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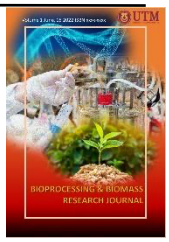

Research Article

Prediction of Artificial Sweetener Adulteration in Commercial Stevia Using FTIR-Based Chemometrics

Farah Najeeha Saiful Bahri[a], Zanariah Hashim[a*]

[a] *Food and Biomaterial Engineering Research Group, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*

ARTICLE INFO

Article History:
Received 07 December 2025
Received in revised form 23 December 2025
Accepted 23 December 2025
Available online 30 December 2025

Keywords:
FTIR,
Chemometrics,
Stevia adulteration,
PLS regression,
Principal component analysis.

ABSTRACT

Stevia is widely used as a natural sweetener, yet its high cost has led to increasing cases of adulteration with cheaper artificial sweeteners. Rapid and reliable detection methods are therefore essential for quality control. This study evaluates the use of Fourier Transform Infrared (FTIR) spectroscopy combined with chemometric modelling to predict the adulteration level of artificial sweeteners in commercial stevia products. FTIR spectra of pure stevia, three artificial sweeteners (aspartame, sodium saccharin, sodium cyclamate), and their mixtures were analysed. Principal component analysis (PCA) successfully distinguished pure stevia from adulterated samples based on characteristic wavenumbers. Partial least squares (PLS) regression models were developed to predict adulterant levels, yielding R^2 values of 0.88–0.95 which indicate a strong correlation between FTIR spectral features and adulterant concentration, and RMSEP values of 33.72–43.28% that reflect moderate prediction errors that are acceptable for screening purposes. Application of the models to three commercial stevia products revealed varying levels of adulteration, particularly with sodium saccharin and sodium cyclamate. Although some predictions exceeded 100%, indicating model extrapolation, the overall results demonstrate that FTIR coupled with chemometrics provides a rapid, non-destructive approach for screening stevia authenticity. This method shows strong potential for routine detection of sweetener adulteration in commercial products.

INTRODUCTION

Due to the growing popularity of stevia, counterfeit stevia sweeteners have appeared on the market in recent years. Stevia is also adulterated because of the desire of organisations to maximise earnings in a short amount of time (Sharma and Kaushal, 2021). Stevia is often falsified through addition of less expensive artificial sweeteners such as aspartame, sodium saccharin and sodium cyclamate; such undeclared additions not only undermine product authenticity but have prompted health-related concerns in some studies and regulatory scrutiny in several markets (Peteliuk et al., 2021).

Stevia, a plant-derived sweetener, is low in calories and has a negligible glycaemic index, making it attractive for people with diabetes, pre-diabetes, hypertension, and for consumers seeking to reduce sugar intake (Peteliuk et al., 2021). Given its widespread use in health-oriented foods and beverages, reliable and rapid methods for verifying stevia purity are essential for consumer protection and quality control. Conventional approaches such as HPLC and Raman spectroscopy have proven effective but can be time-consuming, require extensive sample preparation or specialised instruments, and in some cases have been applied only to single analytes or limited sample sets.

The purpose of this study was to evaluate whether FTIR spectroscopy, combined with chemometric tools, can reliably predict the adulteration level of stevia by multiple artificial sweeteners. For analysis we applied principal

*Corresponding Author
E-mail address: zanariahhashim@utm.my
DOI address: 10.11113/bioprocessing.v4n2.87

component analysis (PCA) to extract discriminating spectral features, and partial least squares (PLS) regression to build quantitative prediction models between FTIR spectral variables (X-matrix) and known adulterant concentrations (Y-vector). While earlier work has demonstrated FTIR-based screening for various food adulterants and methods for single-sweetener quantification (Roosmayanti et al., 2021), there is a relative paucity of studies that (1) apply FTIR–chemometrics to simultaneously identify and quantify several common artificial sweeteners in stevia, and (2) use PLS models directly on commercially available products from a specific market. By focusing on simultaneous multianalyte detection with rapid, non-destructive FTIR measurements and multivariate calibration, this work aims to offer a practical, low-cost approach suitable for routine quality-control screening (Galvin-King et al., 2019).

MATERIALS AND METHOD

Raw Materials and Sample Preparation

Pure stevia powder was procured from EvaChem (Selangor, Malaysia), while three commercial stevia products (Commercial Stevia 1–3) were obtained from different manufacturers in Malaysia and Singapore. Aspartame (EvaChem, Selangor, Malaysia), sodium saccharin (Tianjin Changjie, China), and sodium cyclamate (Jincheng Chemical, China) were all food-grade powders. Each artificial sweetener was individually mixed with pure stevia to obtain 30 g of powder samples with target adulterant concentrations of 0%, 30%, 70%, and 100% (w/w). The mixture compositions used for FTIR analysis are summarised in **Table 1**.

Table 1 Composition of pure stevia–artificial sweetener mixtures used for FTIR analysis

Class	Stevia		Artificial Sweetener	
	Wt. %	Weight (g)	Wt. %	Weight (g)
0%	0	0	100	30
30%	30	9	70	21
70%	70	21	30	9
100%	100	30	0	0

Note: Each class was prepared separately for aspartame, sodium saccharin, and sodium cyclamate, resulting in three sets of mixtures.

Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were collected using a Thermo Fisher Scientific Fourier Transform Infrared (FTIR) spectrophotometer. Spectra were recorded over the range of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1}. All samples were analysed in their original form without any pre-treatment. For each sample, at least three replicate spectra were obtained, and the resulting FTIR data were expressed in absorbance units.

Chemometric and multivariate data analysis

In this work, principal component analysis (PCA) was used for exploratory multivariate analysis of FTIR spectra, while partial least squares (PLS) regression was applied as a chemometric calibration method to relate spectral features to adulterant concentrations.

PCA of the FTIR spectra, using absorbance values across the full wavenumber range of 4000–400 cm^{-1}, was performed using MetaboAnalyst 4.0 (Chong and Xia, 2020). PCA was applied to explore the hidden structure within the dataset, including clusters, trends, and multivariate outliers. The method reduces the dimensionality of the spectral dataset by transforming it into a smaller set of principal components that capture the major sources of variation.

PLS regression models were developed in Minitab Statistical Software to quantitatively analyse the artificial sweeteners based on their spectral profiles. After model calibration, a separate test set was used to evaluate predictive performance and minimise the risk of overfitting. Model accuracy was assessed using the coefficient of determination (R^2) and the Root Mean Square Error of Prediction (RMSEP) for both calibration and prediction sets. RMSEP was calculated using:

$$\mathrm{RMSEP} = \sqrt{\frac{\mathrm{PRESS}}{n}}$$

where PRESS is the Predictive Error Sum of Squares and *n* is the number of samples in the prediction set.

RESULTS AND DISCUSSION

Quantification of FTIR spectra

To construct prediction models for quantifying adulteration levels in commercial stevia products, the significant FTIR wavenumbers that characterise each artificial sweetener were first identified. These key wavenumbers were selected as the primary variables for model development. By incorporating these informative spectral regions into the multivariate analysis, the accuracy of both identification and quantification was enhanced (**Figure 1**). As adulteration increases, corresponding changes in peak intensities or band positions can be observed in the FTIR spectra. **Table 2** summarises the major characteristic peaks obtained from pure stevia and the three artificial sweeteners.

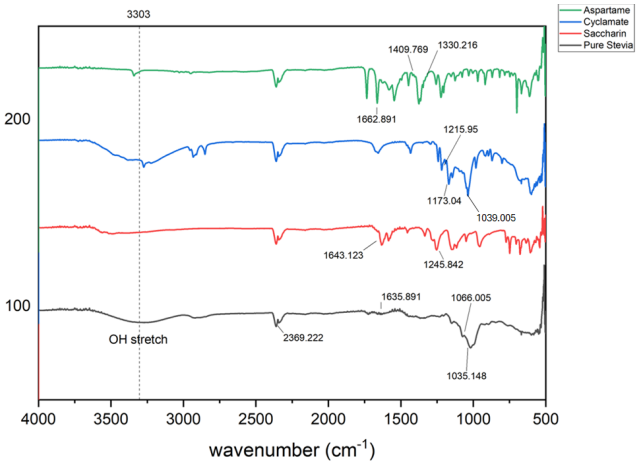

Figure 1 FTIR spectra of aspartame, sodium cyclamate, sodium saccharin and pure stevia

Table 2 Major FTIR absorption bands identified in pure stevia and artificial sweeteners

Sample	Wavenumber (cm^{-1})	Functional Group Assignment	References
Aspartame	1410-1330	C-C stretching; C-O bonds	Wang et al., 2020
	1662	C=O stretching; aromatic C–H stretching	Buyukgoz et al., 2015
Sodium cyclamate	1173	Symmetric SO_2 stretching	Wang et al., 2020

	1258–1119	SO_2 characteristic absorption range	
	1039	Deformation of C-H ring and vibration of C-C	Guven et al., 2019
Sodium saccharin	1245	Asymmetric SO_2 stretching	Wang et al., 2020
	~1635	C=O stretching; aromatic ring vibrations	
Pure stevia	~1030–1070	C–O stretching (carbohydrates)	Guven at al., 2019
	~3400	O–H stretching (hydroxyl groups)	Martono et al., 2016

Note: Peak positions may vary slightly due to sample matrix effects, instrumental resolution, and baseline conditions.

Principal Component Analysis (PCA)

Figure 2 (a) and **(b)** illustrate the separation between aspartame-adulterated samples and pure stevia. PCA enables the spectral data to be visualised in a reduced-dimensional space, allowing similarities and differences between sample groups to be clearly distinguished. Aspartame mixtures at 30%, 70%, and 100% showed clear separation from pure stevia. This difference corresponds to wavenumbers 1662-1663 cm^{-1} associated primarily with C=O and C–H functional groups. According to Buyukgoz et al. (2015), these regions are characterised by strong C=O stretching vibrations and moderate aromatic C–H stretching, which contribute to the distinct spectral features observed in the PCA score plots.

PCA score plot in **Figure 3 (a)** demonstrates a separation between sodium cyclamate and pure stevia, with slight overlap between the pure stevia and 30% cyclamate groups. This might be attributed by similar FTIR spectral pattern between stevia and sodium cyclamate (as seen from **Figure 1**) which becomes more evident at low sodium cyclamate concentrations. As shown in **Figure 3 (b)**, the peak at 1173.52 cm^{-1} corresponds to the symmetric SO_2 stretching vibration, consistent with characteristic bands of sodium cyclamate. Wavelengths in the range of 1258–1119 cm^{-1} are also indicative of cyclamate, as reported by Wang et al. (2020).

Similarly, **Figure 4 (a)** and **(b)** show the distinction between pure stevia and samples containing sodium saccharin at 30%, 70%, and 100%, with a characteristic band at 1245.36 cm^{-1} attributed to asymmetric SO_2 stretching (Wang et al., 2020). While the 30% samples are well separated from pure stevia, partial overlap is observed between the 70% and 100% sodium saccharin groups, likely due to the high saccharin content in the 70% mixture (70% sodium saccharin + 30% stevia), which produces spectral features very similar to the 100% sample.

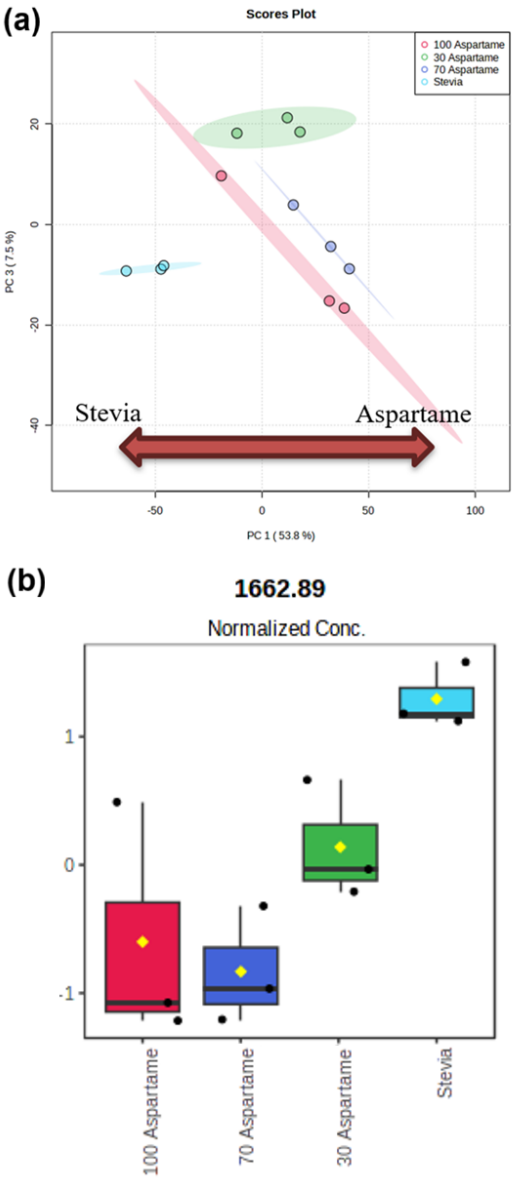

Figure 2 (a) PCA score plot showing the separation between pure stevia and stevia samples adulterated with aspartame at 30%, 70%, and 100%. **(b)** Corresponding FTIR loading plot highlighting the key wavenumber at 1662.89 cm^{-1}

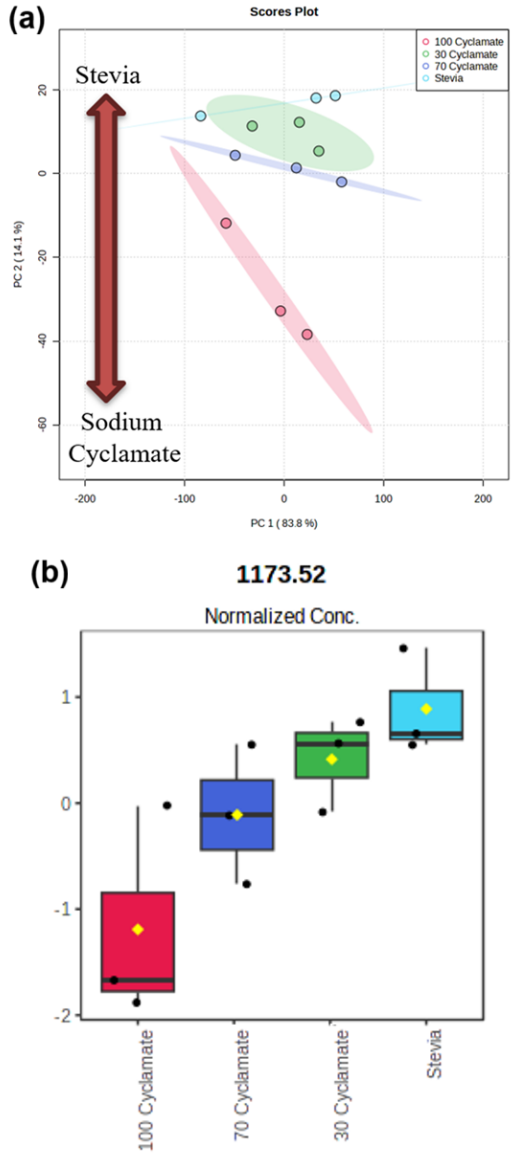

Figure 3 (a) PCA score plot showing the separation between pure stevia and samples adulterated with sodium cyclamate at 30%, 70%, and 100% concentrations. **(b)** Corresponding FTIR loading plot highlighting the key wavenumber at 1173.52 cm^{-1}

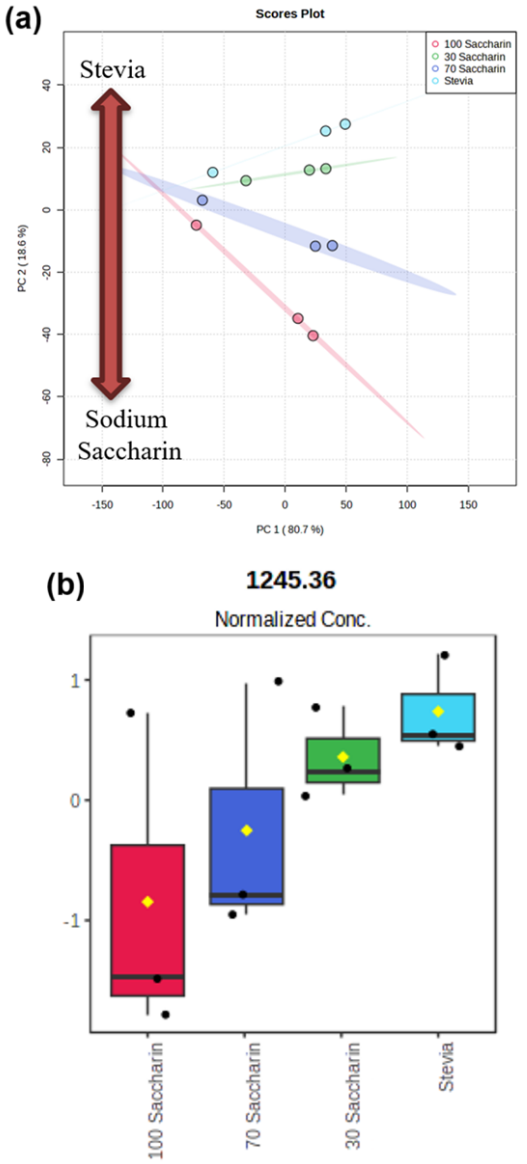

Figure 4 (a) PCA score plot showing the separation between pure stevia and samples adulterated with sodium saccharin at 30%, 70%, and 100% concentrations. **(b)** Corresponding FTIR loading plot highlighting the key wavenumber at 1245.36 cm^{-1}

Partial Least Square (PLS) Regression

Partial Least Squares (PLS) regression models were developed to quantitatively analyse the artificial sweeteners using the FTIR spectral data. After calibration, a separate test set was evaluated to determine the predictive capability of the models and to minimise the risk of overfitting. Model performance was assessed using the coefficient of determination (R^2) and the Root Mean Square Error of Prediction (RMSEP) for the prediction sets, following the criteria described by Wang et al. (2020).

As shown in **Table 3**, all models produced *p*-values below the significance threshold of 0.05, indicating statistically meaningful relationships between the spectral predictors and adulterant concentrations. The three-component PLS models explained 0.96, 0.98, and 0.95 of the variance in the predictor (X) matrix, respectively. X-variance values close to 1 indicate that the selected latent variables effectively capture the relevant spectral information.

Table 3 Statistical parameters of PLS calibration models for artificial sweeteners in stevia

	Aspartame	Sodium Saccharin	Sodium Cyclamate
P value	0.000	0.001	0.001
X-variance	0.965	0.983	0.956
R^2	0.880	0.875	0.859
PRESS	7744.3	5685.1	9365.1
R^2 predicted	0.555	0.673	0.462
RMSEP	39.36%	33.72%	43.28%

The R^2 values reported in **Table 3** demonstrate strong goodness-of-fit, with higher R^2 indicating better agreement between predicted and actual concentrations. In addition, the models yielded relatively low RMSEP values, all below 50%, suggesting acceptable prediction accuracy for this type of multivariate calibration. Taken together, the high R^2 and low RMSEP values indicate that the PLS models were capable of predicting the concentrations of aspartame, sodium saccharin, and sodium cyclamate in stevia samples.

Therefore, the models successfully captured the variation associated with adulteration across all three sweeteners.

The developed PLS models were subsequently applied to predict adulteration levels in three commercially available stevia products. **Table 4** summarises the predicted percentages of artificial sweeteners detected in each sample. Pure stevia is generally considered non-adulterated when impurity levels are below 20%, corresponding to an acceptable purity range of 80–100%. Based on this criterion, Commercial Stevia 1 was found to be adulterated with sodium saccharin (87.75%) and sodium cyclamate (167.02%). Commercial Stevia 2 was predicted to contain all three artificial sweeteners included in this study. Commercial Stevia 3 also appeared to be adulterated, with notable levels of sodium saccharin (84.68%) and sodium cyclamate (157.20%).

Table 4 Predicted adulterant levels in commercial stevia products using PLS models

Product	Aspartame, %	Sodium Saccharin, %	Sodium Cyclamate, %
Pure Stevia	-5.75 ± 0.89	6.63 ± 4.43	1.66 ± 6.60
Commercial Stevia 1	-60.48 ± 2.89	87.75 ± 1.23	167.02 ± 2.44
Commercial Stevia 2	35.25 ± 0.39	53.31 ± 0.03	58.61 ± 0.92
Commercial Stevia 3	-46.80 ± 0.57	84.68 ± 0.47	157.20 ± 0.50

Note: Values above 20% indicate potential adulteration (Olazar et al., 2022). Predictions exceeding 100% reflect model extrapolation beyond the calibration range.

When the FTIR spectra of the commercial stevia products were compared with those of the adulterants, several characteristic absorption bands were clearly present, supporting and validating the prediction results (**Figure 5**). In Commercial Stevia 1, cyclamate-associated absorption bands, particularly those related to deformation of C-H ring and vibration of C-C (1039 cm^{-1}) were prominent in the sample's spectral profile. Sodium saccharin was also detected at a high level (87.74%), supported by characteristic saccharin-associated C=O and aromatic vibrations (1635 cm^{-1}). In contrast, aspartame produced a negative predicted value (–60.48%), which indicates that its spectral contributions were negligible; thus, its concentration was effectively interpreted as zero.

Commercial Stevia 2 showed evidence of all three artificial sweeteners. The relatively high predicted level of sodium cyclamate is consistent with the dominance of cyclamate-related bands in the low-wavenumber region, while the additional presence of aspartame (1330 cm^{-1}) and sodium saccharin (1635 cm^{-1}) reflects a more complex mixture. The combination of multiple characteristic absorption features across the mid-IR region contributed to the model identifying all three adulterants simultaneously.

For Commercial Stevia 3, the model again indicated substantial adulteration, particularly with sodium cyclamate (157.20%) and sodium saccharin (84.68%). Strong deformation of C-H ring and vibration of C-C characteristic of cyclamate (1039 cm^{-1}) supported the high predicted value, while distinctive saccharin-related carbonyl and aromatic peaks (1635 cm^{-1}) aligned with the model's estimation of saccharin content. When considered together, these spectral indicators provided a clear basis for classifying Commercial Stevia 3 as adulterated with both sweeteners.

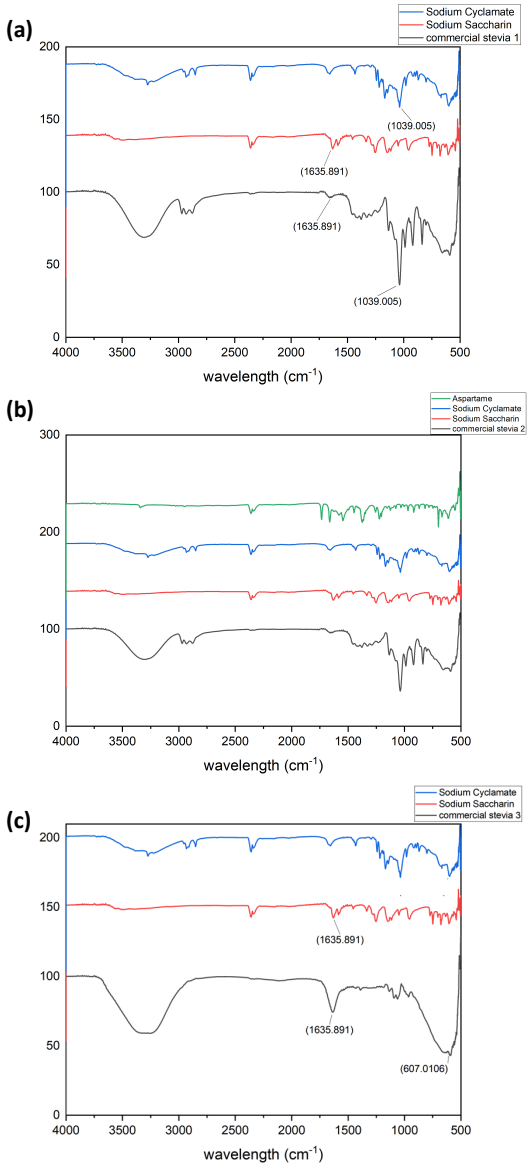

Figure 5 Comparative FTIR spectra of **(a)** sodium cyclamate, sodium saccharin and Commercial Stevia 1, **(b)** aspartame, sodium cyclamate, sodium saccharin and Commercial Stevia 2, and **(c)** sodium cyclamate, sodium saccharin and Commercial Stevia 3

Overall, the PLS predictions were coherent with the expected spectral signatures of the artificial sweeteners. Although some concentrations exceeded 100%, indicating model extrapolation, the consistent presence of characteristic SO_2 and aromatic bands across the commercial samples confirms the occurrence of adulteration by sodium cyclamate and sodium saccharin.

The inflated estimates exceeding 100% can be attributed to model extrapolation beyond the calibration range. This occurs when the spectral characteristics of a commercial sample fall outside the variability represented in the training mixtures, as the commercial stevia may contain other ingredients such as fillers or bulking agents. Several factors can contribute to this: (i) matrix effects arising from additional ingredients in commercial stevia products, which can alter absorbance patterns relative to laboratory-prepared mixtures; (ii) strong non-linear spectral responses at high adulterant levels, particularly for compounds with intense SO_2 functional groups such as cyclamate and

saccharin; and (iii) dominance of certain high-intensity absorption bands that cause the model to project concentrations beyond the upper calibration boundary of 100%. Despite this numerical overshoot, such values still provide reliable qualitative evidence of adulteration, which may be verified by further analysis such as HPLC to determine the actual concentration of adulterants. Overall, the PLS models demonstrated clear capability in detecting and differentiating artificial sweeteners in commercial stevia products available in Malaysia.

In this study, FTIR spectroscopy provided the chemical fingerprints required to differentiate pure stevia from samples adulterated with artificial sweeteners, based on characteristic absorption bands associated with SO_2, C=O, and aromatic functional groups. PCA was first employed as an exploratory tool and demonstrated clear separation between pure stevia and adulterated samples, confirming that the FTIR spectra contained sufficient discriminatory information. Building on this qualitative separation, PLS regression was applied to establish quantitative relationships between spectral variation and adulterant concentration. The resulting models showed strong correlations, with R^2 values ranging from 0.88 to 0.95 and RMSEP values between 33.72% and 43.28%, indicating that while the models provide moderate quantitative accuracy, they are suitable for screening and authentication purposes. In accordance with reported purity criteria for stevia-based sweeteners, adulterant levels exceeding 20% were considered indicative of fraud, as acceptable purity typically falls within the range of 80–102% (Olazar et al., 2022). As explained previously, some PLS predictions exceeded 100%, which can be attributed to several factors including matrix effects in commercial products, the presence of additional formulation components not included in the calibration set, and non-linear spectral responses at high adulterant levels. These effects are further amplified when the full FTIR spectrum is used for modelling, as irrelevant or overlapping bands may interfere with regression coefficients. Model performance could therefore be improved by restricting calibration to a smaller set of chemically meaningful wavenumbers identified through PCA, which would reduce noise, minimise extrapolation, and enhance quantitative robustness.

The predictive performance obtained in this study is comparable to that reported in similar FTIR–chemometric investigations of sweetener and food adulteration. For example, Wang et al. (2020) reported R^2 values above 0.85 with RMSEP values typically in the range of 20–45% for FTIR–PLS quantification of artificial sweeteners, while Roosmayanti et al. (2021) demonstrated that FTIR-based PLS models with RMSEP values exceeding 30% were still effective for screening and authentication of sugar adulteration. Likewise, Martono et al. (2016) noted that FTIR–PLS models applied to complex food matrices often show moderate prediction errors due to matrix effects, yet remain suitable for detecting and differentiating adulterated products. In this context, the R^2 values of 0.88–0.95 and RMSEP values of 33.72–43.28% obtained in the present study fall within the expected performance range for FTIR-based screening models and support their applicability for rapid authenticity assessment rather than precise quantitative determination.

CONCLUSION

FTIR spectroscopy combined with chemometric analysis proved effective for detecting adulteration in commercial stevia products. Distinct spectral features, particularly SO_2 stretching bands for sodium cyclamate and sodium saccharin, and C=O and aromatic vibrations for aspartame enabled PCA to clearly differentiate pure stevia from adulterated samples, while PLS regression provided good performance with significant *p*-values, high explained variance, and acceptable RMSEP values. Application of the models to commercial stevia products revealed substantial adulteration, especially with sodium cyclamate and sodium saccharin; although some predicted concentrations exceeded 100%, this reflects model extrapolation due to matrix differences and dominant functional-group intensities rather than literal values. Importantly, the commercial spectra exhibited characteristic absorption bands matching those of the adulterants, validating the prediction results. Overall, the findings demonstrate that FTIR–chemometrics offers a rapid, non-destructive, and reliable approach for screening stevia authenticity.

Acknowledgement

The authors acknowledge the Ministry of Higher Education Malaysia and Universiti Teknologi Malaysia (grant no. R.J130000.7851.4L945) for the support of this research.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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