Honey Authenticity – A Challenge for Quality Control

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Content

- Definitions Authenticity and Adulteration = Fraud
- Legal basis for honey authenticity judgement
- Definition Authentic Honey and Adulterated Honey
- Foreign sugars of interest (enzymatic production)
- Analytical methods for honey authenticity testing
- Standardization of Methods - Comparability of Results
- Quantification of foreign sugars in honey
- Challenge for Quality Control: Feeding or Fraud?
- Challenges for the honey trade
- QSI Recommendation German authorities / Eco Control Bodies
- Outlook

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Definitions Authenticity and Adulteration

Authenticity

“Food authenticity is about ensuring that food offered for sale is of the nature, substance, and quality expected by the purchaser”

Adulteration = Fraud

“Deliberate and intentional substitution, addition, tampering or misrepresentation of food, food ingredients or food packaging: of false or misleading statements made about a product, for economic gain.”

Source: https://www.foodauthenticity.global/definitions

Legal basis for honey authenticity judgement

EU Honey Directive 2001/110/EC

(based on Codex Alimentarius)

„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.**

… it must not have any foreign tastes or odours, have begun to ferment, have an artificially changed acidity or have been heated in such a way that the natural enzymes have been either destroyed or significantly inactivated.”

Definition Authentic Honey

Natural, ripe and mature honey which complies with all the provisions of the EU Honey Directive 2001/110/EC

(nature, composition, production, processing, filling, labeling etc.)

Comments:

- Honey can be blended only with honey (labeling must be in accordance)
- Harvesting immature, unripe honey and selling as honey after moisture reduction (vacuum drying by human intervention) is subject to discussions

  Problem: moisture reduction is difficult to prove by analytical means in a moisture reduced honey, current analytics are not feasible for law enforcement, only indicator is low proline, which is also the case for some natural enzyme weak honeys)

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Definition Adulterated Honey

„Adulteration" is strong wording and implies a criminal act, must be carefully used

Examples for honey adulteration include:

- Active addition of foreign sugars to honey for economic reasons
- Sugar feeding of bees during the harvest to optimize yields
- Artificial ion exchange resin treatment
  to remove undesirable substances from honey (pesticides/antibiotics residues, ethanol/other fermentation products, HMF, other chemicals, foreign odour, foreign taste or to change the colour) and to finally pretend a better quality
- Intentional manipulation of the composition, for example by artificial addition of pollen, enzymes (diastase, saccharase), to mask adulteration or heat damage (processing) or addition of other substances for economic reasons
- Intentional removal of pollen or mixing with filtered honey without proper labeling to mask the origin of a honey, with the consequence that the honey origin cannot be classified by microscopy
- Intentional mislabeling of the botanical or geographical origin for economic reasons

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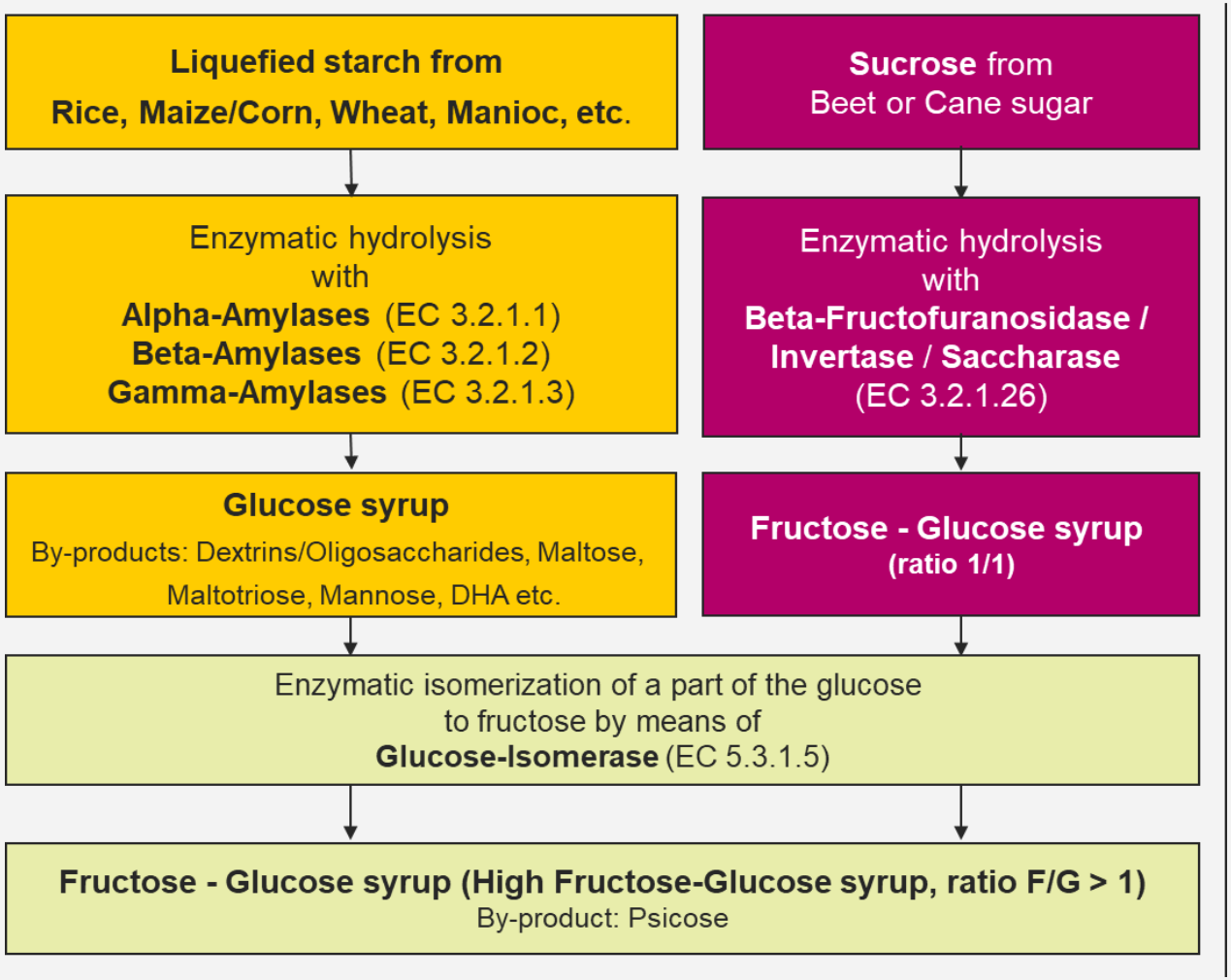
Foreign sugars of interest (enzymatic production)



Bee enzymes

Diastase – alpha-Amylase (EC 3.2.1.1)

➡ Starch depletion

Saccharase / Invertase (EC 3.2.1.20)

➡ Sucrose depletion

Glucose Oxidase (EC 1.1.3.4)

➡ Glucose depletion, H_2O_2, gluconic acid built (prevents honey from fermentation, „peroxide activity“)

further enzymes (Katalase, acidic Phosphatase)

Analytical methods for honey authenticity testing (in order of appearance)

- Fellenberg test (1911), Fiehe´s test (1936), (foreign dextrins, inverted sugar)
- Pollen Analysis (1978, Louveaux et al., Melissopalynology)
- ^{13}C-EA-**IRMS** (C4 sugars; AOAC method 998.12, SCIRA) (1998; White et al.)
- ^{13}C-EA-**LC-IRMS** (C4/C3 sugars) (2008)
- Beta-Fructofuranosidase (foreign enzyme) (2008)
- Beta-gamma-Amylases (2008)
- Honey-foreign Oligosaccharides (from starch-based syrups) (ca. 2008)
- Thermostable alpha-Amylases (2009)
- Caramel color E150d (2010)
- SM-R (Specific Marker Rice) (2013)
- TM-R (Trace Marker Rice, Arsenic) (2013)
- SM-B (Specific Marker Beet /starch-based syrups from plant roots (manioc)) (2013)
- Famyp (Foreign alpha-amylase profiling) (2015)
- ^{1}H-NMR Bruker Honey Profilingv (Nuclear Magnetic Resonance Spectometry,) (2015)
- HRMS (High Resolution Mass Spectrometry) (after 2015)
- Psicose (ca. 2018)

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Standardization of Methods - Comparability of Results I

- **Pollen analysis/sensory**
  comparable, standardized, but final judgement of results depends on literature and expert opinion, especially for sensory: subjective (unifloral honeys, botanical and geographical origin), validated in independent proficiency testing schemes
- **13C-IRMS (C4-sugars)**
  standardized and harmonized, also judgement criteria, only „official method“ (worldwide), validated in independent proficiency testing schemes,
  QSI positive rate = foreign sugars detected (2021): 1.6%
- **13C-LC-IRMS (C4/C3 sugars)**
  method standardized (also CEN/TC/WG6, EU JRC), judgement +- comparable, but slightly different decision criteria used by different labs, partly exceptions (e.g. fresh Acacia, Lavender, Sorghum), validated in independent proficiency testing schemes,
  QSI positive rate: 4.2% (2020), 12.6% (2021, increase due to natural Sorghum honey)
- **1H-NMR Bruker Honey Profiling ™**
  method and Bruker database evaluation standardized, highly comparable results in different labs, but NMR expert opinion (subjective) required in certain cases, thus differences between labs in certain cases possible, validated in independent proficiency testing schemes, Bruker database is continuously improved and expanded, problems are critically and regularly discussed within Bruker consortium (Bruker, QSI, AB Labo) since >7 years, but also with other labs (Intertek) regularly

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Standardization of Methods- Comparability of Results II

- **NMR Eurofins (France): not yet involved in Bruker database and discussions**
  no information available about size of database and authenticity evaluation, comparable?
  Sensitive enough? Would need to be compared in ring trials, not yet done, FEEDM?
  Published results of Eurofins Ritterhude, Germany (Apimondia Istanbul 2022):
  Eurofins NMR (France)positive rate : 2% (2020) and 4% (2021) all lab samples tested,
  QSI NMR Bruker positive rate: 17.3% (2020) and 17.4% (2021) + 2% suspicious in both years
- **HRMS**
  different methods, instruments, HRMS-databases, not standardized and harmonized,
  not yet validated in independent proficiency testing schemes, so far just small ring trials between certain labs and 2 recent ring trials in the FEEDM (5 labs)
  QSI positive rates: 19.1% (2020), 13.3% (2021)
  for comparison Eurofins Ritterhude positive rates: 15% (2020), 11% (2021)
- **Oligosaccharides (from starch-based syrups)**
  different methods, instruments, not standardized and harmonized, but +- comparable between labs offering the tests, not validated in independent proficiency testing schemes
  QSI positive rates: 13.6 % (2021), for comparison Eurofins Ritterhude: 17% (2021)
- **Other specific enzyme and marker methods**
  mostly inhouse methods, mostly not published, not standardized and harmonized,
  but +- comparable between labs offering the tests, in parts recently evaluated in independent proficiency testing scheme (DRRR, beet syrup, rice syrup)

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Quantification of foreign sugars in honey, possible?

- Quantification only possible for C4 sugars (maize/corn, cane) determined by 13C-IRMS (harmonized AOAC method 998.12): **> 7.00% = „adulterated“**

  Comment: calculation assumes a certain isotopic value for C4 sugar, quantification also unsharp, as isotopic value of the pure C4 syrup present in a honey sample is not known

- **For all other methods: no quantification of level of foreign sugars in honey possible**, meaning it depends on the method, the syrup and the honey, how sensitive foreign sugars are detected
- Quantification of foreign sugars in honey would be only possible for some methods if a particular syrup used for feeding / adulteration is known to the lab as reference syrup (syrups contain different marker levels and are differently produced)

Challenge for Quality Control: Feeding or Fraud?

- In the lab, foreign sugars from remaining bee feeding cannot be differentiated from intentionally added foreign sugars
  (directly added / fed „through the bee“ during the nectar flow to improve yield)

- Foreign sugars and certain markers or enzymes used for authenticity testing can be possibly also naturally introduced into honey (for example by honeydew insects, yeasts, rotten fruits, certain plants) or unintentionally via inadequate feeding (yeasts, proteins, feeding supplements like SuperDFM® for example)

- The decision limits for analytical tests are difficult to harmonize between the labs and there is no legal limit for a level of foreign sugars to be tolerated (typically 5%, but again: quantification is not possible!)

- Positive test results must be critically reviewed in the context of all test results as already small feeding remains might cause a failing of a specific test (technically unavoidable, starting from about 1% foreign sugar)

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Challenges for the honey trade I

- How many and which tests are required and accepted by the trade partners to adequately demonstrate the quality and authenticity of a honey batch?
- Does a purchase specification of a buyer contain sufficient and transparent requirements for authenticity tests? Are specific methods and limits defined?
- Does a seller (beekeeper/collector) accept the rejection of a product by the buyer if certain authenticity tests fail?
- What if one or more analyses indicate foreign sugars, even if the honey is authentic? Could inadequate feeding be the reason, or a false-positive result?
- Which level of foreign sugars is accepted by a buyer, 0%, 1%, 5%, 7%, 20%?
- What about contradictory results from different labs, which results are accepted (different techniques or databases, analytical measurement uncertainty)?
- What if not accredited methods were used or methods that are not fit-for-purpose or not validated in independent ring trials / proficiency testing?
- What about different results for pre-shipment samples vs. after shipment?
- Was the testing performed in homogenized goods or raw honey and were the tested lab samples representative for a honey batch?

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Challenges for the honey trade II

- Quality control is finally always a compromise between cost efficiency and safety/risk and directly impacts the profit margin of both buyer and seller
- A beekeeper knows how the honey he sells was produced, if feeding was necessary or when the bees were fed and which bee feeding was used, but what about unintentional contamination with foreign sugars or foreign enzymes?
- Highest profit margin for all trade partners can be achieved with highest quality products: the better the quality, the lower the testing effort required
- A thorough risk-based quality control is necessary not only to comply with trade specifications but also to meet legal requirements for all FBO (required due diligence under Regulation 178/2002/EC, traceability)
- Further pressure on all FBO is caused by consumer organizations (Stiftung Warentest, Öko-Test, Test Achat etc.)

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QSI Recommendation

German Authorities / Eco Control Bodies 2021

Test Methods Triple Screening Authenticity	Test Results Triple Screening *	Examples borderline results	Maßnahme / Measure
NMR Honey Profiling (Bruker)	**0 results positive** or **max. 1 result borderline positive/ suspicious** (based on expert opinion of lab)	**NMR** suspicious **13C-LC-IRMS** borderline positive: C4 sugars indicated, but difference max. close to limit (could be positive starting from 1% to 3% C4 sugar already) **HRMS** borderline positive, for example SM-B slightly above threshold of 5%	**Kein Handlungsbedarf/ No need for action**
13C-LC-IRMS (C3/C4 sugars)	**max. 1 result positive** or **max. 2 results borderline positive/ suspicious** (based on expert opinion of lab)	see examples above	**Weitere Untersuchungen erforderlich / Erneute Probenahme empfohlen** **Further investigations required / New sampling recommended**
LC-HRMS / LC-MS/MS	**min. 2 results positive** or **all 3 results borderline positive/ suspicious** (based on expert opinion of lab)	see examples above	**Verdacht einer Verfälschung / unsachgemäße Fütterung während der Tracht, Handlungsbedarf** **Suspicion of adulteration / improper feeding during the harvest, need for action**

* Analyse und Bewertung nach derzeitigem Kenntnisstand / Analysis and evaluation according to current knowledge

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Outlook

- Recommended by QSI for authenticity testing is a screening including a pollen analysis/sensory, NMR Bruker Honey profiling, 13C-LC-IRMS, HRMS and optionally other suitable test (risk-oriented), to get a good picture of a honey
- Analytical methods require further harmonization and evaluation in independent proficiency tests to assure that the methods are „fit-for-purpose“ and accepted by trade partners for quality control
  (harmonized is only the IRMS C4 sugar method, more or less harmonized are 13C-LC-IRMS and NMR Profiling (Bruker database with > 28,000 references) and least harmonized is HRMS, the most recent screening technique
- The rate of false positives must be further reduced including special honeys (like Manuka, Lavender, Bracatinga, Avocado etc.) and the sensitivity of the methods to detect foreign sugars must be improved as well (continuous, databases)
- Harmonization will take many years (example 13C-LCIRMS: ca. 10 years)

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Thank you for your attention!

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