FAQS ON HONEY TESTING METHODS FOR DETECTING ADULTERATION WITH SUGAR SYRUPS

WHAT TESTING METHODS ARE USED TO DETECT HONEY ADULTERATION?

Ensuring honey authenticity is one of the great challenges facing the honey industry today. Over the past half century, a number of honey testing methods have been developed to detect food fraud. To date, there is no single universal analytical method available which is capable of detecting all types of adulteration with adequate sensitivity. A variety of methods are used to detect honey adulteration, each test has strengths and weaknesses; when used in combination these tests offer the most reliable predictor of honey's purity from sugar syrup adulteration .

1. **[13]C Stable Carbon Isotope Ratio Method (SCIRA) or EA/IRMS.**
   Limit of C4 Syrup Detection 7%
   Originally developed in the 1970's and the only AOAC Official method – 998.12 C4 Plant Sugars in Honey. This method can detect only corn or sugar cane derived syrups (C4 sugars), it cannot detect syrups made from beets, rice, wheat or other plants (C3 sugars).

2. **[13]C Stable Carbon Isotope Ratio Method paired with a Liquid Chromatograph or EA/LC-IRMS.**
   Limit of Syrup Detection 3-5% C4, 10-30%* C3
   Developed in 2008, this method is also good for directly determining adulteration with C4 sugar syrups; but can also indirectly detect adulteration with C3 sugar syrups, though with less sensitivity than for C4. *(some custom C3 syrups are undetectable by this method).

3. **Nuclear Magnetic Resonance Profiling or NMR.**
   Limit of Syrup Detection 10-40%
   This is a rapid screening method designed to test for both known and unknown syrups, as well as to detect prohibited processing methods and other potential manipulations of pure honey. NMR compares each sample against a database of curated pure honey samples using an array of 53 parameters to detect sugar syrups and a quantitative analysis of 36 compounds. This technique is not as sensitive as other methods, but can also be used to determine the country of origin and botanical source of honey.

4. **Liquid Chromatography-High Resolution Mass Spectrometry analysis or HRMS.**
   Limit of Syrup Detection as low as 5%
   Similar to NMR, this method can test for both known and unknown syrups in honey, although in the case of HRMS the focus is on the identification of specific markers typical of sugar syrups. HRMS is highly sensitive, detecting very low concentrations of telltale syrup markers where they may be present in otherwise pure honey.

WHAT ARE THE DIFFERENCES BETWEEN THESE TESTS?

13C EA/LC-IRMS (C3/C4 sugar detection)

This test was developed and validated in 2004-2007 to detect the presence of added sugars both from C4 plants (e.g. corn and cane syrup) and C3 plants (e.g. rice, wheat, beet syrup). It has been used commercially for honey adulteration analysis since 2007. The first ISO 17025 accreditation was achieved in 2008.

- This method differs from the AOAC SCIRA method in that it makes use of a liquid chromatograph to separate each major sugar and evaluate individually. This improves the sensitivity and makes possible the detection of some C3 sugars.
- In 2015-2017, this method was used in an EU Commission monitoring program to check honey products for adulteration on the European market (~2300 samples, ~14% non-compliance).
- Limit of Detection for C4 sugars: 3-5 % (depending on honey type and adulterant)
    - C4 sugars > 7% can be quantified by this method.
- Limit of Detection for C3 sugars: 10-30 % (depending on honey type and adulterant)
    - C3 sugars percentage cannot be quantified by this method and some are undetectable.
- Note: This method cannot detect the tailor-made syrups which are adapted to the honey type or a host of other illegal processing methods.

NMR Profiling

NMR is short for nuclear magnetic resonance. NMR-spectroscopy has been used since the 1970s for quantification and structural analysis and is becoming a reliable method to officially test the authenticity of food (e.g. fruit juice, wine, edible oils, and honey).

- Research and Development for the application of NMR to honey began in the late 1990s, and was introduced for routine analysis (honey profiling) in 2013.
- NMR compares the spectra of commercial honeys with a reference database of authentic honeys. At present, there are several reference databases. The most well-known are owned and maintained by Bruker and Eurofins.
- No harmonized and publicly available reference database exists for NMR at this time. (under discussion at the EU Joint Research Centre)
- New additions to existing databases can result in changes to interpretations of spectra and the determination of sample purity.
- Each NMR lab must use the same analytical procedure, instrumentation and reference database to achieve identical results (e.g. the Bruker Honey Screener). If different NMR methodologies and databases are used, cross-check benchmarking is necessary to achieve comparable result interpretations. Status quo: >98% agreement between the main commercial databases (Bruker, Eurofins).
- Most applicable for verifying the authenticity of honeys that have known, singular botanical and geographical origin; in the case of blends of different botanical and geographical origins, only the purity of honey can be checked.

- Subjective "expert interpretation" is often utilized to conclude a sample is adulterated, even when the honey is determined to be acceptable by comparison to the database alone.
- Not the most sensitive method for detection of honey dilution with syrups (LOD 10-15% in the best case and as high as 40% with tailored syrups), but well suited to general screening for adulteration, botanical/geographical origin verification and detection of illegal processing practices (e.g. resin technology).
- May give false positive results for unique honeys not yet included in the database (a false positive is the finding that a pure honey appears adulterated according to the database).
- Once measured, data for each sample is retained and can be compared against future additions to the syrup database without re-processing the original sample.

Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS)

LC-HRMS is the most recently developed method and is not yet in widespread use. There are two approaches to the application of the method, one uses a stand-alone HRMS instrument and the second uses a paired approach with HRMS to identify new markers and LC-MS/MS to process routine samples once markers are identified.

- HRMS first became viable with the development of highly sensitive and affordable instruments in 2015.
- Does not require an extensive reference database of authentic honeys for foreign sugar detection, but uses targeted screening for adulterant trace marker compounds intrinsic to sugar syrups (exact mass determination, publicly available databases).
- The method uses a screening approach to identify syrup-specific target compounds in a sample. This approach will confirm the presence of known compounds and identify any new or unusual compounds foreign to honey and suspect as indicators of sugar syrup. More than 15,000 compounds can be simultaneously screened.
- Once LC-HRMS has identified known adulterants (>400 known adulterant markers identified to date), future analysis can be done using another instrument; LC-MS/MS. Each syrup has characteristic multi-marker profiles allowing for an identification with a high confidence level. LC-MS/MS is not well suited to screening for new or unusual compounds but is very capable of targeting those first identified using HRMS.
- HRMS methods offer excellent sensitivity for adulteration markers (better than IRMS and NMR), but it is not capable of accurately quantifying the level of sugar syrup addition, nor the exact source of the syrup addition. For the method to successfully quantify the amount of added sugar it is necessary to have a sample of the syrup used to adulterate the honey which is not usually the case.
- HRMS replaces older single-marker methods such as:
  - SM-B (beet syrup)
  - SM-R/TM-R (rice syrup)
  - E150d (caramel color)
  - Oligosaccharides (syrups produced from starch)
  - Mannose/Psicose: tailor-made and refined syrups
- Once measured, data for each sample is retained and can be compared against future additions to the syrup database without re-processing the original sample.

WHAT TEST IS BEST FOR DETECTING HONEY ADULTERATION?

The current best recommendation is to apply all three methods in combination in order to leverage the inherent strengths of each.

Strengths and Weaknesses of each Testing Method

Determination Of	13C EA/ LC-IRMS	LC-HRMS	NMR-Profiling
C4 Sugar	🟩	🟩	🟨
C3 Sugar	🟨	🟩	🟨
Tailor-made syrups	🟥	🟩	🟥
Illegal (Resin) Processing	🟥	🟥	🟩
Moisture Reduction	🟥	🟥	🟨
Botanical and Geographical Origin	🟥	🟥	🟩

🟩 Very Effective in Detecting
🟨 Somewhat Effective in Detecting
🟥 Not Effective in Detecting

The National Honey Board is committed to supporting the development and refinement of all honey testing methods as we work towards the common goal of eliminating food fraud in our industry. If you have any additional questions, please email or call the National Honey Board.

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