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HONEY'S MOST COMMON TYPES OF ADULTERATION AND TYPES OF ADULTERANT DETECTION TECHNIQUES – A REVIEW

CELE MAI FRECVENTE TIPURI DE FALSIFICARE A MIERII ȘI TIPURILE DE TEHNICI DE DETECTARE A ADULTERANȚILOR – UN REVIEW

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ABSTRACT | **REZUMAT**

Honey is described as a naturally sweet mixture made by bees (*Apis mellifera*) from nectar, plant secretions, or excretions of insects that feed on living plants. Honey is more expensive than other sweeteners like cane sugar syrup because of the unique flavour character and health benefits supplied by these chemical components. Honey's quality and pricing also vary greatly. Due to these characteristics, imitation honey is a common practice. There are two types of honey adulteration: direct and indirect. A material that is directly introduced to the honey is referred to as direct adulteration. While feeding the honeybees an adulterant result in indirect adulteration. Bee feed can be made artificially from a variety of foods, including sugar syrup and sherbet, cough syrup, powdered sugar mixed with yeast, powdered milk, maize pollen, different cereal flours, medications, and herbal supplements. Directly, honey can be adulterated with sugars such as high fructose corn syrup, glucose syrup, corn syrup, invert sugar syrup and cane syrup. Detection of adulterants in honey is possible by various techniques such as mass spectrometry, chromatography, spectroscopy and biosensors. The purpose of this review is to systematize the main types of forgeries along with existing detection methods.

Keywords: honey adulteration, adulterants, detection techniques

Mierea este descrisă ca un amestec dulce natural produs de albine (*Apis mellifera*) din nectar, secreții vegetale sau excreții ale insectelor care se hrănesc cu plante vii. Mierea este mai scumpă decât alți îndulcitori, cum ar fi siropul de zahăr din trestie, din cauza caracterului unic al aromei și a beneficiilor pentru sănătate oferite de aceste componente chimice. De asemenea, calitatea și prețul mierii variază foarte mult. Datorită acestor caracteristici, falsificarea mierii este o practică comună. Există două tipuri de falsificare a mierii: directă și indirectă. Daca un material care este introdus direct în miere vorbim despre adulterarea directă. În timp ce hrănirea albinelor cu un adulterant se practică în falsificarea indirectă. Hrana albinelor poate fi fabricată artificial dintr-o varietate de alimente, inclusiv sirop de zahăr și șerbet, sirop de tuse, zahăr pudră amestecat cu drojdie, lapte praf, polen de porumb, diferite făinuri de cereale, medicamente și suplimente pe bază de plante. În mod direct, mierea poate fi falsificată cu zaharuri precum siropul de porumb cu conținut ridicat de fructoză, siropul de glucoză, siropul de porumb, siropul de zahăr invertit și siropul de trestie. Detectarea adulteranților în miere este posibilă prin diverse tehnici, cum ar fi spectrometria de masă, cromatografia, spectroscopia și biosenzorii. Scopul acestei analize este de a sistematiza principalele tipuri de falsificări împreună cu metodele de detecție existente.

Cuvinte cheie: falsificarea mierii, adulteranți, tehnici de detectare

Honey is mostly composed of sugars, but it also contains certain acids, phenolic compounds, hydroxymethylfurfural (HMF), minerals, and water in trace amounts. Due to its effects on the economy, trade, and public health, adulterating honey is a problem that affects the entire world (10, 18). The composition of honey can change based on the source of the flowers as well as other outside variables including the time of year, the processing method, and the storage climate (3, 12). Biologically active substances, macro- and microelements, carbohydrates, water, organic acids (gluconic acid, acetic acid, etc.), enzymes (inverts, glucose oxidase, catalase, and phosphatases), minerals, vitamins (ascorbic acid, niacin, pyridoxine, etc.), proteins, pigments, antioxidant substances, aromatics and flavourings substances, sugar alcohols, colloids, and phytochemicals are all present in this product (25). However, depending on the floral source and other outside circumstances, such as seasonal and environmental conditions, processing, and storage times, the composition of honey might change (25).

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ADULTERANTS AND THEIR IMPACT

Due to the high cost of natural honey, beekeepers and merchants around the world are encouraged to adulterate honey, which lowers the quality of the honey while increasing the quantity that is sold for the same price as natural, authentic honey. Honey may contain cheaper components and commercial syrups, such as high fructose corn syrup, cane sugar syrup, glucose syrup, inverted sugar syrup, high fructose inulin syrup, rice syrup, and wheat syrup. The quality and value of honey are diminished by adulteration, which also alters the chemical components and damages the bioactive compounds that are helpful to health (6, 30, 36). In fact, honey that has been altered by the addition of sugar may differ from a control in a number of chemical and/or biological aspects, such as enzymatic activity, electrical conductivity, and chemical concentrations (18). Other adulterating substances, like molasses inverted by corn, sugar beet, and syrups of natural origin, including maple, corn flour, and substances like soil, have been documented (14, 16, 20). According to some writers, honey adulteration may result in a decrease in the amount of protein in the honey. Additionally, honey's protein level might be decreased or eliminated as a result of adulteration, warming, or lengthy storage. A significant contributor to market fluctuations in honey prices is adulteration of honey (39, 4).There have been several efforts made both domestically and internationally to identify fraud and address the issue, but there is still no real way to stop the manufacturing of tainted honey (12, 7).

TYPES OF ADULTERANTS

Corn syrup

High fructose corn syrup (HFCS), sometimes known as corn syrup, is a viscous, tasteless, and colourless liquid that is significantly denser than water. Foods are sweetened with corn syrup, a liquid sweetener made by hydrolysing cornstarch. Corn syrup is divided into three categories according to its fructose content: HFCS-42 (42% fructose), HFCS-55 (55% fructose), and HFCS-90 (90% fructose). Fructose from high fructose corn syrup must be stored in the liver as fat or glycogen since it cannot be used immediately to make energy. As a result, the body is unable to handle the excessive amounts of fructose from HFCS in a healthy way (12, 40).

Cane sugar

Sucrose, or cane sugar, is made up of the monosaccharide's glucose and fructose, two sugar molecules. The monosaccharides glucose and fructose, which have the same chemical formula but a distinct chemical conformation, are joined by a weak glycosidic bond to generate the disaccharide sucrose. The sugar cane, a perennial C_4 grass*, is typically juiced, then purified using chemical and physical methods; the water is evaporated, and the sugar crystals are separated to produce cane sugar. While nectar comes from plants with the C_3metabolic route (Calvin cycle)*, cane sugar comes from plants with the C_4 metabolic pathway (Hatch-Slack cycle) (12,38).

*C3-type plants, so named because the main stable intermediate is the 3-carbon molecule, glyceraldehyde-3-phosphate, are home to the most prevalent set of carbon fixation processes. These reactions attach CO2 to the pentose, ribulose 1,5-bisphosphate (RuBP), a compound well known as the Calvin cycle.

*The term "C_4 Plants" refers to warm-season grasses. This is due to the fact that they carry out photosynthesis via the phosphoenolpyruvate carboxylase (PEP carboxylase), a four-carbon molecule. PEP carboxylase, a photosynthetic enzyme, helps grasses to "attract" CO_2 more effectively than C_3 plants and to seal their stomates more frequently.

Palm sugar

Palm flower buds are used to extract palm sugar. It is a natural sweetener that goes through the bare minimum of steps in the purification process (free chemicals). According to one study, sucrose was the main carbohydrate in palm sugar, followed by glucose and fructose. Due to palm sugar's low glycaemic index, there is no blood sugar spike, which is a considerable benefit. The most common honey adulterant in India is jaggery syrup, which is made by extracting the sap from palm trees and then evaporating it (8, 12).

Rice Syrup

One of the most common honey adulterants in China is rice syrup (RS), a byproduct of rice polysaccharide hydrolysis and derived from a C_3 plant (similar to beet syrup). Maltotriose (52%) is the main sugar, followed by maltose (45%) and glucose (3%), in rice syrup. Because maltose and maltotriose each contain two and three glucose molecules, rice syrup behaves in the body like 100% glucose. Recently, rice syrup-tainted honey has started to appear on the market. Similar to natural honey, rice syrup undergoes photosynthesis using the Calvin cycle, which is a C_3 syrup adulterant (8,12).

The AOAC 998.12-1998 official method can distinguish adulteration with this type of C4 plant adulterants since bees create honey from C_3 plants, making it easy to detect these adulterants because they are produced from C_4 plants (19).

Since the available analysis techniques for the control of honey adulteration are based on the identification of the sugars that come from the C_4 type of plant with which they are manufactured, the discovery in the past ten years of the presence of rice syrup in honey produced in numerous nations poses a significant analytical challenge (42). Because China is the world's top producer of honey, the discovery that commercial honey contains rice syrup recently has considerably worsened the problem of honey adulteration with rice syrup (8, 30, 11).

Inulin Syrup

A polysaccharide that is found naturally and belongs to the class of fructans is inulin. A chain of fructose residues connected to glucose at the end of the chain makes up these dietary fibres. The type of fructan is determined by the fructose molecules bond configurations. For instance, in the case of inulin, glucose has broken the chain of β2-1 connected fructose (33). Furthermore, studies deliberately mimicked honey adulteration by adding various ratios (5, 10, and 20%) of high fructose inulin syrup to a nectar honey sample. Inulin is a tiny component, making it challenging to apply traditional toxicological testing to it (12).

Maple syrup

Malic acid (up to 0.7%), glucose (up to 9.59%), fructose (up to 3.95%), other minor acids less than 0.06 ppm, moisture (26.5 to 39.4%), and primary minerals (Ca, Mg, and K) with approximate ranges of 10 to 3900 mg/L are the principal components of maple syrup (34). Due to its distinctive flavour, seasonality, manufacturing limitations, naturally fluctuating product, and concentration expenses in comparison to the relatively affordable sweeteners like cane or corn syrup, maple syrup is an expensive food item (8, 26, 12).

INDIRECT ADULTERATION

The addition of sugars to honey through bee-feeding is known as indirect adulteration of honey. The manual feeding of bees with artificial sugar at a stage at a time of scarcity is blamed for indirect adulteration. This form of adulteration can occur accidentally, such as when beekeepers make mistakes or misuse their technology, especially when it's necessary to feed the honeybee colonies prior to the winter or the honeyflow season (44). Another technique of adulteration is to overfeed bees with sugar. The water and HMF content, diastase activity, free acidity, and pH value of honeys have all been demonstrated to change when supplemental feeding with sucrose and inverted sucrose syrup is used. Direct adulteration, or feeding such solutions or even crystalline sucrose to honeybees, has recently grown to be a significant issue (12, 9, 41).

Blending

In this process, pure and rare honey of high quality is combined with less expensive honey of lower quality and nutritional value. Pure honey has increasingly been adulterated with synthetic honey in recent years. A common honey industry scam in China and Venezuela involves mixing expensive acacia honey with rape honey (cheaper honey) to boost marketing profits because the pale amber colour of rape honey closely resembles that of the yellow-coloured acacia honey (12, 27).

Feeding Bees with Syrups

Bee colonies are typically fed syrups over the winter to ensure their survival, treat disease by combining veterinary medicines with sugar syrups, or encourage the queen to produce more eggs. Despite being legal, this method is nonetheless regarded as honey adulteration. Researchers tested the capacities of various techniques to identify this kind of adulteration (29).

Honey generated by bees given a sucrose solution and displaying signals from 5-HMF, citric acid, and ethanol was differentiated using proton nuclear magnetic resonance (^{1}H NMR). To assess the variations in the saccharides composition of honey generated by feeding bees HFCS, ^{1}H NMR analysis was also carried out. The levels of endogenous sugar may significantly drop if honey bee colonies are overfed sugar syrups during the major nectar flow period (35, 23, 12).

Different syrups supplied to bee colonies can have their adulterants detected using the ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC/QTOF-MS) technique. Because extensive bee-feeding can have chemical modifications similar to those caused by the direct addition of sugar syrups to honey, it is likely that this type of adulteration was not commonly examined. Amino acids and organic acids in honey may be diminished if bees are fed sugar syrups to increase their weight (35, 23).

DETECTION METHOD IN HONEY ADULTERATION

Since honey adulterants and adulteration techniques have already been covered, the priority in honey food safety now shifts to detection methods to look into the authenticity of honey. To help the government regulate the market and safeguard the rights of the consumer, these detection techniques must be able to distinguish between pure and contaminated honey.

To identify direct adulteration, traditional studies of the chemical makeup and physical characteristics of honey are frequently utilised (44). Although they are frequently used in the honey industry, these analytical techniques take a lot of time and necessitate labourious sample preparation in addition to sophisticated analytical tools. A number of contemporary approaches can also be used to identify adulterated honey (Table 1) (12, 40).

Detection technique (markers)

· Chromatographic techniques: High-performance liquid chromatography- mass spectrometry (HPLC-MS), Gas chromatography–mass spectrometry (GC-MS): specific for bioactive compounds;

· Stable carbon isotope ratio analysis (SCIRA) ($^{13}C/^{12}C$ isotope ratio), Spectroscopic: Nuclear magnetic resonance (NMR), RAMAN, Near-infrared spectroscopy (NIR), infrared spectroscopy (IR), Others: polymerase chain reaction (PCR), Biosensors;

· Chromatographic (impurities): High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) for oligosaccharides, polysaccharides, High-performance liquid chromatography with diode-array detection (HPLC-DAD): 2-acetylfuran-3-glucopyranoside (AFGP) for rice syrup, UHPLC/Q-TOF-MS for difructose anhydrides (DFAs) (15, 30, 37, 39).

Table 1

Detection methods, along with some of their advantages and disadvantages

Method	Advantages	Disadvantages	References
Mass spectrometry (MS, Isotope-ratio mass spectrometry-IRMS)	The primary benefit of using a mass spectrometer is its exceptional sensitivity, which allows it to detect unknown chemical markers in a sample.	The system requires complicated maintenance, and the equipment is costly to buy.Additionally, in order to prepare for the studies, mass spectrometers require laborious steps including washing containers and diluting materials.	(39, 33, 30)
Chromatography	A method called gas chromatography (GC) is used to analyse the mono-, dis-, and trisaccharides in honey with a comparatively high sensitivity and resolution. This helps identify adulterants, particularly HFCS. GC has been used to identify maltose, isomaltose, and HFCS-adulterated honey. Due to its great sensitivity, GC is the method of choice for identifying HFCS adulteration. Thin-layer chromatography is useful for identifying the constituents in a given mixture, tracking the course of a reaction, and figuring out how pure a substance is.	The process of authenticating adulteration typically involves comparing measured-compound profiles with pre-established target values. Proteins, amino acids, carbohydrates, vitamins, phenolic compounds, triglycerides, chiral compounds, and pigments were all identified using LC, while naturally volatile or semivolatile molecules were analysed using GC. Chromatographic techniques call for laborious sample preparation and run the risk of reagent contamination	(39, 40, 23, 20, 10, 12, 22, 1)
Spectroscopy	NMR's non-invasive features, great repeatability, and sensitivity have led to its widespread recognition as a potent technique. Its primary benefit over other spectroscopic techniques is its ability to assign signals to particular chemical markers more powerfully, producing information on a wide range of molecules even in a single experiment. Its data collecting process is also comparatively faster and easier.	Typically, researchers would dissolve honey samples in various chemicals to prepare samples for NMR examination. While NMR can quantify numerous chemical constituents simultaneously from a single spectrum, it is not able to process multiple honey samples at once. MR requires highly skilled experts to be present on site and is more costly than other spectroscopic techniques.	(39, 43)
Infrared Spectroscopy (IR)	Because spectroscopic techniques are non-invasive, intact food samples can be analysed. Rapid and non-destructive, NIR spectroscopy is a valuable approach for assessing sample adulteration in honey and may find application as a screening method in honey quality control. This device is used to obtain the spectra of samples and compositions in order to identify and measure the amount of adulteration present in honey samples.	Because multiple chemicals are present, the results obtained from this type of examination are quite complex. A limitation shared by infrared spectroscopy is the ability to examine only one spot of the studied sample at a time.	(11, 14, 39, 40, 2, 31)
Spectroscopy using Raman	An effective, quick, affordable, and efficient method for assessing the chemical characteristics of honey and ensuring its quality is Raman spectroscopy. The primary benefits of Raman spectroscopy include the minimal sample amount needed, the quickness of analysis, the excellent data repeatability, and the ability to eliminate interference from the water molecule.	Raman spectroscopy is limited to processing one sample's spectrum data at a time, and the spectrum data only pertains to one sample point—which may not be homogeneous throughout. In the event that additional data points are needed from a single sample, the laser light may result in extreme heating that destroys the sample or masks the Raman spectrum.	(44, 38)
Fluorescence Spectroscopy	Its quickness and non-destructiveness, along with its straightforward operation. An effective method for determining the authenticity of honey. Fluorescence spectroscopy provides 100–1000 times more sensitivity than other absorption-based spectroscopic techniques	It hasn't become very popular yet, particularly when it comes to tracking down adulterated honey. A disadvantage is the requirement for the use of potent multivariate data techniques combined with extensive fluorescence spectrum cleaning.	(43, 21)
Classical methods	Due to the professionalization of the panel of tasters and statistical interpretation, sensory analysis is still somewhat subjective and relies on human judgment. Nevertheless, it is a valuable instrument that occasionally becomes indispensable for evaluating quality.	Sometimes standard analytical techniques—such as sensory analysis, physicochemical characterization, melissopalynological analysis, etc — are insufficient to make a preliminary determination about the authenticity of honey	(16, 4, 24)
Hyperspectral Imaging (HSI)	HSI permits the simultaneous measurement of spatial and spectral data, in contrast to the other approaches. The primary benefit of HSI is its ability to capture hyperspectral data from several samples which may be heterogeneous in terms of size and shape in a single picture scan. Several samples can be gathered simultaneously thanks to the features of hyperspectral imaging.	Hyperspectral imaging can identify adulteration, but with restricted use and non-public availability. Hyperspectral cameras are expensive compared to spectroscopy For data processing tasks including picture segmentation, spectral band selection, and calibretion, HSI demands a significant amount of processing power.	(28, 13)
Biosensor	A number of benefits, including high sensitivity, adaptability, robustness, and simplicity, make voltammetry an effective electroanalytical method. Numerous researchers have thus far created cyclic, stripping, pulse, and alternating current voltammetry techniques for the examination of various organic and inorganic substances as well as antioxidative activity. Because e-noses are simple to use, affordable, and—most importantly—provide a quick analysis, they have become a popular non-destructive technique for determining the quality of food products. E-nose and e-tongue instruments are capable of being calibrated to provide objective data for crucial tasks such as quality and safety control.	There is still more research to be done, particularly in the areas of sensor technology, data processing, findings interpretation, and validation studies. The fact that the e-nose and e-tongue systems are influenced by their surroundings is a drawback. For example, humidity and temperature can affect the e-nose system and cause sensor drift, though built-in algorithms and calibration systems can help offset this.	(32, 43, 5, 3, 17)

CONCLUSIONS

Sugars like sucrose, fructose, glucose, inverted sugar syrup, maltose, high fructose corn syrup (HFCS), beet syrup, cane syrup, rice syrup, corn syrup, and barley syrup are the most often used adulterants. For each method there are advantages and disadvantages; in addition, SCIRA can only identify sugar syrups made from C_4 plants; C_3 plants are not detectable. Each of these methods adds to understanding of the different elements of authentic honey. The HMF, ash, moisture, pH, electrical conductivity, total acidity, reduction sugar. and diastase activity are among the criteria that are used to confirm that honey is natural. Aspects of adulteration, like the identification of the botanical or geographic source, the addition of sugars or water, the feeding of bees with sweets, and the mixing of high-quality and low-quality honey, can all be addressed by analytical and screening procedures. The authors recommendations, based on the review of numerous previous scientific investigations,call for the combined use of detection methods for the degree of honey adulteration analytical results. Since honey adulteration has a major negative effect on honey quality, research into various honey adulterants, adulteration techniques, and detection strategies is crucial.

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