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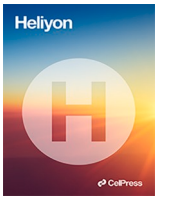

Ultraviolet–visible spectroscopy combined with machine learning as a rapid detection method to the predict adulteration of honey

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ABSTRACT

Honey is often adulterated with inexpensive and artificial sweeteners. To overcome the time-consuming honey adulteration tests, which require precision, chemicals, and sample preparation, it is needful to develop trustworthy analytical methods to assure its authenticity. In the present study, the potential of ultraviolet–visible spectroscopy (UV–Vis) in predicting the sucrose content was evaluated by using Support Vector Regression (SVR) and Partial Least Square Regression (PLSR). To predict the sucrose content based on diagnostic wavelengths, a Point Spectro Transfer Function (PSTF) was evaluated using Multiple Linear Regression (MLR). For this purpose, the spectra of authentic (n = 12), commercial (n = 12), and adulterated (n = 16) honey samples were recorded. Four distinguished wavelengths from correlation analysis between sucrose content and spectra absorption were 216, 280, 316, and 603 nm. The SVR performed better calibration model than the PLSR estimations (RMSE = 0.97, and R^2 = 0.98). The predictive models result revealed that both models had high accuracy for the sucrose content estimation. This study proved that UV–Vis spectroscopy provides an economical alternative for the rapid quantification of adulterated honey samples with sucrose.

1. Introduction

Honey is a highly important natural sweet agent produced by honeybees (*Apis mellifera*) from the nectar of flowers or plant secretions. In terms of composition, honey is a rich source of sugars, predominantly glucose and fructose, organic acids, vitamins, proteins, enzymes, bioactive compounds, and mineral [1–3]. The health properties of honey, like antioxidant capacity, prebiotics, and decreasing cardiovascular risks, besides the desirable taste, are the reasons for the expensive cost of honey compared to other popular sweeteners. It makes honey prone to the adulteration [4,5].

Over the last few years, adulteration of honey has become a significant subject as a consequence of growing global trade for economic increment [3]. Honey fraud, whether adding or removing any kind of substance [6], is an illegal activity, and it can cause health adversity in unaware consumers. Honey is the third most adulterated product after olive oil and milk worldwide [3].

Finding a non-destructive and reliable analytical method to detect honey adulteration is extremely important. Several analytical methods including gas chromatography (GC), volumetric electronic tongue, near-infrared spectroscopy (NIR), mid-infrared spectroscopy (MIRS), high-performance liquid chromatography (HPLC), infrared (IR), mass spectrometry (MS), and nuclear magnetic resonance (NMR), have been applied for the quantification of honey [2,3,5,7–16]. These methods are expensive, time-consuming,

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destructive, require a skilled operator, and complicated sample preparation. They are not easily enforced in routine honey laboratories where swift analysis is more appropriate [1,17,18]. UV–Vis spectroscopy method is easy to use, low cost, environmentally friendly, in situ implementing, and non-destructive which preserves the integrity of the sample and the possibility to re-analyze with another technique if necessary. In this sense, the proper chemometric methods besides UV–Vis spectroscopy have increased the reputation in food quality monitoring. It can be propounded as an acceptable alternative to the chemical generally used methods for food control. Chemometrics play a vital role in the quantification of complex samples. The functions of chemometrics are to find well statistical correlations between chemical parameter data and spectral data for the model development process [17,19–21].

The calibration success is up to the choice of the calibration method and its performance in modeling spectra. The quantitative multivariate models that are often used to describe a system with a hidden relationship between available data and generated information are Support Vector Regression (SVR), Partial Least Square Regression (PLSR), Hierarchical Cluster Analysis, Linear Discriminant Analysis, Qualitative Data Analysis, Soft Independent Modeling by Class Analogy and Principal Component Analysis [2,5,19,20,22].

PLSR is a regression method commonly used in the field of spectral analysis. It is very suitable for high-dimensional spectral datasets and can establish a possible mathematical relationship between the data and the labels based on a linear multivariate model to obtain the predicted values of the target [23]. SVR has the capability to solve both linear and nonlinear multivariate regression problems with a simple process. These modeling approaches predict unknown samples by constructing one model [24].

From the studies that have been conducted using UV–Vis spectroscopy on honey, the authentication and classification of honeys according to their botanical, entomological, and geographical origins using ultraviolet (UV) spectroscopy and SIMCA (soft independent modeling of class analogy) [25], honey adulteration detection by UV–Vis spectroscopy in combination with factorial design, response surface methodology and supervised machine learning classifiers [26], UV–Vis spectroscopy and Raman spectroscopy together with principal components analysis [18], UV–Visible spectroscopy coupled with linear discrimination analysis to discriminate between monofloral and multifloral honey [27], Classification of the Botanical Origin for Honey [28], characterization of Brazilian floral honey using UV–vis, near-infrared (NIR), and nuclear magnetic resonance (NMR) spectroscopy [29], and honey authentication with sugar syrups using UV–Vis spectroscopy and one-class classifiers [2] can be mentioned. What is certain is that UV–Vis spectroscopy was not used alone in any of these studies to detect honey adulteration, and used along with other equipment. On the other hand, the studies that used UV–Vis spectroscopy alone were not for adulteration detection and were mostly classification. In this research, the sucrose content is measured using UV–Vis spectroscopy, which both shows adulteration in honey and can replace time-consuming and expensive chemical tests for measuring sucrose content.

To the authors' knowledge, no previous study on the application of UV–Vis spectroscopy combined with a machine learning algorithm to evaluate the sucrose content of honey has been reported. Such studies are needed for rapid quantitative detection of honey adulterated. The results of this study can eliminate the need to conduct chemical and time-consuming tests in laboratories and honey quality verification centers. The purpose of this study is to evaluate the potential of the UV–Vis spectroscopy assay in estimating sucrose content, to develop a point Spectro transfer function for sucrose content prediction, and to compare PLSR, and SVR models for optimal estimation of sucrose content using spectral absorption as a rapid screening method to detect the honey adulteration.

2. Materials and methods

2.1. Samples and sample preparation

For this study, a total of 40 samples were used. There were 12 commercial samples purchased in the supermarket (Sari, Mazandaran, Iran) on April 2022, 12 authentic spring honey samples from different providers (Sari, Mazandaran, Iran) on April 2022, and 16 adulterated honeys. Sucrose syrup was prepared by mixing 750 g of sugar, 375 g of distilled water, and 1.125 g of citric acid. Then, it was heated at 40 °C for 20 min. The ratio and preparation conditions of sugar syrup were chosen according to pre-tests, and the final syrup had standard pH (>3.5), acidity (<40 meq/kg), and hydroxy methyl furfural content (<40 mg/kg). To prepare adulterated samples, the authentic honey with highly quality and standard range parameters were mixed with sucrose syrup at different ratios: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, and 1:16 w/w. Adulterated samples were mixed using a magnetic stirrer at 20 °C for 24 h to ensure homogeneity before analysis [3,30].

2.2. Determination of sucrose content

To determine the total sugars after and before hydrolysis, the Fehling test was carried out. Fehling reagent was prepared by mixing 5 mL of each of Fehling A (7 g $CuSO_4.5H_2O$ dissolved in 100 mL of distilled water) and Fehling B (35 g of potassium tartrate and 10 g of NaOH in 100 mL of distilled water). 1 g of honey sample was diluted in 100 mL of distilled water, and then 50 mL of diluted honey was taken in the burette. 15 mL of diluted honey was mixed with 10 mL of Fehling reagent and 10 mL of distilled water. The mixture was heated until it started boiling, then 1 mL of methylene blue was added, and titration was completed during boiling. Titration was carried out while heating the solution until the indicator was discolored. The percentage of total reducing sugar before hydrolysis was calculated by the following Eq. (1):

$$S_1 = \frac{2 \times 1000}{V \times W} \quad (1)$$

Where: S_1 = g of total reduced sugar before hydrolysis, V = volume of diluted honey solution consumed, and W = weight of the honey sample.

50 mL of honey dilution was added to 2 mL of HCL in a volumetric flask and heated at 70 °C for 10 min. After cooling to room temperature, phenolphthalein was added to the solution as an indicator, titration was performed by NaOH (0.1 M), and the volume was adjusted to 100 mL. Afterward, 25 mL of the prepared mixture was mixed with 10 mL of Fehling reagent and 10 mL of distilled water. The mixture was heated until it started boiling, then 1 mL of methylene blue was added, and titration was completed during boiling. Titration was carried out while heating the solution until the indicator was discolored. The percentage of total reducing sugar after hydrolysis was calculated by using Eq. (2):

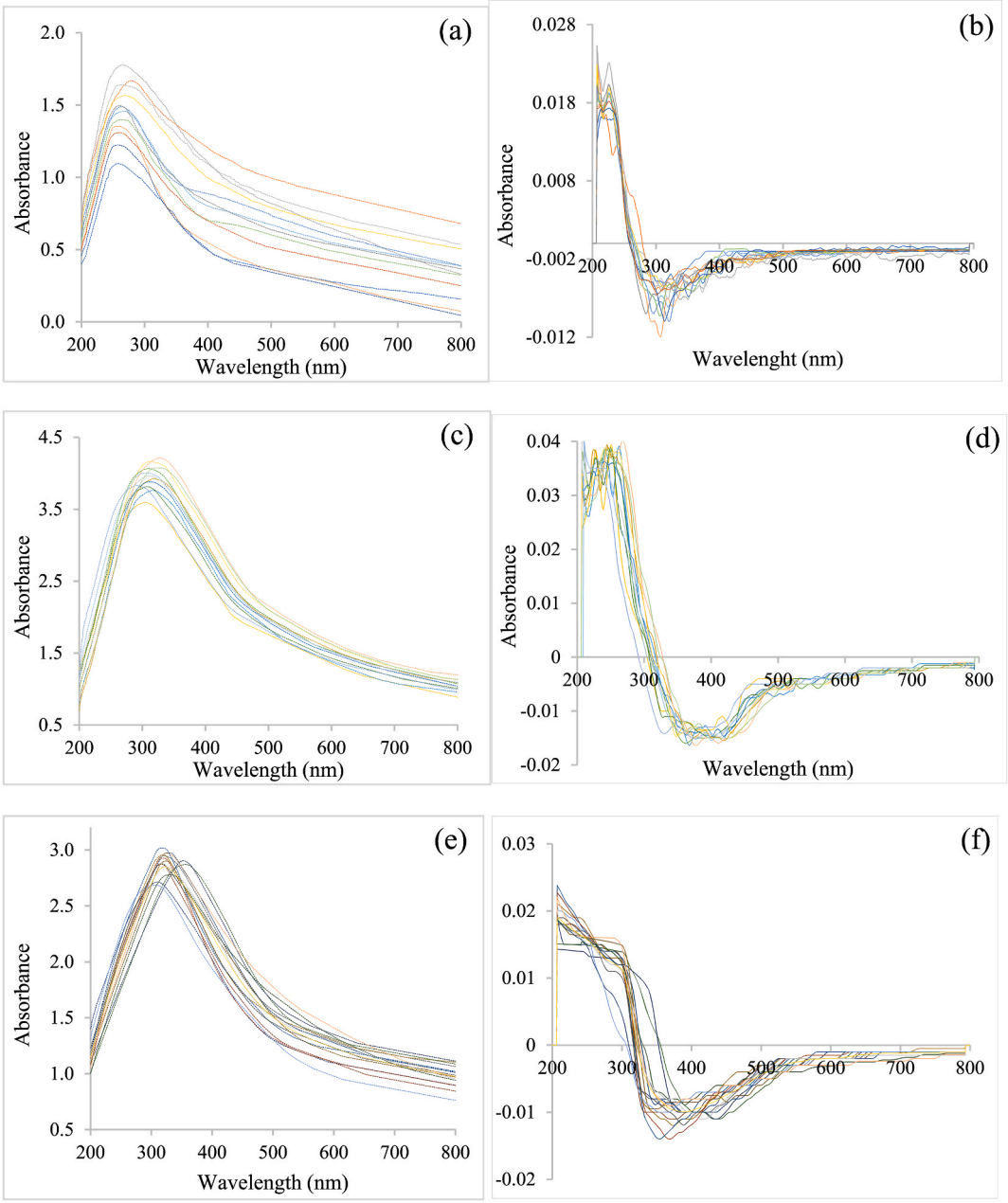

Fig. 1. The raw absorption data of the authentic (a), commercial (b), and adulterated (c) honey samples. The preprocessed absorption data of the authentic (d), commercial (e), and adulterated (f) honey samples by 1st Derivative.

$$S_2 = \frac{4 \times 1000}{V \times W} \quad (2)$$

Where: S_2 = g of total reduced sugar after hydrolysis, V = volume of diluted honey solution consumed, and W = weight of the honey sample. The sucrose content was calculated according to S_2–S_1 [31].

2.3. Instrumentation

The UV–Vis spectra were recorded in the region from 200 to 800 nm using a PG instrument spectrophotometer (PG instrument T80+, USA) equipped with a quartz cuvette, and with a photodiode array in the region from 190 to 1100 nm with a resolution 1 nm. All measurements were performed at room temperature (20 ± 1 °C). The average spectrum of each sample, considering its quadruplicate, was used to construct of the chemometric models.

2.4. Chemometric procedures

The spectra between 200 nm and 800 nm of UV–Vis spectra were recorded. Then, four different pre-processing methods were applied to the spectral pre-processing algorithm: a) first derivative Savitzky -Golay smoothing with a polynomial of second-order (1st Der), b) standard normal variation (SNV), c) linear baseline correction (LBC), d) offset correction (OFF). Then, 40 honey samples were randomly divided into validation and calibration datasets. The calibration set was composed of 28 (70%) honey samples to construct the models, while the other 12 (30%) honey samples were included in the validation set [32]. The *t*-test was applied to evaluate the mean difference between validation and calibration datasets for spectral analysis. Then, PLSR and SVR models were employed for authentication purposes. To predict the sucrose content based on diagnostic wavelengths, a Point Spectro Transfer Function (PSTF) was evaluated using Multiple Linear Regression (MLR) using SPSS 20 software (IBM Corp, Armonk, New York, USA). The coefficient of determination (R^2), root mean squared error (RMSE), and the ratio of predicted deviation (RPD) was determined to evaluate the efficiencies of the predictive models. The RPD estimations were classified as: excellent with RDP >2.5, very good with RDP = 2–2.5, good with RDP = 1.8–2, moderate with RDP = 1.4–1.8, weak with RDP = 1–1.4, and very poor with RDP <1 [33]. All chemical procedures were performed using the Unscrambler X (version 10.4) software (Camo Software As, Oslo, Norway). Statistical analyses were applied by SPSS 20 software (IBM Corp, Armonk, New York, USA).

3. Results and discussion

3.1. UV–vis spectra analysis

UV–Vis spectroscopy is traditionally used for analysis according to the height and position of characteristic peaks. Fig. 1 illustrates the average raw (a, c, e) and preprocessed (b, d, f) UV–Vis spectra of three different honey samples in the region from 200 to 800 nm. As can be observed, in Fig. 1 (a, c, e), molecular absorption bands are exhibited in three regions. The commercial, adulterated mixture, and authentic honey samples had the highest absorption, respectively. Fructose, glucose, and sucrose are the most abundant carbohydrate in honey, which is used as a marker for authenticity [2]. The region from 220 to 310 nm had the highest absorbance peak for authentic honey samples. Similarly, Suhandy et al. (2021) reported the peak absorbance intensity at 270 to 300 for Indonesian honey samples. The distinctive peaks observed at 270–300 nm are associated with the absorbance of salicylic, benzoic, and aryl-aliphatic acids in honey [34]. These spectral results are consistent with previously reported works for Indonesian [1], Iranian [12], and Italian honey [17].

The commercial honey samples presented an intense absorption peak of around 280–330 nm. Parri et al. (2020) reported a vast peak between 250 and 340 nm for the Sulla honey sample, which is in accordance with our spectra [17]. The enlargement in the absorbance is due to the presence of sugar and other components generated during syrup preparation and adulteration. The adulterated honey samples presented a higher peak than authentic honey samples, and the wavelength was around 300–370 nm. The peak at 325–400 nm is related to Maillard reaction products, mainly HMF and furosine [12]. Aliaño-González et al. (2019) measured the Vis-NIR spectra for pure and adulterants honey. They reported different intensities in some spectral zones are relevant to detecting and determining the adulterants used [5].

Although differences in the spectra obtained are observed, it is necessary to apply chemometric tools to extract useful information related to the presence of sucrose in honey [5]. Chemometrics can accomplish the quantitative analysis of complex samples like honey.

Table 1
Statistical analysis of the sucrose content of honey samples.

Honey type (Sucrose content (%))	Min	Max	Mean	Median	STD	Q_1	Q_3	CV (%)
Authentic (0.0–11.5)	0.06	11.44	7.77	10.09	4.36	4.61	11.21	56.19
Commercial (15–23)	15.27	22.66	20.66	21.26	2.33	20.07	22.50	11.31
Adulterated (11.5–15	11.57	14.81	12.87	12.71	1.08	11.88	13.70	8.42

STD= Standard Deviation, Q_1, and Q_3 = First and Third quartile, respectively, CV= Coefficient of Variance, where >35 % = high variability, 15–35 % = moderate variability, <15 % = low variability.

As said before, four pre-processing methods were applied on a raw data, and the 1st Der pre-processing was used to cancel the baseline drifts and to enhance small spectral differences. The lower RMSE of 1st Der pre-processing caused other analyses to be performed on it, and the other pre-processing was ignored.

3.2. Sucrose content

The statistical summary for sucrose content of honey samples is shown in Table 1. The CV of commercial and adulterated samples varied very close to each other (11.31, and 8.42%, respectively). Furthermore, authentic honey sucrose contents were low, with an average of 7.77%. This sucrose content is very close to the highest sucrose content allowed by the Food and Agriculture Organization of the United Nations (FAO Code: 1182-Honey, natural) sucrose content (<5 g/100 g) for the honey sample [2]. The commercial honey sample showed the highest coefficient of variations (11.31%) among all samples, is related to higher sucrose content. Among the honey properties, the percentage of sucrose is very important. As can be seen in Fig. 1S, the adulterated honey samples showed lower sucrose content than commercial samples. It is related to lower sucrose content of pure honey sample which are used to prepare the adulterated honey samples.

3.3. Correlation analysis and prediction of sucrose content

The possibility of quantifying the level of adulteration using UV–Vis spectroscopy was evaluated. A Pearson's correlation coefficient (Pearson's r) between sucrose content and the spectral absorbance in the region from 200 to 800 nm was applied and illustrated in Fig. 2 (a). There is a high correlation between the honey spectral absorbance and the actual values of sucrose content. Generally, the relationship between spectra and sucrose content developed at 250–350 nm.

The incidence effects of varied parameters in each wavelength and repeated information in adjoining wavelengths are one of the vital problems in processing spectral data [35]. The overfitting will increase when predictive variables (601 wavelengths), are greater than the actual samples (40 honey samples). The multiple linear regression (MLR) analysis was conducted to consider the correlation between the sucrose content and initial spectra wavelengths. In this study, important wavelengths and sucrose content were included

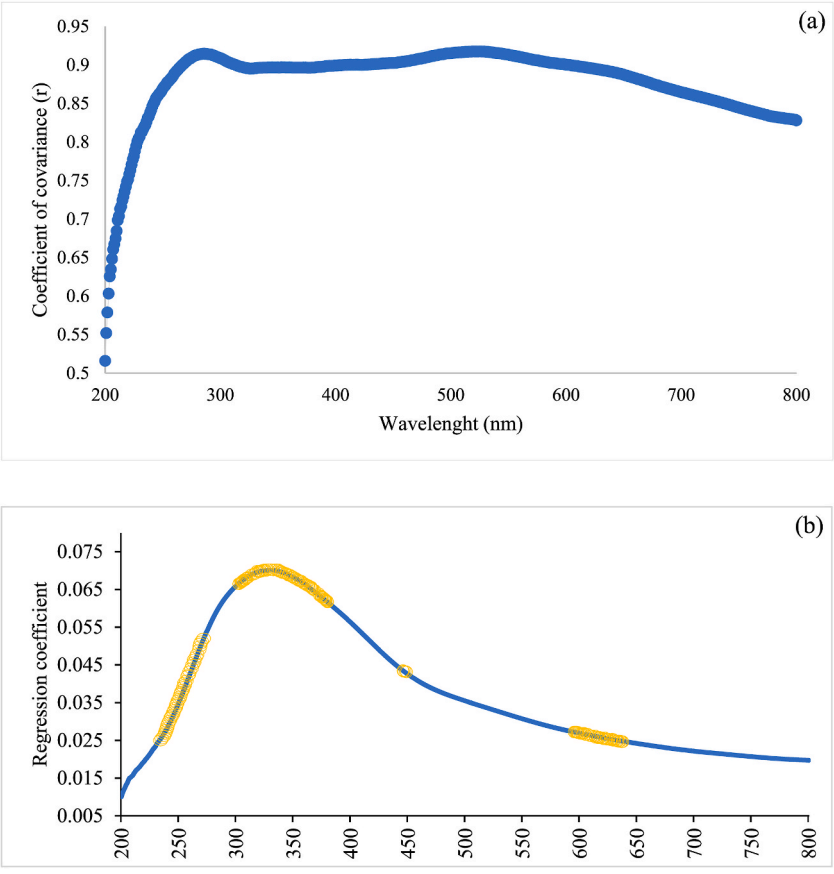

Fig. 2. Pearson's correlation coefficient (r) between spectral absorbance values across the UV–Vis range and sucrose content (a) and important wavelengths of spectral absorbance according to MLR (b).

in the regression model. According to Fig. 2. (b), the wavelengths in the region from 240 to 280, 308–382, 448–452, and 603–644 nm were the important wavelengths and were used to predict the sucrose content. Fig. 2. (b) showed the correlation between wavelengths (200–800 nm) and sucrose content for selecting effective spectra for the development of PSTF. The selected spectra had the highest correlation with sucrose content and were significant at the 95% level of confidence. Eq. (3) provides the PSTF for estimating sucrose content based on spectral absorbance.

$$\text{Sucrose content (\%)} = -3.1 - 17.41R_{261} + 34.75R_{280} - 21.13R_{316} + 23.48R_{603} \quad (3)$$

Where, R_x is the spectral absorbance at a wavelength x. At mentioned wavelengths, sucrose content is detectable. Considering the significant positive correlation between honey spectral absorbance and sucrose content, the utilization of these spectra is reasonable.

3.4. Prediction of sucrose content

Fig. 3(a–d) illustrates the scatter plots of predicted sucrose content against actual sucrose content using PLSR and SVR approaches. It indicates that the actual and predicted sucrose content are greatly consistent. In both validation datasets, PSTF ($R^2 = 0.84$, RMSE = 1.97 for validation datasets and $R^2 = 0.95$, RMSE = 1.32 for calibration datasets) showed high performance. Calibrations were better than validations. It indicates that performance of the model during calibration was better than validation. The lower values of RMSE obtained for the validation (RMSE = 1.97) and calibration (RMSE = 1.32) than standard validation of the actual data (Standard Deviation = 5.23 for the validation and Standard Deviation = 6.05 for the calibration) exhibited the acceptability of these spectral function for the prediction of sucrose content based on key wavelengths. Considering the time and cost involved in sample preparation and experimental tests of sucrose content of honey and the good accuracy of the PSTF, the application of PSTF for honey adulteration

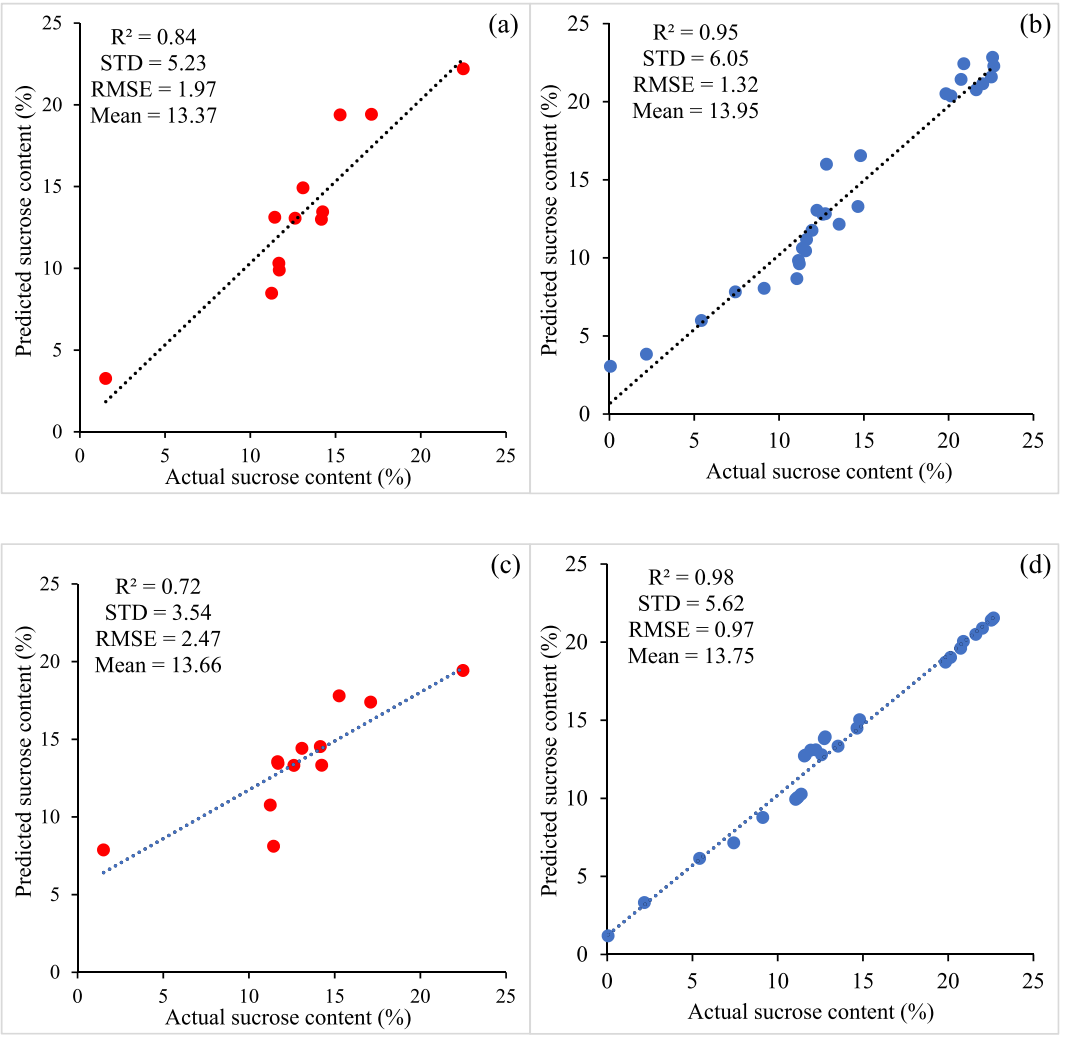

Fig. 3. Prediction results by PLS models for validation (a) and calibration (b) datasets and SVR models for validation (c) and calibration (d) datasets.

studies has been proved.

Results for both models for sucrose content prediction using spectral absorbance are presented in Table 2. The results indicate that the predicted and actual values are largely consistent. Underestimated and overestimated predicted values considering RMSE and RPD weren't seen. Comparing both models for sucrose content, the SVR model showed the largest R^2 (0.98) and lower RMSE (0.97), with a RPD of 5.80, demonstrating a superior prediction compared to the PLSR model ($R^2 = 0.95$, RPD = 4.58). The results of the student t-test showed no statistically significant difference ($P > 0.05$) between prediction and actual sucrose content values which exhibits the efficiency of the model to predict. Aliaño-González et al. (2019) evaluated the capability of models by checking the RMSE of Calibration (RMSEC) and RMSE of Prediction (RMSEP). They reported the coefficient of determination above 0.98 for all models and really low errors (RMSEC and RMSEP values were below 3%) [5]. The RMSE under 3% demonstrated the accuracy and robustness of the only adulterant model, which is in accordance with the results of previous studies [5,36].

The results of sucrose content were according to previous studies showing the excellent efficiency of the SVR model in comparison to PLSR. In a study conducted by Truong et al. (2022), chemometric models using PLSR and SVR were developed to predict manuka honey potency, and purity. The accuracy of the SVR (89 %) was higher than PLSR (74 %) model [37]. Valinger et al. (2021) evaluated the potential of UV–Vis and NIR spectroscopy coupled with PLSR modeling and ANN modeling for detecting the adulteration and quantifying of the physical and chemical properties of pure and adulterated honey samples. Although, they didn't measure the sucrose content as a honey fraud, their results indicated that the PLSR model could be used efficiently for screening. However, ANN modeling provided an accurate and simultaneous predictions of the honey properties [38]. Raypah et al. (2022) reported that the PLSR model by high-value coefficient of correlation and low RMSE value of validation and prediction effectively quantified the percentage of water and apple cidar vinegar adulteration [14].

The results indicate that the SVR is more efficient than the PLSR method due to its ability to incorporate nonlinear interactions and relationships. Boateng et al. (2022) used the chemometric technique as an easy, fast, and cheaper alternative for detecting of syrup adulteration in honey. The performance of the regression algorithm based on the RMSE of the external validation set in the PLSR was more than SVR [22]. Chen et al. (2019) measured the performance of the NIR spectroscopy for detecting cAMP content in red juice using SVR and PLSR models. Their results indicate that the SVR model can largely enhance predictive performance [39]. In a study conducted by Guelpa et al. (2017), the PLSR was applied to NIR spectroscopy data to verify the authenticity and detect adulteration of South African honey by glucose and fructose syrup [21]. Li et al. (2017) used the PLSR to predict the extent of honey adulteration. Their result showed that PLSR couldn't be used to quantify adulteration with high fructose corn syrup, but it could be used to quantify adulteration with maltose syrup [40]. However, the initial results (Table 2) illustrated that SVR is a considerably favorable model for predicting the sucrose content. It's statistically essential to determine the statistically significant difference between RMSE and R^2 values of models. Thus, the P-value from the randomized student t-test of both models with 40 replicated simulations. The statistical difference between SVR and PLSR models for the prediction of sucrose content wasn't significant ($P > 0.05$). This proves that both models have a high predictive accuracy in quantifying sucrose content. The results of the predictive models confirm that UV–Vis spectroscopy is extremely useful for estimating the sucrose content of honey samples. More importantly, spectroscopy provides a fast and non-destructive method for collecting information about honey adulteration, a technique that can be a valuable tool for estimating sucrose content.

4. Conclusion

This paper compares the prediction results of PLSR and SVR models for the assessment of sucrose content in honey samples using UV–Vis spectroscopy. The distinguished spectral for sucrose content prediction was the wavelengths of 261, 280, 316, and 603 nm. The performance of the SVR model was better than the PLSR in evaluating sucrose content. The results demonstrated that spectral absorbance is a hopeful tool for impressive assessing unknown honey samples and sucrose content estimation. With the application of PLSR and SVR, quantitation models of UV–Vis spectroscopy were established for the first time. A major correlation between the sucrose content and honey spectral absorbance was observed. Chemometric techniques offers fast, simple and unexpensive method to verify the authenticity and detect the fraudulence of honey. It is a non-destructive and efficient method for the determination of sugar adulterants without the use of reagents and the generation of harmful residues, which protects the environment.

Authorship statement

The authors declare no competing financial interests or personal relationships that could influence this work.

Table 2
Comparison of quantitative models for sucrose content in honey.

Model	Calibration			Validation		
	Rc^2	RMSEC	RPD	Rp^2	RMSEP	RPD
PLS	0.95	1.32	4.58	0.84	1.97	2.65
SVR	0.98	0.97	5.80	0.85	2.47	1.43

Rc^2: Coefficient of calibration, Rp^2: Coefficient of prediction, RSMEC: Root Mean Squared Error of Calibration, RSMEP: Root Mean Squared Error of prediction, RPD: Ratio of Prediction to Deviation.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Razie Razavi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Reza Esmaeilzadeh Kenari:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20973.

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