Journal of Undergraduate Chemistry Research, **2026**, 25 (1), 19

DIFFERENTIATING HONEY AND SYRUP USING ATR-FTIR SPECTROSCOPY AND CHEMOMETRIC ANALYSIS

Thomas Panzera*, Natalie D. Rodrigues* and Elmer-Rico E. Mojica†

Department of Chemistry and Physical Sciences, Pace University, New York, NY 10038

Abstract

Honey adulteration with inexpensive sugar syrups compromises product quality and consumer trust, creating a need for reliable analytical screening methods. In this study, attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy was used to differentiate authentic Grade A honey samples from a commercial syrup reference. ATR-FTIR spectra were collected for three honey products and one syrup sample and analyzed using correlation coefficients, derivative spectroscopy, difference spectra, and multivariate statistical methods. The honey samples exhibited near-identical spectra ($r \geq 0.9996$), while comparison with syrup showed reduced similarity ($r = 0.9785$). First- and second-derivative analyses and difference spectra highlighted the fingerprint region (900–1200 cm^{-1}) as the primary source of spectral differentiation. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) further confirmed clear separation between honey and syrup samples. These results demonstrate that ATR-FTIR spectroscopy combined with straightforward chemometric approaches provides an effective, rapid, and non-destructive method for qualitative honey authenticity assessment suitable for undergraduate research and instructional laboratory applications.

†Corresponding author: emojica@pace.edu *Undergraduate researchers and co-authors

Keywords: ATR-FTIR spectroscopy; honey authentication; syrup adulteration; derivative spectroscopy; principal component analysis (PCA); hierarchical cluster analysis (HCA)

Received: January 6, 2026 Accepted: January 27, 2026 Revision Received: January 30, 2026 Published: February 1, 2026

Introduction

Honey has been consumed by humans for thousands of years, with archaeological evidence suggesting its use as early as 8000 years ago. Beyond its role as a natural sweetener, honey has long been valued across cultures for its medicinal properties. Chemically, honey is a complex natural matrix composed of more than 200 distinct compounds, consisting primarily of carbohydrates predominantly fructose and glucose along with amino acids, vitamins, minerals, organic acids, enzymes, and phenolic constituents.[1,2] This chemically diverse composition underpins honey's documented anti-inflammatory, antimicrobial, antifungal, and antioxidant activities, supporting its traditional and modern use in applications such as wound treatment, gastrointestinal relief, and general health supplementation.[1,3]

Despite its nutritional and therapeutic value, not all commercially available honey products are fully authentic. Honey adulteration, commonly achieved through the addition of inexpensive sugar syrups such as corn syrup or high-fructose corn syrup, is a widespread practice driven by economic incentives.[4] While such adulterants may mimic the taste and appearance of genuine honey, their inclusion dilutes naturally occurring bioactive compounds, thereby diminishing honey's nutritional and medicinal quality.[4,5] Because adulterated honey is often difficult to distinguish from authentic honey using sensory evaluation alone, the development and application of reliable analytical methods for honey authenticity assessment are essential for consumer protection and quality control. Honey adulteration and its detection have been long-standing concerns in food chemistry, and a wide range of analytical approaches have been proposed over the past several decades.[6]

Infrared (IR) spectroscopy is a widely used analytical technique for characterizing chemical composition based on molecular vibrational behavior. In IR spectroscopy, infrared radiation is passed through a sample across a range of wavenumbers, typically from 650 to 4000 cm^{-1}. Absorption occurs when the frequency of the incident radiation matches the vibrational frequency of a molecular bond that undergoes a change in dipole moment. As a result, IR spectra provide characteristic absorption patterns associated with specific functional groups, generating a molecular "fingerprint" that can be used for compound identification and sample comparison.[7] The fundamental principles of infrared spectroscopy and molecular vibrational analysis are well established within analytical chemistry and are commonly introduced at the undergraduate level.[8] This capability makes IR spectroscopy particularly well suited for the analysis of complex food matrices, including honey, where multiple overlapping functional groups contribute to the overall spectral profile.

In recent years, attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy has emerged as a powerful and practical variant of IR spectroscopy for food authentication studies. ATR-FTIR employs total internal reflection within a high-refractive-index crystal to generate an evanescent wave that interacts with molecules at the sample surface. This configuration minimizes sample preparation, enables rapid and reproducible measurements, and reduces interference from bulk sample effects, making ATR-FTIR especially advantageous for viscous liquid samples such as honey.[9] ATR-FTIR is a well-established analytical technique with a long history of application in chemical analysis, and its methodological foundations have been extensively described in the spectroscopy literature.[10] Numerous studies have demonstrated that ATR-FTIR spectroscopy can capture subtle compositional differences related to honey authenticity, botanical origin and processing when combined with appropriate data analysis strategies.[4,11]

Recent work has shown that the discriminatory power of ATR-FTIR spectroscopy is significantly enhanced when coupled with chemometric techniques such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). For example, Rodrigues et al. (2025) applied ATR-FTIR spectroscopy together with PCA and HCA to differentiate commercially available honey types, demonstrating that although honey samples share dominant carbohydrate-related vibrational bands, subtle variations within the fingerprint region enable reliable discrimination among honey varieties.[12] Similar findings have been reported in studies examining honey adulteration with sugar syrups, where multivariate analysis revealed systematic spectral differences that were not readily apparent in raw FTIR spectra.[4,11]

Building on this foundation, the present study focuses on the qualitative differentiation of authentic Grade A honey samples from a syrup-based reference using ATR-FTIR spectroscopy combined with correlation analysis, derivative spectroscopy, difference spectra, and multivariate statistical methods. Rather than emphasizing botanical or geographical classification, this work examines whether systematic spectral comparison and complementary chemometric approaches are sufficient to distinguish authentic honey from common sugar-based adulterants despite their closely related carbohydrate profiles. By integrating univariate and multivariate analyses, this study aims to contribute to the growing body of ATR-FTIR–based honey authentication research by demonstrating a robust yet practical framework for identifying syrup-related adulteration in commercially available honey products.

Materials and Methods

Samples.

Three commercially available Grade A honey samples were analyzed as representative authentic honey products: Golden Blossom Honey (GBH), Gunter's Pure Honey (Clover) (GPHC), and Life Everyday Honey (LEH). A commercially available pancake syrup (Great Value Original Syrup) was used as a reference adulterant, as such syrups commonly contain corn syrup or high-fructose corn syrup, which are frequently used in honey adulteration.[4,5] The syrup sample was intentionally treated as a reference material rather than as a statistically representative population of adulterants, consistent with the qualitative and screening-focused scope of this study.

All infrared spectra were collected using a PerkinElmer Frontier Fourier-transform infrared (FTIR) spectrometer equipped with an attenuated total reflectance (ATR) accessory. ATR-FTIR spectroscopy was selected due to its minimal sample preparation requirements, high reproducibility, and widespread use in food authentication studies. No additional chemicals or reagents were required for sample preparation.

Infrared spectra were acquired using ATR-FTIR spectroscopy over the mid-infrared region from approximately 650 to 4000 cm^{-1}. For each measurement, a small aliquot of the liquid sample was placed directly onto the ATR crystal, ensuring full contact with the crystal surface. No dilution or chemical pretreatment of the samples was performed, allowing direct analysis of the samples in their commercial form.

Background spectra were collected prior to each set of measurements and automatically subtracted from the sample spectra by the instrument software. After each measurement, the ATR crystal was thoroughly cleaned to prevent cross-contamination between samples. All spectra were recorded under identical instrumental conditions to ensure comparability among samples.

Spectral Processing and Data Analysis.

Individual ATR-FTIR spectra were collected for each of the three honey samples and the syrup reference sample. All spectra were overlaid to enable qualitative comparison of peak positions, intensities, and overall spectral profiles. Major functional group absorptions were assigned by comparison with published FTIR spectra of honey reported in the literature.[2,4,6]

To quantitatively assess spectral similarity, Pearson correlation coefficients were calculated for all pairwise combinations of the honey spectra. Because the honey samples exhibited extremely high spectral similarity, the spectra of the three honey samples were averaged to generate a single representative honey spectrum for subsequent analyses. Spectral averaging is commonly employed in FTIR-based food authentication studies to reduce redundancy while preserving representative spectral features.[11,12] A correlation coefficient was then calculated between the averaged honey spectrum and the syrup spectrum to assess overall spectral similarity between authentic honey and the adulterant reference.

To enhance subtle differences between spectra that were not readily observable in the raw FTIR data, first- and second-derivative spectra were generated for both the averaged honey spectrum and the syrup spectrum. Derivative spectroscopy was used to accentuate changes in slope and curvature across the spectral range, particularly within the fingerprint region.

Finally, a difference spectrum was generated by subtracting the averaged honey spectrum from the syrup spectrum at each wavenumber. This approach was used to identify regions of maximum spectral divergence between authentic honey and syrup, thereby highlighting wavenumbers that may be most useful for differentiating between the two sample types. These univariate mathematical transformations are commonly used to improve discrimination between chemically similar samples in infrared spectroscopy.[11]

Multivariate Analysis.

Multivariate statistical analyses were applied to the ATR-FTIR spectral data to evaluate similarities and differences among the honey samples and the syrup reference. Principal component analysis (PCA) was performed as an unsupervised dimensionality-reduction technique to transform the original spectral variables into a smaller set of orthogonal principal components that capture the majority of variance in the dataset. PCA was used to visualize clustering behavior among samples and to identify systematic spectral differences that may not be readily apparent from univariate analysis of the raw ATR-FTIR spectra.[11,12] PCA is widely used in analytical chemistry and chemometrics to visualize patterns and clustering behavior in complex spectral datasets.[13]

Hierarchical cluster analysis (HCA) was conducted to further examine relationships among samples based on spectral similarity. HCA groups samples using a distance metric derived from their spectral data and presents the results as a dendrogram that illustrates relative similarity without requiring predefined class assignments. The clustering outcomes were used to validate and complement the grouping trends observed in the PCA score plots. HCA is commonly paired with PCA in spectroscopic authentication studies to provide an independent assessment of sample grouping.[13]

All multivariate statistical analyses were performed using Origin data analysis and graphing software (OriginLab Corporation). Identical preprocessing and analytical conditions were applied to all spectra prior to PCA and HCA to ensure consistency and comparability across samples. These chemometric techniques were selected due to their widespread application in ATR-FTIR–based food authentication studies and their demonstrated effectiveness in differentiating chemically similar samples based on subtle spectral variations.[4,11,12]

Results and Discussion

ATR-FTIR Spectral Comparison of Honey and Syrup Samples. The ATR-FTIR spectra of the three Grade A honey samples and the syrup reference are shown in **Figure 1**. All samples exhibit broadly similar absorption features, reflecting the dominant carbohydrate composition shared by honey and syrup-based sweeteners. Prominent bands were observed near 3275 cm^{-1}, corresponding to O–H stretching vibrations; around 2930 cm^{-1}, attributed to aliphatic C–H stretching; and near 1643 cm^{-1}, commonly associated with H–O–H bending related to moisture content. These assignments are consistent with previously reported ATR-FTIR analyses of honey samples from diverse botanical and geographical origins.[2,7]

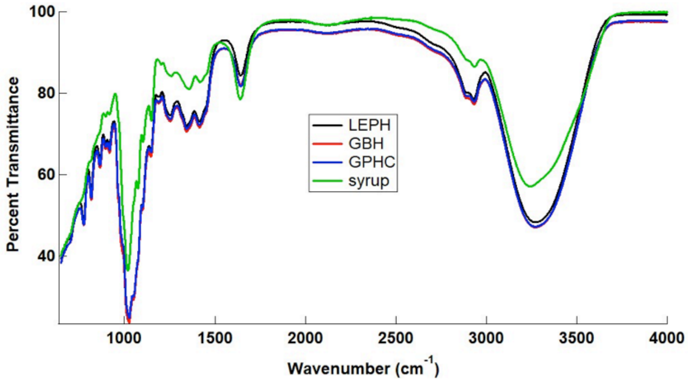

Figure 1. ATR-FTIR spectra of the three Grade A honey samples and the syrup reference, illustrating the extensive overlap in major carbohydrate-related absorption bands.

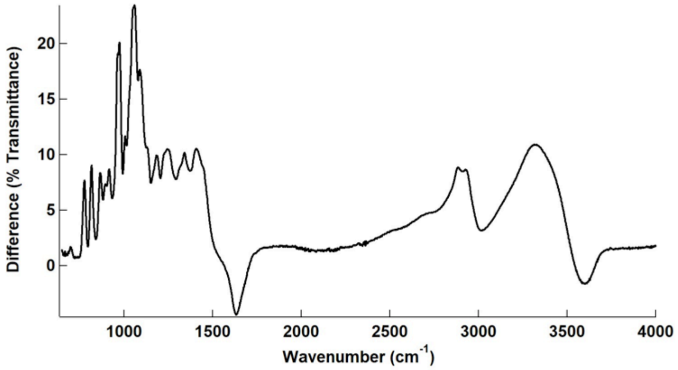

Figure 2. ATR-FTIR difference spectrum obtained by subtracting the averaged honey spectrum from the syrup spectrum, highlighting wavenumber regions of maximum spectral divergence.tion bands.

Table 1. Correlation coefficients between FTIR-ATR spectra of different honey samples, and between average honey spectra and syrup.

Comparison	Correlation Coefficient
LEPH vs. GBH	0.999936
LEPH vs. GPHC	0.999627
GBH vs. GPHC	0.999845
Avg. Honey vs. Syrup	0.978468

The substantial spectral overlap between honey and syrup is expected given their shared monosaccharide composition, primarily glucose and fructose. Corn syrup and high-fructose corn syrup contain many of the same functional groups present in natural honey, resulting in highly similar infrared absorption profiles. This overlap underscores the difficulty of relying on visual inspection of raw FTIR spectra alone for honey authentication. To visualize subtle differences masked by this overlap, a difference spectrum was generated by subtracting the averaged honey spectrum from that of the syrup, as shown in **Figure 2**.

As reported in multiple FTIR-based honey authentication studies, visual inspection of raw spectra alone is often insufficient to reliably distinguish honey from sugar-based adulterants.[4,5] Similarly, one study[9] demonstrated that even authentic honeys of differing botanical origins display nearly identical dominant ATR-FTIR absorption bands, emphasizing the need for quantitative and chemometric analyses beyond simple spectral comparison.

Correlation Coefficient Analysis and Spectral Averaging. Quantitative evaluation of spectral similarity was performed using Pearson correlation coefficients, with results summarized in **Table 1**. All honey–honey comparisons yielded correlation coefficients exceeding 0.9996, indicating near-identical spectral profiles among the commercially available Grade A honey samples analyzed. This high degree of similarity supports prior findings that authentic honeys of comparable quality cluster tightly when evaluated using infrared spectroscopy.[2,5]

In contrast, comparison between the averaged honey spectrum and the syrup spectrum produced a lower correlation coefficient (r = 0.9785), indicating increased dissimilarity relative to honey-honey comparisons. Although this value remains relatively high, it reflects the well-documented challenge that syrup adulterants closely mimic honey in their infrared signatures due to shared carbohydrate chemistry.[4] Similar reductions in correlation have been reported when honey spectra are compared with syrup adulterants, even at elevated adulteration levels.[5]

Given the near-perfect similarity among honey samples, averaging the individual honey spectra was justified for subsequent

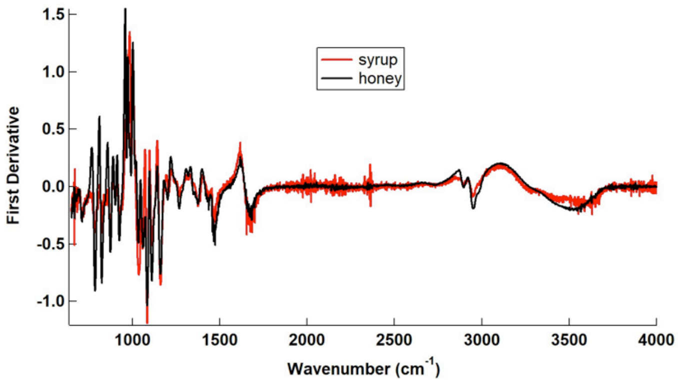

Figure 3. First-derivative ATR-FTIR spectra of averaged honey and syrup samples, emphasizing slope differences in the fingerprint region (900–1200 cm^{-1}).

analyses. Spectral averaging is a common strategy in FTIR-based food authentication studies to reduce redundancy while preserving representative spectral features.[11,12]

First Derivative Spectral Analysis.
The first-derivative spectra of the averaged honey and syrup samples are shown in **Figure 3**. Derivative spectroscopy enhances subtle differences in slope and peak shape that may be obscured in raw FTIR spectra. Clear distinctions between honey and syrup emerge within the fingerprint region (900–1200 cm⁻¹), where the honey spectrum exhibits sharper and higher-magnitude derivative features than the syrup spectrum.

The fingerprint region is widely regarded as the most diagnostically informative portion of honey FTIR spectra, as it encompasses overlapping C–O, C–C, and O–H vibrations associated with carbohydrate structures.[2,7] Derivative processing accentuates differences in band shape and intensity arising from variations in molecular environment and hydrogen bonding, rather than the presence of entirely new functional groups. Ciursă et al. (2021)[4] similarly reported that derivative processing significantly enhanced the discrimination of honey adulterated with various syrups, even when raw spectra appeared nearly indistinguishable.

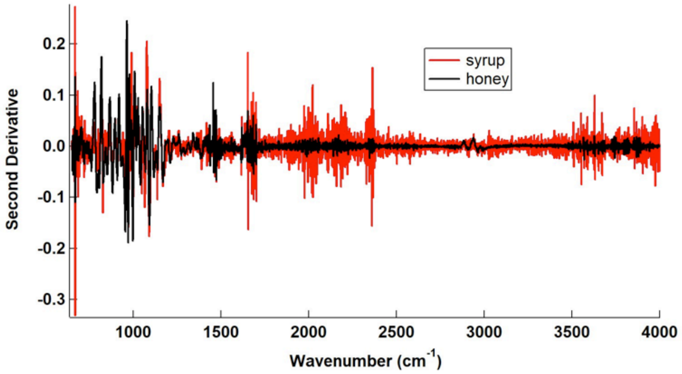

Figure 4. Second-derivative ATR-FTIR spectra of averaged honey and syrup samples, revealing enhanced curvature differences associated with carbohydrate vibrations.

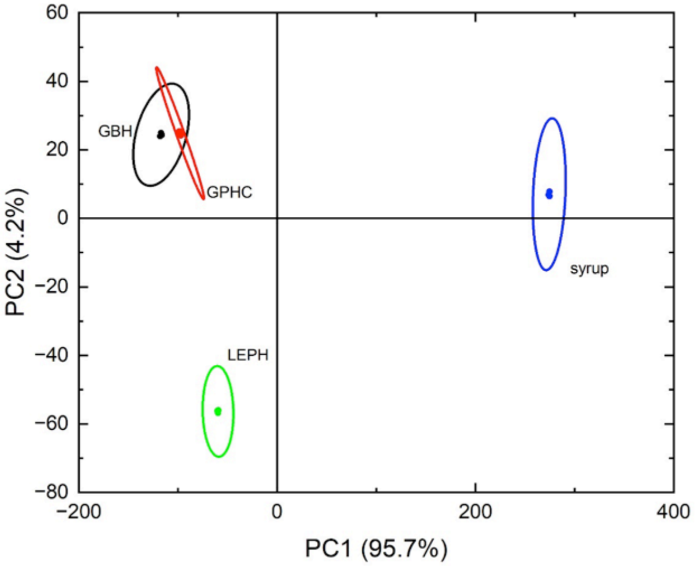

Figure 5. Principal component analysis (PCA) score plot of ATR-FTIR spectra showing tight clustering of honey samples and clear separation of the syrup reference.

In addition, the syrup sample exhibited a distinct derivative pattern between 2000 and 2500 cm⁻¹ that was less pronounced in the honey spectrum. These differences may reflect variations in molecular organization, hydrogen bonding, and processing-related structural features between natural honey and syrup-based products, rather than the presence of entirely new functional groups.

Second Derivative Spectral Analysis.
Second derivative spectra, shown in **Figure 4**, further amplified the differences observed between honey and syrup. Compared to the honey spectrum, the syrup spectrum displayed higher-amplitude oscillations across much of the spectral range, particularly within the fingerprint region. This behavior indicates greater curvature changes in syrup-related absorption bands, consistent with differences in sugar composition and processing.

Previous studies have demonstrated that second-derivative spectroscopy improves resolution of overlapping carbohydrate bands and increases sensitivity to compositional variation in complex food matrices.[5,9] Although full multivariate calibration models were not applied at this stage, the derivative patterns observed here parallel findings from PCA- and HCA-based ATR-FTIR studies, including those reported by Rodrigues et al. (2025)[12] where subtle differences became discernible only after advanced data processing.

Principal Component Analysis (PCA).
The PCA score plot derived from the ATR-FTIR spectral dataset is shown in **Figure 5**. PCA was applied to reduce data dimensionality and to visualize clustering behavior based on overall spectral variance. As commonly observed in FTIR-based food authentication studies, the first two principal components accounted for the majority of the variance, indicating that most relevant chemical information was captured in two dimensions.[5,11]

The averaged honey samples cluster tightly in the PCA score plot, reflecting their high spectral similarity as previously demonstrated by correlation analysis (Table 1). This clustering behavior supports the conclusion that the Grade A honey samples analyzed

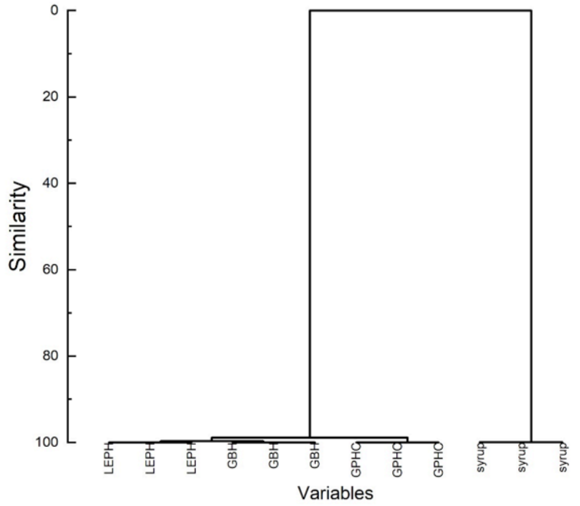

Figure 6. Hierarchical cluster analysis (HCA) dendrogram based on ATR-FTIR spectra, illustrating distinct clustering of honey samples relative to the syrup reference.

share nearly identical chemical compositions. Similar clustering of authentic honey samples has been widely reported and is attributed to the dominant influence of carbohydrate-related vibrational bands on spectral variance.[2,12]

In contrast, the syrup reference sample is clearly separated from the honey cluster along the first principal component (PC1), indicating systematic spectral differences between honey and syrup-based sweeteners. This separation suggests that, despite shared functional groups, differences in relative band intensities and molecular environments particularly within the fingerprint region, contribute sufficiently to overall variance to enable discrimination by PCA.[4,5]

Hierarchical Cluster Analysis (HCA).
Hierarchical cluster analysis results are presented as a dendrogram in **Figure 6**. HCA groups samples based on spectral distance, providing an independent assessment of similarity relationships without requiring predefined class labels. This technique is commonly paired with PCA to validate clustering behavior in ATR-FTIR food authentication studies.[7,12]

The HCA dendrogram shows that the averaged honey samples cluster together at a low distance threshold, confirming their strong spectral similarity and reinforcing the conclusions drawn from correlation and PCA analyses. In contrast, the syrup reference sample forms a distinct branch separated from the honey cluster, indicating greater spectral dissimilarity relative to honey–honey comparisons. Similar HCA-based separations between honey and syrup adulterants have been reported in previous FTIR studies.[4,5]

The strong agreement between PCA and HCA results demonstrates that the observed spectral differences between honey and syrup are systematic rather than random, strengthening the reliability of the multivariate findings.

Collectively, these univariate and multivariate analyses demonstrate that while raw ATR-FTIR spectra of honey and syrup are highly similar, mathematical enhancement and chemometric modeling reveal reproducible differences driven primarily by variations in carbohydrate-related vibrational features. While raw ATR-FTIR spectra exhibited extensive overlap between honey and syrup, mathematical enhancement and chemometric modeling revealed reproducible differences driven primarily by variations in carbohydrate-related vibrational features.

This integrated analytical strategy aligns with established approaches in the literature, where PCA and HCA are used to corroborate trends identified through derivative or difference-based FTIR analyses.[4,11,12] The agreement observed across correlation analysis, derivative spectroscopy, PCA, and HCA supports the internal consistency of the analytical approach.

Conclusion

This study demonstrates that attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy, when combined with complementary univariate and multivariate data analysis approaches, provides an effective qualitative method for distinguishing authentic Grade A honey from syrup-based sweeteners. Although raw ATR-FTIR spectra of honey and syrup exhibited substantial overlap due to their shared carbohydrate composition, quantitative correlation analysis revealed near-identical spectra among honey samples and measurably reduced similarity between honey and the syrup reference.

Derivative spectroscopy and difference spectra enhanced subtle spectral variations, particularly within the fingerprint region (900–1200 cm^{-1}), highlighting carbohydrate-related vibrational features and hydrogen-bonding environments as primary contributors to spectral differentiation. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) further confirmed these distinctions, with honey samples clustering tightly and the syrup reference forming a separate group, supporting the internal consistency of the analytical approach.

While the present investigation employed a single commercial syrup as a reference adulterant rather than controlled honey–syrup mixtures, this design reflects the qualitative and screening-focused scope of the study. The results are consistent with previously reported ATR-FTIR–based honey authentication studies and demonstrate that even relatively straightforward chemometric tools can reliably distinguish authentic honey from syrup-based sweeteners. Future work may extend this approach by incorporating multiple adulterant types, controlled adulteration series, and quantitative calibration models to establish detection thresholds and predictive capability.

Acknowledgements

This material is based upon work supported by the Pace University Course-Based Undergraduate Research Experiences Award and the Undergraduate Research and Creative Inquiry Award obtained by Thomas Panzera during Summer 2024 and Natalie Rodrigues during Summer 2025.

References

1. Eteraf-Oskouei, T.; Najafi, M. *Iranian Journal of Basic Medical Sciences* **2013**, *16*(6), 731–742.
2. Mail, M. H.; Ab Rahim, N.; Amanah, A.; Khawory, M. H.; Shahudin, M. A.; Seeni, A. *Biomedical and Pharmacology Journal* **2019**, *12*(4), 2011–2017. https://dx.doi.org/10.13005/bpj/1833
3. Khan, S. U.; Anjum, S. I.; Rahman, K.; Ansari, M. J.; Khan, W. U.; Kamal, S.; Khattak, B.; Muhammad, A.; Khan, H. U. *Saudi Journal of Biological Sciences* **2018**, *25*(2), 320–325. https://doi.org/10.1016/j.sjbs.2017.08.004
4. Ciursă, P.; Pauliuc, D.; Dranca, F.; Ropciuc, S.; Oroian, M. *Food Control* **2021**, *130*, 108266. https://doi.org/10.1016/j.foodcont.2021.108266
5. Sahlan, M.; Karwita, S.; Gozan, M.; Hermansyah, H.; Yohda, M.; Yoo, Y. J.; Pratami, D. K. *Veterinary World* **2019**, *12*(8), 1304–1310. https://doi.org/10.14202/vetworld.2019.1304-1310
6. Zhang, X.H., Gu, H.W., Liu, R.J., Qing, X.D., Nie, J.F., *Food Chemistry*, X **2023,** 19, 100850.
7. Svečnjak, L.; Biliskov, N.; Bubalo, D.; Barišić, D. *Agriculturae Conspectus Scientificus* **2011**, *76*, 191–195.
8. Stuart, B., *Infrared Spectroscopy: Fundamentals and Applications,* Wiley: Chichester, U.K., 2004, pp 1-13.
9. Fahelelbom, K. M. S.; Saleh, A.; Al-Tabakha, M.; Ashames, A.

Reviews in Analytical Chemistry **2022**, *41*, 21–33. https://doi.org/10.1515/revac-2022-0030

10. Mirabella, F.M. *Applied Spectroscopy Reviews*, **1985**, 21, 45-178.
11. Nieuwoudt, H.H., Prior, B.A., Bauer, F.F., J*ournal of Agricultural and Food Chemistry*, **2004**, 52 (12), 3726-3735.
12. Rodrigues, N.; Palomares, S. C.; Mojica, E.-R. E., *JUCR*, **2025**, *24*(1), 21–24.
13. Miller, J.N., Miller, J.C., *Statistics and Chemometrics for Analytical Chemistry,* 7th ed., Pearson, **2018**, 224-228.