The Complexity of Honey Fraud Detection

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Abstract: This article aims to be a useful guidance for authorities and stakeholders of the honey chain understand the complexity of detecting honey fraud, a problem that threatens not only the beekeeping industry globally, but also the food security of many countries.

Keywords: Traceability; EA-IRMS; LC-IRMS; ^{1}H-NMR; LC-HRMS

Food Fraud Characteristics

Food Fraud or Economically Motivated Adulteration (EMA) is a criminal act defined in some regional and national laws such as the European Commission Regulation (EU) 2019/1715 (European Commission Regulation, 2019) and, in the case of the U.S., by Title 21 – Food and Drugs, Chapter 9 – Federal Food, Drug and Cosmetic (FD&C) Act, Subchapter IV – Food, United States Code (U.S.C.) §342 (USCODE, 2011).

EMA can occur in all food products and is often referred to as food fraud. Food fraud is generally considered to be the intentional misrepresentation of the identity or contents of a food product or food ingredient for economic gain (Everstine et al., 2020). It is characterized by the following facts: (i) unlawful action (ii) intentionality (iii) purpose of economic gain, and (iv) consumer´s disappointment (European Commission Regulation, 2019).

It is also worth emphasizing that it is the very nature of fraud that the illegal action should remain unrecognized, which includes a constant innovation of the fraud practices.

Food fraud risk-mitigation has emerged as a necessary action for food producing companies during the past decade and has become a core element of food safety audit programs. In 2020, The United States Pharmacopeia (2020) developed its Food Fraud Mitigation Guidance to assist the food industry in assessing their vulnerability to fraud and developing mitigation plans. Recently, the United States Pharmacopeia Honey Quality and Purity Expert Panel has been preparing a Honey Fraud Mitigation Guidance (Food Chemicals Forum, n.d.) to specifically address the problem in honey.

The Composition of Honey

Honey has a highly complex and variable composition derived from the source of nectar and/or honeydew that the bees collects, the geographic region where the hives are located, the climate conditions of the year of production, and also the beekeeping practices. This naturally variable composition of honey makes characterization and authenticity testing particularly complex.

Although all honeys have a quite similar composition regarding their main components (e.g., 95% sugars of the dry weight), they show quite big differences in the content of minor substances (Figure 1). In fact, those hundreds of molecules in low concentration give each honey its most interesting characteristics like color, flavor, aroma, and antioxidant, antimicrobial, anti-inflammatory properties.

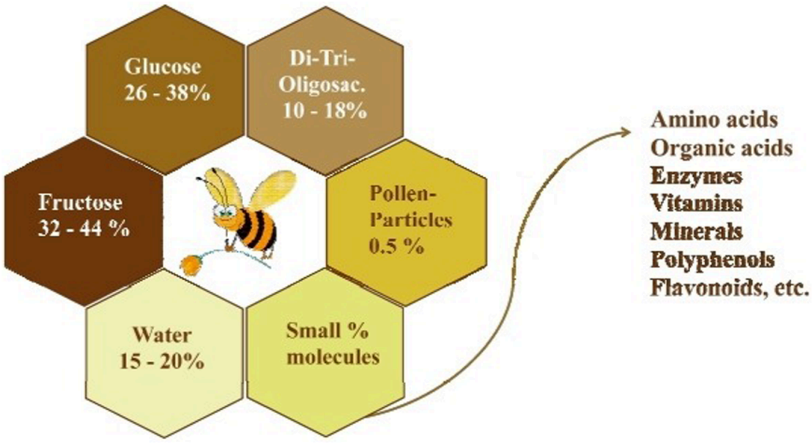

Figure 1. Average composition of honeys.

An adequate knowledge of the typical characteristics of a particular honey (color, moisture, conductivity, pH, free acidity, H.M.F., sugars profile and F/G, microscopic and sensorial analysis, enzymes activity, proline, flavonoids, polyphenols profiles, etc.) should be a prerequisite when testing for its authenticity in order to avoid a false interpretation of results. It should also be noted that hundreds of molecules in low concentration may also interfere when looking for markers of adulteration.

Honey Fraud

Although it is historically well documented that honey has long been subject to fraud, the conditions for honey fraud have become particularly well aligned in recent years (García, 2018; García & Schwarzinger, 2020). Some of the factors underlying the current situation include outdated honey standards, inappropriate testing, inefficient traceability and auditing systems, and collusion between sellers and buyers.

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Honey fraud schemes and modes are multiple and constantly evolve, but in all cases involve a deviation of internationally accepted standards and their limits (Dübecke & Schwarzinger, 2021). They include, but may not be limited to:

1. Dilution or total replacement of honey with sugar syrups.
2. Feeding sugar syrups to bees during a nectar flow to increase the production volume.
3. Harvesting of immature honey, which must be later dehydrated through the use of equipment such as vacuum dryers.
4. Intentional manipulation of the composition of honey, e.g., artificial addition or removal of pollen; addition of enzymes (diastase and saccharase); blending with an ultra-filtered product; intentional alteration of honey properties (color, fructose/glucose ratio, etc.); use of ion-exchange resins to lighten the color of honey or to remove unwanted residues (e.g., pesticides, heavy metals, antibiotics), etc.
5. Mislabeling the geographical and/or botanical origin of honey.

Modes of adulteration constantly vary and several modes may be present in the market at the same time requiring different responses.

A special remark should be dedicated to the dilution of honey with syrups. The existence of foreign substances in honey contradicts all internationally accepted honey standards (Codex Alimentarius, 1981; European Honey Council Directive 2001/110/EC, 2001; United States Pharmacopeia, 2021). Nowadays, there is a manifold of syrups available in the market which substantially differ in their physical and chemical properties. Furthermore, new syrups are constantly developed in response to novel analytical methods in a very dynamic learning process.

It should also be noted that the same syrups used to intentionally adulterate honey for economic purposes can also be used by beekeepers to artificially feed their bees. Both practices may render similar quality deviations, although quantitatively different.

Hence, when investigating the authenticity of a honey, it becomes essential to differentiate a natural compositional variation from:

- An intentional fraudulent practice for economic gain.
- An accidental contamination derived from a bad beekeeping practice, e.g., incorrect supplemental feeding of bees (close to or at the beginning of a nectar flow), bad harvesting or processing practices, etc.

Syrups are made from different plant sources and through different and constantly varying processes. According to the photosynthetic cycle of the plant that produced the sugar, syrups can be classified in:

- C4 Syrups: from corn and sugar cane.
- C3 Syrups: from rice, wheat, beet, and cassava.

Depending on the plant source of the syrup and the process used to obtain it (hydrolysis of starch or inversion of sucrose) different markers can remain in the syrup that can be used for fraud detection.

First Step of Honey Fraud Detection: The Use of Sample Information Tools Derived from Quality Management

Due to the complexity of honey fraud, there is no single tool nor method that can detect the multiple modes of adulteration.

During the last years, probably the greatest emphasis has been put on a constant development of increasingly complex laboratory methods trying to face a very dynamic and sophisticated adulteration industry. However, there are also available many other tools derived from quality management which should be used as a **first step** to mitigate the vulnerability of honey to fraud and as a prerequisite before analytical testing. These type of tools may include (but are not limited to) the traceability of honey, the audit strategy used to assure the integrity of the product, an assessment of the supply chain, an evaluation of the fraud history and the eventual existence of economic anomalies in the production region of the honey (see García & Schwarzinger, 2020).

In fact, every honey manufacturer or trader should have a documented multi-pronged fraud mitigation plan, which should include a vulnerability assessment, a mitigation strategy, and its implementation (Food Chemicals Forum, n.d.; United States Pharmacopeia, 2020).

When making decisions about a specific honey sample, at any level of the supply chain (be it producers, traders, exporters, importers, packers, government officers, auditors) reports on **Traceability**, **Audits,** and **Extra reports** described below should be the first step before lab testing.

In fact, some of these Sample Information Tools can be more efficient than the typical Analytical Testing Tools for the detection of some kinds of fraud (Table 1).

Traceability

The traceability of honey is critical in making the process transparent, especially in more complex chains where the authenticity of the product may become more vulnerable to fraud. The purpose of a strong traceability system should always be to strengthen the honey chain, adding value to the product through streamlining and real-time control of the events that occur in the production and commercialization of honey (Pietropaoli et al., 2020). A falsified origin of a honey may constitute a "red light" to suspect fraud. Apimondia (n.d.) recommends that honey should be traceable to the beekeeper, the source from

Table 1. Overall detection capacity of the different modes of honey fraud by Analytical Testing and Sample Information Tools derived from quality management.

Mode of adulteration	Effectiveness of detection by analytical testing tools	Effectiveness of detection by sample information tools
Dilution with Syrups	⬢⬢⬢⬢	⬢
Feeding of Bees (GBP)	⬢⬢⬢	⬢⬢
Mislabeling the Origin	⬢⬢⬢	⬢⬢
Intentional Manipulation and Un-allowed Processing	⬢⬢	⬢⬢⬢
Harvesting Immature Honey	⬢	⬢⬢⬢⬢

which bees produced their honey, and the apiary's geographic location.

A modern online traceability system should include the formal registration of all beekeepers, geo-referenced apiaries, movement of colonies, and honey extraction, trading, blending, and packing. The traceability system should also involve honey containers, which should be easily identifiable by means of electronic codes. In such way, the whole honey chain could be perfectly transparent, and auditable.

The traceability system should also serve as a great source of information to quickly locate a problematic lot, immobilize unsafe products and, if necessary, withdraw them from the market, thus giving greater peace of mind to consumers and customers in case of any fraud or safety issue.

Audit Schemes

As previously mentioned, strong auditing programs can efficiently detect some modes of honey fraud like the production of immature honey and the use of illicit processing methods (e.g., the existence of honey dryers, ion-exchange resin technology, etc.). Immature honey, one of the main current modes of honey fraud, is difficult to be efficiently detected by the only use of analytical testing, however, it could easily be detected by adequate auditing.

In all cases, audits which should always be unannounced to randomly chosen beekeepers and processing facilities, be performed by third-party or official properly trained auditors, and should include multiple anti-fraud measures.

Auditors should be able to detect the production of immature honey (in the field and in the extraction facilities), to search for evidence of artificial feeding during the honey season, to assess the production capacity and hive yields of the beekeeper in comparison with the yields of the region, and to take samples of the recently extracted honey for moisture and purity determinations.

Auditors should also be able to inspect the integrity of the methods used at honey processing facilities, to perform mass and financial balances, to perform traceability exercises, to interview the staff of factories searching for any suspicious activities, to detect the presence of honey-drying devices and/or ion-exchange resin technology, the existence of sugar syrups at the processing plants, and also to collect samples of honeys arriving and exiting the facilities.

Extra Reports

As a general principle, honey should be sourced from suppliers without a documented history of fraud activity. The closer the relationship between buyer and supplier, the lower the risk of having fraud problems (United States Pharmacopeia, 2020).

The use of statistical information has been shown to be another valuable tool for authorities and stakeholders to detect economic anomalies. Less-than-market pricing, honeys offered at different prices according to the test/s they pass, the ability to maintain surprisingly more stable pricing than competitors, the sharp increases of export volumes of countries without parallel increments of productive capacities, and the increase of importing and re-exporting activities are good indicators of potential fraud problems (García, 2018; García & Schwarzinger, 2020).

Second Step of Honey Fraud Detection: Analytical Testing

All the background information provided by the previously mentioned sample information tools mainly derived from quality management of a honey should be the first step for laboratories when deciding the bundle of tests to assess purity and authenticity of a sample and for the final interpretation of results. As for other analytical samples of biological origin, the variability of the honey matrix and the variable acceptable limits according to the honey type make it extremely necessary to have sufficient background information (at least the geographical origin) about the sample before testing it. "Blind testing" without any information about the sample may be inefficient, may lead to improper selection of tests, and also to a false interpretation of results, thus unfairly bringing troubles to honest traders and beekeepers (Kilpinen & Vegsnæs, 2021).

Laboratories that perform the tests should always be accredited by ISO 17025 in order to be comparable and to improve reproducibility of tests.

Available tests to study honey authenticity should always be considered as complementary since no unique test that detects all modes of adulteration is currently available. There are specific single marker tests – or targeted tests- as well as multiple-parameter screening tests. The latter also provide an untargeted mode for honey adulteration detection. Specific marker tests detect an individual analytical marker, which is typically a compound or property that indicates the likely presence of syrups. In contrast, the multi-parameter screening tests provide information on many parameters in one test.

Targeted Tests: How to Distinguish If a Sugar Is Foreign from Honey through Single-Parameter Testing?

Different plant sources and production processes of syrups may render different marker molecules used to detect fraud. It should also be noted that the presence of a certain marker may constitute a proof of the occurrence of exogenous sugars in honey, while its absence cannot rule out some kind of honey fraud (García & Schwarzinger, 2020). On the counterpart, some modes of fraud may be detected by the loss of some natural constituents of honey, e.g., the use of resin technology removes not only residues and also lightens the natural color of honey.

Analytical methods based on specific marker molecules in a targeted manner can detect specific syrups through e.g., $\delta^{13}C/^{12}C$ratio, a rice syrup marker, a beet syrup marker, honey-foreign enzymes, etc. The use of these markers involves a previous validation with sufficient number of honey varietals to determine if the compound is not naturally present in any type of honey, or indeed present but in low concentrations in some special honey types.

EA-IRMS

A well-known example of analytical test targeting an analytical marker is the Internal Standard Stable Carbon Isotope Ratio method (EA-IRMS) (White & Winters, 1989). This method measures the ratio $\delta^{13}C/^{12}C$ for the total honey and for its extracted proteins. The ratio is different in substances produced by nectar producing C3-plants than by C4-plants (corn, sugarcane, sorghum). When a foreign C4-sugar is added to honey, the ratio of the sample becomes less negative and allows detection.

This normalized method detects and quantifies foreign C4-sugars with a 7% Limit of Quantification and delivers no false positives (FP). Low C4-sugar

residues derived from excess supplemental feeding of bees and C3 foreign sugars (from rice, beet, wheat, cassava) are not detected by EA-IRMS, however, this method still remains as the official one for many authorities to detect honey fraud.

Experience of countries where only C4 sugars are available has shown that the detection level of this method provides a good benchmark to detect intentional adulteration while giving a sufficiently ample tolerance for any accidental presence of foreign C4 sugars derived from artificial feeding of bees. An economic gain purpose of a C4 syrup presence can be discarded at lower levels of detection of EA-IRMS.

Establishing official methods typically requires quite long times, which contrasts with the quick development of new adulteration modes/materials. A serious testing strategic for honey adulteration mitigation should include both official methods and additional newly established methods (Apimondia, n.d.; United States Pharmacopeia, 2021).

LC-IRMS

An improvement of EA-IRMS was achieved by LC-IRMS, which measures the $\delta^{13}C/^{12}C$ values of the individual sugar fractions of honey, namely the $\delta^{13}C/^{12}C$ of mono-, di-, tri- and oligosaccharides (Elflein & Raezke, 2008). If the maximum $\delta^{13}C/^{12}C$ difference between the different fractions (D-maximum) is higher than 2.1 or 2.5 ‰ (not yet normalized and different decision limits now exist among laboratories) it is indicative of a foreign sugar presence. LC-IRMS relies on several decision criteria, thus allowing an improved limit of detection of syrup addition.

Currently, EA and LC-IRMS are performed in combination by most laboratories. The sensitivity of LC-IRMS is higher than EA-IRMS and may detect C4-sugars derived from bee feeding practices even below 7% (between 1% and 5% depending on the syrup).

LC-IRMS is not a quantitative method, unless the exact composition of the pure honey and the syrup are known (and even only an estimation may be possible). The method, however, may also render some false positives:

- Forest honeys may contain oligosaccharides > 1%, which can be interpreted as a syrup addition.
- Blossom honey with honeydew from C4 plants like sorghum (as low as 0.2 to 0.5% of honeydew) can yield high Delta maximum values.
- A low accidental contamination of honey withC4 syrups used to feed bees may be detected by LC-IRMS due to its high sensitivity. In such a case, a retention sample of the bee feed used should be tested. If the quantity of bee feed in honey results to be below 5%, then the accidental nature of the deviation could be assumed and the honey should be considered as acceptable.

Finally, LC-IRMS may also render some false negatives:

- The dilution of honey with some C3 syrups (through the oligosaccharides fraction) is sometimes detected but with a low sensitivity (≥20%), and in other occasions the method seems not useful for the detection of tailored syrups available and offered in the market to specifically pass this test.

Other targeted methods

As previously mentioned, highly-hydrolyzed and purified or bioengineered syrups are now offered in the market that pass one or more of the most widely used marker tests. In fact, markers show a limited useful life because when marker substances for specific artificial sugars are revealed and published, fraudsters then develop methods to specifically purify their adulterating products.

Normally, marker tests are currently used as complement of more developed screening tests or when these give inconclusive results.

There are several available marker tests offered by internationally recognized laboratories (Table 2).

The Detection of Fraud by Screening or Non-Targeted Methods

As opposed to marker tests, which show a limited useful life and a one-parameter scope of detection, the use of multi-parameter screening non-targeted tests like 1H-NMR (Schwarzinger et al., 2016;

Table 2. Most frequently used marker tests by internationally recognized laboratories. FN=false negatives, FP=false positives.

Test/Marker	Methods	LOD/LOQ of markers	Advantages	Disadvantages
Oligosaccharides (e.g., Zhou et al., 2014)	HPLC-ELSD/HPIC-PAD	LOD > 1% depending on the syrup	Low sensitivity for HFCS	Not Normalized. **FP:** feeding under GBP. **FP:** presence of natural Oligosaccharides. **FN:** highly hydrolyzed syrups and beet-sugar-based syrups.
Mannose (Missler et al., 2016)	NMR/HRMS/HPIC-PAD	LOQ > 250 ppm	Detects bad practices	Not Normalized. **FP:** floral honey with honeydew (may contain up to 1,400 mg mannose/kg)
Psicose (Kämpf, 2018)	HPLC-ELSD/HPIC-PAD	LOD > 0.1%	Highly specific. Low sensitivity for some syrups.	Not Normalized. Detects only a few syrups. **FP:** honeydew honey
Rice marker (SM-R)/Beet marker (SM-B)/Colorant (E150-4-MEI) (FoodQS, 2019; Xue et al., 2013)	LC-MS/MS/HRMS	Low LOD	Highly specific. Low sensitivity for some syrups.	Not Normalized Detects only a few syrups. **FP:** SM-B plant sources.
Foreign Enzymes (β-Fructofuronidase, α–β-γ–Amylases, Heat-stable amylases) (e.g., Raezke et al., 2019)	Enzymatic/ Spectrophoto-HPLC	LOD > 5 U/kg	Additional complement test and indirect test for foreign sugars.	Not Normalized **FP:** Honey dew containing foreign enzymes from aphids. **FP:** Feeding bees with brewers´ yeast patties. **FN:** syrups with inactivated enzymes.

Spiteri et al., 2017) and LC-HRMS (Du et al., 2015; Senyuva et al., 2015) provide information on multiple parameters in one single test and have the advantage to detect new adulterations or fraudulent practices even without specifically looking for them. Furthermore, they allow a retrospective investigation of samples tested in the past in case a new maker is discovered.

These methods involve the comparison of the signal profile of the sample with that of authentic honeys and syrups included in a database. Through such comparison, screening tests can detect both the absence or dilution of honey-typical compounds as well as the presence of signals not naturally found in honey.

A novel and promising DNA-based analytical testing method was recently applied for honey. This method comprehends a massive sequencing of the complete DNA of honey from different groups of organisms like plants, humans, bees, viruses, bacteria, etc. A variety of authenticity models or criteria are applied and if the composition, proportions and concentration of DNA do not match with pure honey profiles, it is a FAIL. At this point, it should be emphasized that before any new test is released, it must have the complete recognition of the scientific community in order to avoid confusions and an eventual unfair damage to the image of honey.

^{1}H-NMR (proton nuclear magnetic resonance)

^{1}H-NMR profiling is the result of hundreds of NMR signals, i.e., "a fingerprint." ^{1}H-NMR has the capacity to perform: (i) targeted testing for honey authenticity by utilizing many adulteration markers as well as by qualifying and quantifying many natural honey compounds, and (ii) non-targeted testing for unexpected, or still unknown adulteration modes or products by statistical comparison with reference group models.

It is currently also possible to use ^{1}H-NMR to test for the geographic and the botanical origin of honey types for which patterns have been already established, even in the absence of pollen in the sample.

As previously said, the method requires a database for comparison, which is built with authentic and tested honeys of different types from all over the world.

^{1}H-NMR is fast, easy to use, and can detect both C4 and C3 syrups as well as some bad beekeeping or processing practices. However, it should be noted that when unexpected deviations are found, the possibility of being a natural variation should always be considered and further testing and an expert interpretation is usually required.

As a disadvantage,^{1}H-NMR has low sensitivity for some foreign sugars (in some cases over 20%) and may lead to false identification of geographical/botanical origins or untypical honeys in case of insufficient samples of those varietals are included in its database or in case of blends. The method is not normalized.

LC-HRMS: (liquid chromatography coupled to high resolution mass spectroscopy)

LC-HRMS is a screening method for adulteration markers and, similarly to^{1}H-NMR, it has targeted and non-targeted detection modes.

In its targeted mode, LC-HRMS measures (quali and quantitatively) hundreds of many known molecules or markers. The non-targeted mode compares the screening signal with statistical models. This is accomplished by creating a database containing information of authentic honey samples and syrups.

Compared to ^{1}H-NMR, LC-HRMS can be more sensitive and more markers be detected, covering a greater variety of syrups and, in some way, closes the gap for syrups which cannot be detected by other techniques.

Due to its high sensitivity, very low quantities (sometimes less than 3%) of some syrups can be detected, e.g., residues of supplemental feeding of bees even under Good Beekeeping Practices. For that case, and similarly to what happens with LC-IRMS, a reasonable threshold level should probably be defined to discard an economic gain purpose of the syrup presence. If the presence of a feeding syrup wants to be estimated quantitatively, the exact composition of the syrup must be known.

Unfortunately, LC-HRMS has not been harmonized between laboratories. There are processes/projects ongoing that will make the method become more comparable in the future, which will also facilitate standardization and regulation.

The Choice of the Best Combination of Analytical Tests

As previously stated, and due to the nature, complexity, and dynamism of honey fraud, there is no single method or approach able to detect all modes and products used for adulteration. At least two main scenarios may be found:

1) If the traceability of the sample is known and the background information indicates no history of honey adulteration cases and no economic anomalies from that origin, a targeted test chosen after a risk-assessment + one screening test could be a recommendable approach. For example, in a country of origin with no history of adulteration, where only C4-sugars seem to be available, EA/LC-IRMS would be initially sufficient. However, and in order to prevent surprises, the addition of a screening test could be recommendable. In case a non-conclusive result appears, then other available tests can be used before a final conclusion.
2) If the traceability of the sample is not known, or if the origin of the honey indicates cases of fraud and economic anomalies from that origin, a combined approach using state-of-the-art targeted and non-targeted analytical methods is considered the best practice to detect honey adulteration. The election of the appropriate combination of test methods to be used should be the result of a detailed risk-assessment, where the appropriateness of the test methods applied has to be periodically checked and revised according to new developments (United States Pharmacopeia, 2021). In such a case, the currently accepted best practice is the application of a portfolio of the following three tests, since they are considered to be the best available ones:
   - EA/LC-IRMS
   - ^{1}H-NMR
   - LC-HRMS.

Interpretation of Analytical Results

Table 3 shows all possible combination of results from the four most frequently used or recommended tests. Due to the compositional variability of both natural honeys and industrial syrups, the constant intentional adaption of syrup composition to testing regimes, the low reproducibility

Table 3. All possible combination of results and conclusions after using the best currently available tests.

Case	EA-IRMS	LC-IRMS	NMR	LC-HRMS	Conclusion	Observations and possible explanations
1	Pass	Pass	Pass	Pass	Pure honey	
2	Fail	Pass	Pass	Pass	Adulterated	A failure of official method EA-IRMS currently means adulteration. Adulterated with a highly-hydrolyzed C4 syrup not detected by LC-IRMS or HRMS.
3	Pass	Fail	Pass	Pass	Inconclusive	Maybe a presence of honeydew from a C4-plant (additional tests required) Another possibility a false positive (FP), the test should be repeated by another accredited laboratory.
4	Pass	Pass	Fail	Pass	Inconclusive	If the failure is a false declared botanical or geographical origin, pollen testing is recommended to confirm. Other possibilities: A FP by NMR; presence of C3-syrup not included in the LC-HRMS database; or illegal practice like immature honey. Confirmation needed by marker tests.
5	Pass	Pass	Pass	Fail	Inconclusive	Confirmation by another laboratory recommended. If confirmed, the cause can be the presence of a C3-syrup not detected by NMR, or an excess of bee feeding with C4-syrup not detected by LC-IRMS. Confirmation by markers or enzymes recommended.
6	Fail	Fail	Pass	Pass	Adulterated	A strange case since a detection of a C4-sugar by EA-IRMS and LC-IRMS should always be also detected by more sensitive LC-HRMS.
7	Fail	Pass	Fail	Pass	Adulterated	Similar to 2 with respect to EA-IRMS failure + NMR fail.
8	Fail	Pass	Pass	Fail	Adulterated	Similar to 2, adulterated with a well-hydrolyzed C4-syrup but in this case detected by LC-HRMS.
9	Pass	Fail	Fail	Pass	Adulterated	Could be a mix of a C-4 honeydew + illegal practice or a C3-syrup not detected by LC-HRMS: rare case.
10	Pass	Fail	Pass	Fail	Inconclusive	Probably a moderate accidental excess of feeding (< 7%) with a C4-syrup.
11	Pass	Pass	Fail	Fail	Adulterated	Typically adulterated with a C3-syrup or a bad practice.
12	Fail	Pass	Fail	Fail	Adulterated	Similar to 2 plus NMR and LC-HRMS fails.
13	Fail	Fail	Pass	Fail	Adulterated	Adulterated with a C4-sugar but below the limit of detection of NMR.
14	Fail	Fail	Fail	Pass	Adulterated	Very strange case. Probably a false FP of HRMS.
15	Pass	Fail	Fail	Fail	Adulterated	Adulterated with a C3 syrup also detected by LC-IRMS.
16	Fail	Fail	Fail	Fail	Adulterated	Adulterated with C4 syrup or a blend of C4 and C3 syrups.

and limitations of some tests, and the lack of harmonization among laboratories, there may be times when the testing results may be inconclusive and problematic for beekeepers (Kilpinen & Vejsnæs, 2021).

In general, when one test yields an intermittent failure, it may warrant consideration as a false positive or further investigation using other methods or background sample information. On the counterpart, when a single test consistently fails, or multiple tests fail, it is indicative of adulteration in some form.

In cases of inconclusiveness, other targeted tests may be necessary to determine the origin of the failure. The targeted tests should be chosen based on a risk-assessment that takes into consideration factors such as the most common modes of adulteration and adulterants used in the country/region of origin of the product.

As previously discussed, both LC-IRMS and LC-HRMS may be sensitive enough to detect foreign C4-sugars derived from bee feeding as low as 1%, which may mean a problem even for honest beekeepers with no intention to adulterate their honey. As previously said, it would be desirable to normalize those methods and establish a reasonable benchmark of syrup presence. It should also be said that when using good beekeeping practices for artificial feeding of bees (e.g., Dini & García, 2023), the great majority of positive results by LC-IRMS and LC-HRMS derived from the feeding of bees can be prevented.

Final Conclusions

Probably one of the biggest challenges in authenticity testing of honey is to differentiate natural compositional variations of honey from intentional fraudulent alterations of its composition. The necessary multi-pronged approach to detect fraud, with large number of available tools, reflects both the complexity and diversity of honey as an analytical matrix and the multiple modes of honey adulteration.

The syrups and fraudulent practices are constantly evolving as well as the methodologies to detect them. A multi-pronged approach is necessary using all available tools: analytical tools and tools derived from quality management to detect fraud.

In the case of analytical tests, they should be considered as complementary and sophisticated enough to be always performed by an ISO17025 accredited laboratory. Furthermore, for a better and more efficient combat against honey fraud, a global normalization of tests should be promptly reached.

In such a complicated scenario, it must be expected that sometimes analytical testing may give inconclusive results.

However, the current tools to detect honey adulteration are so ample and effective that the mere possibility of finding some cases of inconclusiveness or contradicting results should never be an impediment to use the best available methods and tools to protect consumers, the sustainability of beekeeping, and the very existence of bees as the main pollinators of our planet.

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