

ADVANCES AND FUTURE DIRECTIONS IN HONEY AUTHENTICATION TECHNOLOGIES: A MULTINODAL ANALYTICAL PERSPECTIVE

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ABSTRACT:

Food authenticity has become a critical global concern due to increasing commercialization, complex supply chains, and rising consumer demand for high-quality natural products. Honey, a nutritionally rich natural sweetener with significant medicinal value, is among the most frequently adulterated food commodities worldwide, with fraudulent practices ranging from simple sucrose addition to sophisticated sugar syrup blending, indirect bee feeding, and geographical mislabeling that often evade single-parameter detection. This review critically evaluates conventional and advanced analytical techniques for honey authentication, encompassing chromatographic methods (TLC, HPTLC, GC, HPLC, HPAEC), spectroscopic approaches (FTIR, NIR, Raman, aquaphotomics), mass spectrometric platforms (LC-MS, Q-TOF-MS, EA-IRMS, LC-IRMS), and DNA-based methods. The review found that no single technique offers universal detection capability; chromatographic and isotopic methods excel in identifying specific adulterants but are costly and infrastructure-intensive, while spectroscopic tools combined with chemometrics offer rapid screening but lack confirmatory power for low-level or concealed fraud. A multi-layered framework combining rapid non-destructive screening followed by targeted chromatographic-MS and isotopic confirmation emerges as the most robust strategy for reliable authentication. Critical gaps remain, including the absence of standardized reference databases, limited validation of methods across diverse honey matrices, inadequate regulatory harmonization between FSSAI, Codex, APEDA, and EU frameworks, and insufficient detection tools for C3 syrup adulteration and indirect feeding practices. Addressing these gaps through integrated, internationally harmonized analytical approaches is essential for strengthening honey quality assurance and protecting global trade integrity.

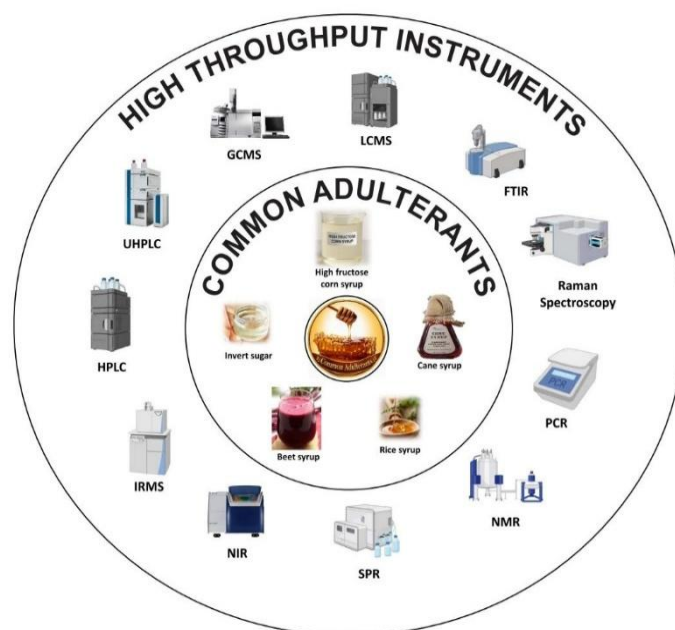


Figure 1: High-throughput instruments for reliable detection of honey adulterants

KEYWORDS: Honey authentication, Food adulteration, Quality parameters, Spectroscopic techniques, Chromatographic analysis, DNA-based methods, Chemometrics

1. INTRODUCTION:

Food authenticity is a critical dimension of food quality, increasingly challenged by the globalization of food trade and the structural complexity of agri-food supply chains. Food fraud prevention encompasses regulatory policies, risk-based control measures, and coordinated monitoring systems aimed at maintaining food safety and integrity. In the European Union, structured surveillance programs and enforcement actions are implemented to detect, deter, and mitigate fraudulent practices across the food chain (European Commission, 2021).

Honey is the naturally produced organic sweetener composed primarily of carbohydrates (70-80%), chiefly fructose and glucose, water, organic acids, proteins, enzymes, minerals, vitamins, etc., (Puscion-Jakubik et al., 2020). Its global market was valued at approximately USD 8.58 billion in 2021 with a projected annual growth rate of 5.2% (Grand View Research, 2022).

The increasing global demand for honey, coupled with constrained production has created supply chain vulnerabilities that have encouraged fraudulent practices, resulting in the circulation of adulterated honey in global markets (Jaafar et al., 2020). According to Codex Alimentarius standards, honey intended for commercial sale must be free from added ingredients, flavoring, artificial aroma, or any evidence of fermentation.

Adulteration alters the chemical composition of honey, reduces its bioactive constituents, and diminishes both product quality and commercial value, with consequent economic harm to producers and erosion of consumer confidence (Geana and Ciucure, 2020). Accordingly, accurate and reliable analytical techniques are essential for large-scale honey authentication. Various methodologies for honey authentication and adulteration detection have been extensively reviewed by different authors and physicochemical extraction methods including protein extraction and characterization by mass spectrometry (Chua et al., 2013); sugar syrup adulteration and geographical origin determination (Ulberth, 2016); isotope ratio mass spectrometry and electrochemical approaches (Trifković et al., 2019); nuclear magnetic resonance (NMR) applications (Siddiqui et al., 2017); comparative assessment of conventional and modern tools for detecting sugar adulteration (Wu et al., 2017); and portable, field-deployable detection strategies (Naila et al., 2018).

The present review provides a critical evaluation of regulatory frameworks governing honey authenticity, conventional and advanced analytical techniques for detecting adulteration and origin misrepresentation, and emerging rapid approaches supported by chemometrics and molecular tools. The aim is to identify current methodological limitations and propose directions for strengthening honey authentication within food safety and regulatory systems.

2. Honey Standards

According to Codex Alimentarius (2001) (CODEX STAN 12-1981), honey moisture content must not exceed 20%, at least 60g of total sugars per 100g and not more than 5g of sucrose per 100g. Free acidity should not exceed 50 milliequivalents (meq) acid per 100g, while the diastase activity must be ≥ 8 Schade units. The level of 5-hydroxymethylfurfural (HMF) is limited to 40 mg per kg. The electrical conductivity must be ≤ 0.8 milliSiemens (mS) cm⁻¹ and the water insoluble fraction should not surpass 0.1g per 100g of honey

2.1 Honey Quality

Honey quality is determined by key physicochemical parameters including water content, sugar composition, HMF, acidity and diastase activity (Olugbenga and Obasanmi, 2014; Pasias et al., 2017). Honey's density, extraction technique, and level of maturity are all strongly correlated with its water content. Enzymatic and thermal quality indicators such as invertase activity, diastase number, and hydroxymethylfurfural (HMF) content are frequently used to assess freshness and heat-induced deterioration (Pasias et al., 2017). However, depending on the botanical origin, naturally low diastase levels may also exist (Ouchemoukh et al., 2007). Increased HMF readings typically indicate inappropriate storage conditions, aging, or excessive heating (Khalil et al., 2010). Electrical conductivity (EC) is impacted by the amount of organic and mineral acids (Sohaimy et al., 2015). Honey with pH of less than 3.5 is more vulnerable to microbial deterioration and fermentation and the primary source of acidity is organic acids such as gluconic acid and their related lactones and ester derivatives, along with inorganic ions such as phosphates and chlorides (Sohaimy et al., 2015).

2.2 Quality parameters for honey importation

The import quality parameters of honey are presented in Table 1.

FSSAI: Food Safety and Standards Authority of India

Codex: Codex Alimentarius

Table 1. Import quality parameters of honey

Parameter	FSSAI Limit	Codex Limit	Notes
Moisture Content	$\leq 20\%$	$\leq 20\%$ (Heather 23%)	Prevents fermentation

Hydroxymethylfurfural	≤80 mg/kg	≤40 mg/kg (tropical 80 mg/kg)	Indicator of freshness and heat damage
Diastase Activity	≥8 Schade units	≥8 Schade units (≥3 low enzyme)	Enzyme integrity
Sugar Content	Sucrose ≤5%, reducing sugars ≥60%	Sucrose ≤5%, reducing sugars ≥60%	Adulteration detection
Free Acidity	≤50 meq/kg	≤50 meq/kg	High acidity may indicate fermentation
Electrical Conductivity	Blossom honey ≤0.8 mS/cm	Blossom honey ≤0.8 mS/cm	Differentiates blossom from honeydew honey
	Honeydew honey ≥0.8 mS/cm	Honeydew honey ≥0.8 mS/cm	Higher conductivity in honeydew due to mineral content
Water insoluble matter	≤0.3%	≤0.1% (pressed honey ≤0.5%)	Purity assurance
Proline Content	≥180 mg/kg	≥180 mg/kg	Marker of honey maturity and authenticity
Ash Content	≤0.6%	≤0.6%	High ash content may indicate adulteration or contamination
Microbial Contamination	<1000 cfu/g (aerobic plate count), absence of pathogens	Absence of pathogens and limits on microbial loads	Ensures hygienic quality and safety
Sensory Characteristics	Clear, free of fermentation taste/odour	Clear, free from fermentation and off-flavours	Ensures organoleptic quality

Source: FSSAI, 2023; FSSAI, 2025; Codex 1981; Fakhlaei et al., 2020.

2.3 Quality parameters for honey exportation

The export quality parameters of honey are presented in Table 2.

FSSAI: Food Safety and Standards Authority of India

APEDA: Agricultural and Processed Food Products Export Development Authority

Codex: Codex Alimentarius

EU: European Union

Table 2. Export quality parameters of honey

Parameters	FSSAI Standards	APEDA requirements	Codex Standards	EU Standards	Notes
Moisture Content	≤20%	≤20%	≤20%	≤20%	Prevents fermentation and quality degradation
Hydroxymethylfurfural	≤40 mg/kg	≤40 mg/kg	≤40 mg/kg	≤40 mg/kg	Ensures freshness, prevents heat damage

Diastase activity	≥8 Schade units	≥8 Schade units	≥8 Schade units	≥8 Schade units	Indicator of enzymatic activity and freshness
Sugar profile	Reducing sugars ≥65%, sucrose ≤5%	Compliant with FSSAI and Codex	Reducing sugars minimum 60%	Sucrose ≤5%, fructose + glucose ≥60%	Detects adulteration and confirms honey type
Microbiological limits	Absence of pathogens (fungi, bacteria), <1000 cfu/g total count	Must comply with importing country standards	Limits on pathogenic bacteria	Limits on microbial contamination	Ensures hygienic and sanitary quality
Labelling requirements	Floral source, origin, grade, net weight, processing date	Mandatory adherence to FSSAI & Codex	Codex labelling guidelines	EU labelling based on EU regulation 1169/2011	Traceability and consumer information
Packaging & Storage	Food grade packaging, storage <25°C	Food-grade & hygienic packaging	Protective packaging standards	Protective packaging & cold chain, if applicable	Preserves quality during transport

Source: FSSAI, 2023; FSSAI, 2025; APEDA, 2025; EU, 2001; Codex 1981.

3. Honey Adulteration

The substances commonly used for honey adulteration are low-cost sugars and commercial syrups such as corn syrup, high-fructose corn syrup, glucose syrup, sucrose syrup, inverted syrup and high fructose inulin syrup (Ismail and Ismail, 2018). The selection of honey adulterants is influenced by three factors: geographical origin, economic benefits and the local availability of sugars or sweeteners (Fakhlaei et al., 2020).

3.1 Sugar Syrups

3.1.1. Cane Sugar

Cane sugar made from perennial C4 grass sugarcane consists of sucrose, a disaccharide composed of two monosaccharides: fructose and glucose. Honey authentication frequently exploits differences between C3 and C4 plant photosynthetic pathways, which produce distinct carbon isotope signatures (Hatch-Slack cycle). Cane sugar is used for both direct and indirect adulteration of honey (Li et al. 2017). During direct adulteration, cane syrup is added to the honey and in the indirect method, the bees are fed with syrup.

3.1.2 Corn Sugar

High-fructose corn syrup, is a thick, colourless, odourless liquid with a density higher than that of water. Corn syrup is a liquid sweetener used in cuisine that is produced by hydrolyzing corn starch (ECA, 2010). The fructose from high fructose corn syrup is stored in the liver as fat or glycogen as it cannot be used immediately to produce energy and over consumption can lead to metabolic abnormalities in humans (Záborská et al., 2015).

3.1.3 Palm Sugar

Palm sugar is derived from the blossom buds of palm trees. It is a natural sweetener produced through minimal processing without the use of chemicals. Its primary carbohydrate constituents include sucrose, glucose, and fructose. Due to its low glycemic index (~35), palm sugar is advantageous as it prevents rapid spikes in blood glucose levels. Jaggery syrup, which is produced by evaporating palm tree sap, is the most widely used honey adulterant in India (Luis et al., 2012).

3.1.4 Invert Sugar

Invert sugar is produced by the hydrolysis of sucrose into its constituent monosaccharides; fructose and glucose (dextrose). The inversion process is typically achieved by heating sucrose syrup in the presence of acids, alkalies, or the enzyme invertase (ECA, 2010). Invert sugar derived from beet and cane plants mimics the carbohydrate profile of pure honey (Gehlawat, 2001). A prominent honey adulterant is inverted beet syrup; because sugar beets are C3 plants, their syrup closely resembles the natural glucose-fructose profile of honey, making the detection difficult (Veana et al., 2018).

3.1.5 Rice Syrup

Rice syrup, composed of glucose (3%), maltose (45%), and maltotriose (52%), is a commonly used honey adulterant in China (ECA, 2010). As a C3 plant, rice produces carbon isotope ratios when compared to those of authentic honey, rendering the syrup adulteration analytically challenging for detection (Wu et al., 2017).

3.1.6 Inulin Syrup

Inulin is a naturally occurring polysaccharide found predominantly in wheat, onions, bananas, garlic, asparagus, sunchoke, and chicory (Matute et al., 2007).

3.2 Methods of Adulteration

Commercial honey adulteration is classified into direct adulteration, indirect adulteration, and blending (ECA, 2010).

3.2.1 Direct Adulteration

Direct adulteration typically involves the addition of sucrose syrup or other sugar-based adulterants like sugar beet, high fructose corn syrup, maltose syrup, or industrial sugar syrups (glucose and fructose) which are produced by heat, enzyme, or acid treatment of starch directly to pure honey (Mehryar et al., 2011). This practice adversely affects both producers and consumers by compromising product integrity and economic value (Wu et al., 2017). Additionally, pure honey is adulterated with various proportions of inverted sugar syrups resulting in directly contaminated honey (Guler et al., 2014).

3.2.2 Indirect Adulteration

Indirect adulteration occurs when bees are fed with sugar syrups during the nectar flow period (Chen et al., 2014). Through this process, substances such as industrial sugars and pesticides are processed through the bees' digestive tracts and incorporated into the honey (Bogdanov et al., 2008). During the major nectar flow phase, bee colonies are fed with excessive amounts of sugar syrup (Guler et al., 2014). The colonies are placed in empty beehives with bees, brood, and honey frames and standard bee-feeding techniques are used during early spring (Chen et al., 2014). Over 95% of the sugar syrup feed is converted to fructose and glucose, which may not significantly alter the primary sugar profile of the final honey (Guler et al., 2008). Excessive sucrose levels in honey samples are the indicator of adulteration. Prolonged sugar syrup feeding provides the same outcomes as direct adulteration, (Damto, 2019).

3.2.3 Blending

High-quality honey is combined with less expensive and less nutritious honey. In recent years, the adulteration of pure honey using synthetic honey has become common (Cordella et al., 2005). Since the light amber color of rape honey is quite similar to that of the yellow-colored acacia honey, combining the expensive acacia honey with the less expensive rape honey is a well-known deception in the honey industry in China and Venezuela (Irawati et al., 2017).

4. Analytical techniques for honey authentication

4.1 Advanced chromatographic techniques

4.1.1 Thin-layer chromatography and High-performance thin layer chromatography

Thin-layer chromatography (TLC) is the first analytical method for detecting high-fructose corn syrup (HFCS) adulterated honey. TLC method with charcoal–Celite column was used to isolate the oligo and polysaccharides (Kushnir, 1979). In silica-gel TLC, adulterated honey displays more spots or streaks, whereas pure honey produces just one or two blue-grey or blue-brown spots (Kushnir, 1979). The increasing variety of adulterants, reduced the use of TLC but set the stage for contemporary methods (Wu et al., 2017). High-performance thin-layer chromatography (HPTLC) provided improved detection limits, greater separation efficiency, and wider applicability (Ullah and Mohammad, 2020). HPTLC enhanced the chromatographic separation of glucose, fructose, and sucrose. HPTLC-PCA techniques enhanced the phenolic compound separation and HPTLC fingerprinting of lipophilic fractions identified the botanic origin of monofloral honey (Stanek et al., 2019). Artificial Neural Network (ANN) models and High-performance thin layer chromatography-Partial least square regression (HPTLC-PLSR) methods are employed to identify adulteration with syrups like rice, corn, glucose, and maple at different quantities (Milojkovic-Opsenica et al., 2022).

4.1.2 High-performance anion-exchange chromatography

High-performance anion-exchange chromatography (HPAEC) resolved a wide range of alditols, amino sugars, mono-, oligo-, and polysaccharides and was considered as a cost-effective alternative for identifying HFCS or corn syrup in honey (Morales et al., 2008). HPAEC coupled with pulsed amperometric detector (PAD) is used to determine the proportion of oligosaccharides such as maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7) (Wu et al., 2017) and gel permeation chromatography (GPC) for monosaccharides and disaccharides in honey (Morales et al., 2008). The oligosaccharide fraction analysis can identify the adulterants when HPAEC-PAD is coupled with multivariate analysis like Principal Component Analysis (PCA)/ Linear Discriminant Analysis (LDA) and Artificial Neural Networks (ANN) and effectively classifies floral honey species (Wu et al., 2017).

4.1.3 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) detects higher levels of HMF, ash, and calcium in syrup adulterated honey (Aal et al., 1993). HPLC coupled with refractive index detection (HPLC-RID) is easy and affordable to detect both C3 and C4 starch syrups. (Wang et al., 2015; Cotte et al., 2003). Changes in viscosity and viscoelastic qualities of honey due to adulteration with sucrose and fructose syrups can be identified by rheological analyses and HPLC-RID. HPLC coupled with diode array detector (HPLC-DAD) quickly identifies honey contaminated even with 10% rice syrup (Xue et al., 2013). HPLC coupled with electrochemical detection (HPLC-ECD) identifies the adulteration of expensive acacia honey with inexpensive rape honey because rape honey has larger amounts of ellagic acid (27.16 mg/kg) than acacia honey (0.27 mg/kg) (Wang et al., 2014).

4.1.4 Advanced liquid chromatography techniques

Advanced liquid chromatography techniques are pivotal in detecting honey adulteration as they identify complex syrup-based adulterants. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) identifies organic acids like gluconic acid, malic acid, citric acid, succinic acid, and tartaric acid in honey (Suto et al., 2020). Ultrahigh-performance liquid chromatography in conjunction with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) with difructose anhydrides (DFA) is used to detect beet and HFCS adulterants (Du et al., 2015). The combination of LC with IRMS can authenticate honeys containing non-extractable proteins (Dong et al., 2018). Furthermore, LC-IRMS is capable of detecting C4 sugar adulteration even in samples with anomalous sugar levels (Dong et al., 2016).

LC-MS and LC-IRMS techniques provide strong chemometric discrimination, reduced detection limits, and precise molecular fingerprints. Together, these characteristics establish mass spectrometric techniques based on liquid chromatography as crucial instruments in the developing field of honey authentication (Brar et al., 2023).

4.1.5 Gas Chromatography

Gas chromatography (GC) is an extensively used, highly sensitive method for detecting mono-, di-, and trisaccharides in honey, allowing for the accurate identification of syrup adulteration, especially from maltose-based sweeteners and high-fructose corn syrup (HFCS) (Doner et al., 1979). GC can identify the addition of industrial sugar syrups to monofloral honeys such as lavender, acacia, and chestnut (Cotte et al., 2003). HFCS and invert sugar syrups contain difructose anhydrides (DFAs) which are formed during caramelization and heating processes but are absent in pure honey (Saito and Tomito, 2000).

GC-MS analysis detects high-fructose inulin syrups (HFIS) and since inulotriose is absent in real honey, its presence indicates the adulteration (Matute et al., 2010). Additionally, GC-MS is able to differentiate honey produced from bees fed with high fructose corn syrup, based on the presence of fructosyl-fructose isomers, which are absent in honeys from free-flying bees. Headspace gas chromatography ion mobility spectrometry (HS-GC-IMS) in conjunction with chemometrics like Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), analyzes honey authenticity as an alternative to ¹H NMR profiling. Combination of Box-Behnken design with response surface technique (BBD-RSM) and Ion mobility sum spectrum (IMSS) are suitable for identifying and measuring the adulterants such as brown cane sugar syrup, fructose syrup, rice syrup, invert syrup, and HFCS. IMSS is also used to differentiate the composition of volatile organic compounds (VOCs) of the pure and contaminated honey (Brar et al., 2023).

4.2 Spectroscopic and Spectrometric Approaches

4.2.1 Infrared-based Spectroscopy

Infrared (IR) spectroscopy, encompassing both near-infrared (NIRS) and mid-infrared (MIRS) techniques, has emerged as a rapid, non-destructive, economical, and highly reliable tool for detecting honey adulteration. Advanced chemometric methods are crucial for precise classification and quantification of honey, as honey is a chemically complex matrix with overlapping spectral signatures (Wu et al., 2017). NIR diffuse reflectance spectroscopy using a Discriminant Partial Least Squares (DPLS) model detects pure and adulterated honey (Chen et al., 2011). NIR transreflectance spectroscopy using soft independent modeling of class analogy (SIMCA) and PLS accurately categorize the beet syrup and HFCS adulteration (Kelly et al., 2006).

NIRS coupled with PCA and aquaphotomics combined with NIR spectroscopy were used to examine the adulteration of stingless bee honey with apple cider vinegar and water (Raypah et al., 2022; Brar et al., 2023). AI-enhanced aquaphotomics detects greater H-bond structured water molecules in pure honey compared to sugar syrups (Yang et al., 2020). IR-based spectroscopy offers a rapid, accurate, and non-destructive approach for assessing C3 and C4 sugar adulteration in honey. Its portability and minimal sample preparation requirements make it a promising technique for on-site honey authentication (Brar et al., 2023).

4.2.2 Quadrupole time-of-flight mass spectrometry

Quadrupole time-of-flight mass spectrometry (Q-TOF-MS) is a sophisticated analytical tool for identifying pollutants and adulterants in honey at low levels. Targeted adulterants as well as unknown metabolites, degradation products, and contaminants can be identified and quantified (Wang and Leung, 2007). Q-TOF-MS is used to distinguish botanical and geographical origins of honey using metabolomics. UPLC-Q-TOF-MS effectively distinguishes monofloral and polyfloral honeys within regions as well as monofloral honeys from various geographical locations (Jandric et al., 2015). This method successfully detects residual syrups in honey even after bees are fed with syrups (Du et al., 2015).

4.2.3 Elemental analyzer isotope ratio mass spectrometry

Elemental Analyzer Isotope Ratio Mass Spectrometry (EA-IRMS) is the most popular isotopic technique for confirming the authenticity of honey, particularly when adulteration involves C4-plant-derived syrups like cane or maize sugar. This approach analyzes the distinct ¹³C/¹²C isotope ratios of carbohydrates that replicate the natural honey sugar profile. Since honey protein originates exclusively from bees, its $\delta^{13}\text{C}$ signature acts as a reliable internal reference (Kawashima et al., 2019). Adulteration using C4 derived syrups shifts the $\delta^{13}\text{C}$ values of bulk honey towards less negative values, while the protein $\delta^{13}\text{C}$ signature remains unchanged, enabling detection of adulteration through this isotopic discrepancy (Vetrova et al., 2017).

4.2.4 DNA-based methods

Botanical and geographical origin authentication

Botanical authenticity of lavender honey is determined by DNA barcoding combined with high-resolution melting (HRM) (Soares et al., 2018) and rape honey by real-time loop-mediated isothermal amplification (LAMP) assay (Wang et al., 2020). Adulteration of honey with corn syrup is detected by an ultra-rapid real-time PCR (R-qPCR) system (Truong et al., 2022) and with rice molasses is detected by agarose gel electrophoresis and real-time PCR (Gregorio et al., 2017). DNA metabarcoding and metagenomics are used to investigate plant, bacterial, and fungal taxa in honey which discriminate honey origins through DNA-based profiling (Wirta et al., 2021).

Entomological origin authentication

Honey produced by different *Apis* species is discriminated by exon-primed intron-crossing (EPIC) markers targeting the single-copy adenine nucleotide translocator (ANT) gene (Moskric et al., 2021). Further validation of entomological origin is achieved using mitochondrial ND2 gene primers that produced species-specific amplicon sizes for *A. cerana*, *A. mellifera* and *A. dorsata* (Mohamadzade et al., 2022).

4.3 Isotope and elemental profiling techniques

4.3.1 Stable carbon isotopic ratio analysis

One of the most popular methods for identifying honey adulteration with C4 derived sugars such as cane and corn syrups is stable carbon isotopic ratio analysis (SCIRA). This method relies on natural differences in the $^{13}\text{C}/^{12}\text{C}$ isotope ratios of plants with different photosynthetic pathways (Cinar et al., 2014). By measuring $\delta^{13}\text{C}$ in bulk honey and comparing it to the $\delta^{13}\text{C}$ value of the honey protein fraction, which was unaffected by added sugars, SCIRA can identify beet sugar adulterants (Cinar et al., 2014). SCIRA was approved as an AOAC Official Method to identify C4 sugar adulteration and is used to identify the botanical and geographical origins of honey (Kropf et al., 2010).

4.3.2 Inductively coupled plasma mass spectrometry

Inductively coupled plasma quadrupole mass spectrometry (ICP-MS) revealed the mineral fingerprint profile of honey dominated by K, Ca, Mg, and Na which predicts the origin and botanical identity (Voica et al., 2020). Al, Cd, K, Ni, Ba, Na, Pb, Cu, Mg, and Zn were identified as the main elements in monofloral honeys (Chudzinska and Baralkiewicz, 2010). Mineral components in honey as a possible pollution indicator are analyzed using the HPLC-ICP-MS technology (Jakkielska et al., 2024).

4.4 Magnetic and mass-based techniques

4.4.1 Nuclear magnetic resonance

Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as a powerful analytical tool for honey authentication as it provides structural information for detailed characterization (Cagliani et al., 2022). This technique is quick, non-invasive, requires minimal sample preparation, and is capable of detecting all organic compounds in their native state without extraction or separation (Bertelli et al., 2010). NMR is a versatile method for assessing purity, quality, botanical and geographical origin, and adulteration of honey (Kuballa et al., 2018; Balthazar et al., 2021).

A typical ^1H -NMR spectrum of honey revealed a dominant sugar region corresponding to major mono- and disaccharides, as well as distinctive aliphatic and aromatic regions (Rachineni et al., 2022). Using spectrum deconvolution tools like Chenomx and AMIX and internal standards like DSS and TSP, quantitative NMR (q-NMR) accurately determines glucose, fructose, sucrose, mannose, proline, and 5-HMF. Magic sandwich echo and fast-field cycling NMR identified high-maltose and glucose syrup adulteration (Berk et al., 2022). Targeted and untargeted NMR measures adulterants like inulin, invert syrup, and corn/malt syrup (Cagliani et al., 2022). ^1H -NMR metabolomics identifies C3 and C4 adulterants in stingless bee honey (Yong et al., 2022) and quantifies sugars, amino acids, and carboxylic acids (Spiteri et al., 2020). Non-sugar components (NSC) in monofloral honeys, are measured using combined HPLC-NMR techniques (Ren et al., 2019).

4.4.2 Stable isotope ratio mass spectrometry

Stable isotope ratio mass spectrometry (SIRMS) detects honey adulterants, like cane and corn syrups, due to its analytical sensitivity and dependability. Carbon isotope ratios $^{13}\text{C}/^{12}\text{C}$ result from different photosynthesis cycles (C3 and C4 plants) and can be differentiated using element analyzers (EA), liquid chromatography (LC), and isotope ratio mass spectrometry (IRMS). The authenticity of honey can be determined by using the isotope ratio of various sugars such as monosaccharides (glucose and fructose), disaccharides, and trisaccharides, where a larger $\delta^{13}\text{C}$ max value denotes greater adulteration. Additionally, honey and honey protein levels of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ offer important information to differentiate distinct mono- and polyfloral honey origins (Aries et al., 2021). Combining IRMS with two-dimensional liquid chromatography (2D-LC) further improves the sensitivity and selectivity of stable isotope analysis of organic acids in honey, making 2D-LC/IRMS a reliable analytical technique for separating samples contaminated with exogenous sugars from genuine honey (Suto et al., 2019; Adhikari et al., 2025).

4.5 Vibrational Spectrometric Approaches

4.5.1 Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) has become a straightforward, quick, and affordable analytical method for identifying botanical or geographical origin and detecting adulteration (Prata and Costa, 2024). FTIR spectroscopy operating in the MIR region enhances the detection of subtle compositional differences in adulterated honey

(Blanco and Villarroja, 2002). It also offers molecular fingerprints based on the vibrational transitions of functional groups, allowing differentiation among honey samples with subtle chemical variations (Riswahyuli et al., 2020). Water content reflects harvesting maturity or dilution, while elevated sucrose indicates early harvesting or sucrose feeding (Pauliuc et al., 2021). FTIR successfully identifies adulterants like sucrose, high-fructose corn syrup, and rice syrup (Dumancas and Ellis, 2022) and also quantifies adulterants, detected thermal degradation products, and is capable of monitoring contaminants such as antibiotics using chemometrics or machine learning (Hassoun et al., 2020). FTIR-based chemometric models such as linear discriminant analysis (LDA), canonical variate analysis (CVA) and DPLS categorized honey samples adulterated with beet invert sugar, and cane medium invert sugar (Sivakesava and Irudayaraj, 2001; Irudayaraj et al., 2003). FTIR in conjunction with PCA and CA allows for the quick, non-destructive analysis of organic functional groups and inorganic ions in honey (Coury et al., 2009).

Attenuated total reflectance Fourier Transform infrared (ATR-FTIR) spectroscopy combined with principal component analysis (PCA) and partial least square (PLS) distinguishes adulteration with glucose, fructose, sucrose, and inexpensive syrups (Corripio et al., 2012). ATR-FTIR distinguishes genuine Indonesian *A. dorsata* honey from cane, and coconut sugar adulterated honey when combined with PCA (Riswahyuli et al., 2020; Brar et al., 2023) and identifies rice syrup adulteration in acacia, jujube, and linden honeys when combined with PLS (Li et al., 2020). ATR-FTIR offers a quicker substitute for pollen analysis and facilitates the characterization of honey's authenticity and crystallinity (Sivakesava and Irudayaraj, 2001). Due to varying carboxylic acid concentration, natural honey shows spectrum changes in infrared spectroscopy indicating floral, regional, and contaminant-related variability (Matysek et al., 2018). Chemical composition, sugar profile, saccharide structure, water content, and crystallization properties are consistently disclosed by ATR-FTIR, facilitating routine categorization and authentication.

4.5.2 Raman Spectroscopy

FT-Raman spectroscopy is a simple, rapid, cost-effective, and non-destructive technique for detecting adulteration, and has been used to quantify glucose, fructose, sucrose, and maltose in honey. Raman spectroscopy combined with multivariate analysis determined sugar concentrations without sample preparation or chromatographic techniques (Ozbalci et al., 2013). Raman spectroscopy was found to be capable of detecting HFCS, maltose syrup, beet invert sugar, and inverted cane sugar, and superior to IR-based methods due to reduced fluorescence interference, making it suitable for routine quality analysis and field applications (Li et al., 2012).

The combination of Raman spectroscopy and powerful machine-learning technologies increased detection capability. This can be integrated into larger spectroscopic authentication schemes due to its speed, minimal sample preparation, and compatibility with chemometrics and AI-based models (Molnar et al., 2020). HFCS and other syrup sweeteners in honey can be identified by phytochemical profiling and adulterant identification, using Raman spectroscopy. Raman spectra distinguish between pure and contaminated honey using PLS, PCA, PCR, and PLS-DA methods (Xu et al., 2020). Surface-enhanced Raman spectroscopy (SERS) with silver-coated gold nanoparticles amplifies Raman signals for quick methylglyoxal assessment in honey (Xia et al., 2021). Green Raman analysis also distinguished raw honey from heat-treated and diluted honey. It also combines the benefits of IR and NIR spectroscopy. Raman spectroscopy can examine honey samples contaminated honeys with fructose (F), glucose (G), inverted sugar (IS), hydrolyzed inulin syrup (IN), and malt must (M) (Oroian et al., 2018).

4.5.3 Near-infrared spectroscopy

Near-infrared (NIR) spectroscopy is a rapid, non-destructive analytical technique widely applied for honey authentication and adulteration detection. Unlike conventional chemical methods, NIRS analysis requires minimal sample preparation and delivers results within minutes, making it particularly suited for routine quality control screening. The technique works by measuring the absorbance of NIR radiation across specific wavelength regions, generating spectral fingerprints that reflect the physicochemical composition of the honey matrix. When combined with chemometric tools such as partial least squares regression (PLS) and principal component analysis (PCA), NIR spectroscopy can quantify adulterant levels, distinguish floral and geographical origins, and detect the addition of exogenous syrups including high-fructose corn syrup, rice syrup, and sugar cane syrup (Sivakesava and Irudayaraj, 2000; Tura and Seboka, 2000).

4.6 Biological and molecular authentication tools

4.6.1 DNA Metabarcoding

species-specific floral preferences of honey bees can be investigated by comparative metabarcoding (Namin et al., 2022). Detection of pathogens and botanical and geographical origins of honey is achieved through the amplification and sequencing of many genetic markers (Pathiraja et al., 2023).

4.6.2 Enzyme-linked immunosorbent assays

Antibiotic contaminants in honey cause hypersensitivity reactions and development of antibiotic-resistant microorganisms, are all detectable using immunoassay-based analytical methods and enzyme-linked immunosorbent assays (ELISAs). These methods are preferred for large-scale screening due to their specificity, sensitivity, low detection limits, ease of operation, and affordability. Indirect competitive ELISA (ic-ELISA), can detect chlortetracycline (Poungmalai et al., 2021), sparfloxacin (SPFX) (Li et al., 2018), salinomycin (SLM) and methyl salinomycin (MLN) (Xu et al., 2019). A semi-quantitative ELISA method detects sulfonamide residues in honey (Mehrabi et al., 2022). Immunochromatographic test strip is used to identify tulathromycin (TULA) (Liu et al., 2018), tetracyclines (TCs) and sulfonamides (SAs) (Li et al., 2021). Thiocloprid, neonicotinoid pesticide residues can be detected in honey using immunoassay method IC-ELISA (Zhu et al., 2024).

4.7 Machine Learning

Conventional adulteration detection techniques, such as melissopalynology, physicochemical analysis, and chromatographic and spectroscopic methods, are frequently destructive, labor-intensive, time-consuming, and expensive, with limited sensitivity to low-level adulteration (Maione et al., 2019; Goyal et al., 2022). Combining machine learning (ML) and hyperspectral imaging (HSI) detected the minute compositional variations linked to adulteration (Wu et al., 2022). Machine learning algorithms like Artificial Neural Networks (ANN), Support Vector Machines (SVM), K-Nearest Neighbors (KNN), Random Forests (RF), and Decision Trees (DT) process high-dimensional spectral data and classify the honey (Goyal et al., 2022). ML models in conjunction with hyperspectral imaging, successfully differentiate pure and contaminated honey (Mozaffari et al., 2019).

4.8 Sensor-based and rapid detection techniques

Sensor-based and rapid detection techniques have gained increasing attention as field-deployable alternatives to laboratory-based honey authentication methods. Among these, surface plasmon resonance (SPR) sensors utilizing silver nanoparticles have been applied to detect glucose and fructose adulteration through variations in resonance angle (Jaafar et al., 2021), while paper-based amperometric biosensors have demonstrated utility in measuring glucose levels in stingless bee honey (Julika et al., 2020). Microwave-based approaches, particularly self-complementary dipole antennas (SCDA) integrated with open-loop resonance (OLR), enable adulteration detection through dielectric changes in sugar concentration. Electronic tongue (E-tongue) systems represent another promising platform, generating electrochemical digital fingerprints for honey authentication (Pauliuc et al., 2020). E-tongue configurations incorporating silver, gold, glass, and platinum electrodes have been used to identify adulteration with corn syrup, maple syrup, inverted sugar, and rice syrup in monofloral honey (Ciursa and Orion, 2021), while zinc oxide, titanium dioxide, and silver electrode-based systems have similarly demonstrated authentication capability (Pauliuc et al., 2020). Collectively, these sensor-based platforms offer rapid, cost-effective, and minimally invasive approaches; however, further validation under real-world conditions is needed before their adoption in regulatory frameworks.

Table.3 Summary of the analytical techniques/instrumentations used for honey adulteration detection

S.No	Instruments/techniques	Adulterants detected	References
1.	Thin Layer Chromatography (TLC)	High fructose corn syrup (HFCS), rice syrup, beet sugar, jaggery	(Kushnir, 1979; Wu et al., 2017)
2.	High Performance Thin Layer Chromatography (HPTLC)	Rice, corn, maple and glucose syrups	(Stanek et al., 2019; Milojkovic-Opsenica et al., 2022)
3.	High Performance Anion Exchange Chromatography (HPAEC)	High fructose corn syrup	(Morales et al., 2008; Wu et al., 2017)
4.	High Performance Liquid Chromatography (HPLC)	Cane syrup, rice syrup, rape honey.	(Aal et al., 1993; Wang et al., 2015; Xue et al., 2013; Wang et al., 2014)
5.	Liquid Chromatography – Mass Spectrometry (LC-MS)	Syrup adulteration indicators through metabolite profiling	(Suto et al., 2020)
6.	Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MS)	Cane syrup, beet syrup, HFCS, rice syrup, starch syrups	(Du et al., 2015; Wang et al., 2015; Brar et al., 2023)
7.	Gas Chromatography (GC / GC-MS)	HFCS, high fructose inulin syrup (HFIS), palm and cane syrups	(Doner et al., 1979)
8.	Infrared Spectroscopy (NIRS / MIRS / ATR-FTIR)	Glucose, fructose, sucrose syrups, HFCS, beet syrup, apple cider vinegar, water	(Sivakesava and Irudayaraj, 2001; Kelly et al., 2006; Raypah et al., 2022)
9.	Ultra-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UPLC-QTOF-MS)	Rice syrup, beet syrup	(Wang and Leung, 2007; Du et al., 2015)
10.	Elemental Analyzer – Isotope Ratio Mass Spectrometry (EA-IRMS)	Cane sugar syrup, maize syrup	(Kawashima et al., 2019; Vetrova et al., 2017)
11.	DNA-based methods	Rice molasses, corn syrup	(Truong et al., 2022)

	(PCR, LAMP, metabarcoding)		
12.	Stable Carbon Isotope Ratio Analysis (SCIRA)	Corn syrup, cane syrup	(Cinar et al., 2014)
13.	Nuclear Magnetic Resonance (¹ H-NMR)		(Spiteri et al., 2015; Cagliani et al., 2022)
		Corn syrup, malt syrup, invert syrup, inulin syrup, glucose syrup	
14.	Stable Isotope Ratio Mass Spectrometry (SIRMS)	Detection of exogenous sugars (Corn sugar, cane syrup)	(Suto et al., 2019; Adhikari et al., 2025)
15.	Isotope Ratio Mass Spectrometry (IRMS)	Cane sugar syrup, corn syrup	(Aries et al., 2021)
16.	Fourier Transform Infrared Spectroscopy (FTIR)	Sucrose, HFCS, rice syrup	(Sivakesava and Irudayaraj, 2001; Dumancas and Ellis, 2022)
17.	Raman Spectroscopy	HFCS, maltose syrup, beet syrup, cane syrup	(Li et al., 2012; Oroian et al., 2018)
18.	Near Infrared Spectroscopy (NIR)	Corn syrup, cane syrup, rice syrup	(Sivakesava and Irudayaraj, 2000)
19.	Machine Learning + Hyperspectral Imaging	Rice syrup, beet syrup, corn syrup	(Wu et al., 2022)
20.	Sensor-based techniques (SPR, Biosensors, E-tongue)	Glucose, fructose, corn syrup, maple syrup, rice syrup adulteration	(Jaafar et al., 2021; Ciursa and Oroian, 2021)

5. Research gaps and future perspective

Despite significant progress in the development of analytical tools for honey authentication, several critical research gaps remain that limit the reliability, harmonization, and regulatory implementation of existing methodologies. Addressing these challenges is essential to counter increasingly sophisticated adulteration practices and to ensure consumer protection and market transparency. A major limitation is the inadequate detection of adulteration involving C3 plant-derived sugars such as rice syrup, beet sugar, wheat syrup, jaggery, and enzymatically modified invert syrups. Stable carbon isotope ratio analysis (SCIRA) and elemental analyzer–isotope ratio mass spectrometry (EA-IRMS) remain as the reference techniques for identifying C4 sugar adulteration. This highlights the urgent need for alternative molecular, isotopic, or metabolomic markers capable of differentiating C3-based adulterants from authentic honey matrices.

In parallel, the emergence of novel and “designer” syrups engineered to mimic the sugar composition, isotopic signatures, and oligosaccharide profiles of natural honey poses a growing challenge. Most recent studies focus on conventional adulterants such as high-fructose corn syrup or cane sugar, whereas systematic evaluations of newly developed syrups, including high-fructose inulin syrup and blended plant-based syrups, remain scarce. Future research must prioritize comprehensive characterization of these evolving adulterants across multiple analytical platforms.

Another critical gap lies in the lack of standardized and harmonized analytical protocols. The absence of unified thresholds, reference materials, and validation criteria significantly restricts global comparability and regulatory acceptance of advanced methods such as LC-IRMS, Q-TOF-MS, metabolomics, and DNA-based assays. Currently, research on honey authentication also relies heavily on single-technique approaches and there is an urgent need to move toward integrated, multi-technique authentication frameworks that combine complementary information from isotopic ratios, carbohydrate profiling, phenolic and metabolomic fingerprints, spectroscopic signatures, elemental composition, and DNA markers, all of which offer greater robustness and resilience against complex or concealed adulteration practices. While chemometric and artificial intelligence-based models have significantly improved classification and quantification accuracy, their real-world applicability remains uncertain. Future studies should emphasize large-scale, multi-origin datasets, external validation, and model interpretability to ensure reliable deployment in routine authenticity testing. The development of comprehensive, open-access reference databases represents another unmet need. High-resolution techniques such as NMR, Q-TOF-MS, and metabolomics depend on extensive libraries of authentic honey profiles and known adulterant signatures. The absence of globally harmonized spectral, isotopic, and metabolomic databases currently limits reproducibility, data sharing, and broader adoption of these advanced tools.

Challenges associated with DNA degradation during syrup processing and the low abundance of amplifiable DNA in refined adulterants necessitate further methodological refinement and validation before routine implementation. Additionally, the influence of processing, storage, filtration, thermal treatment, and fermentation on key analytical markers remains insufficiently understood. These factors can significantly alter isotopic signatures, sugar profiles, volatile compounds, and spectroscopic fingerprints, potentially leading to false positives or negatives. Future research should focus on disentangling natural and processing-induced variability from genuine adulteration signals.

Finally, most authentication studies focus primarily on analytical detectability, with minimal integration of toxicological and public health risk assessment. Future research should adopt a risk-based framework that links adulteration detection limits with potential health impacts, supporting evidence-based regulatory decision-making. Moreover, region-specific

adulteration practices, particularly those prevalent in developing markets, remain underrepresented and warrant focused investigation.

6. CONCLUSION

Honey adulteration has evolved from simple sucrose addition to sophisticated strategies involving C3/C4 syrups, indirect bee feeding, and blending practices that consistently evade single-parameter testing. Modern chromatographic, spectroscopic, isotopic, and DNA-based tools have substantially improved detection, yet each carries inherent limitations in cost, infrastructure, and discriminatory power. No single technique is sufficient. A multi-layered framework combining rapid non-destructive screening with targeted chromatographic-MS and isotopic confirmation, aligned with FSSAI, Codex, APEDA, and EU standards, represents the most robust and practical approach to ensuring honey authenticity and safeguarding international trade.

CRediT authorship contribution statement

Sahana Fathima T: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. Preetha G: Conceptualization, Supervision, Investigation, Writing – review & editing. Saminathan V R: Conceptualization, Supervision, Writing – review & editing. Rajanbabu V: review & editing. Mahalingam A: review & editing. Malathi J: review & editing. Praveenkumar C: review & editing.

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Declaration of competing interest

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Data availability

No datasets were generated or analysed in the current study.

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