www.beecome2017.it

Beehive nutrition and honey contamination

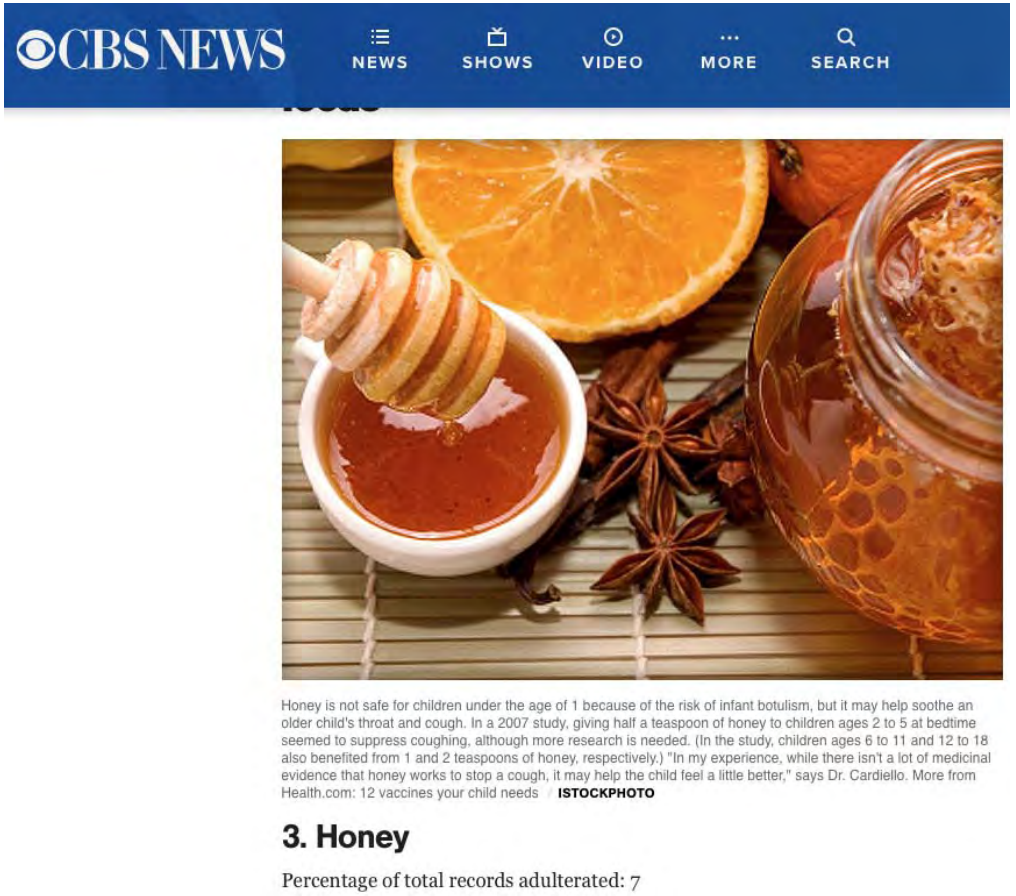
Etienne Bruneau

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



European Commission

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.

Health and Food Safety

Context of adulteration

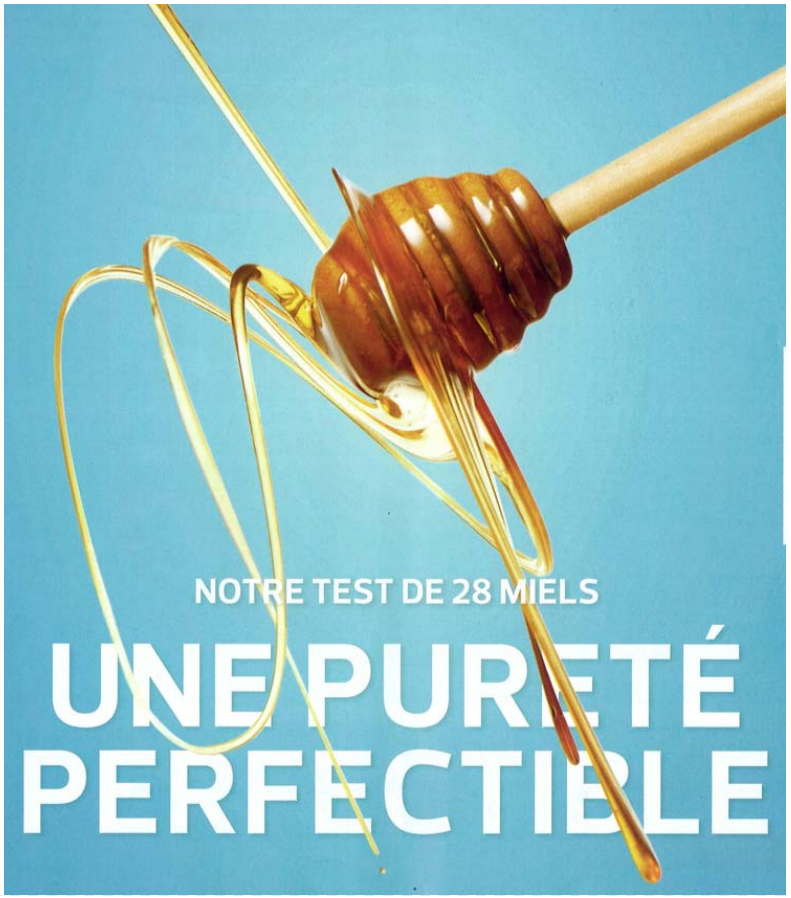
- Results of the JRC on 893 honeys

Origin	Samples (n)	Suspicion of non-compliance (n)	(%)
Blend of EU honeys	96	19	19.8
Blend of EU and non-EU honeys	426	40	9.4
Blend of non-EU honeys	30	3	10.0
Single EU Member State	275	53	19.3
Single non-EU country	55	11	20.0
Unknown	*11*	*1*	*9.1*
TOTAL	893	127	14.2

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



Context of adulteration

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

Parameter	Result	Unit	Method
Foreign oligosaccharides	0.20	%	LC-ELSD (a)

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.
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Interpretation:

Honey-foreign o
Therefore, the s
20/12/2001).
The presence of

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

Parameter	Result	Unit	Method
Foreign oligosaccharides	0.13	%	LC-ELSD (a)

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.
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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.

Context of adulteration

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)

Parameter	Result	Unit	Method
beta/gamma amylase activity	5.1	units/kg	PM DE01_115 (a) [1]

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)

Parameter	Result	Unit	Method
ß-fructofuranosidase activity	n.d.	units/kg	PM DE01_102 (a) [1]

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

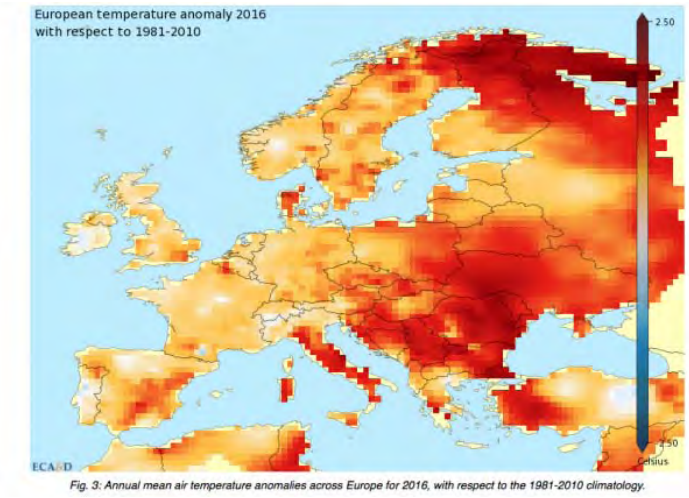


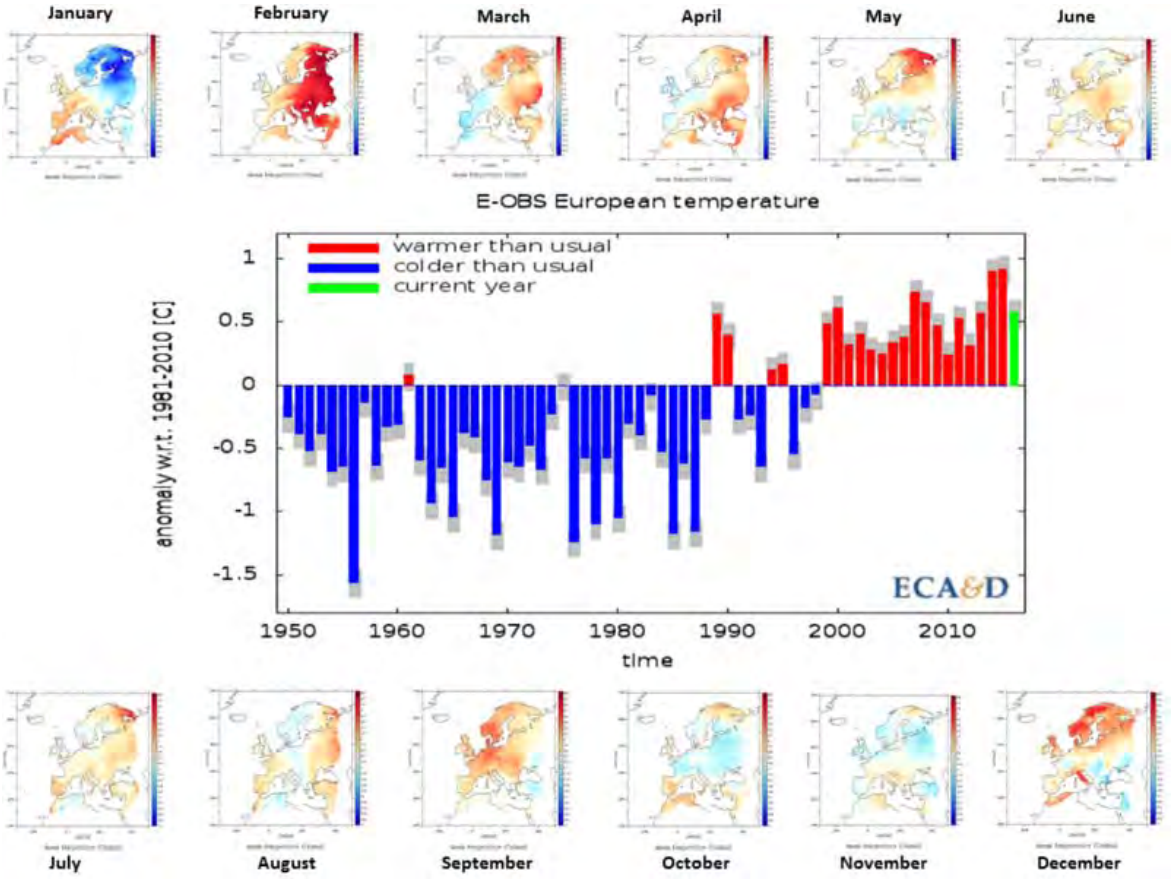
Fig. 3: Annual mean air temperature anomalies across Europe for 2016, with respect to the 1981-2010 climatology.

Annual mean air temperature anomalies across Europe for 2016, with respect to the 1981-2010 climatology

http://cib.knmi.nl/mediawiki/index.php/European_climate_in_2016

The syrup and nutrition of beehives

- Climate change: annual mean air temperature anomaly



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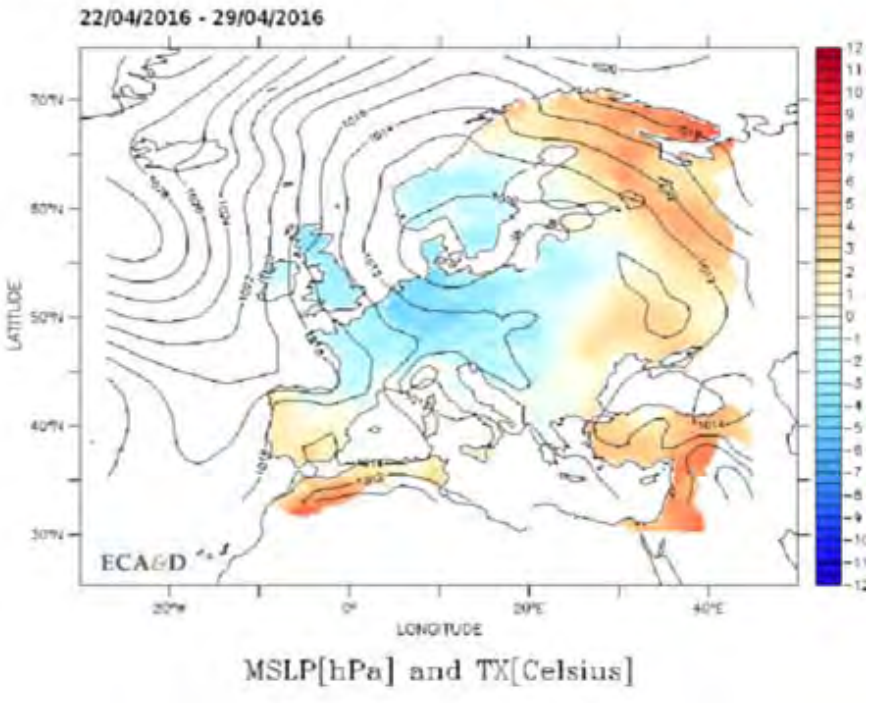
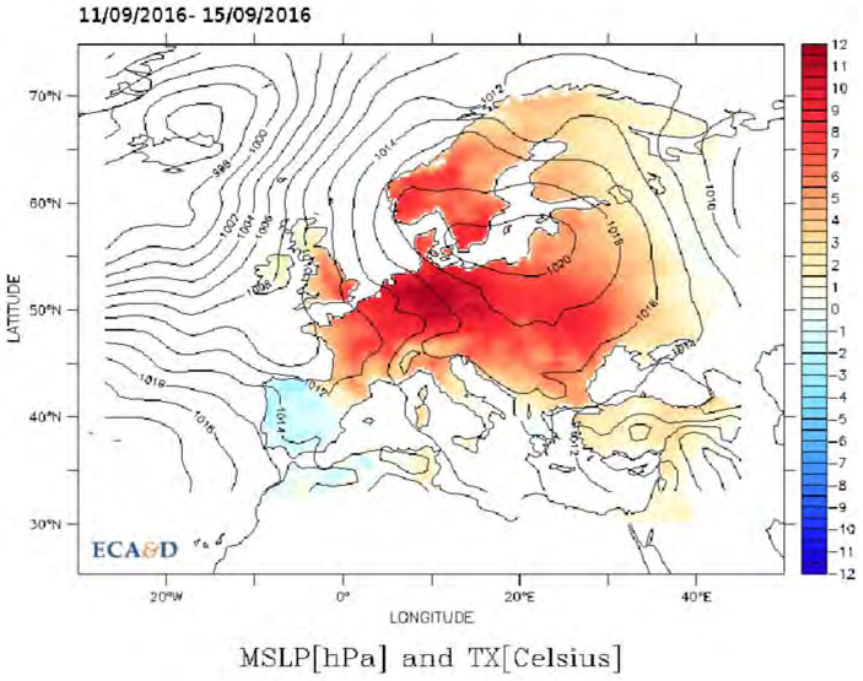
The syrup and nutrition of beehives

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





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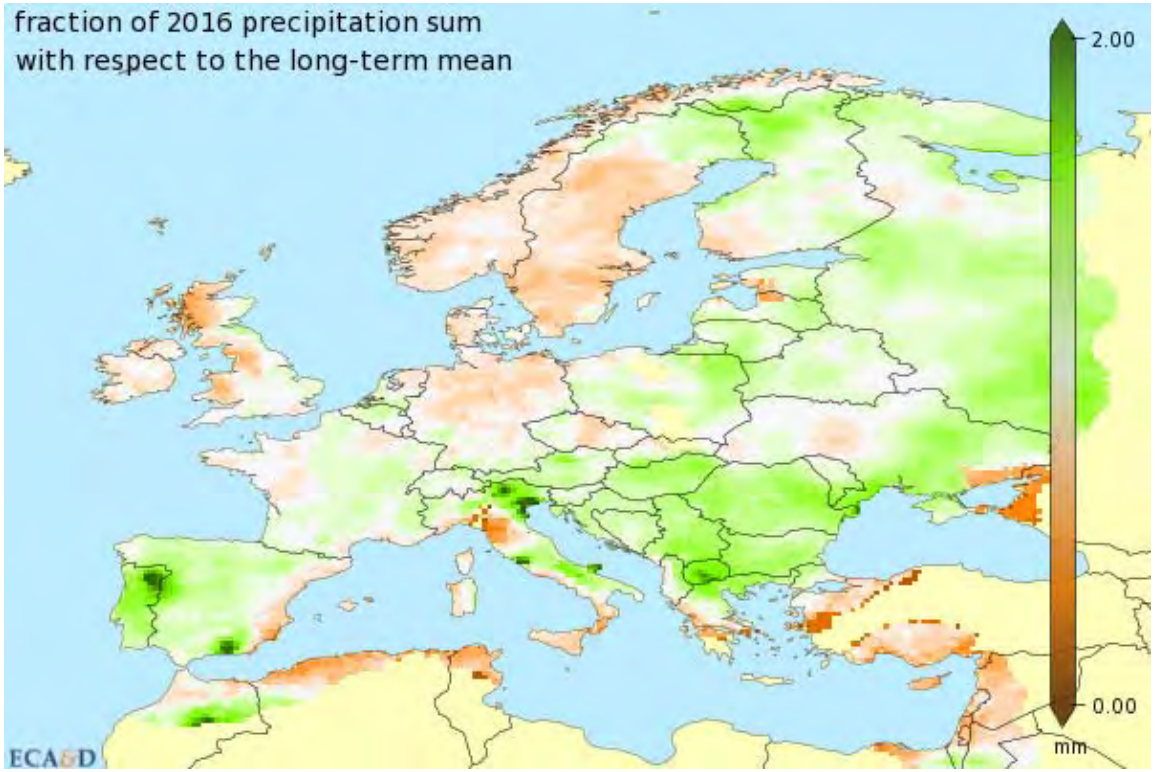
The syrup and nutrition of beehives

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



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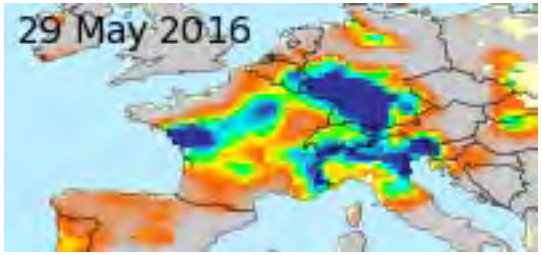
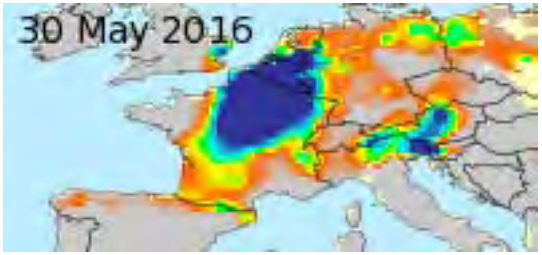
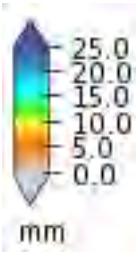
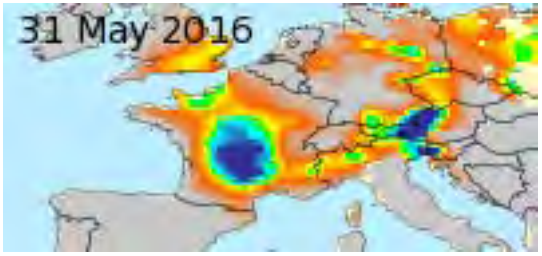
The syrup and nutrition of beehives

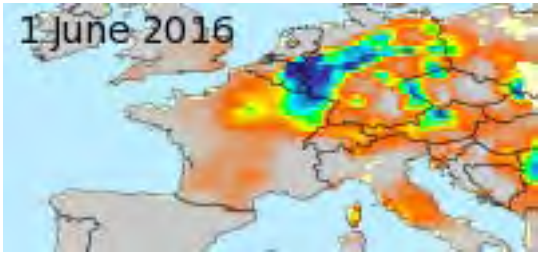
- Climate change: *daily precipitation amounts in the period 29 May - 1 June 2016*

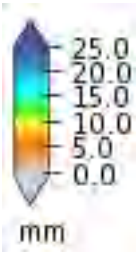












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The syrup and nutrition of beehives

- 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

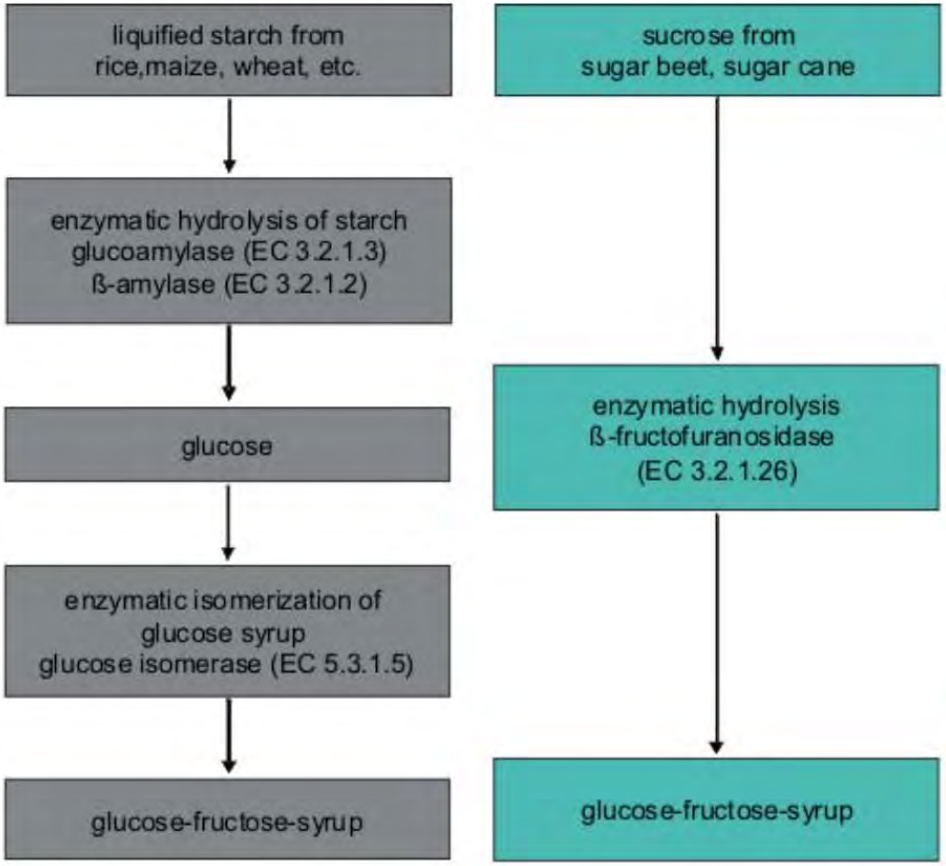
The adulteration residues analyzes

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: a, ß, y amylase - photometrisch
- Foreign oligosacacharides - 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
  - ß-fructofuranosidase activity
    - Syrup: >1000U/kg
    - Detection: 20 U/kg
    - Honey: 20U/kg
  - ß/gluco amylase activity
    - Syrup : 200 U/kg
    - Detection 1U/kg
    - Honey : < 5 U/kg

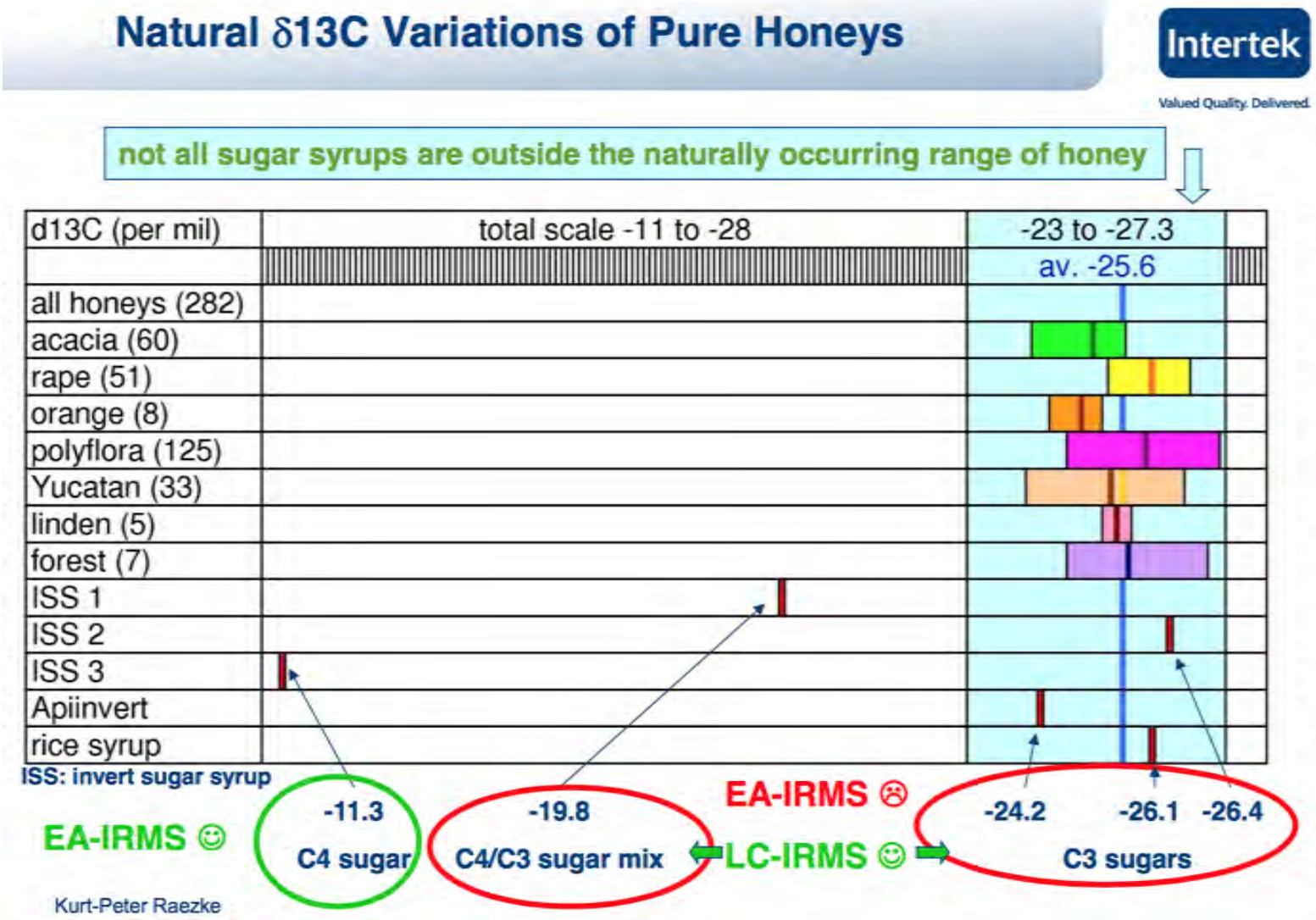


http://www.pcelinjak.hr/OLD/images/stories/test2/aaa/publication_foreign_enzymes_05.02.10.pdf

The residues analyzes

- Cane, corn, millet syrup

Limit of detection > 7%



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 %

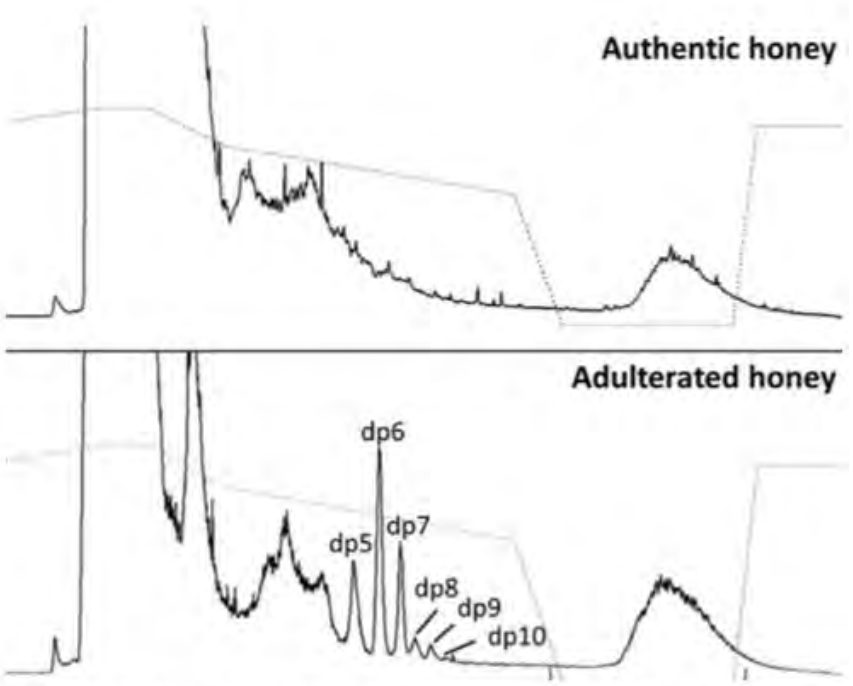


Fig. 3: LC-ELSD chromatogram of authentic and adulterated honey. The adulterated sample shows honey-foreign oligosaccharides (DP 5 to DP 10) which serve as marker substances to detect adulteration.

www.intertek.com/food

European Commission

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.

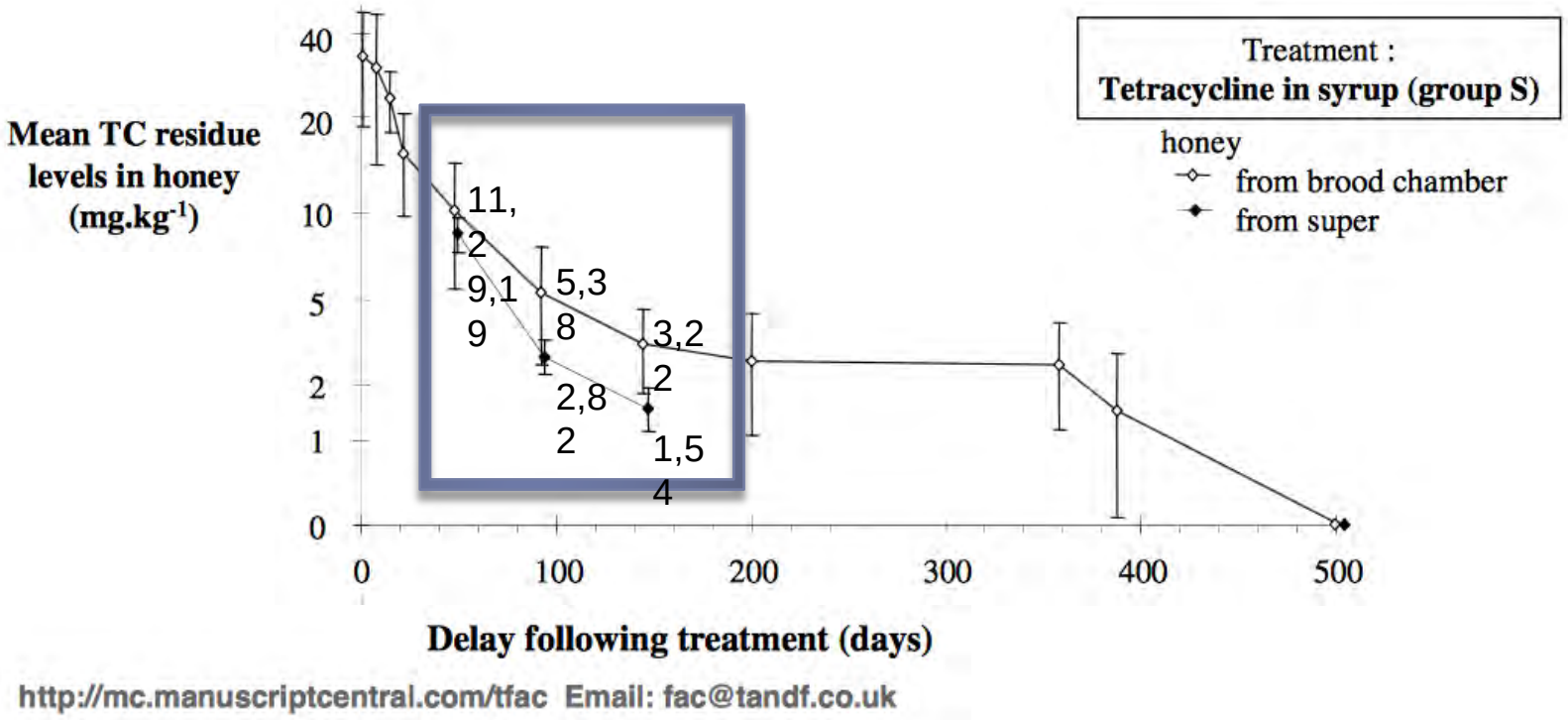
The biological explanation

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)

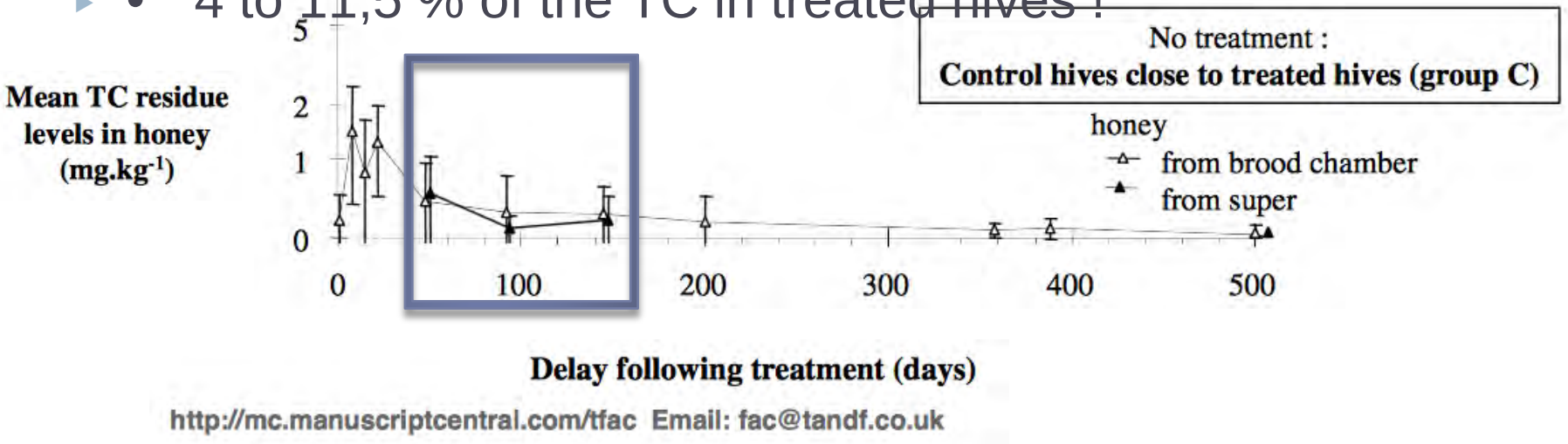
The biological explanation

- 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

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