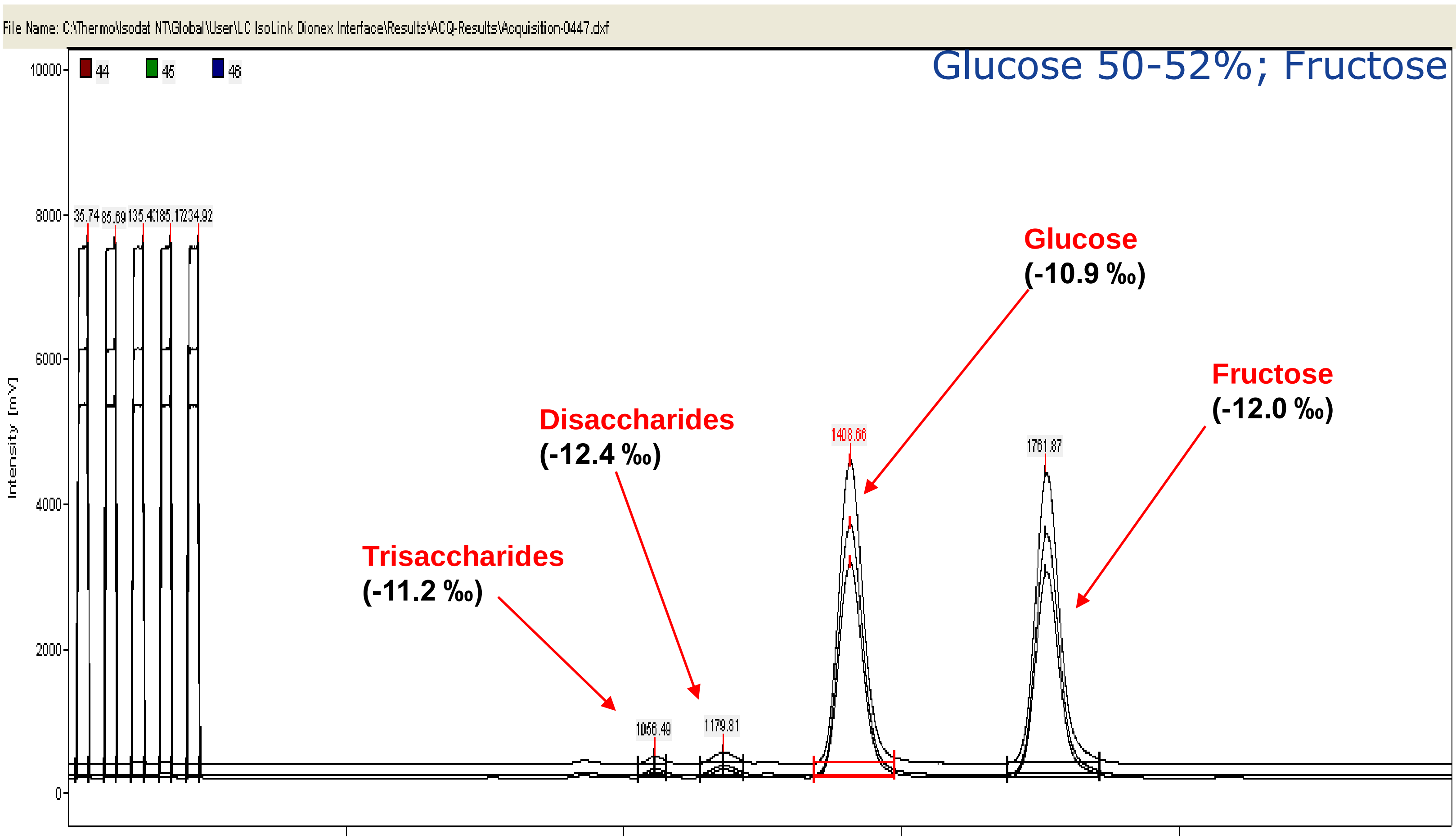
# High Fructose Corn Syrup (C4 plant)

Glucose 50-52%; Fructose 42%

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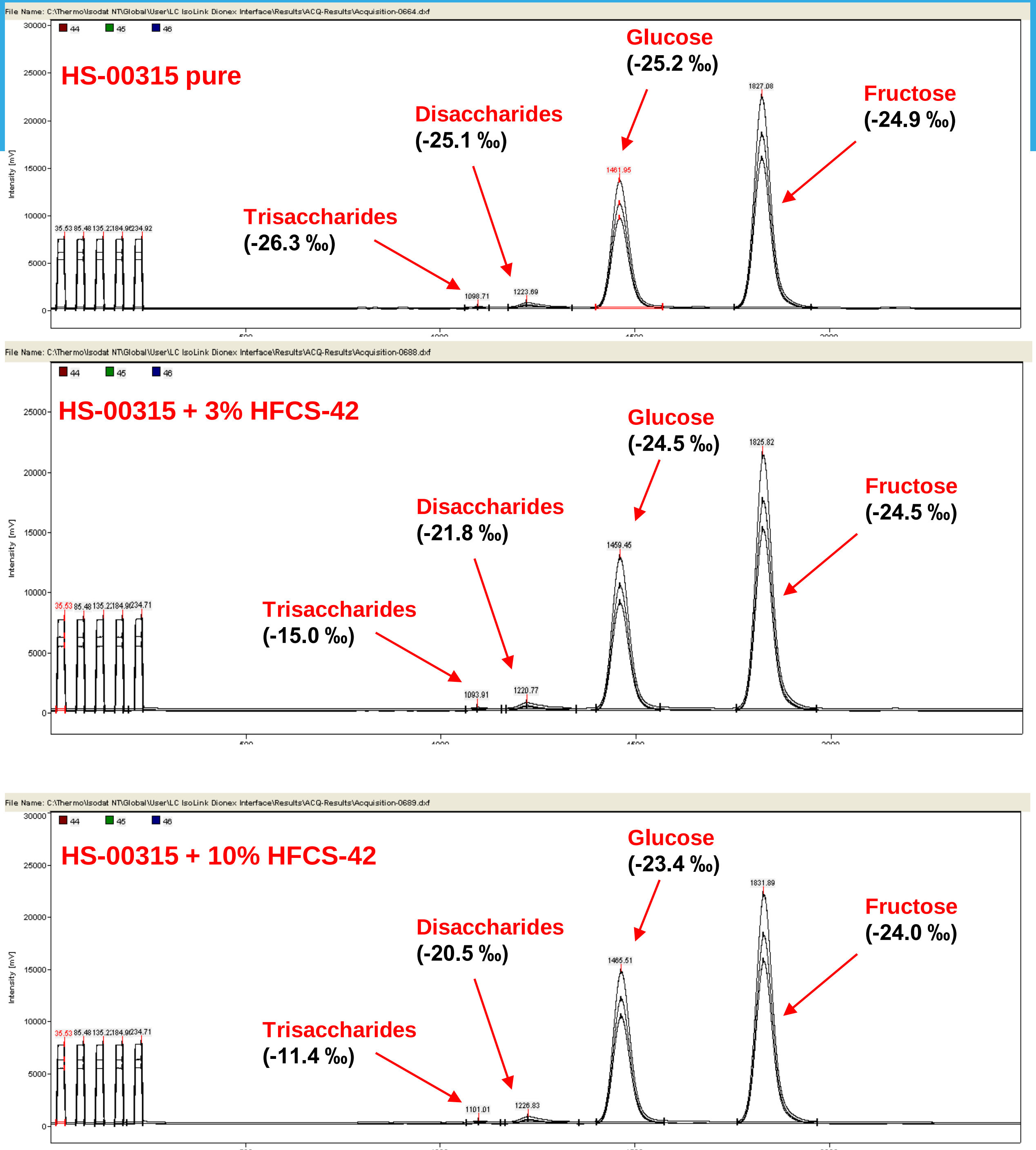
# Adulteration Experiment




**Protein honey: -25.12 ‰**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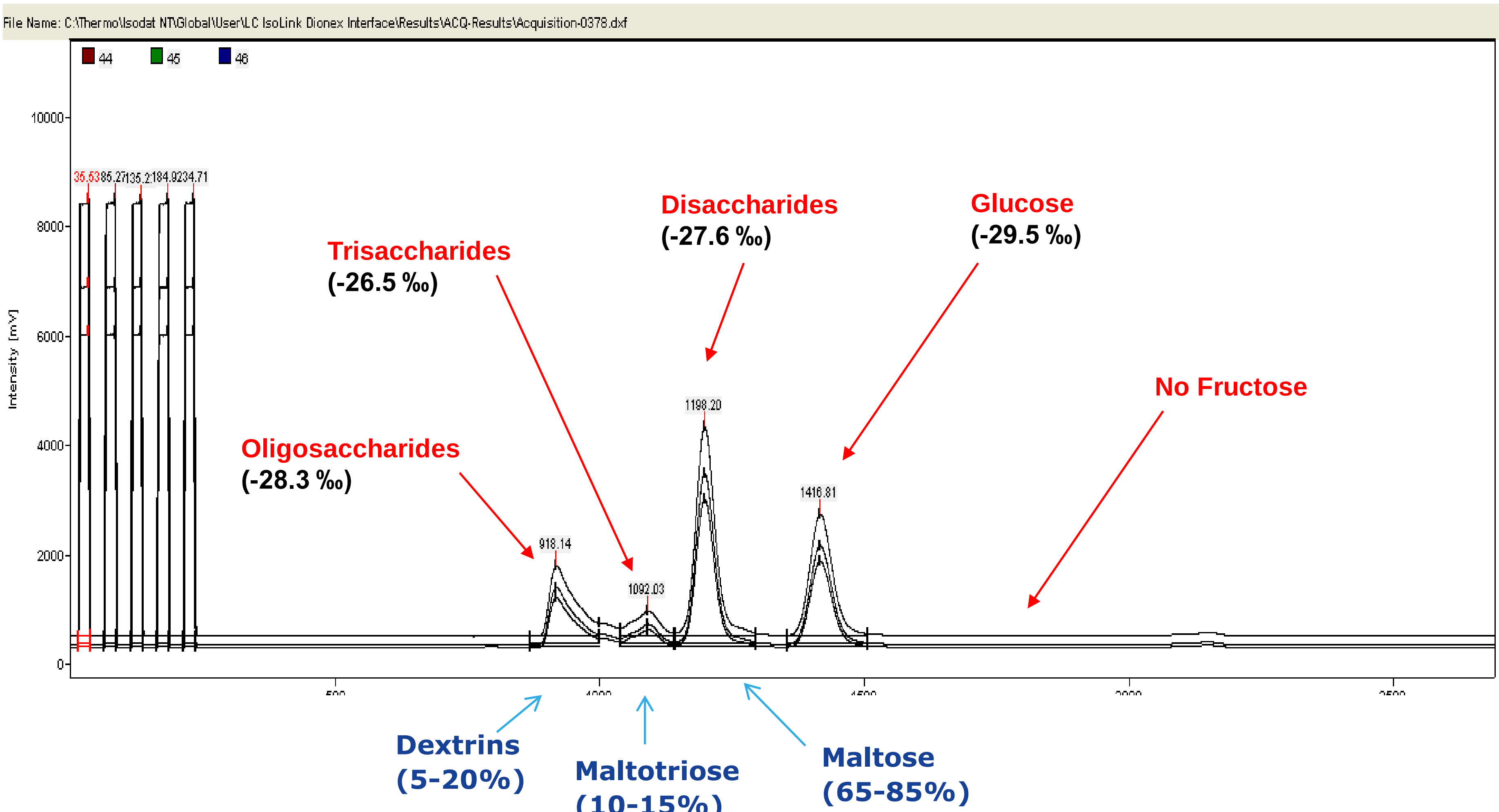
# Rice Syrup (C3 plant)

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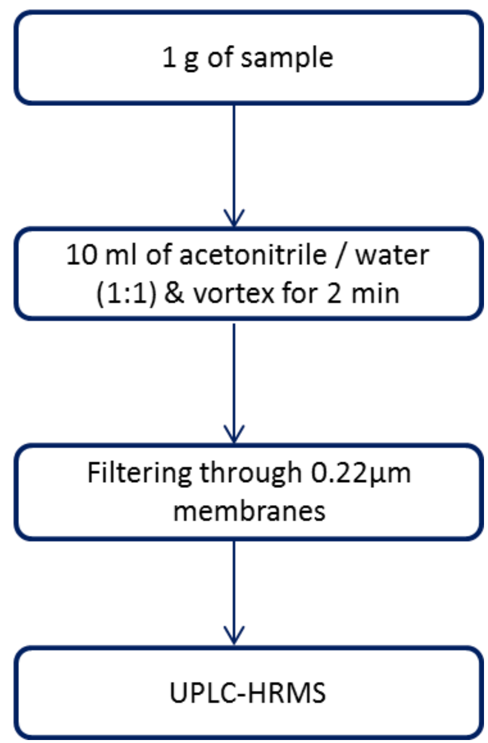
# Sugar metabolomic profiling by UPLC-HRMS

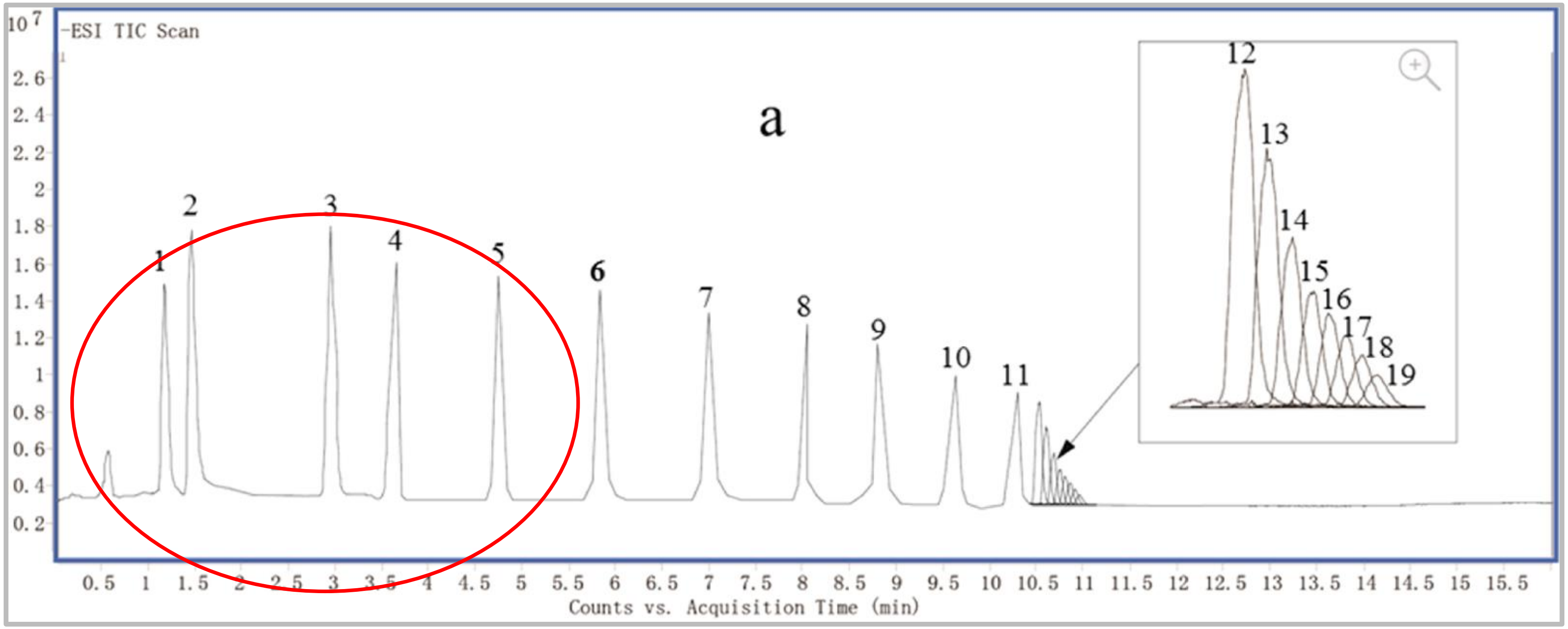

Thermo Scientific Exactive Plus with Ultimate 3000 Rapid Separation LC system

- **Simple sample preparation**




- **Rapid UPLC-HRMS analysis (16 min)**
  - Obtain profiles from standards & -saccharides
  - Obtain profiles from sugar syrups
  - Confirm adulteration by fortifying authentic samples
  - Confirm adulterated honey samples

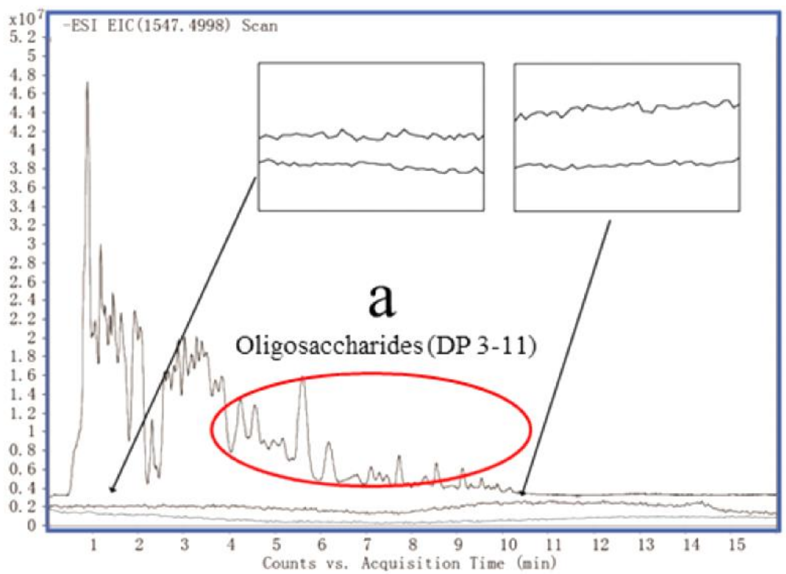
# Screening sugar profiles by UPLC-HRMS



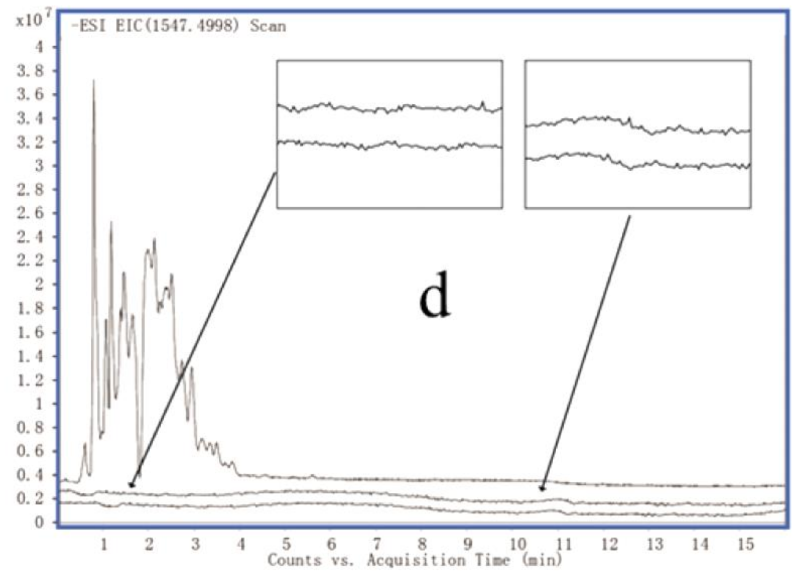

- *When honey is adulterated with syrups you obtain degradation products from the enzymatic hydrolysis of starch*

- *Peaks 3-11 to oligosaccharides (DP 3-11)*
- *Peaks 12-19 to polysaccharides (DP 12−19)*

# Screening sugar profiles by UPLC-HRMS

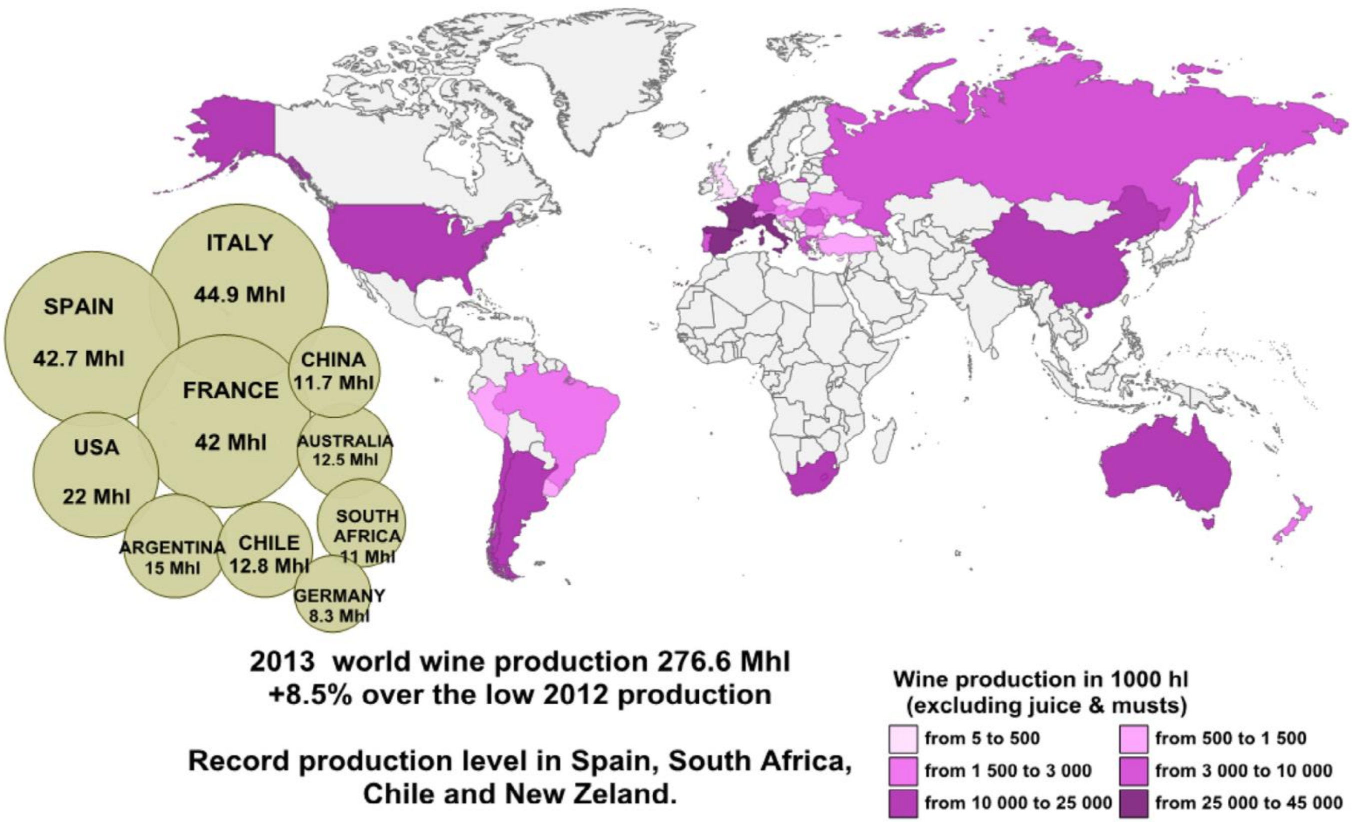



**Adulterated honey sample**




**Authentic honey sample**

# Global Wine production




**2013 world wine production 276.6 Mhl**
**+8.5% over the low 2012 production**

**Record production level in Spain, South Africa, Chile and New Zeland.**

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# Wine adulteration

- Isotopic analysis for detection of sugaring (chaptalisation) and watering of wine, and geographical origin of wine
- • SNIF®-NMR
- • $\delta$ $^{13}C$ of ethanol
- • $\delta$ $^{18}O$ of wine water

# Wine authenticity

- European Reference Centre for Control in the Wine Sector
- Select the most promising methods that are in use or in development in the national control laboratories for the determination of geographical origin, varietal composition and vintage of wines
- Apply targeted and untargeted metabolomics approaches (NMR, MS etc.)

# Untargeted metabolomics - Varietal and geographical origin identification

**UPLC-MicrOTOF II analysis**

UPLC
Ultimate 3000 Dionex

Waters Acquity UPLC
BEH C18 column (100 x 2.1 mm, 1.7 μm)

Flow rate = 300 μl/min
Injection volume = 2 μl
Column T = 30°C

**Mobile phase**

**(A)** water + 0.1% Formic acid
**(B)** Acetonitrile + 0.1% Formic acid

**Gradient program**

| Time [min] | Flow [ml/min] | Solvent A [%] | Solvent B [%] |
|---|---|---|---|
| 0.0 | 0.3 | 99 | 1 |
| 1.5 | 0.3 | 99 | 1 |
| 14.0 | 0.3 | 65 | 35 |
| 16.0 | 0.3 | 20 | 80 |
| 17.0 | 0.3 | 1 | 99 |
| 19.0 | 0.3 | 1 | 99 |
| 20.0 | 0.3 | 99 | 1 |
| 28.0 | 0.3 | 99 | 1 |

MicrOTOF II

**MS conditions**

Scan range 50-1000 m/z, R = 16,500
Mass accuracy 1-2 ppm

**(+) and (-)-ESI Ionisation conditions**

Capillary voltage: 2500 V
Nebulizer pressure: 1.7 bar
Dry gas flow: 10 l/min
Dry gas T: 215°C
Capillary exit: 90 V
Skimmer 1: 30V
Spectra rate :1 Hz

**1st Group:** 21 wine samples (12 red & 9 white)

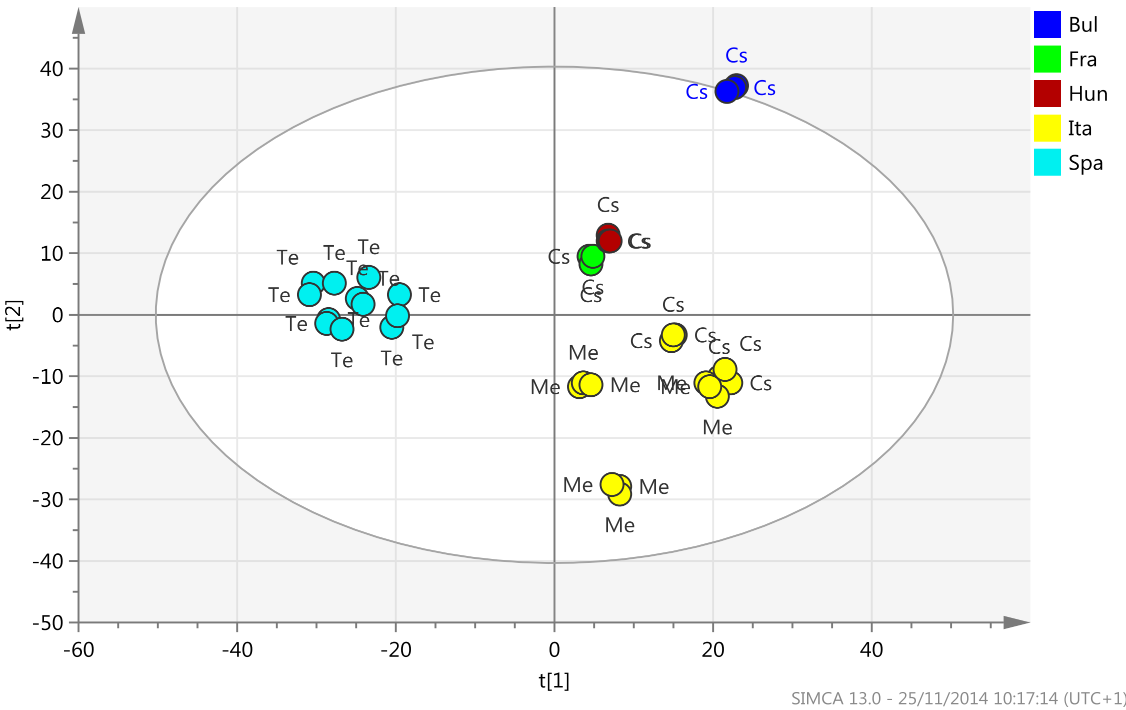
Bruker Data Analysis & Profile Analysis

**2nd Group:** 60 wine samples (red &white)

Bruker Data Analysis R and XCMS package

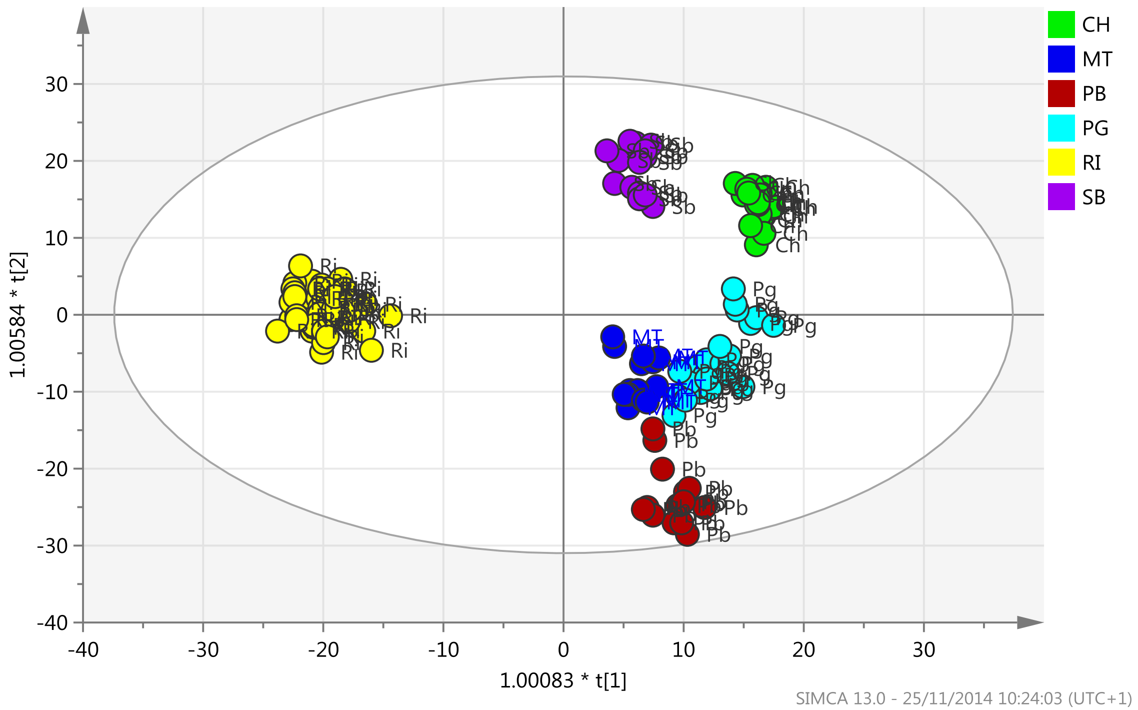
**SIMCA-P**
PCA-X,
PLS-DA,
OPLS-DA

# Geographical origin




**PLS-DA of red wines according to country of origin. Te - Tempranillo, Me - Merlot, Cs - Cabernet Sauvignon; Fra - France, Bul - Bulgaria, Hun - Hungary, Ita - Italy, Spa - Spain.**

# Varietal origin




➤ **OPLS-DA model of white wines according to their cépage. Ch – Chardonnay, MT – Müller-Thurgau, Pb - Pinot Blanc, Pg – Pinot Gris, Ri – Riesling, Sb – Sauvignon Blanc.**

# Metabolomic approaches in food-Discussion

- The demonstrated potential of metabolomic approaches suggests that metabolomics could play a major role in many aspects of food authenticity.
- In food authentication, one of the difficulties is that because the issue under study is linked to a legal requirement, standard or guidance, the interpretation of the results has to be made taking into account measurement uncertainty, natural variation, tolerance permitted and the conclusion must be beyond reasonable doubt.

# Metabolomic approaches in food-Discussion

- **At the moment many studies are based on relatively small sample sizes. Transition of these techniques to routinely used methods would require the establishment of larger databases that take into account greater variability…How large?**
- **Does the development of robust classification models based on chemometrics be based on well-designed experimental studies that include all sources of variation:**
  - individual variability, variety or breed, feeding or fertilisation practice, geographical location or climate, etc.?
  - sampling process and storage (wine and honey)?

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# Metabolomic approaches in food-Discussion

- In order to account for variability to obtain models with a global applicability, should large studies where sample collection and analysis is done through a number of years and different locations need to be organised?

- In terms of instrumentation, should different type of analytical techniques be used in a complementary way (NMR, UPLC-HRMS, GC-MS, GCxGC-TOFMS or LCxLC-HRMS) to expand metabolite coverage?

- Can the creation of food databases (e.g. wine-metabolome database) for assignment of metabolites in food matrices help and encourage the application of food metabolomics?

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# Metabolomic approaches in food-Discussion

- **Should money and time be invested to create large projects to improve sample sets, the technology and databases available?**
- **What is needed at legislation level in order to enforce and facilitate the implementation of food metabolomics for routine control in the future?**

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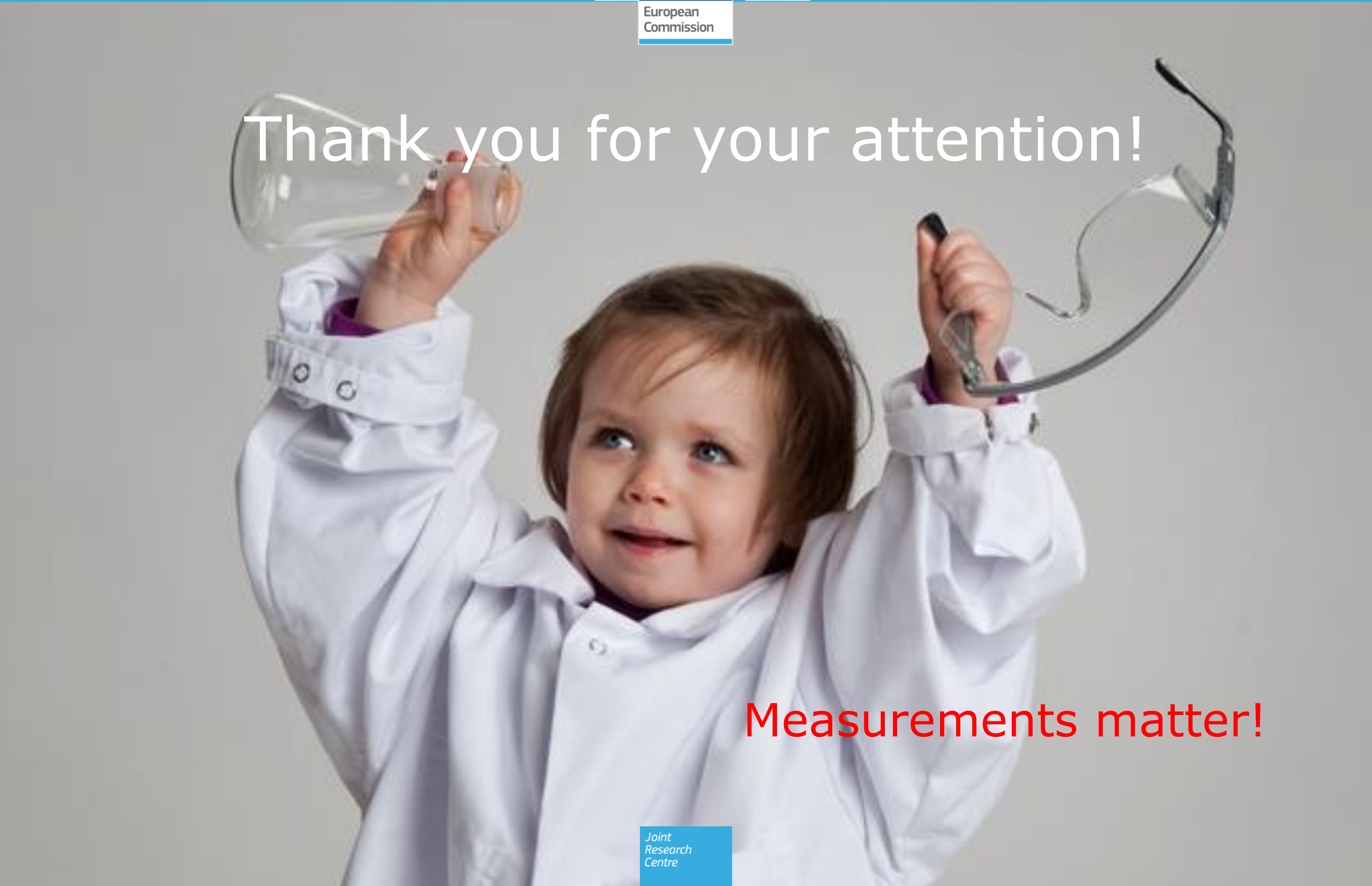

# Thank you for your attention!

Measurements matter!

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