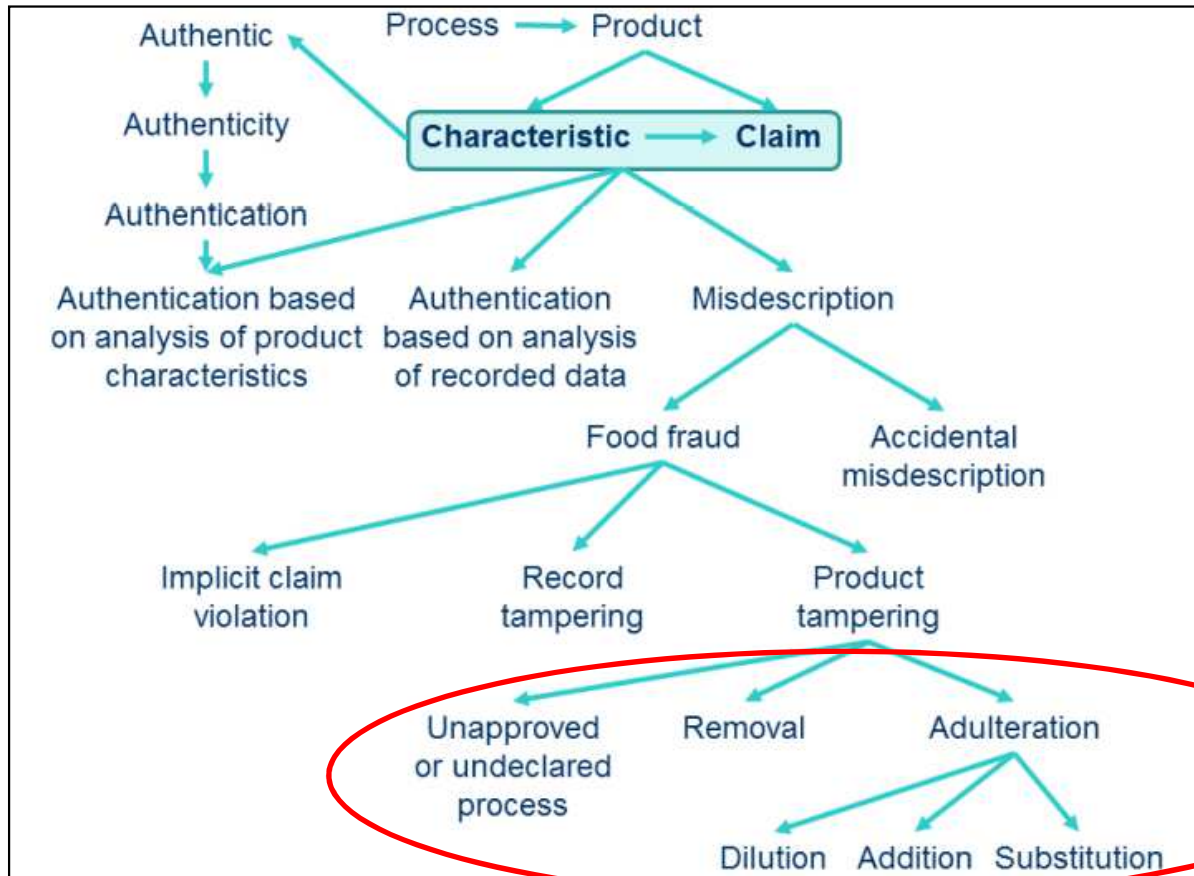


Authenticity of Honey

Current Issues and Update on New Analytical Trends



Food Fraud Definition



CEN CWA 17369:2019
(CEN Workshop 86)

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EU FOOD LAW

Article 8 of Regulation (EC) No. 178/2002 (the “General EU Food Law”)

Prevention of:

1. Fraudulent and deceptive practices
2. Adulteration of food
3. Any other practices that may mislead the consumer

EU Honey Directive 2001/110/EC, Article 1/Annex II

When placed on the market as honey or used in any product intended for human consumption, **honey shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey.** Honey must, as far as possible, be free from organic or inorganic matters foreign to its composition.

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Current Issues and Update on New Analytical Trends



Intentional (FRAUD):

- Mislabeling of geographical and/or botanical origin
- Deliberate adulteration with sugar syrup
- Excessive sugar feeding
- Technical processing (*water reduction, “resin technology” to remove drug and pesticide residues, contaminants and HMF, artificial improvement of desired properties e.g. improvement of Manuka honey antibacterial activities*)
- Honey blends produced by man are not labeled as such (“Blend of...”)

Untinentional/accidental:

- Bees forage on unnatural sugar sources (*e.g. sugar sap honey*)
- Contamination with improper bee feeding products due to bad beekeeping practice (sugar, protein, yeast, flour, starch) causing failure in in honey authenticity assessment

Authenticity of Honey

Current Issues and Update on New Analytical Trends



EU Commission: Coordinated Control Plan for Honey (2015-2017)

Final Report:

https://ec.europa.eu/food/sites/food/files/safety/docs/oc_control-progs_honey_jrc-tech-report_2016.pdf

- 2264 honeys sampled by authorities in the EU member states
- 14% found to be non-complying with LC-IRMS benchmark purity criteria

EU Commission: JRC Round Table Honey Adulteration (January 2018)

Meeting Report:

https://ec.europa.eu/jrc/sites/jrcsh/files/ares181569074-1_technical_round_table_on_honey_adulteration_report.pdf

Discussed authenticity methods (JRC):

- **¹³C EA/LC-IRMS:** applied by the JRC for the Coordinated Control Program)
- **LC-HRMS:** evaluated by the JRC (no official use)
- **¹H NMR:** applied by some member states for evaluation (no official use)

Authenticity of Honey

Current Issues and Update on New Analytical Trends



Stable Isotope Analysis (^{13}C EA/LC-IRMS)

- Developed and validated in 2004-7 (new interface available for hyphenation of liquid chromatography and stable isotope mass spectrometry)
- Established since 2007 in routine analysis, ISO 17025 accreditation since 2008
- 2015-17: used in EU Commission monitoring program to check honey products for adulteration on the European market (~2300 samples, ~14% non-compliance)
- Subject to standardization (harmonized method) at EU level for use in official controls
- Good detection of common adulterations with different types of foreign sugars (C4 and C3 plant source)
- Difficulty in detection of very sophisticated adulterations done with high technical effort (purified and honey-tailored syrups e.g. from China)
- LOD C4 sugars: 3-5 % (depending on honey type and adulterant)
- LOD C3 sugars: 10 -30 % (depending on honey type and adulterant)

Authenticity of Honey

Current Issues and Update on New Analytical Trends



¹H NMR Profiling

- R&D since the late 1990s, introduced for routine analysis (honey profiling) in 2013
- Non-target approach: compares NMR spectra with reference database of authentic honeys.
- No harmonized and publicly available reference database yet (under discussion at the EU Joint Research Centre)
- Comparability: Each lab needs to use the same analytical procedure, instrumentation and reference database to achieve comparable results
- “Living” databases, new releases can cause change in interpretation
- Status quo: >98 % agreement between the main commercial databases (Bruker, Eurofins), regular database updates necessary
- Not the most sensitive method for adulteration detection (LOD >15 %, but well suitable as a general screening tool for adulteration, type/origin verification and detection of illegal processing (resin technology)
- Confirmation of non-compliant results necessary by complementary methods
- Recent findings in 2018: certain custom-tailored Chinese syrups may still pass at 30-40% levels despite the database update!

Authenticity of Honey

Current Issues and Update on New Analytical Trends



LC-HRMS screening (new in 2018, first ISO 17025 accreditation 10/2018)

- Newest analytical tool with first affordable (400k) and sensitive systems.
- Combined non-target / target approach (screening and confirmation)
- Works similar to NMR, but does not require extensive databases of authentic honeys to be used for authenticity testing (exact mass determination, publicly available databases)
- Non-target: screening of adulterants, identification of markers, monitoring for “unknown or unusual” compounds in known adulterated honeys (simultaneous screening of 30k+ compounds by LC separations)
- Multi-target screening to check for identified/known adulterants (Eurofins: 434 markers to date)
- Allows to identify yet unknown and known adulterants (syrups) due to the use of both universal syrup markers and syrup-specific markers
- Very sensitive (ppb vs. ppm level of NMR)! Threshold LOD for routine application: 5%
- Allows to replace existing single-marker methods (multi-method covering universal and specific sugar syrup markers)
- Database updates: retrospective re-evaluations of already measured samples possible

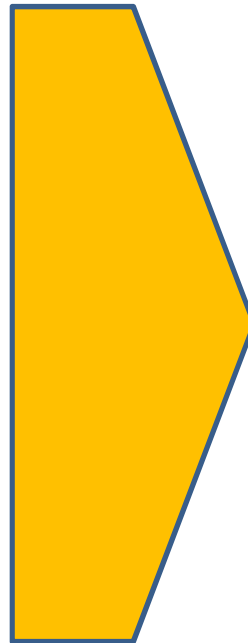
Authenticity of Honey

Current Issues and Update on New Analytical Trends



Past - Present

1. ^{13}C EA/LC-IRMS
2. ^1H NMR profiling
3. Foreign Oligosaccharides
4. Beta-fructofuranosidase
5. beta/gamma-amylases
6. FAmP
7. Mannose/Psicose
8. SM-R
9. TM-R
10. SM-B
11. E150d



Present - Future

- ^{13}C EA/LC-IRMS
- LC-HRMS (including 3,7,8,9,10,11)
- ^1H NMR profiling

40% cost reduction

Higher certainty in result assessment

Foreign enzymes, pollen analysis / sensory

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Current Issues and Update on New Analytical Trends



Combination of four methods for the state-of-the-art approach:

1. ¹³C EA/LC-IRMS: Foreign Sugar Adulteration
2. LC-HRMS: Foreign Sugar Adulteration
3. Pollen / Sensory: Variety / Origin
4. ¹H NMR Profiling: Physicochemical, Variety /Origin (Verification Only), Illegal Processing

<u>Method</u> <u>Capabilities:</u>	¹³ C EA/LC-IRMS	¹ H NMR Profiling	LC-HRMS	Pollen / Sensory
C4 Sugars	😊	😐	😊	😞
C3 Sugars	😐	😐	😊	😞
Tailor-made Syrups	😞	😞	😊	😞
Illegal Processing	😞	😊	R&D	😐
Moisture Reduction	😞	😐	R&D	😐
Type/Origin	😞	😐	R&D	😊

Authenticity of Honey

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Many thanks for your attention!



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