# **Detection of Adulteration of Honey**

Subjects: Food Science & Technology

Contributor: Marinos Xagoraris, Nefeli Sofia Sotiropoulou, Panagiota-Kyriaki Revelou, Eleftheria Kaparakou

Nowadays, adulteration of honey is a major concern among authorities in order to ensure its quality by imposing specific standards that allow the honey to be competitive in the market. Traditionally, the identification of adulteration of honey is performed by physicochemical methods. However, spectroscopic techniques are considerably more practical when detecting impurities in honey due to fraudulent acts, as these techniques are easy to execute, more rapid, and more reliable than physicochemical methods.

Keywords: honey adulteration ; IR ; Raman

### 1. Introduction

A main topic concerning the beekeeping sector, the honey industry, and researchers is the adulteration of honey. According to European Union regulations, the addition or removal of any kind of honey substance is illegal <sup>[1]</sup>. Honey adulteration is achieved by adding lower-quality honey and artificial adulterants <sup>[2]</sup>. Honey's health benefits and its unique flavor and aroma make it more expensive in comparison to other sweeteners. Therefore, in an attempt to reduce production costs and simultaneously increase profit, honey is a product usually subjected to adulteration <sup>[3][4][5]</sup>. Starch and inverted syrup fed to bees, the addition of sugars such as high fructose, glucose, and saccharose syrups, and low-quality honey added to high-priced honey are considered the most common ways of honey adulteration <sup>[4][6]</sup>. Honey adulteration can occur in any step of production or processing. It is also difficult to detect due to the fact that the adulterated honey is similar to the pure one <sup>[Z]</sup>. Moreover, the classical methods that certify honey quality, such as physicochemical analyses, are incapable of detecting adulteration accurately. Thus, it is essential to develop and adopt a new process for honey quality control. For the aforementioned reasons, many analytical techniques have been applied, characterized by high effectiveness, accuracy, and sensitivity for the detection of honey adulteration <sup>[3]</sup>.

In recent years, vibrational molecular spectroscopy techniques such as infrared (IR) and Raman are used to identify and quantify the chemical composition of various food products with flexibility, efficiency, and low cost [54]. These techniques also provide an easy, reliable, environmentally friendly, non-destructive, and prompt way for honey quality control <sup>[8][2][5][9]</sup> [10][11][12][13].

#### 2. Detection of Honey Adulteration using IR spectroscopy

Infrared-based spectroscopy can be used for the detection of different adulterants in honey at different ranges of absorption. (Table 1).

Chen et al.<sup>[14]</sup> used near-infrared (NIR) spectroscopy on blossom honey to determine adulteration with high fructose corn syrups. The characteristic bands of the blossom honey spectrum were around 6851 cm<sup>-1</sup> (O–H stretch), 5607 cm<sup>-1</sup> (CH<sub>2</sub> group), 5201 cm<sup>-1</sup> (O–H stretch and bend band), 4782 cm<sup>-1</sup> (O–H deformation band, and C–O stretch band), 4686 cm<sup>-1</sup> (C–H stretch and deformation band), and 4182 cm<sup>-1</sup> (CH<sub>2</sub> stretch and deformation band). For the determination of high fructose corn syrup in honey, Ferreiro-González et al. <sup>[9]</sup> applied visible (Vis)-NIR spectroscopy. In another study, Fourier transform infrared (FTIR) spectroscopy was used to quantify corn syrup in honey to detect the adulteration based on sugar content. The differentiation between pure and adulterated honey was obtained clearly at the spectral range of 1150–650 cm<sup>-1</sup>, which was characteristic of pure honey <sup>[15]</sup>. Moreover, in a study by Li et al. <sup>[11]</sup>, mid-infrared (MIR) spectroscopy successfully quantified high fructose syrup (HFGS) in honey samples. The absorption maxima of pure honey and HFGS were achieved at 3285, 2930, 1642, 1370–1420, 1200–1350, and 1025 cm<sup>-1</sup>. The characteristic band at 3285 cm<sup>-1</sup> (OH– stretching vibrations of water) raises by increasing HFGS concentration due to its high moisture.

NIR spectroscopy seems to be effective to classify honey in both cases of adulteration, with high fructose corn and maltose syrup. The characteristic peaks of absorbance were the same for pure and adulterated honey: 6891, 5619, 5155, 4778, 4395, and 4231 cm<sup>-1</sup> [16]. The same spectroscopic technique (NIR), using three different NIR instruments (a

laboratory, as well as portable and mobile instruments), was applied to South African honey. Particularly good classification accuracies were obtained between the non-adulterated and adulterated honey and verified the capability of NIR spectroscopy to detect the addition of sugars and cheap imported honey, irrespective of the type of instrument  $\frac{117}{1}$ . NIR spectroscopy was used to detect adulterants (corn, sucrose, high fructose, beet, and rice syrups) in Manuka honey  $\frac{18}{1}$ .

Aliaño-González et al. <sup>[2]</sup> used Vis-NIR spectroscopy in order to guarantee the quality of multi-floral Granada Protected Designation of Origin (PDO) honey by determining common adulterants (rice and fructose syrups, invert and brown cane sugars). Thirteen significant wavelengths (465.5 nm, 499.0 nm, 559.5 nm, 675.5 nm, 736.0 nm, 1104.5 nm, 1170.5 nm, 1253.0 nm, 1324.5 nm, 1423.5 nm, 1467.5 nm, 1544.5 nm, and 1958.0 nm) were selected for the discrimination. Most of the bands are characteristic regions (550–600 nm, 1190 nm, and 1700–1900 nm) of the Vis-NIR spectra.

In another study, FT-MIR technique was employed to characterize and quantify sugar adulterated honey from different varieties. Specifically, the addition of sucrose syrup was detected by the increase in absorbance in the region of 1800–650 cm<sup>-1</sup> and the Full-Width-at-Half-Maximum (FWHM) was found at 1056 cm<sup>-1</sup> for all honey samples, related to C–O, C–C, and O–H stretching and was increased by increasing the concentration of the adulterant <sup>[19]</sup>. FT-MIR analysis was also carried out for pure and adulterated *Trigona* spp. and *Apis* spp. honey by Mail et al. <sup>[20]</sup>. The characteristic peaks of *Trigona* spp. and *Apis* spp. honey were 3272, 2934, 1643, 1416, 1345, 1256, and 1026 cm<sup>-1</sup>. In the case of Apis honey, the characteristic spectra were changed in all the regions with the addition of vinegar, even at low percentage due to the dilution by the amounts of water in the vinegar. The adulterated *Trigona* spp. honey with water also shift away from pure honey at most of the spectral regions. Thus, the spectroscopic data showed that this technique could rapidly detect the adulterants in both honey types.

Attenuated total reflectance (ATR)-FTIR spectroscopy was used in a study on stingless bee (*Heterotrigona itama*) honey from Malaysia for its capacity to detect adulteration by five adulterants including fructose, glucose, sucrose, corn syrup, and cane sugar. Especially, the absorption peaks at 1054, 876, and 779 cm<sup>-1</sup> were attributed to the increasing percentages of fructose. The characteristic peaks at 1022, 991, and 898 cm<sup>-1</sup> were assigned to the presence of glucose, and at 991 and 921 cm<sup>-1</sup> to the presence of sucrose <sup>[21]</sup>. In another study of honey adulteration with sugar, FTIR spectrometer with an ATR device was applied to honey produced in different places of Ecuador <sup>[13]</sup>. The ATR-FTIR technique has been also used for the estimation of the adulteration with commercial sugars of aren (*Arenga pinnata*), coconut, and cane sugar of Indonesian honey <sup>[22]</sup>.

Pure (105 samples) and adulterant (154 samples) honey were analyzed by NIR and MIR spectroscopies to detect adulteration by rice and corn syrups <sup>[23]</sup>. In another study, natural and syrup-adulterated honey from China were analyzed using both spectroscopies, NIR and ATR-FTIR. Two types of adulterants were studied: type 1, rice and beet syrup, and type 2, high fructose corn, corn, maltose, and sucrose syrup. Between NIR and ATR-FTIR, more characteristic peaks were observed in the second technique. The spectral region at 750–1500 cm<sup>-1</sup> was related to the absorption of major monosaccharides (such as fructose and glucose) and disaccharides (such as sucrose) and the region at 750–900 cm<sup>-1</sup> was attributed to anomalous peaks corresponding to the characteristic absorptions of sugars <sup>[5]</sup> (**Table 1**).

Type of Spectroscopy	Type of Adulterants	References
ATR-FTIR	Fructose syrup, glucose syrup, sucrose syrup, corn syrup, cane sugar	[21]
ATR-FTIR	Commercial sugars of aren (Arenga pinnata), coconut, cane sugar	[22]
ATR-FTIR and Raman	Sucrose, reducing sugars	[13]
MIR and Raman	High fructose corn syrup, maltose syrup	[11]
NIR	High fructose corn syrup	[14]
NIR	High fructose corn syrup	[16]
NIR	Glucose syrup, fructose syrup, cheap imported honey	[17]
NIR	Corn syrup, sucrose syrup, high fructose corn syrup, beet syrup, rice syrup	[18]
NIR and MIR	Rice syrup, corn syrup	[23]

Table 1. Application of vibrational spectroscopic techniques coupled with chemometrics in detection of honey adulteration.

Type of Spectroscopy	Type of Adulterants	References
NIR and ATR-FIIR	Type 1: rice and beet syrup, type 2: high fructose corn syrup, corn syrup, maltose syrup, sucrose syrup	[5]
Raman	Glucose, fructose, sucrose, maltose	[12]
Raman	High fructose corn syrup, maltose syrup	[24]
Raman	Molasses, date molasses, grape molasses, high fructose corn syrup, corn syrup (dark and light), sucrose, inverted sugar	[25]
NIR	High fructose corn syrup	[9]
NIR	Inverted sugar, rice syrup, brown cane sugar, fructose syrup	[2]

## 3. Detection of Honey Adulteration using Raman spectroscopy

Raman spectroscopy can be successfully used to detect adulteration of honey (Table 1).

Raman technique coupled was applied at honey to identify and quantify sugars (glucose, fructose, maltose, and sucrose contents) and further to characterize them as adulterants. The characteristic spectral bands that correlated to sugars of honey were 314, 341, 415, 530, 617, 744, 776, 790, 838, 856, 911, 933, 1028, and 1106 cm<sup>-1</sup> [12]. Moreover, Raman technique was used by Salvador et al. <sup>[13]</sup> to detect the sugar content and the type of adulteration in commercial honey of Ecuador. The main observed bands of honey from Pichincha and Loja provinces were 326, 338, 419, 516, 630, 707, 817, 862, 918, 1062, and 1126 cm<sup>-1</sup>. These bands were assigned to the presence of sugar (glucose, fructose, and sucrose) in honey samples. The bands of pure honey at 817 and 862 cm<sup>-1</sup>, in the case of adulteration with sucrose, were overlapped with strong absorptions at 822 and 834 cm<sup>-1</sup>.

In another study, Raman spectroscopy was also used to detect adulteration of honey with high fructose corn syrup and/or maltose syrup. The characteristic bands corresponding to authentic and adulterated honeys were observed: 351, 425, 517, 592, 629, 705, 778, 824, 865, 915, 981, 1065, 1127, 1264, 1373, and 1461 cm<sup>-1</sup> <sup>[24]</sup>. Raman spectroscopy was successfully employed for the quantification of HFGCS (high fructose syrup) in adulterated honey, as well. At the band of 2791 cm<sup>-1</sup>, the absorption was increased by increasing the HFGS concentration, while at 1130 cm<sup>-1</sup>, the absorption was reduced due to the decrease in protein and amino acid content in the adulterated honey <sup>[11]</sup>.

Non-invasive techniques using a handheld and compact benchtop Raman system were employed to detect honey adulteration by molasses, date molasses, grape molasses, high fructose corn syrup, corn syrup (dark and light), sucrose, and inverted sugar. The characteristic spectroscopic bands found at 424, 517, 629, 706, 824, 1067, 1127, 1265, 1373, and 1461 cm<sup>-1</sup> were concerning the presence of sugars  $\frac{[25]}{2}$ .

## 4. Other Techniques

The technique of isotope ratio mass spectrometry (IRMS) is used to detect honey adulteration based on the difference of  ${}^{13}C/{}^{12}C$  carbon isotopes. The botanical origin of honey is from C3 plants, while the sugar cane, corn, and other plants from which syrups are used in the adulteration of honey belong to the C4 plants. The difference is that they follow different photosynthetic pathways resulting in differentiation in the metabolic pathways of the compounds containing the  ${}^{13}C$  isotope. Therefore it is possible to detect the addition of cheap syrups from C4 plants due to the difference  $\delta^{13}C$  which ranges from -22 to -338 % for honey from C3 plants, while for syrups from C4 plants range from -10 to -208 %. Also when sugar (C4 plants) is added to pure honey the  $\delta^{13}C$  value changes, with the indication -23.5 % indicating the existence of adulteration<sup>[26][27]</sup>.

Nuclear magnetic resonance spectroscopy (NMR) provides information on the structural and chemical properties of food ingredients. It is used to certify the geographical and botanical origin of honey as well as to adulterate honey with syrups of different origins. The NMR experiments performed are one-dimensional (1D NMR) with 1H and 13C spectra and two-dimensional (2D NMR) such as heteronuclear two-dimensional spectroscopy (<sup>1</sup>H-<sup>13</sup>C HMBC). Nuclear magnetic resonance spectroscopy is a technique that provides us with many possibilities, however, the equipment and maintenance required are of high cost so it is used mainly for research purposes or by large industries<sup>[28][29][30]</sup>.

DNA-based techniques are reliable and are used to certify the geographical and botanical origin of honey as well as to detect microorganisms and allergens in honey. The approaches that have been performed are the DNA barcode with the combination of universal primers and mass parallel sequencing, the targeting of ADH1 genes to detect the botanical origin of flower honey, the extraction of DNA from pollen, the detection of fungi and DNA-based bacteria, the isolation of cetyltrimethylammonium bromide (CTAB) precipitation buffer DNA and the detection of markers for the polymerase chain reaction (PCR). Finally, entomological identification of the origin of honey has been performed based on the mitochondrial 16S rRNA of the bee<sup>[31][32][33]</sup>.

## 5. Conclusion

Honey consumption gradually raises mainly because of its health benefits. However, an important issue that consumers, producers, industries, and researchers must deal with is the cases of adulteration. Consequently, it is urgent to develop low-cost, simple, and reliable techniques that will ensure the quality of honey. Thus spectroscopic methods, namely IR and Raman, can evaluate the quality of honey and are able to detect adulteration, mainly with sugar syrups.

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