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Optimizing Optical Alignment and Repeatability in SBH Spectroscopy Using a Custom Mechanical Module

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ABSTRACT

Stingless bee honey (SBH) has gained prominence in the Malaysian honey industry because of its distinct chemical composition and bioactive properties. However, the high market value of SBH makes it particularly vulnerable to economic adulteration, raising concerns over product authenticity among consumers. Adulteration in SBH is challenging to detect because conventional detection methods are often time-consuming, laboratory-bound and unsuitable for on-site screening. This study aims to evaluate how a portable Visible Near-Infrared (Vis-NIR) spectroscopic system, REVA INVITRO, can be applied for the detection of adulteration in SBH, with emphasis on the role of mechanical stabilization. The system is equipped with a custom-designed mechanical jig that holds cuvette in place, ensuring a consistent optical path length between Vis-NIR sensor and the sample. In this work, the effectiveness of REVA INVITRO was evaluated by analyzing spectral information of SBH samples contaminated with three widely used substances, distilled water (DW), apple cider vinegar (ACV), and fructose syrup (FS), at varying concentrations level. Spectral measurements acquired with and without mechanical jig were compared to assess the contribution of mechanical stabilization. Spectral trends were examined qualitatively and quantitatively to evaluate the measurements reproducibility and sensitivity. Spectral measurements obtained from using the jig achieved a classification model of 100% accuracy on the evaluation dataset, with a mean five-fold cross validation accuracy of 94.55%, indicating its robustness and consistent performance. In contrast, the classification accuracy dropped to 89.39% when measurements were acquired without using jig. For the regression models, the coefficient of determination (R^2) for honey content quantification across all adulterant type ranged from 0.995 to 0.999, with predictions error between 0.34% and 1.96%. The jig improved the regression performance by reducing the prediction error (RMSE) by approximately 45-79% across all adulterant types. These findings showed a significant improvement in spectral uniformity and dependability with use of the jig. The jig integration enables the REVA INVITRO system to deliver a stable measurement suitable for SBH analysis. Overall, the ability of the system to detect adulteration trends both visually and quantitatively supports its potential as a portable tool for on-site honey quality assessment and authentication.

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1. Introduction

Honey is a valuable natural product in the world, and commonly known due to its nutritional, medicinal and therapeutic advantages. These advantages are primarily explained by its rich composition, that contains enzymes, amino acids, vitamins, minerals and other bioactive substances [1]. Each honey has a different chemical whose composition differs based on a number of factors, which includes botanical origin, seasonal variations, mode of production and storage conditions [2]. On average, honey comprises of 38.19 g of fructose, 31.28 g of glucose, 7.97 g of maltose, 4.5 g of sucrose, 0.86 g of higher-order sugars, 17.2 g of water, and trace minerals per 100 g [3].

Bees of different species produce honey with unique physical and chemical properties from each. *Apis spp.*, such as *Apis mellifera* and *Apis cerana* which are commonly farmed, produce honey that is typically sweet, viscous, and dark brown in color. In contrast, *Trigona spp.* (stingless bees), which are native to Malaysia, produce honey that is lighter in color, more watery in texture, and characterized by a distinctive sweet-sour flavour [4,5].

Stingless bee honey (SBH) from *Trigona spp.* has been receiving an increase in attention in Malaysia over the years contributed by its unique composition and therapeutic potential. It is rich in phenolic compounds, organic acids, and antioxidants, which contribute to its reported antimicrobial, anti-inflammatory, and antioxidant activities [6,7]. These attributes of SBH made it popular among the consumer, increasing the demand for it both locally and globally.

However, despite this high demand, SBH production in Malaysia remains limited. This cause is largely contributed to the fact that stingless bee species have a biological constraint when it comes to producing honey. They naturally produce smaller quantities of honey compared to other species such as *Apis mellifera* [8,9]. Furthermore, SBH harvesting is more labor-intensive and time-consuming, resulting in a much higher cost of production. As a result, SBH is often sold at a significantly higher price that can be up to 20 times more expensive than conventional honey [7].

The combination of limitation in supply and premium pricing made SBH an attractive target for economic adulteration. To capitalize on market demand, some unethical individuals intentionally dilute *Apis spp.* honey with inexpensive sweeteners and sell the adulterated product at prices similar to the genuine SBH or even higher [10,11]. In some cases, other cheaper honey is mixed with sour-tasting liquid such vinegar to produce a sour taste resembling the taste of pure SBH. More than that, adulteration of SBH by dilution of water also occurs to increase the yield volume of the honey [4].

This practice directly violates the Codex Alimentarius Standard for honey, which defines authentic honey as a natural product to which no food ingredients may be added and from which no constituent may be removed [12]. Adulteration of food products is unethical as it deceives consumers, leads to loss of trust, and threatens product integrity, which in turn complicates quality assurance efforts on the supplier's end. Therefore, quality control becomes even more essential to reassure consumers regarding stingless bee honey.

Various methods are available in the detection of honey adulteration, one in which using the traditional methods such as chemical analysis. These methods are indeed effective but often requires laboratory facilities, are time-consuming and may not be suitable for rapid, on-site evaluation [13]. Not to mention that direct sugar analysis of adulterated honey with invert sugar or syrup may not be effectively detected because majorly, the constituents of the natural components of honey and the adulterated product would also have similar physical properties to natural honey [14].

In recent years, spectroscopy has been increasingly emerged as one of the methods used in honey adulteration detection due to its fast, simple, environmentally friendly and non-destructive in nature which preserve the honey after testing [15-17]. From previous papers, spectroscopy methods such as fourier transform infrared spectroscopy (FTIR) [4], Raman spectroscopy [18], Ultraviolet-Visible

(UV-Vis) spectroscopy [19] and more. Aliaño-González *et al*., [20] reported to have successfully done a full discrimination between non-adulterated honey and adulterated honey with invert sugar, brown sugar cane, rice syrup and fructose syrup by using Linear Discriminant Analysis (LDA). Another study by Raypah *et al.,* [21] employ the same method which is Vis-NIR spectroscopic approach but utilizing a different analytical technique, Principal Component Analysis (PCA) combined with Partial Least Square Regression (PLSR) in classifying and quantifying SBH adulteration. Despite these advances, many spectroscopic systems remain laboratory-based or rely on bulky equipment, limiting their accessibility and practicality for non-specialist users.

To address this gap, REVA INVITRO, a custom-built, portable, solvent-free, and user-friendly Vis–NIR spectroscopic device specifically designed for in-vitro measurement of stingless bee honey was introduced. The system integrates an optical sensor with a precision-engineered mechanical jig that ensures stable cuvette alignment, minimizes ambient light interference, and standardizes the optical path. This design supports consistent and accurate spectral acquisition while requiring no chemical preparation and minimal user training, making it suitable for non-destructive, field-deployable honey analysis by small-scale producers, sellers, and consumers who lack access to laboratory-based testing facilities.

The main objectives of this work are twofold:
(1) to demonstrate the device's effectiveness in detecting and classifying stingless bee honey adulteration using multivariate analysis techniques.
(2) to evaluate the impact of the jig on spectral measurement consistency.

By validating both the mechanical stability and analytical performance of REVA INVITRO, this study establishes its potential as a practical and reliable solution for on-site honey authentication.

2. Methodology

2.1 Hardware Configuration

The REVA INVITRO device employed in this study combines optical and mechanical components designed specifically for in-vitro spectral measurements. A primary mechanical feature is a specially fabricated jig that provides stable positioning and light isolation to ensure consistent spectral data acquisition. The jig incorporates a magnetic cuvette slot that securely positions the cuvette along a fixed optical path between the sensor and reflectance standard.

The modular design of the jig allows for interchangeable cuvette holders to accommodate different experimental requirements, with available optical path lengths of 2 mm, 5 mm, and 10 mm. In this study, a 2 mm cuvette slot was utilized, yielding an optical path length of 2 mm. The reflectance standard is housed within a magnetic enclosure that attaches to the opposite end of the jig, maintaining proper alignment with the sensor while reducing interference from external light sources. A fitted lid covers the cuvette slot during measurements to further minimize ambient light intrusion and improve signal stability.

2.2 Cuvette Conditioning

A quartz cuvette with a 2 mm path length was employed for sample containment throughout the experiment. Prior to each measurement, cuvettes underwent cleaning using an ultrasonic cleaner. The cleaning protocol required filling the cleaner with distilled water before activation, a critical step to prevent transducer damage from overheating. Subsequently, a beaker containing isopropyl alcohol was positioned inside the cleaner, and the cuvettes were immersed in the alcohol solution. The ultrasonic cleaning cycle operated for approximately 5 minutes. Following completion, the

cuvettes were removed from the beaker and allowed to air-dry, ensuring no cross-contamination occurred with samples during subsequent spectral measurements.

2.3 Cuvette Conditioning

To evaluate the spectral behavior of adulterated honey, a structured series of binary mixtures was prepared using SBH as the primary constituents. The honey was adulterated with three commonly used substances of adulterants, easily found in market, which are distilled water (DW), apple cider vinegar (ACV) and fructose syrup (FS). SBH was combined with each adulterant in varying volumetric ratios to simulate different levels of adulteration. A total of nine concentration levels for each adulterant were mixed with the SBH, ranging from 10% - 90% adulterant mixed with 90% - 10% SBH respectively. Thus, making the total samples of SBH-adulterant that need to be prepared to be 27 samples. In addition, six pure samples which comprise of pure adulterant for each type and three pure SBH were also prepared.

All volumes of SBH and the adulterants were measured precisely by using pipette to ensure volumetric accuracy. Each was pipetted into a beaker according to its respective ratio and stirred using a glass stirrer to ensure homogeneity. The resulting mixture was then transferred into a cuvette for spectral measurement using REVA INVITRO device. The complete composition of each mixture is provided in Table 1 below:

Table 1
Table of ratios of honey and adulterant for each sample (total volume fixed at 0.35 ml)

SBH		Adulterants		Total
%	ml	%	ml	ml
0	0	100	0.350	0.35
10	0.035	90	0.315	0.35
20	0.070	80	0.280	0.35
30	0.105	70	0.245	0.35
40	0.140	60	0.210	0.35
50	0.175	50	0.175	0.35
60	0.210	40	0.140	0.35
70	0.245	30	0.105	0.35
80	0.280	20	0.070	0.35
90	0.315	10	0.035	0.35
100	0.350	0	0	0.35

2.4 Spectral Measurement

Once the sample had been prepared, REVA INVITRO device was powered on and connected to the REVA mobile application via Bluetooth Low Energy (BLE). Two main steps were carried out where background calibration will be done and followed by sample measurement. Spectral measurement for each sample was repeated ten times each. A total of 33 samples were prepared (27 SBH-adulterant mixtures and 6 pure solutions). Thus, a total of 330 spectral measurements were stored in the REVA mobile application. Upon completion of data collection, all the spectral data in the REVA mobile application was transferred to the AINS database for further analysis. In this study, there are

two methods of spectral measurement that were implemented, one was using REVA INVITRO's jig and the other without the jig.

2.4.1 Measurement with REVA INVITRO's Jig

In this setup, background calibration was performed by placing a clean, empty cuvette into the jig's 2 mm slot, with the reflectance standard and lid components already in place as configured. The sample measurement followed by replacing the cuvette with one filled with the prepared mixture, following the same positioning. This configuration ensures consistent optical alignment throughout the process. The spectral data collected using this method was used for model development.

2.4.2 Measurement without REVA INVITRO's Jig

In this configuration, both background calibration and sample measurement were performed without the jig or reflectance standard. The cuvettes were held manually by hand in front of the sensor, which leads to a potential variation in alignment and light interaction. This setup was applied only to SBH–DW mixtures and their corresponding pure samples, serving as a basis for comparison with the jig-assisted setup. The resulting data were not used for model training but served to assess the impact of the jig on measurement reliability.

3. Results

This study aims to evaluate the difference in spectral measurement taken with the jig that is integrated into the REVA INVITRO and those taken without it. The jig was designed to hold the cuvette in a fixed position while maintaining a consistent optical alignment and minimize ambient light interference during spectral acquisition. At the end of the jig, a reflectance standard is positioned to help with the reflection of the light to not be absorbed by the material of the jig and reflect it back to the sensor. From these comparative results, it is established that the presence of the jig produces more repeatable and consistent spectral readings, which was quite essential in model development as compared to without using the jig.

3.1 Justification for Cuvette Dimensions

The proper selection of cuvette thickness plays a vital role in achieving accurate and reproducible spectral data, particularly for applications involving quantitative modeling. This study examined three cuvette thickness options, 2 mm, 5 mm, and 10 mm, to establish which configuration generated spectral outputs with the closest alignment to a golden reference spectrum established using a NIST-traceable Vis-NIR calibration standard.

To evaluate the performance of each cuvette configuration, two key metrics were analyzed which are the Pearson correlation coefficient, which assesses the similarity in spectral trends, and the Root Mean Square Error (RMSE), which quantifies the actual deviation in absorbance values from the reference. Among the three, the 2 mm cuvette showed the highest average correlation score ($r = 0.6825$), demonstrating strong agreement with the reference spectrum in terms of overall trend. Although its RMSE (0.1408) was higher than ideal, it was significantly lower than those for 5 mm and 10 mm cuvettes, indicating more accurate absorbance values.

In contrast, the 5 mm and 10 mm cuvettes showed both lower correlation values and significantly higher RMSEs (0.2342 and 0.4638, respectively), indicating substantial distortion in both spectral

shape and magnitude. These findings confirm that the 2 mm cuvette dimension offers the optimal configuration, delivering the most reliable and NIST-traceable spectral match for downstream analysis.

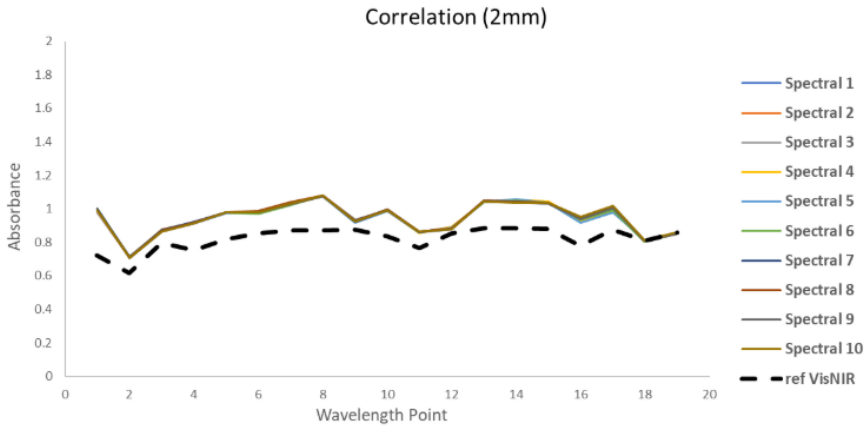

Fig. 1a. Spectral comparison of 2mm Cuvette thicknesses against the golden reference spectrum

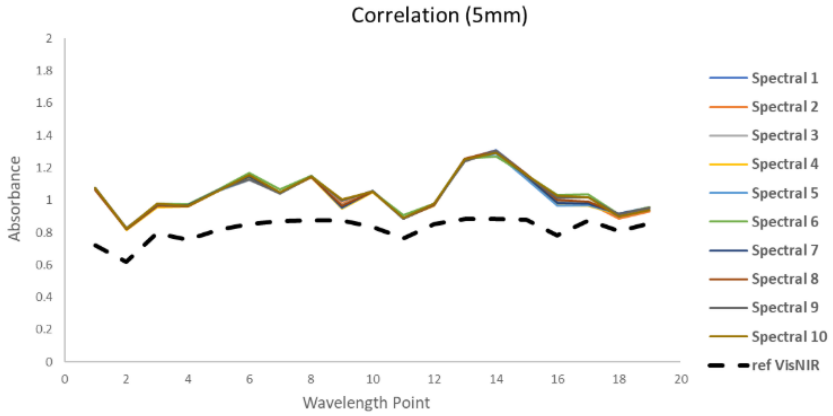

Fig. 1b. Spectral comparison of 5mm Cuvette thicknesses against the golden reference spectrum

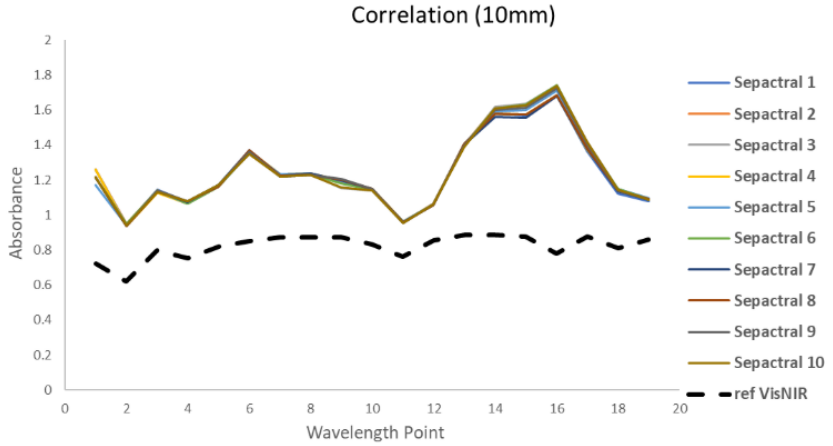

Fig. 1c. Spectral comparison of 10mm Cuvette thicknesses against the golden reference spectrum

Table 2
Table of average correlation and RMSE score for each cuvette size

Cuvette Size (mm)	Average Correlation	Average RMSE
2	0.6825	0.1408
5	0.6069	0.2342
10	0.4537	0.4638

3.2 Spectral Trends with Jig

Ensuring repeatability in spectroscopic measurements is a critical prerequisite for any application involving quantitative analysis, particularly in portable and field-deployable instrumentation. A mechanically engineered cuvette jig was included in the REVA INVITRO system to minimize the sources of variability inherent in handheld spectrometers, including but is also not limited to inconsistencies in cuvette positioning, angular misalignment and ambient light exposure. The cuvette is attached to the jig which maintains a constant position between the light source and detector with the optical path length that has been guaranteed to be standard or of the same length for each use. This significantly reduces the variability caused by the user.

All types of honey-adulterants with jig, display a smooth and consistent trends in the spectral results with increasing concentrations, which is a sign of high repeatability and low-noise measurements. In SBH-DW series (Figure 2a), one can clearly observe an upward shift in absorbance with increasing concentration of distilled water. The curves are closely aligned and stable, reflecting low spectral variation between repeated measurements. SBH–ACV (Figure 2b) shows similar consistency, with slight yet noticeable spectral variation without changing the shape of the curves. As for SBH-FS (Figure 2c), it displays even more pronounced shifts especially in the longer wavelength range, with each concentration clearly separated where it demonstrates the capability of jig to resolve fine spectral changes.

Visual observation alone is insufficient to justify the jig's effectiveness. Quantitative analysis is required to support and confirm these trends. The standard deviation (STD) of the absorbance values at each concentration was computed across ten replicate measurements. These values were then averaged across all concentrations to come up with one mean standard deviation (SD). The average values of the SDs of SBH-DW, SBH-ACV, and SBH-FS were found to be 0.00221, 0.00250, and 0.00532 respectively as shown in Table 3, indicating that a jig-supported setup produces low variability and high internal consistency across repeated measurements.

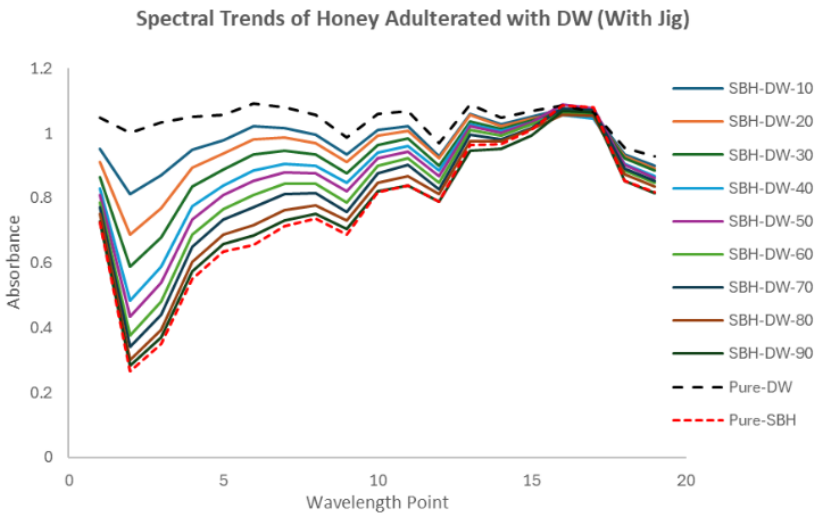

Fig. 2a. Spectral Measurements using REVA INVITRO with Jig for SBH–DW

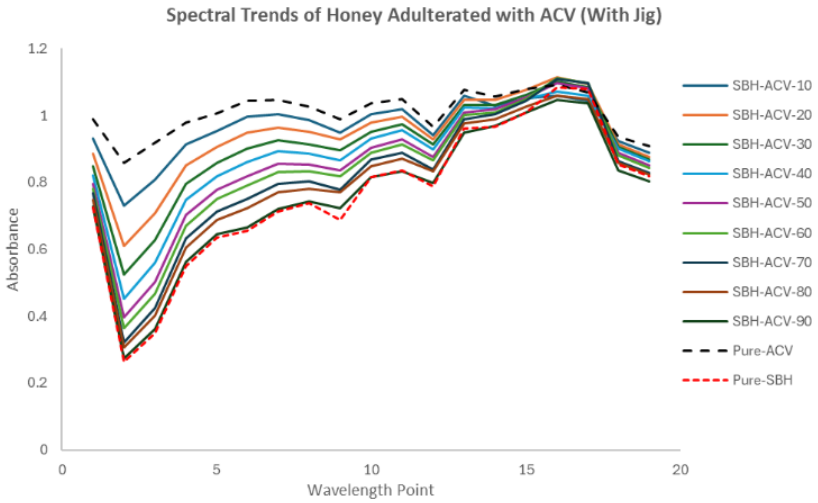

Fig. 2b. Spectral measurements using REVA INVITRO with Jig for SBH–ACV

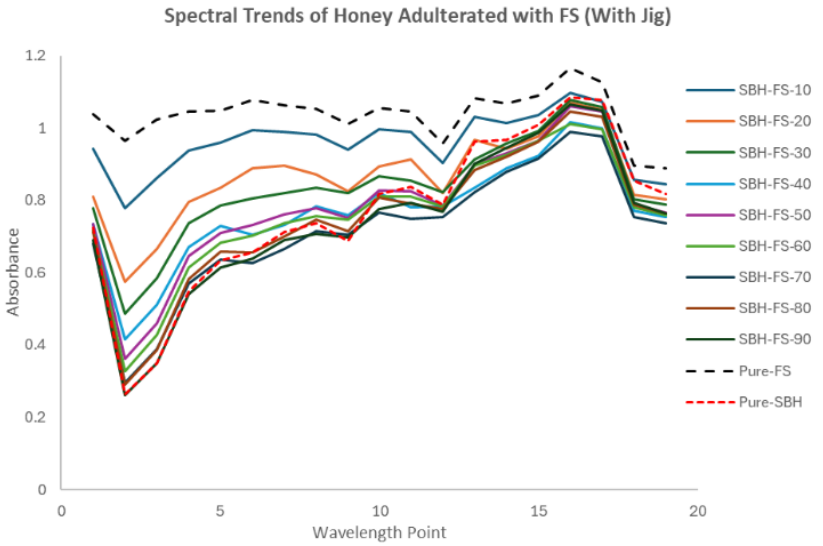

Fig. 2c. Spectral measurements using REVA INVITRO with Jig for SBH–FS

3.3 Spectral Trends without Jig

To isolate and evaluate the mechanical contribution of the cuvette jig, a control experiment was done using the same honey-adulterants sample which are SBH -DW, SBH -ACV, and SBH -FS sample sets, but without the use of jig. The cuvette was manually positioned in front of the sensor in this configuration, and no mechanical support and reflectance standard were used.

On visual comparison of the spectral output, the differences are immediately apparent. Although it is still possible to notice a general increasing trend in absorbance with the increasing concentration of the adulterant, the spectral curves are characterized by significant inconsistency in all the three types of adulterants. The repetitions of each concentration in the SBH-DW series are broadly spread across particularly in the middle to the higher wavelengths and it is hard to see a clear pattern. In the same way, the irregular fluctuations and overlapping curves observed in the SBH-ACV spectra at the various concentrations suggest that the measurements have no alignment and thus the noise is high. The data of SBH-FS shows the baseline shifts and jagged spectral shapes, especially at longer wavelengths, which further adds to the visual impression of instability.

These inconsistencies imply that in the absence of the cuvette jig, the system is prone to the noise of angular misalignment, sensor to sample distance variations, and uncontrolled exposure to ambient light. This is because of the lack of a standard optical alignment or spectral reflectance in the handheld configuration which causes even a minor change in the placement of the cuvettes to cause major changes in the spectral output. As a result, the measurement repeatability is visibly compromised in the absence of the jig across all adulterant types.

To further validate this observation, the mean SD, using the same method as described in section 3.2, was also computed for the no-jig setup. This allows a direct comparison of repeatability between the two setups.

An addition of calculation is also added which is the Improvement Ratio where it is to quantify how much more variable the no-jig set up was than the jig-assisted set up. It is defined as in Eq. (1):

$$Improvement\ Ratio\ = \frac{Mean\ STD\ without\ Jig}{Mean\ STD\ with\ Jig} \quad (1)$$

This ratio explains how many times greater the variability is without the use of cuvette jig in the REVA INVITRO system. A high ratio indicates that the repeatability improved to a greater extent through the usage of the cuvette jig.

The improvement ratios calculated in Table 3 were 14.76 for SBH-DW, 12.18 for SBH-ACV and 5.76 for SBH-FS. From these values, it can be interpreted that the SBH-DW benefits the most from the use of the cuvette jig in terms of spectral repeatability, followed by SBH-ACV and SBH-FS. These findings indicate that the jig configuration would greatly increase spectral repeatability to all the types of adulterants.

In conclusion, a simple visual inspection of the spectral curves confirms that the use of a mechanical jig significantly improves repeatability and signal integrity. This is now quantitatively supported by statistical variability analysis and improvement ratio calculations, further reinforcing the mechanical jig's value as an integral part of the REVA INVITRO system.

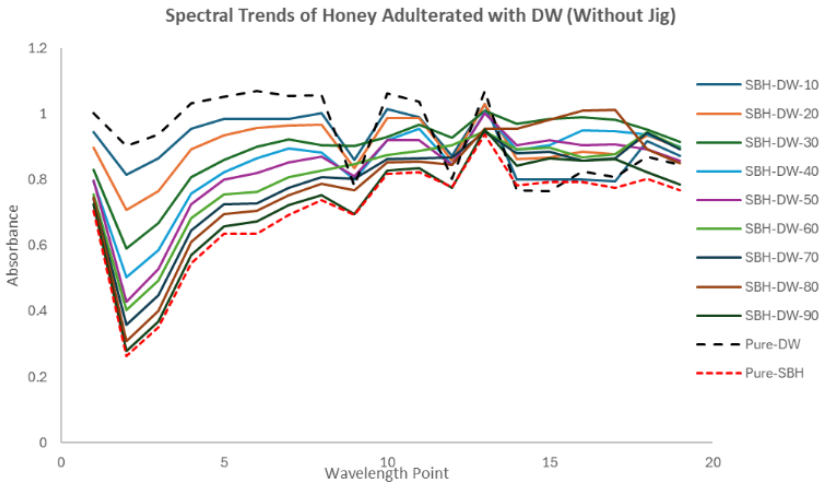

Fig. 3a. Spectral measurements of SBH–DW mixtures without using the Jig

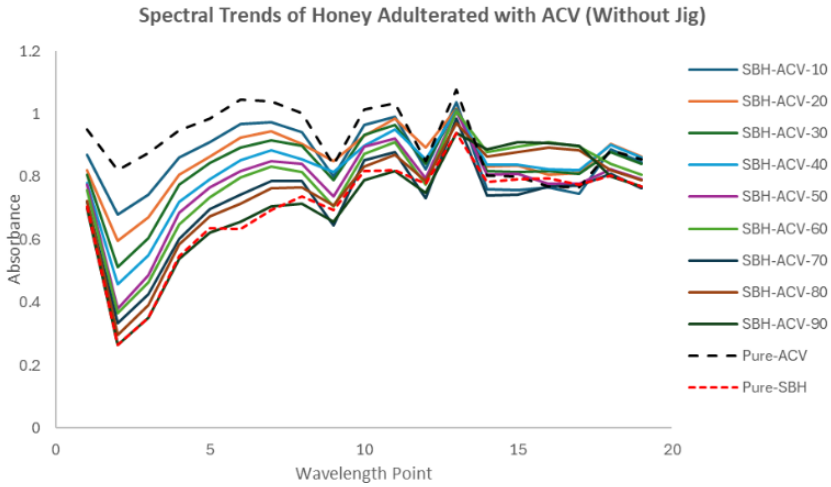

Fig. 3b. Spectral measurements of SBH–ACV mixtures without using the Jig

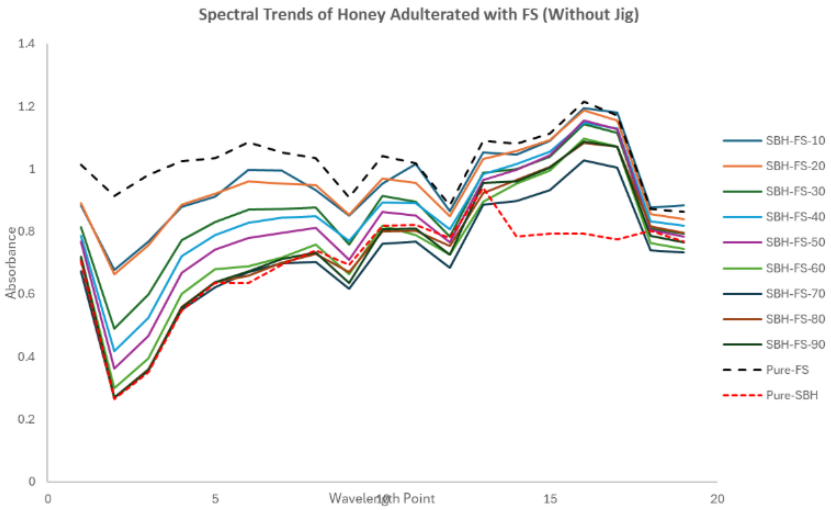

Fig. 3c. Spectral measurements of SBH–FS mixtures without using the Jig

Table 3
Table of mean standard deviation and improvement ratio for honey-adulterant mixtures with and without jig

Honey-Adulterant	Mean STD (With Jig)	Mean STD (Without Jig)	Improvement Ratio
SBH-DW	0.00221	0.03263	14.76
SBH-ACV	0.00250	0.03045	12.18
SBH-FS	0.00532	0.03066	5.76

3.4 Classification Performance Adulterant Type Identification

The classification task aims to utilize exclusively the jig-stabilized spectral data to enable reliable discrimination between four classes: Apple Cider Vinegar adulteration (ACV), Distilled Water adulteration (DW), Fructose Syrup adulteration (FS), and Pure Honey (H). A hierarchical classification pipeline was developed to automatically identify adulterant types from spectral profiles, consisting of StandardScaler preprocessing, Principal Component Analysis (PCA) for dimensionality reduction to 10 components, and OneVsRestClassifier with Logistic Regression for multi-class discrimination.

3.4.1 Classification Performance: Performance with Mechanical Jig Stabilization

The jig-stabilized spectral measurements (n=330 samples: 100 each of ACV, DW, FS, and 30 Pure Honey) demonstrated high classification performance under controlled measurement conditions. The test set evaluation (n=66, 20% holdout) achieved complete class separation with 100% classification accuracy on the held-out test set. The confusion matrix (Figure 4) shows no misclassifications within the held-out test samples, with all 20 ACV samples, 20 DW samples, 20 FS samples, and 6 Pure Honey samples correctly identified.

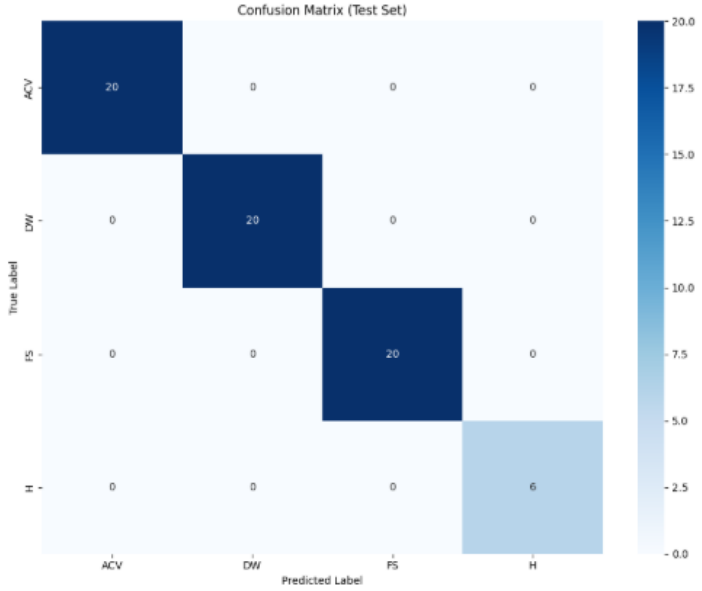

Fig. 4. Classification confusion matrix - perfect classification with jig

The PCA visualization (Figures 5a and 5b) illustrates the spectral basis underlying the observed classification performance. In the reduced 2D and 3D principal component space, the four classes form distinct, well-separated clusters. Pure Honey samples formed the tightest cluster, reflecting

consistent spectral signatures across different honey batches, reflecting improved spectral consistency associated with jig-stabilized measurements. The adulterant classes show clear boundaries with minimal overlap, demonstrating that each adulterant creates a unique, reproducible spectral signature when measured under stable conditions.

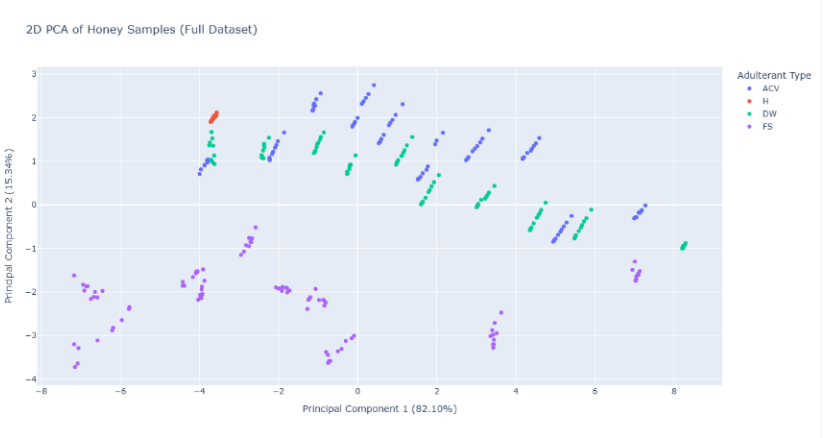

Fig. 5a. PCA 2D Scatter Plot - Showing Distinct Cluster Separation

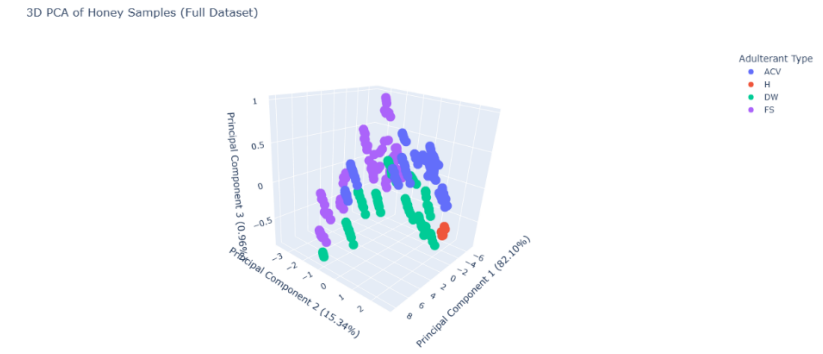

Fig. 5b. PCA 3D Scatter Plot - Demonstrating Spectral Discrimination

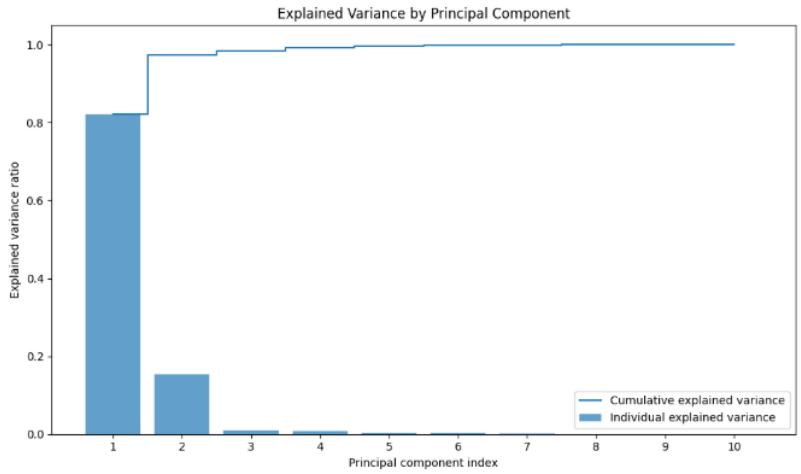

Fig. 6. PCA explained variance plot showing that the first principal component accounts for 85.74% of the total variance, with 10 components capturing 99.97% of the spectral variance

Cross-validation analysis (5-fold stratified) yielded a mean accuracy of 94.55% ± 9.47%, with individual fold scores ranging from 75.76% to 100%. While one fold showed reduced performance (75.76%), this variability indicates sensitivity to data partitioning, highlighting the importance of mechanical stabilization for consistent classification performance.

3.4.2 Classification performance: Performance without jig stabilization identification

In contrast to the jig-stabilized measurements, spectral data acquired without mechanical stabilization showed measurably reduced classification performance. Test set accuracy decreased to 89.39% (n=66), with balanced accuracy of 91.25% and macro F1 score of 91.30%. While this represents a 10.61 percentage point reduction compared to jig-stabilized measurements, the performance remains above 85% accuracy, indicating that basic classification capability is retained even in the absence of mechanical stabilization.

The confusion matrix (Figure 7) reveals 7 misclassifications out of 66 test samples, concentrated among chemically similar adulterants (ACV↔DW, DW↔FS). Notably, all six Pure Honey samples were properly categorized, resulting in flawless precision and recall for this class. This pattern indicates that the spectral signature of unadulterated honey remains sufficiently distinct even under variable measurement conditions, while subtle differences between adulterants are more susceptible to measurement noise.

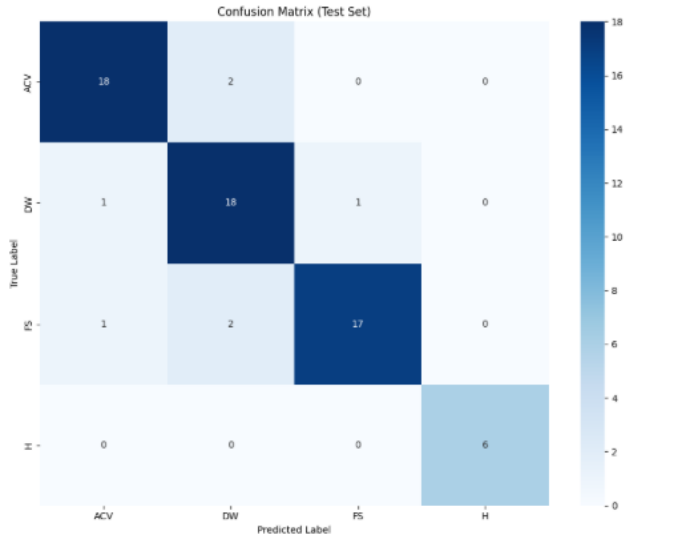

Fig. 7. Classification confusion matrix - without jig (7 errors)

PCA visualizations (Figures 8a and 8b) show increased cluster overlap, particularly at the boundaries between ACV and DW, as well as between DW and FS. This suggests that measurement variability introduced by manual handling obscures subtle spectral differences between dilution-based adulterants.

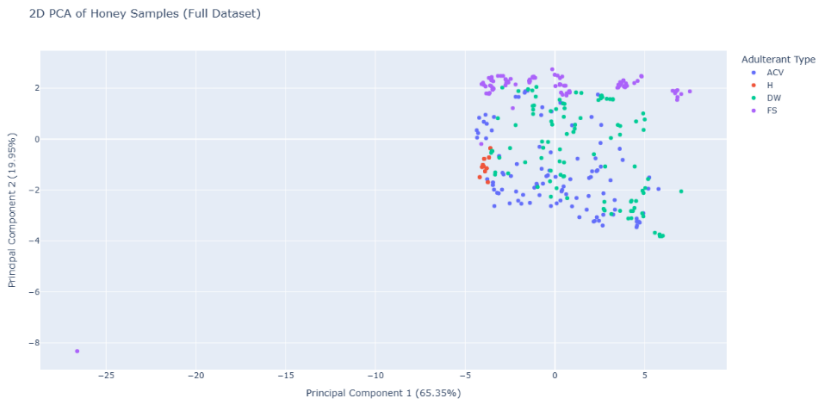

Fig. 8a. PCA 2D scatter plot - increased cluster overlap

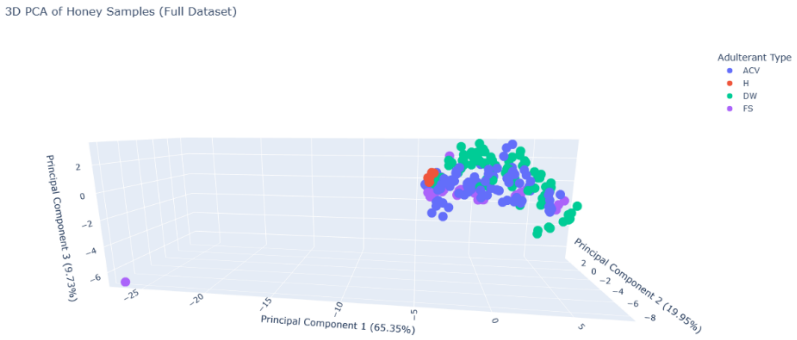

Fig. 8b. PCA 3D Scatter plot - reduced seperation

Cross-validation results (85.15% ± 10.29%) show both reduced mean performance and increased variability compared to jig-stabilized measurements. The fold score range (68.18% to 96.97%) indicates greater sensitivity to data partitioning, with the lowest fold performing 7.58 percentage points worse than the corresponding jig-stabilized fold.

3.4.3 Classification performance: Comparative analysis of jig impact on classification

Table 4 summarizes the consistent improvement in classification performance achieved through mechanical stabilization across all evaluation metrics. The mechanical jig provides a substantial 10.61 percentage point improvement in test accuracy (100.00% vs 89.39%), with consistent improvements observed across accuracy and class-balanced performance metrics.

Table 4
Table of classification performance comparison: with versus without jig

Metric	With Jig	Without Jig	Δ
Test Accuracy	100.00%	89.39%	-10.61%
Balanced Accuracy	100.00%	91.25%	-8.75%
F1 Score (macro)	100.00%	91.30%	-8.70%
Matthews Correlation	1.000	0.853	-0.147
ROC AUC	1.000	0.991	-0.009
Log Loss	0.016	0.270	+0.254
Cross-Validation Mean	94.55%	85.15%	-9.40%

Cross-Validation Std	9.47%	10.29%	+0.82%
Misclassifications			

The misclassification pattern without jig reveals systematic confusion between chemically similar adulterants. All 7 errors occurred among the three adulterant classes (ACV, DW, FS), with no confusion involving Pure Honey. This suggests that the spectral signature of unadulterated honey remains sufficiently distinct even under variable measurement conditions, while subtle differences between adulterants require the enhanced signal quality provided by mechanical stabilization. The predominance of ACV-DW and DW-FS confusion indicates that dilution-based spectral changes are particularly sensitive to measurement noise. model development as compared to without using the jig.

3.5 Regression Performance: Honey Percentage Quantification

Beyond qualitative adulterant type identification, the REVA INVITRO system's ability to quantify adulteration levels was evaluated using Partial Least Squares Regression (PLSR) models. Separate models were built for each adulterant class (ACV, DW, and FS) to account for their different chemical interactions with honey. This quantitative capability allows not only the identification of adulteration but also the accurate quantification of honey purity, which provides critical indicators for regulatory validation and market valuation.

3.5.1 Regression performance with mechanical jig stabilization

The jig-stabilized spectral measurements enabled highly accurate quantification across all three adulterants (Table 5). The DW quantification model achieved the best performance with test RMSE of 0.34% and R^2 = 0.9999, utilizing 19 PLS components. This exceptional accuracy reflects water dilution's systematic spectral effects, which create clear, reproducible patterns when measured under stable conditions. The ACV model demonstrated similarly strong performance (test RMSE = 0.56%, R^2 = 0.9996) using 15 components, while the FS model, facing the inherent challenge of distinguishing added fructose from honey's natural sugars, achieved test RMSE = 1.96% and R^2 = 0.9952 with 14 components.

Table 5
Table of regression performance with jig-stabilized measurements

Adulterant	Components	Test R^2	Test RMSE	Test MAE	Full RMSE	Full MAE
DW	19	0.9999	0.34%	0.28%	0.26%	0.20%
ACV	15	0.9996	0.56%	0.47%	0.40%	0.31%
FS	14	0.9952	1.96%	1.41%	1.23%	0.93%

The actual vs predicted plots (Figures 9a, 9b and 9c) for all three adulterants show tight clustering around the ideal y=x line across the full concentration range (0-100% honey), visually confirming the models' quantitative accuracy. Error analysis reveals consistent performance across concentration ranges for DW and ACV, with no systematic bias at high or low honey percentages. The FS model shows slightly elevated error in the mid-concentration range (40-60%), reflecting the difficulty of distinguishing exogenous fructose from honey's natural carbohydrate content at intermediate adulteration levels.

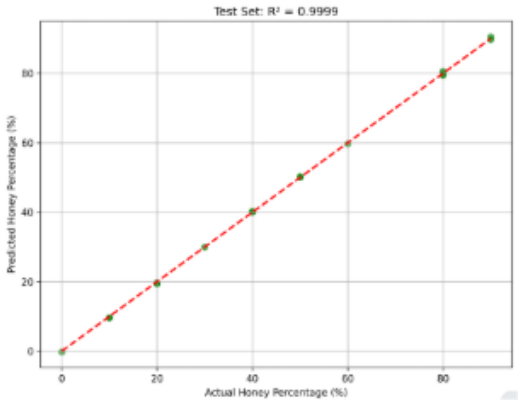

Fig. 9a. Predicted vs Actual Plot - DW Adulterant

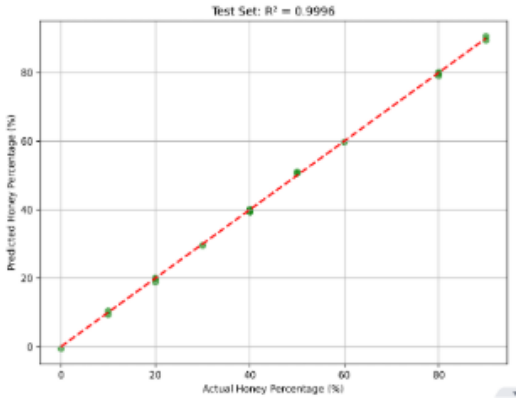

Fig. 9b. Predicted vs Actual plot - ACV adulterant

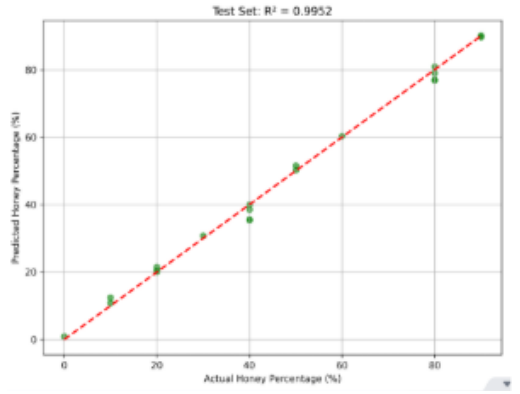

Fig. 9c. Predicted vs Actual plot - FS adulterant

Detailed error analysis across concentration ranges reveals adulterant-specific patterns. DW quantification maintains uniform accuracy across all levels (MAE = 0.15-0.23%), indicating absence of concentration-dependent bias. ACV shows similar uniformity (MAE = 0.20-0.39%) with slightly elevated mid-range error due to pH-dependent spectral changes. FS exhibits more pronounced concentration dependence, with elevated mid-range error (40-60%: MAE = 1.32%) where exogenous and intrinsic fructose spectral signatures overlap most significantly.

3.5.2 Regression performance without mechanical jig stabilization

Regression models trained on unstabilized spectral measurements demonstrated reduced but still acceptable quantification performance. The DW model achieved test RMSE = 1.63% (versus

0.34% with jig), ACV model RMSE = 1.43% (versus 0.56%), and FS model RMSE = 3.57% (versus 1.96%). Notably, the optimal component counts decreased substantially without jig stabilization (DW: 19→10, FS: 14→6), indicating that measurement noise masks fine spectral features and forces the models to rely on coarser, more robust spectral patterns.

Table 6
Table of regression performance without jig-stabilized measurements

Adulterant	With Jig (RMSE/Components)	Without Jig (RMSE/Components)	RMSE Reduction with Jig
DW	0.34% / 19	1.63% / 10	79%
ACV	0.56% / 15	1.43% / 15	61%
FS	1.96% / 14	3.57% / 6	45%

The differential jig impact across adulterants provides insights into detection mechanisms. DW quantification shows the largest benefit (79% RMSE reduction), reflecting water dilution's subtle spectral effects that require stable measurement conditions to resolve. ACV quantification shows moderate improvement (61%), consistent with its more robust acidic signature. FS quantification, while showing substantial improvement (45%), remains the most challenging even with jig stabilization due to the inherent difficulty of distinguishing exogenous fructose from honey's natural carbohydrates.

The RMSE improvements with jig stabilization have direct practical implications. For DW, the improvement from 1.63% to 0.34% represents the difference between approximate screening (±1.6% uncertainty) and regulatory-grade quantification (±0.3% uncertainty). The jig-stabilized configuration achieves high quantitative accuracy (RMSE ≤ 2%) suitable for controlled laboratory analysis, while measurements acquired without jig retain practical performance (RMSE ≤ 3.6%) suitable for field screening where portability is prioritized over ultimate precision.

4. Discussion

The visual spectral trend analysis (Sections 3.2 and 3.3) combined with the classification and regression performance results (Sections 3.4 and 3.5) provides comprehensive validation of the jig's mechanical stabilization impact, directly addressing Objective 2 of this study.

As seen in Sections 3.2 and 3.3, the jig transforms measurement quality from erratic, inconsistent curves into smooth, systematic trends. Section 3.2 shows that with jig stabilization, spectral curves for each adulterant type (DW, ACV, FS) exhibit minimal visible fluctuation between repeated measurements at the same concentration, with tightly grouped curves that progress systematically across concentration gradients. In stark contrast, Section 3.3 demonstrates that without jig stabilization, the same DW samples produce irregular, scattered curves with noticeable baseline shifts, curve distortion, and random fluctuations; visual artifacts caused by angular misalignment, inconsistent cuvette-sensor distance, and ambient light interference during manual handling.

The improvement in signal-to-noise ratio demonstrates a shift from qualitative screening capability toward quantitative analytical performance. Reduced measurement noise allows the detection of subtle spectral differences between adulterants and supports precise honey content quantification, whereas higher noise levels limit analysis to gross adulteration screening.

The jig addresses three critical sources of variability: (1) cuvette positioning, ensuring consistent optical path length across measurements, (2) angular alignment, maintaining fixed sensor–sample geometry, and (3) ambient light interference through the enclosed optical path and reflectance

standard. The result is a transformation from erratic, inconsistent spectral curves to smooth, systematic trends that enable both visual detection and automated machine learning analysis.

This improvement in spectral quality translates directly into consistent gains in both classification reliability and quantification accuracy. Critically, these improvements cannot be achieved through algorithmic sophistication alone; they require reduction of measurement noise at the source through mechanical stabilization. The reproducibility metrics demonstrate that the jig highlights mechanical stabilization as a critical enabling factor for extending the REVA INVITRO system from a screening tool toward quantitative analytical use.

This is in agreement with recent literature, where high classification accuracy (>92–97%) and low quantification error (RMSE < 5–7%) are only achieved when measurement noise is minimized at the source, not just through data processing [22].

The differential impact across adulterants provides additional insights into detection mechanisms. As shown in Table 6, DW quantification benefits most from jig stabilization, reflecting water dilution's subtle spectral effects that are highly sensitive to measurement noise. ACV quantification shows moderate improvement, consistent with its more robust acidic signature, while FS quantification remains the most challenging due to the inherent difficulty of distinguishing exogenous fructose from honey's natural carbohydrates. These adulterant-specific patterns, combined with the accurate classification of Pure Honey even without jig stabilization (Section 3.4.2), demonstrate that the system performance is fundamentally linked to both the chemical nature of the adulterant and the measurement stability provided by the jig.

Similar adulterant-specific detection challenges are reported in recent studies, sugar syrups are more difficult to detect at low levels, and robust model performance depends on both the chemical nature of the adulterant and the stability of the measurement system [23].

4.1 Limitations and Future Directions

Several limitations warrant consideration. First, the training dataset, while sufficient for proof-of-concept, represents limited honey variety diversity (primarily Stingless Bee Honey). Validation across several floral sources, regional origins, and seasonal variations is required to assess model transferability. Second, the system only detects single-adulterant scenarios; real-world adulteration may involve numerous adulterants at the same time, necessitating more complex modeling approaches. Third, the unstabilized measurement performance, while acceptable for screening, shows increased variability that may limit detection of low-level adulteration (<10%).

Future work should focus on: (1) expanding training datasets to encompass diverse honey types and adulterant combinations, (2) developing confidence scoring to flag uncertain predictions for manual review, (3) implementing temperature compensation for field deployment under varying ambient conditions, and (4) miniaturizing the jig mechanism for truly handheld operation while maintaining measurement stability.

5. Conclusion

This study successfully validates both the mechanical stability and analytical performance of the REVA INVITRO portable spectroscopic system for detecting adulteration in Stingless Bee Honey, directly addressing the two main objectives of this work.

Objective 1 – Device Effectiveness and Multivariate Analysis:

The REVA INVITRO system demonstrates strong capability in detecting and classifying SBH adulteration using multivariate analysis techniques. Machine learning–based classification enables

reliable identification of common adulterant types (DW, ACV, FS) from spectral profiles, while regression-based quantification supports estimation of honey content across a wide concentration range. The multivariate approach effectively captures complex spectral patterns associated with chemically similar adulterants, and basic adulterant identification capability is retained even in the absence of mechanical stabilization, supporting its use for preliminary screening applications.

Objective 2 – Jig Impact on Spectral Measurement Consistency:

Quantitative reproducibility analysis provides clear evidence of the jig's role in improving measurement consistency. Mechanical stabilization enhances spectral repeatability, reduces measurement noise, and improves baseline stability by controlling cuvette positioning, sensor–sample alignment, and ambient light interference. These improvements form the foundation for reliable classification and quantification performance, demonstrating that measurement stability is a key enabling factor for robust spectroscopic authentication.

While the results demonstrate the effectiveness of the proposed system, they should be interpreted within the scope of controlled experimental conditions, a limited set of adulterant types, and machine learning models trained on a finite dataset. Further validation using more diverse honey sources, multi-adulterant scenarios, and independent datasets will be required to fully assess model generalizability and robustness under real-world conditions.

From a practical perspective, the portability and non-destructive nature of the REVA INVITRO system support its potential deployment in decentralized testing environments, such as small-scale producers, quality control checkpoints, or field inspections. The use of optical measurements and reusable hardware suggests favourable scalability and operational cost compared to laboratory-based analytical methods. While regulatory adoption would require further validation against established reference techniques and inter-laboratory studies, the proposed system provides a complementary screening approach that can support existing honey authentication workflows

Overall, the findings highlight that integrating mechanical stabilization with portable Vis–NIR spectroscopy substantially improves analytical reliability for honey adulteration detection. The REVA INVITRO system, when combined with a stabilization mechanism, offers a practical pathway toward portable spectroscopic analysis that bridges controlled laboratory measurements and on-site screening, supporting future development of scalable and standardized honey authentication tools.

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