Authenticity of Honey
A Major Analytical Challenge

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Honey is a natural and almost untreated food produced by the bees. The industrial processing for packaging is reduced to only necessary treatments of the raw honeys, like careful warming for liquefaction, homogenization and filtration in order to maintain the valuable properties. Therefore, honey is highly esteemed by consumers as an authentic, naturally pure and healthy product. Honey is commercially offered in many varieties, e.g. as flower honey, forest or honeydew honey, or as monofloral honey of a certain botanical origin like acacia, rape, sunflower, citrus, lavender or chestnut. Furthermore, there are specialty and premium honeys, for example manuka honey from New Zealand which is famous for its high natural antibacterial activity. Honey is not only used in form of table honey (breakfast spread or sweetener for tea), but has also major importance as an ingredient in the food industry replacing plain sugar.

Food which was produced close to nature, organic food and natural food like honey are highly demanded by consumers due to the growing awareness for nature and the environmental and health aspects of food. Thus, both the food industry and the consumers are willing to spend more money for these products. For this reason, it is very important to control the raw and finished products very carefully in order to verify whether the labelled quality and purity is actually true. The production, trade and sale of honey are a globalized business. Those countries showing the highest demand and consumption of honey can usually produce only a small amount of the required quantities. Therefore, the majority has to be imported from other countries. According to the latest statistics [1], Germany imports approx. 83% of the honey from foreign countries, the import quota of the EU and USA are 57% and 66%, respectively. The major export markets are Asia (China, India, Vietnam, Thailand, New Zealand, Australia), South and Central America (Argentina, Mexico, Brazil, Uruguay, Chili, Cuba) and South and Eastern Europe (Spain, Hungary, Romania, Ukraine, Bulgaria, Italy, Poland, France).

While the legal regulations for honey are very stringent in the EU [2], and although the related Codex Alimentarius Standard of Honey [3] is acknowledged worldwide, the individual national regulations and controls all over the world are quite different and not yet harmonized. This leads to different quality levels, different consumer expectations and also different legal opinions regarding the marketability of honey. In the EU, honey can only be sold under the label “honey”, if the product consists of 100% honey. This is not always the case elsewhere, e.g. there are products on the market in the USA labelled as “honey syrup” or “imitation honey”. These products either contain only a small portion of honey or no honey at all. Also, there are different types of honey on the market in Asia, e.g. the “traditional style” honey as it is defined by EU law, but also honey which is produced by feeding bees with sugar syrup. Such a product would not be marketable under the label “honey” in the EU and would be considered as adulterated equal to honey which was deliberately diluted with sugar syrup.

The continuously growing demand for honey is in contrast to the stagnating or partly decreasing production volumes worldwide due to climate changes and the impairment of bee health e.g. by the agricultural use of pesticides and the overall increasing industrialization. This fact and the lack of internationally harmonized rules for honey makes honey adulteration, i.e. diluting honey with cheaper sugar syrups, very attractive in order to increase the available volumes of honey and gain financial profit. The term often used in this context is economically motivated adulteration (EMA) [4, 5], because such adulterated honey can be produced, offered and purchased at significantly lower costs, thus increasing the profit margins. Additionally, the competition for the lowest (best) price in the retail market and the still partly existing consumer attitude “the cheaper the better” contribute to the currently low market value of honey (except premium honeys) which is not in line with the rather high production and trading prices.

As a consequence of the horse meat scandal, the EU Commission published a report about food fraud in December 2013, listing the ten most frequently adulterated food items [6]. According to this ranking, honey is in the sixth place. Numerous cases of honey adulteration have been recorded in the recent years [7, 8]. Sourcing and trading authentic and high quality honey is a difficult task for producers, importers, exporters, packers and dealers under these circumstances. In order to manage this, measures of many kinds are necessary along the supply chain. Besides audits and traceability programs an almost gapless analytical testing scheme is performed on each production batch including a broad spectrum of analytical methods. Therefore, honey can be considered as one of the best controlled food products in general.

The authenticity assessment is one of the most crucial parts of honey testing besides quality para-
Authenticity of honey includes on the one hand the determination of the geographical and botanical origin in order to verify the accurate labeling of the honey type and correct origin declaration according to the legal requirements. On the other hand, it must be evaluated whether honey has been adulterated with foreign sugars or whether honey was produced by excessive sugar feeding of the bees. The microscopic analysis of the pollen spectrum is still the reference method for the determination of the geographical and botanical origin. For this purpose, harmonized and standardized protocols are available [9,10]. However, for adulteration detection such harmonized and standardized methods do not exist so far. Hereinafter, several analytical methods will be discussed which are routinely used nowadays for the assessment of honey adulteration.

Verification of honey authenticity is a complex analytical task. The determination of the common quality parameters like sugar spectrum (fructose, glucose, sucrose, maltose, turanose, melezitose, erlose and other minor sugars), enzyme activities (diastase, invertase), hydroxymethylfurural (HMF), prolin content, pollen spectrum and sensory may give hints at possible manipulation or substantiate them, but are usually not sensitive enough to detect and prove admixtures of foreign sugars to honey. The reason is that honey is a natural product showing large compositional variations depending on the geographical origin, the botanical type and environmental factors which complicate the definition of exact product specifications and the distinction from non-authentic products. Additionally, retail honeys are often commercial blends of various geographical and botanical origins which make it even more difficult to set boundaries. Therefore, the analytical methods applied for adulteration detection must be independent from these variability factors as far as possible. From the historical aspect, the introduction of stable isotope analysis for honey testing was a milestone in this respect. $^{13}$C/$^{12}$C carbon stable isotope ratio mass spectrometric analysis ($\delta^{13}$C-IRMS) can be used to differentiate between different sugar sources. As both the honey sugars (derived from nectar and honeydew collected by the bees) and the sugar syrups may be used as adulterant originate from plants, the isotope ratios of the sugars are influenced by the photosynthetic pathway of the plant species. Thus, it is possible to distinguish different natural or synthetic sugar sources according to their $\delta^{13}$C isotopic values. The first IRMS method for honey was established in the late 1980s and published as AOAC method [11]. The purpose of this method was to detect sugars from C4 plants (sugar cane and corn) in honey. The $\delta^{13}$C values of the isolated honey protein and the bulk honey are determined by combusting the samples in an elemental analyzer (EA) to carbon dioxide, nitrogen and water, and measuring the $^{13}$C/$^{12}$C isotope ratio in the formed carbon dioxide with an isotope ratio mass spectrometer (EA-IRMS). In case of authentic honey, the isotope values of the honey protein and the bulk honey are very similar showing only a slight natural variability of approx. $\pm 1\%$. The isotopic value of the honey protein serves as an internal standard for the pure honey, because it is not altered when sugar syrup is added to honey (syrup does not contain any measurable protein). If C4 sugars ($\delta^{13}$C value approx. -10 \%) are admixed to honey, the isotopic value of the bulk honey ($\delta^{13}$C value approx. -23 to -29 \%, depending on the honey type) will shift towards more positive values while the corresponding $\delta^{13}$C protein value will remain unaltered. The more C4 sugars are added to honey the larger the difference between both values. However, as the calculation of the C4 sugar percentage is dependent on the absolute $\delta^{13}$C values of the protein and the honey, the limit value was not set for the maximum allowable natural variation between both values but for the resulting C4 sugar percentage calculated thereof. Thus, honey is considered to be adulterated, if the C4 sugar percentage is $\geq 7\%$. This refers to a difference of $\delta^{13}$C protein minus $\delta^{13}$C honey showing values of approx. 0.9 to 1.4 \%.

The AOAC method can only prove adulteration in case of negative differences, i.e. the $\delta^{13}$C value of honey is more positive than that of the protein (adulteration with C4 sugars). However, a definitive result interpretation cannot be given in the reverse case of positive differences ($\delta^{13}$C value of protein more positive than that of honey). One possible reason for such a deviation can be the adulteration with so-called C3 sugars derived from plants like wheat, sugar beet, rice or tapioca. But also other reasons can apply. Except for South and Central America, adulteration of honey with C4 sugars plays only a minor role in other regions worldwide, while adulteration with C3 sugars is prevailing in Europe and Asia. As the AOAC method cannot detect C3 sugar adulteration, other and more sophisticated techniques are required which are able to prove this type of adulteration. The absolute $\delta^{13}$C isotopic values cannot be used for differentiation of honeys and C3 sugars in this case, because the isotopic values of nectar and honeydew from which the honey is produced is also derived from C3 plants. Here, a specific feature of honey can be utilized: the $\delta^{13}$C values of honey protein and the individual sugars of honey are almost identical in authentic honeys [12,13]. By comparing the individual deviations between the $\delta^{13}$C values of the different honey fractions it can be evaluated whether the honey is authentic or has been manipulated with foreign sugars (C4/C3). The proper technical solution for this analytical problem is the online hyphenation of liquid chromatography (LC) with IRMS (LC-IRMS), a technique which is still not very common in food analysis. The sugar fractions of honey (fructose, glucose, di- and trisaccharides) are separated by LC and subsequently chemically

![Diagram](image_url)

**Fig. 1:** $\delta^{13}$C EA/LC-IRMS for detection of honey adulteration. The protein isolated by precipitation reaction from the honey is combusted in the elemental analyzer (EA) and the $^{13}$C/$^{12}$C isotope ratio of the formed CO$_2$ gas is measured by isotope ratio mass spectrometry (IRMS). The individual sugar fractions (fructose, glucose, disaccharides and trisaccharides) are separated from each other by liquid chromatography (LC), chemically oxidized to CO$_2$ in the interface, separated from the liquid eluent and the $^{13}$C/$^{12}$C isotope ratios determined by IRMS.
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oxidized to carbon dioxide using an interface specifically designed for this purpose. The carbon dioxide is separated from the eluent with a selective gas-permeable membrane and transferred into the iRMS for δ¹³C measurement using helium as a purge and carrier gas. However, the δ¹³C value of the isolated honey protein cannot be measured in this way and must be determined by conventional EA-I RMS instead (Fig. 1).

A reference database of authentic honeys has been built up in order to set purity criteria and limit values for authentic honeys. This reference database has been continuously expanded over the years and consists nowadays of more than 20,000 samples worldwide. The following limit values were defined: the δ¹³C values of fructose and glucose shall not differ more than ±1‰; the δ¹³C values of protein, bulk honey, fructose, glucose, di- and trisaccharides shall not differ more than ± 2.1‰ [12-14].

If the honey in question exceeds these limit values, it can be assumed that the honey is adulterated with foreign sugars (Fig. 2).

The δ¹³C EA/LC-IRMS technique has become established over the last years and proved successful in the general routine screening for honey adulteration. Thus, it is nowadays an inherent part of the current authenticity assessment of honey. It provides significant advantages compared to the AOAC method, as detection is independent of the sugar type (C4/C3). However, like any other screening technique, there are also some drawbacks that have to be mentioned. In practice, often neither the honey type nor its origin, nor the possible types of adulterant (syrup) are known. This makes it difficult to perform a more sophisticated evaluation and define stricter limit values depending on the honey variety and the type of sugar syrup. Therefore, the defined limit values are a reasonable compromise regarding detection sensitivity and prevention of false positive findings due to the natural variability of honey and considering commercial honey blends.

Furthermore, this screening technique is primarily a qualitative method, because calculation of the percentage of foreign sugar is difficult and can lead to misinterpretations when the exact composition of the pure honey and the syrup, respectively, are unknown. The general detection limits were empirically evaluated by spiking experiments and are typically ≥ 1% for C4 sugars and ≥ 10% for C3 sugars [12, 13]. Nevertheless, sometimes the detection limits can be higher, particularly when honeys and syrups are mixed together which show very similar isotopic patterns (e.g. rape honey and rice syrup). In these cases, where the detection limit of the screening method is not sufficient, isotopic screening has to be complemented with alternative analytical methods which are more specific and more sensitive for these types of adulteration. Examples for such complementary methods are the foreign enzymes methods detecting enzymes like beta-fructofuranosidase and beta-/gamma-amylases which are used in the production of invert sugar syrups from sucrose or starch [15,16]. Additionally, there are methods detecting specific marker substances indicating the presence of sugar syrups in honey [17-20] by GC-MS, LC-MS or LC-ELSD, for example the honey-foreign oligosaccharides (oligosaccharide ≥ DP 4) which are a remainder of the enzymatic starch degradation and do not occur naturally in flower or honeydew honey (Fig. 3).
Of course, the disadvantage of these specific marker methods is that they can only detect and prove one certain type of adulteration. Conclusively, several complementary methods have to be applied in order to perform a comprehensive and reliable assessment of the honey authenticity. To date, there is no universal method available for routine control being able to determine all the different types of honey adulterants with sufficient detection sensitivity and robustness at the same time. The availability of such a universal method has been a long time requirement for a faster and more cost effective control of honey authenticity, but will still be part of current and future research.

As a look out to future developments the current investigation of using NMR profiling technology for honey adulteration detection can be mentioned. NMR profiling has already been applied successfully for authenticity screening of fruit juices and wines. While NMR has been used in the context of honey so far mainly as an alternative or complementary methodology to IRMS in order to determine δ13C/δ2H isotope ratio values of honey (so-called SNIF-NMR) or for the botanical differentiation of different honey types [21-24], the 1H-NMR profiling approach for honey adulteration detection is rather new [25,26]. Here, an untargeted profiling of all substances contained in the honey and their concentration levels is performed and compared with the respective compound spectra of authentic honeys using chemometrics. Honeys showing an ‘untypical’ 1H-NMR profile (transgression of typical concentration ranges found for authentic honeys, detection of syrup marker compounds) are automatically considered as non-authentic or adulterated, respectively. However, there are still major challenges in this context: (i) to build up very comprehensive and representative reference databases which fully reflect the natural variability of the honey composition depending on the botanical and geographical origin, (ii) the consideration of possible compositional variations due to seasonal, productional or climatic factors and (iii) feasibility to detect the relevant adulteration markers despite the fact that NMR is not the method of choice for trace substances (i.e. the marker substances) occurring only at very low concentration levels in adulterated honeys. At present, NMR profiling has to be complemented with other analytical methods like stable isotope analysis and mass spectrometric target screening of adulteration markers in order to achieve reasonable detection levels (5-10% foreign sugars) at all times. Furthermore, there is still the difficulty in proving the causal relationship between an untypical 1H-NMR profile and the admixture of foreign sugars in case no signals of known adulteration markers (e.g. honey foreign oligosaccharides) are visible. Further experience will tell whether this new analytical approach will become a regular feature in the authenticity assessment of honey in future.

References:
[7] Food Fraud Database (www.foodfraud.org)
[9] DIN 10760, Honig - Untersuchung der relativen Pollenhäufigkeit
[19] Rommerskirchen F. and Efflein L., Development and validation of simple, cost effective and easy to use tests which can be used by the honey industry to verify honey authenticity concerning adulterants with sugar syrup. National Honey Board, USDA/AMS Final Project Report, September 2012
[23] Consonni R. et al. (2013), Geographical discrimination of honeys by saccharides analysis, Food Control 32, 543-548
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