Beehive nutrition and honey contamination

Etienne Bruneau
From the feeder to the honey

- Context of adulteration
- The syrup and nutrition of beehives
- The residues analyzes
- The biological explanation
- How can we avoid this problem?
Context of adulteration

- **Top 10 of the adulterated products** *(Journal of Food Science)*
  - 1. Olive oil
  - 2. Milk
  - 3. Honey
  - 4. Saffron
  - 5. Coffee
  - 10. Apple juice
  - 10. Grape wine
  - 10. Maple syrup
  - 10. Vanilla extract
Background

- 2014 – EP resolution: *Food crisis, fraud in the food chain and the control thereof*, listed honey as a food product most vulnerable to fraud;
- 2015 – EU coordinated control plan to assess the prevalence on the EU market of honey:
  - adulterated with sugars;
  - mislabelled (botanical source/geographical origin).
- Initial results published in December 2015:
  - 6% adulterated by sugar with further 11% suspected.
- JRC mandated to support by conducting advanced tests to detect adulteration with sugars.
### Context of adulteration

- **Results of the JRC on 893 honeys**

<table>
<thead>
<tr>
<th>Origin</th>
<th>Samples (n)</th>
<th>Suspicion of non-compliance (n)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blend of EU honeys</td>
<td>96</td>
<td>19</td>
<td>19.8</td>
</tr>
<tr>
<td>Blend of EU and non-EU honeys</td>
<td>426</td>
<td>40</td>
<td>9.4</td>
</tr>
<tr>
<td>Blend of non-EU honeys</td>
<td>30</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>Single EU Member State</td>
<td>275</td>
<td>53</td>
<td>19.3</td>
</tr>
<tr>
<td>Single non-EU country</td>
<td>55</td>
<td>11</td>
<td>20.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>893</strong></td>
<td><strong>127</strong></td>
<td><strong>14.2</strong></td>
</tr>
</tbody>
</table>

Table 7. Prevalence of suspicion of non-compliant honeys depending on their declared origin (n, number of samples).
Context of adulteration

- Consumer magazines present results of honeys collected by beekeepers with low level of adulteration.
Context of adulteration

**ANALYSIS REQUESTED:** Determination of honey-foreign oligosaccharides by LC-ELSD (101606)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Unit</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign oligosaccharides</td>
<td>0.20</td>
<td>%</td>
<td>LC-ELSD (a)</td>
</tr>
</tbody>
</table>

n.a.: not analyzed; n.d.: not detectable < limit of quantification (LOQ), LOQ: 0.1 %;
Reported value represents foreign oligosaccharides with a degree of polymerization > 4

**SPECIFICATIONS OF PURE HONEY:** foreign oligosaccharides: n.d.

(a) : accredited under terms of DIN EN ISO/IEC 17025. (na) : not accredited method.
This document may only be reproduced in full. The results given herein apply to the submitted sample only.

**Interpretation:**
Honey-foreign oligosaccharides were detected in the investigated honey sample.
Therefore, the sample does not meet the requirements for pure honey (Council Directive 2001/110/EC from 20/12/2001).
The presence of honey-foreign oligosaccharides indicates an addition of starch-based sugar syrup.
The gamma-amylase is used to produce syrup from rice. Honey adulterated with 15% rice syrup give a result of 10.2 U/kg.

**ANALYSIS REQUESTED: Determination of beta-gamma-amylase activities by enzyme test (101455)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Unit</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>beta/gamma amylase activity</td>
<td>5.1</td>
<td>units/kg</td>
<td>PM DE01_115 (a)</td>
</tr>
</tbody>
</table>

*n.a.: not analyzed; n.d.: not detected < 1 units/kg honey  
reference value: < 5 units/kg honey  
(a): accredited under terms of DIN EN ISO/IEC 17025. (na): not accredited method. (1) Inhouse procedure  
This document may only be reproduced in full. The results given herein apply to the submitted sample only.*

**Interpretation:**  
The sample does not meet the specifications of pure honey. The activity of the foreign amylases is outside the naturally occurring range and indicates an addition of foreign amylases or inverted sugar syrup produced with these enzymes. This does not apply in case of Metcalfa honey and Quillay honey. The result is close to the action limit.

**ANALYSIS REQUESTED: Foreign enzyme activity by enzyme test (101411)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Unit</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-fructofuranosidase activity</td>
<td>n.d.</td>
<td>units/kg</td>
<td>PM DE01_102 (a)</td>
</tr>
</tbody>
</table>

*n.a.: not analyzed; n.d.: not detected < 20 units/kg Honey  
(a): accredited under terms of DIN EN ISO/IEC 17025. (na): not accredited method. (1) Inhouse procedure  
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**Interpretation:**
The syrup and nutrition of beehives
The syrup and nutrition of beehives

- The use of syrup increase during the last years
- Climate change
  - Climate not in balance with the biological development of bees
  - Feeding at the end of the winter to help the bees to develop or to arrive to the spring
  - Feeding during the season due to bad climate for the survival of the bee colonies


Annual mean air temperature anomalies across Europe for 2016, with respect to the 1981-2010 climatology
The syrup and nutrition of beehives

- Climate change: annual mean air temperature anomaly

The syrup and nutrition of beehives

- The use of syrup increase during the last years
  - Climate change: mean anomalies for daily maximum air temperatures and mean sea-level pressure field

The syrup and nutrition of beehives

- Climate change: annual precipitation sum for 2016 as a fraction of the 1981-2010 long-term mean.

The syrup and nutrition of beehives

- Climate change: daily precipitation amounts in the period 29 May - 1 June 2016

The syrup and nutrition of beehives

- **Type of food change:**
  - In the past most of the beekeepers used sucrose (saccharose)/water solution (2:1 or 1:1)
  - Today they use: starch-based sugar syrups (produced from corn, wheat, rice), sugar cane, sugar beet, ... at a lower price
  - Development of a lot of syrup and food complement for bees ±60 different in a shop
The adulteration residues analyzes
The adulteration residues analyzes

- $^{13}$C-Isotope-Mass-Spectrom. C$_4$ sugar - AOAC - Methode 998.12
- $^{13}$C-Isotope-Mass-Spectrom. + LC, C$_3$/C$_4$ sugars - $^{13}$C-LC-IRMS
- Foreign invertase, beta-fructofuranosidase - HPLC
- Foreign enzym, thermoresistant Amylase (Diastase) - photometrisch
- Foreign amylase: α, β, γ amylase - photometrisch
- Foreign oligosaccharides - HPLC-ECD
- NMR-Screening - H-NMR
- SMR: Special marker in rice syrup - LC-MS/MS
- Special marker in beet sugar syrup - LC-MC
- TMR: Trace marker in rice syrup - TMR, ICP-MS
The residues analyzes

- Enzymatic analyzes for C3 sugars
  - β-fructofuranosidase activity
    - Syrup: >1000 U/kg
    - Detection: 20 U/kg
    - Honey: 20 U/kg
  - β/gluco amylase activity
    - Syrup: 200 U/kg
    - Detection: 1 U/kg
    - Honey: <5 U/kg

http://www.pcelinjak.hr/OLD/images/stories/test2/aaa/publication_foreign_enzymes_05.02.10.pdf
The residues analyzes

Not all sugar syrups are outside the naturally occurring range of honey.

<table>
<thead>
<tr>
<th>d$\delta^{13}$C (per mil)</th>
<th>$\text{Total scale -11 to -28}$</th>
<th>$\text{-23 to -27.3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>all honeys (282)</td>
<td></td>
<td>av. -25.6</td>
</tr>
<tr>
<td>acacia (60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rape (51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>orange (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>polyflora (125)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yucatan (33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>linden (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>forest (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISS 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISS 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISS 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apliinvert</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rice syrup</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cane, corn, millet syrup**

Limit of detection $> 7\%$

**EA-IRMS**: $-11.3$ (C4 sugar)

**C4/C3 sugar mix**: $-19.8$

**LC-IRMS**: $-24.2$

**C3 sugars**: $-26.1$ and $-26.4$

Kurt-Peter Raaezke
The residues analyzes

- Methods detecting specific marker substances (honey-foreign oligosaccharides (• DP 4) which come of the enzymatic starch degradation) honey indicating the presence of sugar syrups in honey:
  - C-MS,
  - LC-MS
  - LC-ELSD
    - Syrup: ± 3 % ? (0-10 %)
    - LOD: 0,1 %
Method used by JRC

- **Advanced method** capable of detecting adulterations of honey with sugar syrups that mimic sugar profiles of authentic honey.

- Method used is a combination of:
  - **EA-IRMS:** Elemental Analysis-Isotope Ratio Mass Spectrometry &;
  - **LC-IRMS:** Liquid-Chromatography coupled to Isotope Ratio Mass Spectrometry.
The residues analyzes

- Adulteration with $C_4$ and $C_3$ sugars can be detected by applying EA/LC-IRMS. The sensitivity of the method is:
  - 1% for detecting adulteration with $C_4$ sugars (7% with EA-IRMS)
  - 10% for detecting adulteration with $C_3$ sugars

- To date, no universal method exists that is able to determine all the different types of honey adulterants with sufficient sensitivity and robustness. As a consequence, several complementary methods have to be applied in order to perform a reliable assessment of honey authenticity.
The biological explanation
The biological explanation

- Very few publications give information about the exchanges between reserve in brood chamber and supers.
- Dissemination of the syrup is very rapid (radioactive substances):
  - all the bees are touch in 24h (Courtois at al 1958)
  - 43 - 60% of the bees in 27h and
  - foragers became radioactive faster and at a higher rate (76%) than the rest of the colony (Nixon, Ribbands 1952)
The biological explanation

Studies about antibiotic treatments give us some more detailed information.
The biological explanation

- Studies about antibiotic treatments give us some information about the contamination between hives (distance between 20 and 35 m):
  - between 0.41 and 1.31 mg.kg⁻¹ (mean of the 5 hives = 0.45 mg/kg)
  - 4 to 11.5 % of the TC in treated hives!
How can we avoid this problem?

It’s all together that we can find a solution and reduce this problem.
I am sure that most of you try to do their best to produce very high quality of bee products.
How can we avoid this problem?

- We have to be very careful with the feeding:
  - Quality
    - Honey is the best food for the bees
    - Sucrose inverted by the bees stay a good solution
    - Avoid all the food coming from starch
    - Avoid HFCS...
    - You must know the composition of the syrup or the patty: the C•4 must be near 0
How can we avoid this problem?

- **Good beekeeping practices**
  - Keep limited reserve (2 frames) in the colony before the installation of the supers
  - Avoid all feeding in presence of supers during potential honey flow
  - Use only good syrup (see before)
  - Be careful with little colony (fecundation nucs…) fed with sugar
  - Avoid the situation of robbing

- **At a legal point of view**
  - We have to fix maximum residue limit of foreign sugars in the honey

- **Applied research**
  - We have to analyze the movement of sugars in the hive and to have a better view of the contamination of honey by the
Thank you for your attention
Questions?

www.cari.be
Butine.info