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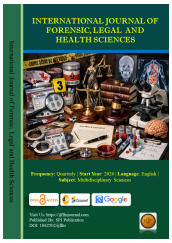

Original Research

Assessment of Adulteration in Commercial Honey Samples Through Physicochemical Analysis and Gas Chromatography–Mass Spectrometry (GC–MS)

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ABSTRACT

Honey is a widely consumed natural product that is frequently subject to adulteration, which lowers its nutritional and therapeutic value and undermines consumer trust. This study assessed the quality and possible adulteration of ten commercial honey samples using established physicochemical methods, namely reducing-sugar content, moisture (water) content, pH, and titratable acidity, complemented by gas chromatography-mass spectrometry (GC-MS) analysis of two selected samples. Reducing-sugar content ranged from 43.2% to 49.6%, indicating variation in purity and the possibility that some samples had been diluted with non-reducing sugar syrups. Moisture content was markedly elevated in Samples 5–10 (87.7%–90.8%), consistent with substantial dilution or poor storage and a heightened risk of fermentation and spoilage. Measured pH ranged from 6.29 to 8.85, with several samples exceeding the range typically reported for pure honey (3.4-6.1), suggesting possible adulteration or contamination. Titratable acidity was notably high in Samples 8, 9, and 10 (4.5, 6.0, and 6.2, respectively), in agreement with their elevated moisture content and indicative of fermentation or the addition of acidic substances. GC–MS analysis of Samples 1 and 2 identified their principal volatile constituents and indicated a comparatively higher level of adulteration in Sample 1. The results reveal considerable variation in honey quality, with a subset of samples showing strong indications of adulteration, and underline the need for rigorous quality control and complementary advanced analytical techniques to safeguard honey authenticity and consumer health.

Keywords: Honey Adulteration, Reducing Sugars, Moisture Content

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INTRODUCTION

Honey is a naturally occurring sweet substance produced by honey bees (*Apis mellifera*) from the nectar of flowers, plant secretions, or the honeydew excreted by plant-feeding insects. During honey production, bees collect these raw materials, mix them with enzymes, and store them in honeycombs, where the nectar undergoes natural ripening through water evaporation and enzymatic conversion of complex sugars into simple sugars. This process gives honey its characteristic thick texture, pleasant flavour, and long shelf life. [1] For centuries, honey has been valued not only as a natural sweetener but also for its nutritional, medicinal, and cultural importance across different civilizations. The composition of honey is highly complex and varies according to floral source, geographical location, climatic conditions, and bee species. [2] It is composed mainly of carbohydrates, with fructose (approximately 38%) and glucose (approximately 31%) accounting for the majority of its sugar content. Water typically constitutes 17–20% of honey and plays an important role in determining its viscosity, stability, and storage quality. [3] Besides sugars, honey contains small quantities of maltose, sucrose, vitamins, minerals, amino acids, enzymes such as invertase and glucose oxidase, and a wide range of bioactive compounds, including antioxidants. These constituents contribute to honey's nutritional value and are associated with its antimicrobial, anti-inflammatory, and wound-healing properties, making it a valuable food as well as a traditional

therapeutic agent. [4] Because of its natural origin, nutritional richness, and premium market value, honey is widely regarded as a symbol of purity and quality. However, its high commercial demand has also made it one of the most frequently adulterated food products. [5] Honey adulteration generally involves the addition of inexpensive substances such as glucose syrup, high-fructose corn syrup, cane sugar syrup, rice syrup, or excess water to increase product volume and maximize economic gain. Such practices not only mislead consumers but also reduce the nutritional and therapeutic value of honey by diluting its natural enzymes, antioxidants, and other beneficial compounds. [6]. Adulterated honey may also exhibit changes in flavour, aroma, colour, viscosity, and overall quality. Ensuring the authenticity of honey has therefore become an important priority for food quality control and consumer protection. A variety of analytical techniques are routinely employed to detect adulteration, including physicochemical analysis, chromatography, spectroscopy, and other chemical methods. [7,8] Physicochemical parameters such as moisture content, pH, electrical conductivity, ash content, hydroxyl-methyl-furfural (HMF), reducing sugars, and acidity provide valuable information about honey quality and can serve as useful indicators of possible adulteration. These methods are relatively simple, cost-effective, and suitable for routine quality assessment.

Recent advances in analytical science have further strengthened the ability to verify honey authenticity. Sophisticated techniques such as nuclear magnetic resonance (NMR) spectroscopy and DNA barcoding provide detailed information on the chemical composition, botanical origin, and geographical source of honey. NMR spectroscopy enables the detection of subtle compositional differences and trace adulterants that may not be identified through conventional methods, while DNA barcoding analyzes pollen-derived plant DNA to confirm floral origin and improve product traceability. [8] Although these advanced techniques offer excellent accuracy, they are often expensive and require specialized instrumentation. Considering the need for reliable yet practical approaches for honey authentication, the present study investigates the quality and possible adulteration of ten commercially available honey samples using standard physicochemical analyses. To further validate the findings, selected samples were analysed by Gas Chromatography Mass Spectrometry (GC–MS), providing a comprehensive assessment of their chemical composition and authenticity.

MATERIALS AND METHODS

Ten commercial honey samples (designated Sample 1 to Sample 10) were procured from local markets and analysed using several complementary analytical techniques, comprising the determination of reducing-sugar content, moisture (water) content, pH, and titratable acidity. Two representative samples (Sample 1 and Sample 2) were additionally examined by gas chromatography-mass spectrometry (GC-MS). The procedures and the corresponding calibration steps are described below.

Reducing-sugar content: For the determination of reducing sugars, one gram of honey was diluted with water in a 250 mL volumetric flask. The Soxhlet modification of Fehling's method was used, in which equal volumes of Solution A (copper sulphate solution) and Solution B (potassium sodium tartrate solution) were mixed. Five millilitres of each solution were transferred to a porcelain dish together with 12 mL of the diluted honey solution(9). The mixture was heated to boiling, and 1 mL of methylene blue indicator was added; boiling promotes the reaction between the reducing sugars in the honey and the copper ions in Fehling's solution. Titration was completed within three minutes, and the volume of honey solution consumed was recorded. [10] The total reducing-sugar contents obtained were: Sample 1, 48.5%; Sample 2, 46.0%; Sample 3, 48.5%; Sample 4, 46.4%; Sample 5, 44.5%; Sample 6, 49.6%; Sample 7, 43.2%; Sample 8, 48.5%; Sample 9, 43.3%; and Sample 10, 47.7%.

Calibration. Fehling's solution was calibrated using standard glucose solutions of known concentration. Titration of these standards confirmed that the solution reacted predictably with known sugar concentrations.

Moisture (water) content: Moisture content was determined by the vacuum-oven method. Two grams of honey were mixed with 5 mL of distilled water and sand in a dish, and the mixture was first heated on a boiling water bath for 30 minutes to evaporate part of the water. [11] The dish was then transferred to a vacuum oven set at 60–70 °C under reduced pressure, which removes moisture without thermal degradation of the honey constituents (12). The weight loss during this process was recorded as the moisture content. The results were: Sample 1, 30.2%; Sample 2, 26.1%; Sample 3, 16.1%; Sample 4, 27.6%; Sample 5, 88.6%; Sample 6, 89.6%; Sample 7, 89.7%; Sample 8, 90.8%; Sample 9, 88.9%; and Sample 10, 87.7%.

Calibration. The vacuum oven was calibrated using a standard moisture-determination procedure with samples of known moisture content, ensuring accurate removal and measurement of moisture [13]

pH determination: The pH of each honey sample was measured with a calibrated pH meter. [14] Pure honey typically exhibits a pH between 3.4 and 6.1, and deviations from this range may indicate adulteration or contamination. [15] The values recorded were: Sample 1, 6.39; Sample 2, 6.35; Sample 3, 8.75; Sample 4, 7.75; Sample 5, 7.54; Sample 6, 6.29; Sample 7, 8.43; Sample 8, 8.85; Sample 9, 7.74; and Sample 10, 7.13.

Calibration. The pH meter was calibrated using standard buffer solutions of pH 4.00, 7.00, and 10.00, ensuring accurate readings across the measurement range. [16]

Titratable acidity: Total titratable acidity was determined by titration. A standard sodium hydroxide (NaOH) solution (0.05 N) was prepared, and phenolphthalein was used as the indicator, turning pink in a basic medium to mark the endpoint. Each honey sample was dissolved in 75 mL of carbon-dioxide-free water and titrated with the NaOH

solution until a persistent pink colour was observed. The volume of NaOH consumed was recorded and used to express the acidity. [17-19] The results were: Sample 1, 2.2; Sample 2, 2.2; Sample 3, 2.3; Sample 4, 2.2; Sample 5, 2.5; Sample 6, 2.0; Sample 7, 2.6; Sample 8, 4.5; Sample 9, 6.0; and Sample 10, 6.2.

Calibration. The NaOH solution was standardised against a primary standard, potassium hydrogen phthalate (KHP), to establish its exact normality and ensure accurate, consistent titration results. [20]

Gas chromatography–mass spectrometry (GC–MS): Two representative honey samples (Sample 1 and Sample 2) were analysed by GC-MS. Volatile and semi-volatile constituents were separated on the gas-chromatographic column and detected by the mass spectrometer; individual compounds were tentatively identified by comparison of their mass spectra with reference spectra in the spectral library, and their relative abundance was expressed as the percentage of the total peak area.

RESULTS

The physicochemical results for all ten honey samples are summarised in Table 1. The individual parameters are discussed in the subsections that follow, and the GC–MS findings for Samples 1 and 2 are presented thereafter.

Table 1. Physicochemical parameters of the ten honey samples

Sample	*Reducing sugars (%)*	*Moisture content (%)*	*pH*	*Titratable acidity*
Sample 1	*48.5*	*30.2*	*6.39*	*2.2*
Sample 2	*46.0*	*26.1*	*6.35*	*2.2*
Sample 3	*48.5*	*16.1*	*8.75*	*2.3*
Sample 4	*46.4*	*27.6*	*7.75*	*2.2*
Sample 5	*44.5*	*88.6*	*7.54*	*2.5*
Sample 6	*49.6*	*89.6*	*6.29*	*2.0*
Sample 7	*43.2*	*89.7*	*8.43*	*2.6*
Sample 8	*48.5*	*90.8*	*8.85*	*4.5*
Sample 9	*43.3*	*88.9*	*7.74*	*6.0*
Sample 10	*47.7*	*87.7*	*7.13*	*6.2*

Titratable acidity is expressed as recorded during titration with 0.05 N NaOH; authors should confirm the reporting unit before submission.

Reducing-sugar content: Reducing-sugar content across the ten samples ranged from 43.2% to 49.6%. Sample 6 exhibited the highest value (49.6%) and Sample 7 the lowest (43.2%). Most samples fell within a comparable range, indicating a reasonable level of authenticity, although the observed variation suggests differences in floral source or minor dilution affecting sugar levels.

Moisture (water) content: Moisture content showed a marked contrast between the two groups of samples. Samples 5 to 10 displayed exceptionally high moisture content, exceeding 87.7%, which is consistent with substantial dilution or improper storage and points to adulteration with water or other liquids. In contrast, Samples 1 to 4 showed moisture contents ranging from 16.1% to 30.2%, more closely matching the levels expected for honey and suggesting better quality and storage conditions.

pH determination: The pH results indicated that Samples 3, 4, 5, 7, 8, 9, and 10 exceeded the range typically reported for honey (3.4-6.1). These elevated values suggest possible contamination or adulteration, for example through the addition of alkaline substances or degradation of the natural acidity of the honey. Samples 1, 2, and 6 lay closer to the expected range, indicating better preservation of natural acidity and a lower likelihood of adulteration.

Titratable acidity: Samples 8, 9, and 10 exhibited higher acidity values (4.5, 6.0, and 6.2, respectively), which may indicate adulteration or fermentation associated with the high moisture content observed in these samples. The remaining samples showed acidity values between 2.0 and 2.6, consistent with the natural acidity of honey and with limited adulteration.

GC–MS analysis: Two of the ten samples (Sample 1 and Sample 2) were selected for GC-MS analysis. The total-ion chromatograms are shown in Figures 1 and 2, and the principal compounds identified, with their retention times and relative peak areas, are listed in Tables 2 and 3.

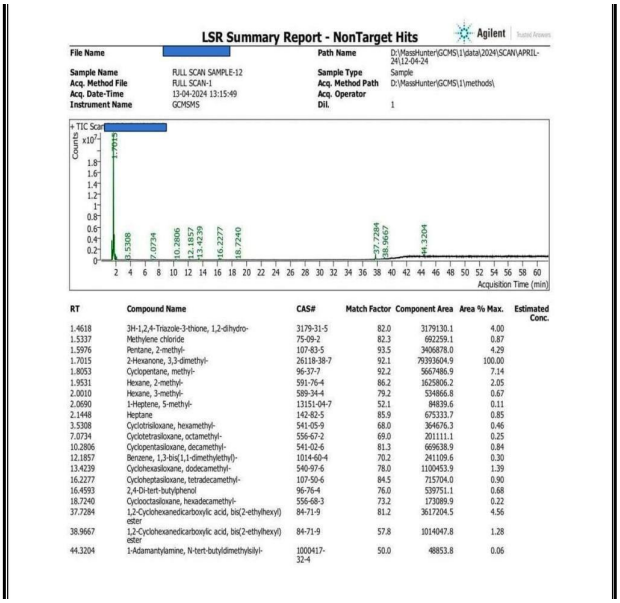

Figure 1. GC–MS total-ion chromatogram of Sample 1

Table 2. Compounds identified in Sample 1 by GC–MS

Retention time (min)	Compound name	Relative peak area (%)
1.7015	2-Hexanone, 3,3-dimethyl-	100.00
3.5308	Cyclotrisiloxane, hexamethyl-	0.46
16.2277	Cycloheptasiloxane, tetradecamethyl-	0.90
18.7240	Cyclooctasiloxane, hexadecamethyl-	0.22

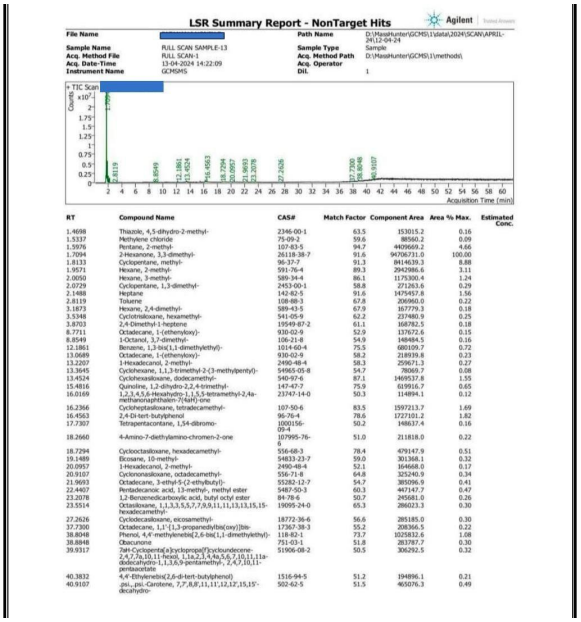

Figure 2. GC–MS total-ion chromatogram of Sample 2

Table 3. Compounds identified in Sample 2 by GC–MS

Retention time (min)	Compound name	Relative peak area (%)
1.7094	2-Hexanone, 3,3-dimethyl-	100.00
2.8119	Toluene	0.22
16.4563	2,4-Di-tert-butylphenol	1.82
38.8848	Obacunone	0.30

On the basis of the GC-MS profiles, Sample 1 was found to show a comparatively higher level of adulteration than Sample 2.

The combined physicochemical findings demonstrated a clear relationship among the evaluated quality parameters and provided strong evidence of differences in honey authenticity. Samples with higher moisture content generally exhibited elevated pH values, suggesting possible dilution with water or other adulterants that altered the natural physicochemical characteristics of honey. Such changes can negatively affect product stability, flavour, shelf life, and antimicrobial properties. Similarly, comparatively lower reducing-sugar content in several samples, particularly those with excessive moisture, is consistent with dilution using water or inexpensive sugar syrups, resulting in reduced sweetness and diminished nutritional quality. Higher titratable acidity observed in Samples 8, 9, and 10, together with their elevated moisture content, further indicates the likelihood of fermentation. Excess moisture creates favourable conditions for yeast growth and microbial activity, leading to the formation of organic acids and subsequent deterioration of honey quality. Based on the overall physicochemical profile, Samples 1–4 exhibited characteristics that were more consistent with authentic honey. These samples showed acceptable reducing-sugar content, comparatively lower moisture content, pH values closer to the expected range, and normal acidity, suggesting minimal adulteration and better product quality. In contrast, Samples 5–10 displayed substantial deviations in moisture content, pH, and acidity, indicating possible dilution, fermentation, and other forms of adulteration. Collectively, these findings suggest that the combined evaluation of reducing sugars, moisture content, pH, and titratable acidity provides a reliable approach for assessing honey quality and identifying adulterated commercial honey samples.

DISCUSSION

The present study evaluated the authenticity and quality of ten commercially available honey samples using physicochemical parameters, with GC–MS analysis performed on two representative samples. The combined findings demonstrated considerable variation among the samples, indicating that not all commercial honey products met the expected quality characteristics of natural honey. Reducing sugar content is one of the most important indicators of honey authenticity because natural honey is predominantly composed of fructose and glucose. Although the reducing sugar values observed in the present study showed only moderate variation, the comparatively lower values recorded in some samples may indicate dilution with sugar syrups or water. Such adulteration decreases the natural carbohydrate composition of honey and reduces its nutritional quality. Similar observations have been reported in previous studies, where reduced sugar content was associated with adulteration using inexpensive sugar-based additives.

Moisture content showed the greatest variation among the analysed samples and emerged as one of the strongest indicators of compromised quality. Samples 5–10 exhibited exceptionally high moisture content compared with the expected range for natural honey. Excess moisture not only suggests possible dilution but also increases the likelihood of microbial growth, fermentation, and reduced shelf life. Honey with elevated moisture is more susceptible to yeast activity, leading to quality deterioration during storage. Therefore, moisture content remains an essential parameter for assessing both authenticity and storage stability. The pH values also varied considerably among the samples. Several samples exhibited pH values higher than those generally reported for natural honey. Since honey is naturally acidic, elevated pH values may reflect adulteration, chemical alteration, or changes occurring during storage. The acidic nature of honey contributes significantly to its antimicrobial activity and preservation; therefore, deviations from the normal pH range may adversely affect both its therapeutic properties and overall quality.

Titratable acidity provided additional evidence supporting the physicochemical findings. Samples exhibiting higher acidity values also showed elevated moisture content,

suggesting the possibility of fermentation. During fermentation, naturally occurring yeasts convert sugars into organic acids, resulting in increased acidity and deterioration of product quality. The combined assessment of moisture content and acidity therefore provides a more reliable indication of honey spoilage or adulteration than either parameter alone. GC–MS analysis further complemented the physicochemical evaluation by providing information on the chemical composition of selected honey samples. The chromatographic profiles revealed differences in the detected compounds between Samples 1 and 2. Sample 1 showed a comparatively higher level of compounds suggestive of adulteration than Sample 2, supporting the physicochemical observations. Although GC–MS alone cannot confirm every type of adulteration, it serves as a valuable analytical tool for identifying unusual chemical constituents and strengthening the assessment of honey authenticity.

CONCLUSION

The present study demonstrated that the combined use of physicochemical analysis and Gas Chromatography–Mass Spectrometry (GC–MS) is an effective approach for evaluating the quality and authenticity of commercial honey samples. Significant variations were observed among the ten samples, with several exhibiting elevated moisture content, abnormal pH values, increased titratable acidity, and comparatively lower reducing-sugar content, indicating possible adulteration through dilution and fermentation. GC–MS analysis further supported the physicochemical findings by revealing differences in the chemical composition of the selected samples, with Sample 1 showing comparatively greater evidence of adulteration than Sample 2. Overall, the study highlights the importance of routine physicochemical screening complemented by advanced analytical techniques for the reliable detection of honey adulteration. Implementing standardized quality control measures and regular authenticity testing will help protect consumers, ensure product quality, and maintain confidence in the commercial honey market.

DECLARATIONS

Ethics Approval and Consent to Participate: Not applicable.
Consent for Publication: Not applicable.
Availability of Data and Materials: The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.
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