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A Primer on Honey and Its Adulteration

Malcolm T. Sanford

Honey is defined via the work of Jonathan White and associates in Composition of American Honeys, 1962. See a copy here. A summary of the findings is found in *Beekeeping in the United States*. Agricultural Handbook 335, Revised October 1980, pp. 82-91 at Bee Source.

Honey, as it is found in the hive, is a truly remarkable material, elaborated by bees from floral nectar, and less often insect residues to produce honeydew. Nectar is a thin, easily spoiled sweet liquid that is changed ("ripened") by the honey bee to a stable, high-density, high-energy food. The earlier U.S. Food and Drug Act defined honey as "the nectar and saccharine exudation of plants, gathered, modified, and stored in the comb by honey bees (*Apis mellifera* and *A. dorsata*); is levorotatory; contains not more than 25% water, not more than 0.25% ash, and not more than 8% sucrose." The limits established in this definition were largely based on a survey published in 1908. Today, this definition has an advisory status only, but is not totally correct, as it allows too high a content of water and sucrose, is too low in ash, and makes no mention of honeydew. The National Honey Board approved the following definition in June 15, 1996, updated September 27, 2003:

"Honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. The definition of honey stipulates a pure product that does not allow for the addition of any other substance. This includes, but is not limited to, water or other sweeteners." See more at The National Honey Board's website.

There is no official definition of honey at the moment. A general one would be that honey is modified nectar, collected by honey bees foraging on blossoms or other parts of living plants. A variant of honey is known as honeydew, modified juices collected by honey bees from insects that are actively feeding on plant tissue. The following is taken from an FDA document dedicated to providing guidance on honey and labeling honey products

"On March 8, 2006, the American Beekeeping Federation and several other honey-related associations submitted a citizen petition requesting that FDA adopt a U.S. standard of identity for honey based on the 2001 Revised Codex Alimentarius Commission's Standard for Honey. The petitioners asserted that a U.S. standard of identity for honey would achieve the following goals: (1) clarify what the term "honey" means with respect to the food's composition and therefore promote honesty and fair dealing in the interest of consumers; (2) combat economic adulteration of honey by aiding enforcement and industry compliance; and (3) promote honesty and fair dealing within the food trade in general, where pure honey is used as an ingredient in other foods.

In a letter of October 5, 2011, we denied the petition because the petition did not provide reasonable grounds for FDA to adopt the Codex standard for honey. We also concluded that the petitioners' goals can be achieved by our existing authorities and a standard of identity for honey would not promote honesty and fair dealing in the interest of consumers. To address the labeling issues relevant to the petition and to reinforce existing laws and regulations to the industry, we are issuing this guidance document, which includes a summary of the current legal authorities that are most relevant to the labeling of honey and questions and answers on the labeling of honey." Honey is an extremely variable product, and what beekeepers do with it also flexible as noted by contributor Rusty Burlew.

One way to ensure that honey is legitimate is to examine the product for pollen. And the preferred way to identify the plant source of any honey is to use pollen analysis, usually accomplished by microscopic examination to determine the amount and specific kind of pollen found in the product. This requires specialized skills and some kind of pollen atlas to use for comparison. There have been books published describing pollen from many parts of the world. This does not work for honeydew, which usually contains minimal pollen, however, this is a specialized product that rarely is produced by beekeepers in the USA but is in other parts of the world (Turkey).

Other issues surrounding honey include honey adulteration, tropical honey, HAACP in processing, and the organic designation. Many are currently examined by the National Honey Board, which has in interesting history in its own right. A unique approach to honey marketing has been developed by Airborne Honey in New Zealand via tracking batch numbers.

Here's a fun explanation of how bees make honey.

Then there's the concept of toxic honey among a big list of all things honey. Read contributor Rusty Burlew's take on poison ivy and poison oak honey. Also see her take on the "raw honey."

Finally, honey has been used as a natural product in human health for centuries, not only for its nutritional value, but also as "nature's first aid kit in a jar." There is no "best" honey as it is infinitely variable. Rather like wine, honey has its own terroir, and many tastings around the world exist to examine the subtleties of this unique sweet material, only available from a unique collaboration of plants and insects.

A final issue concerns something called "raw honey." The definition is often inconclusive here as well. Contributor Rusty Burlew provides her take on this product and how it might be conflated with "pasteurized" and "gluten free," which might also appear on honey labels.

Research continues specifically on detecting honey adulteration in the world's honey as noted in the following information provided at Apimondia 46. Testing for the honey show at that event revealed a "scandal" of sorts, as revealed in this post.

DETECTION AND PREVENTION OF HONEY FRAUD

[Lead-off] Honey: What Is It And How to Ensure Its Authenticity?
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Honey is probably the most natural food on the globe. Beekeepers are allowed only minimal technical intervention during harvesting and processing, while collection of the raw materials, storage in combs, mixing with bee-own substances, conversion, dehydration, ripening, and maturation must be exclusively performed by the bees. The resulting microbiologically stable, energy-rich source of nutrition for the colony has also been esteemed by humans since ages.

Today, often products containing significant portions of industrial sugar syrups are encountered in trade. These syrups closely resemble the composition of honey and can be produced from a number of raw materials (including corn, rice, wheat, …), some matching isotopic patterns in honey. Minor components suitable as markers for adulteration vary greatly as the biotechnological methods, especially the enzymes used to cleave and concert polysaccharides in these processes, as well as clean-up strategies for the syrups change. Also, drying of honey by technical means becomes a problem.

Consequently, there is a multitude of syrups available and adulteration-markers, which have been identified in the past, were absent in other syrups rendering detection of adulterations difficult. Rather than using a number of different methods for adulteration testing it would be desirable to obtain many substances in just one analytical run. This is possible with magnetic resonance (NMR) or high-resolution mass spectrometry (MS) screening. Both methods can derive markers for adulteration based on screening of large sample collections (databases) of authentic honeys and syrups that subsequently allow targeted detection of adulteration. While MS can detect very dilute substance NMR offers the advantage of being quantitative for all parameters detected, including many traditional quality parameters for honey. The high reproducibility of NMR actually allowed establishing multi-parameter ingredient fingerprints from thousands of authentic honeys from many parts in the world. This now

allows targeted verification of geographic origin and variety – even in honey where pollen have been removed. In addition, knowledge of fingerprints of thousands of authentic honey samples also allows untargeted testing for unexpected or even novel deviations. This presentation reviews the current status of developments, highlight merits of technologies but also point out needs for future developments.

DETECTION OF ADULTERATION BY MEANS OF ANALYSIS OF 2H- AND 180-ISOTOPES IN HONEY WATERC.C. Luellmann 1 , A. Duebecke 2, 3 , T. Wiezorek 4 , G.I. Beckh 2 1 Tentamus Group GmbH, Berlin, GERMANY, 2 Quality Services International GmbH, Bremen, GERMANY, 3 Tentamus Center for Food Fraud – TCF2, Bremen, GERMANY, 4 QSI America / Adamson Analytical Laboratories, Corona, USA

Honey is being tested for adulteration already for several decades. Currently the only official method is the 998.12 of AOAC, which targets the ratio of 13C and 12C in the sugar and protein in honey. It works very well for additions of syrups from C4-plants like corn and cane sugar but is practically blind to additions of sugars from C3-plants, e.g. rice and beet sugar. This demonstrates the need for a method independent of the plant-source of the sugar being added to honey. Just like honey, any syrup contains water. The isotopic composition of natural waters from lakes, rivers and sea water is already known worldwide and "isotope maps" readily available.

There is a strong correlation of 2H- and 18O-Isotopes in natural waters, which basically means that if you plot the 2H-values against the 18O-values in a coordination system, any sample of natural waters will be located on a straight line. Thus, if isotopic composition of water is shifted towards lighter isotopes, e.g. due to evaporation, the isotope values of 2H and 18 O are shifted proportionally.

Water samples extracted from honey from different locations worldwide show a different isotopic pattern, which is the basic prerequisite of this method. Additions of water to honey either directly or through the addition of syrup would shift the isotopic composition of honey water towards that of natural water samples and indicates an adulteration or possibly an illicit way of processing.

Results look very promising. The isotopic values of 2H and 18O of water extracted from authentic honeys look very different from those of natural waters. As expected, any addition of natural water or syrup prepared with natural water will cause a shift towards the isotopic composition of natural waters and thus indicate adulteration. The method is prepared for patenting process.

KEEPING HONEY FRAUD AT BAY – HONEY AUTHENTICITY TESTING BY 1H-NMR
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Food fraud is the intentional adulteration of food for financial gain, such as substitution, dilution, counterfeiting of product formulation or packaging. According to the World Customs Organization, food fraud is costing $49 billion annually. Food fraud is a global problem and the consequences are devastating, as the reputation of food companies, regions or countries is damaged and consumer confidence erodes.

Honey is one of the most adulterated food products and the most common frauds are the deliberate addition of sugar syrups and the false declaration of geographical and botanical origins. As the challenges of safeguarding the global food supply chains have increased and frauds become more sophisticated, new and more advanced fraud detection procedures are needed, including new analytical methods that can fill the existing gaps.

Targeted methods for detection of sugar syrups rely on the analysis of foreign enzymes or specific markers of sugar syrups. The adjustment of the process of syrup production and purification can make such methods unable to detect adulterations. The AOAC method 13C EA-IRMS for example is able to detect sugar syrups from C4 plants, like corn and sugar cane but is blind to sugar syrups from C3 plants, like rice, beet or wheat. The 1H-NMR is targeted and non-targeted likewise. The technique is able to simultaneously observe hundreds of components in the honey and due to its high reproducibility acquire a so-called fingerprint of the samples. Combined with a database of authentic and known adulterated samples, the Honey-Profiling method developed by Bruker in collaboration with QSI, Alnumed and Famille Michaud allows to detect adulteration based on a multi-marker analysis.

Pollen analysis is a widely used method; however, pollen can be filtered out and pollen grains from other plants can be added to the honey to disguise the country of origin or botanical variety. For the analysis of country of origin and botanical variety, the combination of 1H-NMR with statistical analysis represents a powerful alternative, as it relies on the chemical composition of the honey. The method will be presented and explained in detail.

HONEY AUTHENTICITY ASSESSMENT EVOLUTION-NEWLY DEVELOPED LC-HRMS ADULTURATION PROFILING FOR IMPROVED DETECTION OF FRAUDULENT ADDITION OF SUGAR SYUPS

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In the last 5 years, tackling food fraud has become a major global issue. Honey is among the top ten food products which are susceptible to economically motivated adulteration. For the consumer, honey is a high-value natural product with an excellent reputation. Therefore, it is essential to safeguard the product honey with a rigid quality control using state-of-the-art methods to verify purity and authenticity.

Over the last 10 years, the number of analytical methods required to detect various kinds of honey adulteration steadily increased, including stable-isotope methods, specific methods for individual adulteration marker compounds and foreign enzymes. Since a few years, 1H-NMR profiling technology has been established to provide a fast and comprehensive authenticity screening of honey, including quality parameters, geographical and botanical origin verification and adulteration detection.

However, the latest experience in the routine testing of honeys from the international markets shows that there is still no universal method to detect all presently occurring sugar syrup adulterations with adequate sensitivity. Even when all existing methods, including NMR, are applied, adulterations with "honey-tailored" sugar syrups may pass the current testing regimes. Therefore, the need for an improved detection of these newly occurring adulterations is required. This was accomplished by method development and application of the LC-HRMS technology at Eurofins for which sensitive and robust instruments have become commercially available.

This technique does not only allow detecting the common and newly occurring adulterations. LC-HRMS also allows combining several individual adulteration marker methods to one single multi-method detecting several hundred different adulteration markers simultaneously. Moreover, due to the combined non-targeted/targeted approach, it is also possible to identify yet unknown adulterations and newly occurring types of sugars syrups.

If new adulterations will pop up in the future, the recorded data can be re-evaluated retrospectively in order to track back their first occurrence and origin. Additionally, a big advantage over other profiling methods like NMR is that no extensive database of authentic honey reference samples is required for result interpretation. As a conclusion, LC-HRMS together with stable-isotope testing (13C-EA/LC-IRMS) and 1H-NMR profiling is considered as the current gold standard for honey authenticity testing.

IDENTIFICATION OF SYRUP MARKERS USING HIGH RESOLUTION MASS SPECTOMETRY (LC-HRMS)
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The ongoing development of different modes of adulteration of honey makes it necessary to find new ways to detect adulteration of honey. There are many examples from the past that demonstrate, that a targeted approach, i.e. focusing on one or few parameters only, does only work for a short period of time until the fraudsters adapted to the new situation. Once they successfully changed the mode of adulteration, the targeted method loses its effectiveness. QSI recognized that in order to overcome this situation it is necessary to increase the number of parameter to make adulteration of honey far more complicated and less profitable for fraudsters.

To tackle this project, the latest cutting-edge technology is being employed, namely high resolution mass spectrometry. First, we analyzed a broad range of syrup and honey samples in a non-targeted way, which means that we were looking basically at any substance entering the mass spectrometer. In the next step we compared syrups with authentic honey samples by using statistical tools like principal

component analysis. This analysis facilitates detection of differences in the datasets. We identified a number of molecule masses that only occur in syrups but could not be detected in authentic honey samples (syrup marker). Thus, detection of such a syrup marker in honey is clearly indicative for the adulteration of a honey sample.

Resin treatment of honey as it is often being done in Asian countries to extract residues, e.g. of antibiotics, is another topic we focused on. We set up own resin treatments of honey in the laboratory and compared the results of LC-HRMS-analysis of honeys with that of a resin blank. The results of this test will also be discussed.

NEW TECHOLOGIES AND BUSINESS/UNIVERSITY COLLABORATIONS IN DETECTION OF HONEY ADULTERATION

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Adulteration of honey for economic benefit has evolved into multiple forms over the last 30 years. In addition to simply adding corn or cane syrup, ultrafiltration has been utilized to attempt to hide country and floral origin, aliphatic resins to remove unpalatable flavors and hide other chemical adulterants, early harvesting to artificially increase production, and bioengineered sugar syrup production to circumvent AOAC standard (C4 sugars) analyses. Recently, the development of NMR techniques in conjunction with other analytical methods has changed how adulteration testing is performed and interpreted. NMR methods now have the capacity to not only determine sugar syrup addition but also aliphatic resin use and immature harvesting. Other analysis methods can be added to further elucidate these methods of adulteration. In addition, collaboration between universities and private laboratories has created a unique opportunity to refine methods and dynamically change creditable testing capacity in pace with the changes in adulteration methods.

And so the search goes on in this critical area. Look for more as time goes on to develop a specific way to detect adulteration, preserving the credibility and future of the global honey crop for generations to come.