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Original Article

FOURIER TRANSFORM INFRARED SPECTROSCOPY AND CHEMOMETRICS FOR AUTHENTICATING CHILI POWDER FROM RHODAMINE B, ERYTHROSINE B, AND PARARED

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ABSTRACT

Objective: The purpose of this study was to identify chili powder adulteration with synthetic dyes of rhodamine B, erythrosine B, and parared using Fourier Transform Infrared (FTIR) spectroscopy and chemometrics.

Methods: The analysis included two types of red chilies, curly red and big red chilies. FTIR and chemometric spectroscopy combination were used to detect synthetic dyes in the chili powders. Principal Component Analysis (PCA), Partial Least Square-Discriminant Analysis (PLS-DA), and Partial Least Square-Regression (PLS-R) methods were applied.

Results: The FTIR spectrum in the range of 700-850, 1100-1700, and 2300-3500 cm^{-1} can detect the adulteration of chili powder with rhodamine B or erythrosine B. Meanwhile, the parared dye can be distinguished at wave numbers of 2000-3000 cm^{-1}.

Conclusion: The combination of FTIR spectroscopy with chemometrics can be used to authenticate red chili powder from rhodamine B, erythrosine B, and parared.

Keywords: Authentication, Chemometrics, Chili powder, FTIR spectroscopy, Synthetic dyes

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INTRODUCTION

Prices of perishable chilies fall when production is abundant. Therefore, the chili is converted into a more durable powder form. Some manufacturers add carrots and garlic in order to increase profits. These ingredients will add weight to the chili powder but may change the color. Hence, banned synthetic dyes such as rhodamine B, parared, or erythrosine B were sometimes added to chili powder [1-4]. Synthetic dyes prohibited for food use have intensive colors, low prices, and stability.

Long-term use of rhodamine B can cause cancer and impair liver function. In addition, rhodamine B also irritates the gastrointestinal, respiratory, and ocular. The dye exposure to the lips leads to chapped lips, itching, and even peeling of the lip skin [5]. Rhodamine B and parared is potentially both genotoxic and carcinogenic [6].

Unlike rhodamine B and parared, erythrosine B can be used at low concentrations in food, cosmetics, and pharmaceuticals. However, at high doses, this dye has been shown to have adverse side effects such as chest tightness, shortness of breath, headache, and skin irritation. Several previous studies reported inhibition of P-glycoprotein [7], inhibition of protein-protein interactions of TNF-R-TNFα, and CD40-CD154 by erythrosine, which are responsible for tumor necrosis and immune responses, respectively [8], toxic effects on spermatogenesis [9], and chromosomal aberrations [10].

Several analytical methods have been employed to detect the presence of red dyes in chili powder and other processed products. The use of these instruments was supported by a variety of extraction techniques. The previous study reported the use of a chromatographic method using a Thin Layer Chromatography/TLC combined with Ultraviolet-Visible (UV-Vis) spectrophotometry [11], High-Performance Liquid Chromatography/HPLC [12], Ultrafast Liquid Chromatography/UFLC coupled with ultra-violet detector [13], Liquid Chromatography-tandem Mass Spectrometry/LC-MS [14, 15], Ultra-Performance Liquid Chromatography/Uplc [16, 17], Ultra-High Performance Liquid Chromatography–High Resolution Mass Spectrometry/UHPLC–HRMS [18], micellar electrokinetic chromatography-mass spectrometry [19], gel permeation chromatography–liquid chromatography–electrospray ionization-tandem mass spectrometry [20].

Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR) is one of the most popular spectrometric methods because of its relatively simple use. The dye analysis in chili has been examined using a variety of spectroscopy methods with various modifications, including UV-Vis spectroscopy [11, 21, 22], fluorescence spectroscopy on the front face [23], near-infrared spectroscopy and raman spectroscopy [24]. However, despite the success of these methods in analyzing dyes in red chilies, there are various disadvantages associated with the need for complicated pretreatments, such as using more than one solvent or reagent, rotary evaporation, and solid phase extraction.

A combination of chromatography/spectroscopy and chemometrics can be a promising method for identifying rhodamine B, erythrosine B, and parared in chili powder. Chemometrics can reduce previously required separation steps, resulting in a faster, more direct, less time-consuming, and relatively inexpensive method [25]. Some studies have used chemometrics to authenticate chili powder by adding a red dye. Liu and coworkers used chemometrics based on fluorescence spectral data [23]. Other studies have constructed a screening method for the adulteration of chili powders using UV-Vis spectra [21, 22]. Ming-Yue Dong used HPLC chromatograms [26]. Another study used near-infrared (IR) spectral data to screen adulteration of chili samples from Sudan [24].

FTIR chemometrics has been used to authenticate food, cosmetics, and pharmaceuticals. In several studies, the method has been applied to detect adulteration in red ginger, gelatin in jelly soft candy, pork in beef meatballs, pure olive oil, edible oil, propolis, and turmeric [27–33]. The application of this method to detect adulteration in chili powder was still limited. In this study, FTIR-ATR was used in combination with chemometrics to detect adulteration of chili powder with potentially adulterated red dyes such as rhodamine B, erythrosine B, or parared.

MATERIALS AND METHODS

Materials

Rhodamine B (R6626, dye content ≥95 %), parared (100994, dye content 95 %), and erythrosine B (200964, dye content ≥95 %) were purchased from Sigma-Aldrich. Two types of *Capsicum annuum* highly marketable commodity in Indonesia, including big red chili (*Capsicum* *annuum* L.) and curly red chili (*Capsicum annuum* L. var. Longum) used in this study. Five different commercial samples of chili powder (four commercial brands/A-D and one commercial non-brand/E) were collected from local market in Tegal, Jawa Tengah, Indonesia.

Preparation of chili powder standard and sample for multivariate analysis

Chili powders were made from fresh Big Red (BR) chili and Curly Red (CR) chili. Seedless chilies were dried at 45 °C for 24 h. Dried chili was powdered and sieved using 80 mesh sieves. A set of standards containing biner mixture of chili powder and rhodamine B, parared, or erythrosine B were prepared. The standard has various concentration with ratio of chili powder and dye, include 100:0; 75:25: 50:50; 25;75; and 0:100 (w/w). A total of thirty standard were prepared to develop the chemometric multivariate analysis. The standard and samples of chili powder were measured by FTIR (Shimadzu IR Tracher-100) with the ATR sampling technique (Specac) without prior treatment. The spectra scan was made with 8 cm^{-1} of resolution, 32 scans, and spectral range from 4000 to 400 cm^{-1}.

Chemometrics analysis

The authentication method of chili powder, includes BR and CR from rhodamine B, parared, and erythrosine B was developed by using absorbance data from FTIR combined with multivariate analysis. FTIR spectra were converted into numerical data prior to multivariate analysis. The wave number data was analyzed by XLSTAT software ver. 2019. PCA was apllied to classify of pure and adulterated chili powder. PLS-DA and PLS-R were used to obtain quantitative analysis. PLS generated by calibration step to obtain the model, internal validation, and external validation. Root mean Square Error (RMSE) and coefficient of determination (R^2) were used as criteria for the calibration model.

RESULTS AND DISCUSSION

Three groups of wave number ranges, such as 700 to 850, 1100 to 1700, and 2300 to 3500 cm^{-1}, were used to classify chili powder containing artificial dyes. This authentication can be carried out simultaneously without prior separation, thereby shortening the analysis time. The different molecular structures make the spectra visually distinguishable from one another. Sometimes, the spectrum differs only in the ratio of signal intensities. For this reason, statistical tools with chemometrics are required to manage the spectrum data generated in order to improve data quality [34]. Chemometric techniques are advantageous in interpreting IR spectra since they allow correlation between a spectral profile and hidden information contained within the sample [35].

FTIR spectra analysis

The FTIR spectra of BR and CR chili powder were not significantly different (fig. 1). The FTIR spectrum of chili powder BR shows the primary peak at wavenumbers of 3275.13, 2924.09, 1743.65, 1624.06, 1543.05, 1458.18, 1269.16, and 1022.27 cm^{-1}. Meanwhile, the most significant CR peaks include 3286.70, 2924.09, 1728.22, 1585.49, 1442.75, 1273.02, 1234.44, 1029.99 cm^{-1}. These results are consistent with previous research, which identified the first peak (3600–3000 cm^{-1}) as OH stretching and the second peak (2950-2800 cm^{-1}) as C-H stretching bound to the methyl and methylene groups of cyclopentane and cyclohexane. Another peak corresponds to carbonyl's stretching vibration absorption peak (C=O) at 1750-1710 cm^{-1}. The fingerprint region strengthens the presence of OH groups by stretching the overtone (1650 cm^{-1}). The scissor's vibration absorption peak of methylene (CH_2) and the anti-symmetric deformation absorption peak of methyl (CH_3) were at 1465±20 cm^{-1}. Finally, the bands in the region of 1200-950 cm^{-1} were assigned to the stretching vibration absorption peaks of C-O and C-C [36, 37]. The spectrum refers to the red pigment of capsicum, which contains the primary compound from chilies, capsaicinoid. Capsaicin, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin, are examples of chemical components from the class of capsaicinoids. About 90% of chilies' capsaicinoids consist of capsaicin and dihydrocapsaicin [38].

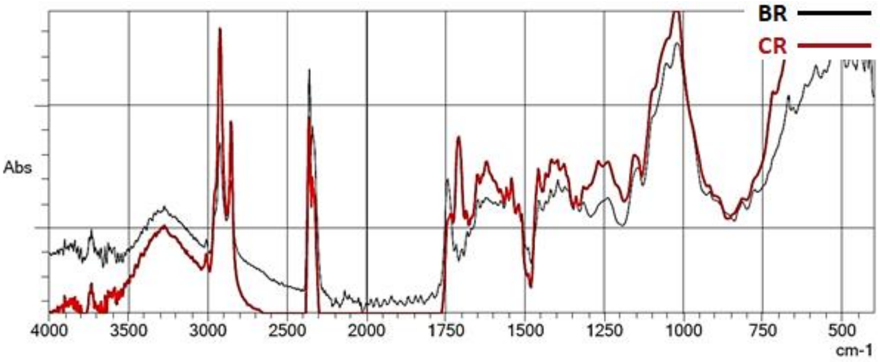


Fig. 1: FTIR spectra of big red (BR) and curly red (CR) chili powder

Fig. 2 displays the FTIR spectra of rhodamine B, erythrosine B, and parared. According to this result, rhodamine B exhibits peaks at wave numbers 3368.61, 3360.00, 2974.23, 1581.63, 779.24, and 678.94 cm^{-1}. The results were similar to those in another study [39]. The peak of 3462.37, 3461.41, and 3459.48 cm^{-1} was associated with the stretching vibrations of the O-H group. As well as the 1630.88, 1634.74, and 1636.67 cm^{-1} peaks caused by O-H vibrations, there were also peaks caused by Al-O vibrations at 775.42, 731.05, and 732.98 cm^{-1} [39]. Furthermore, we observed the spectrum of a red solution of rhodamine B (in water). Compared with rhodamine B powder (green color), the spectrum of rhodamine B solution was not significantly different. Interestingly, the difference is only visible at the peak of wave number 3000-3500 cm^{-1}, which appears to be broad. Spectral peaks in water are responsible for this phenomenon.

Previously, the spectrum of erythrosine B was observed to have peaks at wavenumbers of 3407 (O-H stretching vibration), 1607 (C=C stretching vibration), 1550 (C-C vibration), 1452 (O-H bending vibration), 1341 (C-O-C vibration), 1217 (C-O stretching vibration), 1151 (C-O stretching vibration), and 952, 707, 629, 548, 446 cm^{-1} (C-I vibration) [40]. The data was in line with this research. Erythrosine B showed peaks at 3340.71, 1543.05, 1446.61, 1342.46, 1234.44, 1165.00, and 466.77 cm^{-1}.

Meanwhile, according to the literature, the peak of the parared infrared spectrum was at 1500.52-1521.73 cm^{-1}, which corresponds to the stretching vibration of the azo group (N=N). A stretching vibration was observed at 1328.86, connecting the nitro group (NO_2), 3390 cm^{-1} associated with the O-H group [41]. As a result of the research carried out, this is consistent with the findings. The peak spectrum was at wavenumbers 1500.62, 1327.03, and 3390.86 cm^{-1}.

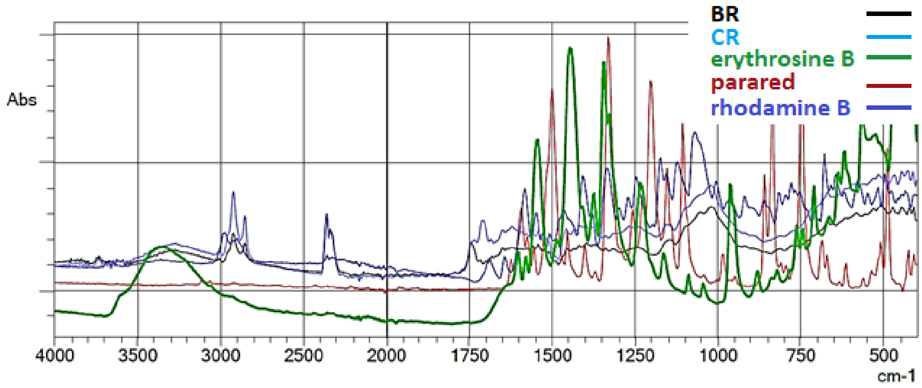


Fig. 2: FTIR spectra of rhodamine B, erythrosine B, parared, big red (BR) chili powder, and curly red (CR) chili powder

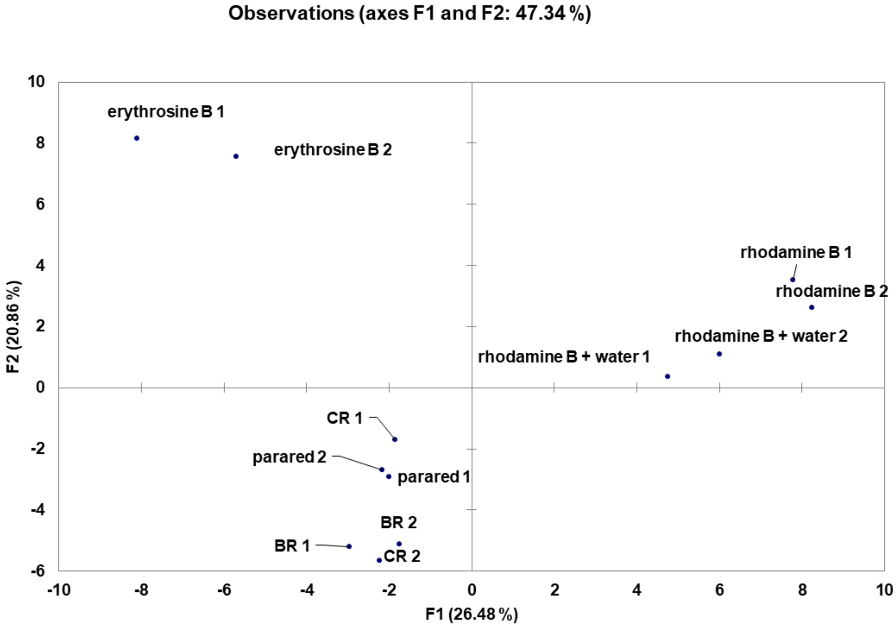


Fig. 3: PCA analysis of FTIR spectra of rhodamine B, erythrosine B, parared, big red (BR) chili powder, and curly red (CR) chili powder obtained using the spectral region of 700 – 850, 1100 – 1700, and 2300 – 3500 cm^{-1}

Principal component analysis (PCA)

As a first step in the spectral region selection for classified the object, the wavenumbers range in which absorbance intensity measurement must be determined. The spectral range should include characteristic regions in which the chemical groups related to the object of interest. Based on analysis correlation spectrum, the regions at 700-850, 1100-1700, and 2300-3500 cm^{-1} showing the region which has capability to classified chili powder and dyes.

PCA results in the creation of new uncorrelated variables from the original variables. Each PC (Principal Components) was a linear combination of the observed variables. The similarities between the samples will be seen when the PC values are closer to each other (fig. 3). Chili powder (BR and CR) was grouped in the same quadrant and can be distinguished from erythrosine B and rhodamine B. Unfortunately, the parared clustered in the same selected spectral region as chili powder. Thus, observations in the range of other wavenumbers were necessary to differentiate chili powder from parared.

A deep examination revealed that the wavenumber used for PCA analysis between CR, BR, and parared ranged between 2000 and 3000 cm^{-1}. The IR spectrum exhibits a more significant difference than the other wavenumber regions. At this wavenumber, CR, BR, and parared can be distinguished (fig. 4).

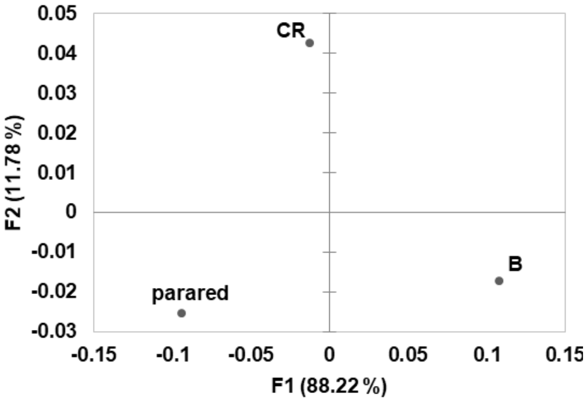


Fig. 4: PCA analysis of FTIR of big red (BR) chili powder, curly red (CR) chili powder, and parared obtained using the spectral region of 2000-3000 cm^{-1}

The selected combination of spectral regions was then used to analyze chili powder samples on the market (A-E) to observe the possibility of counterfeiting. The results of the analysis are shown in fig. 5.

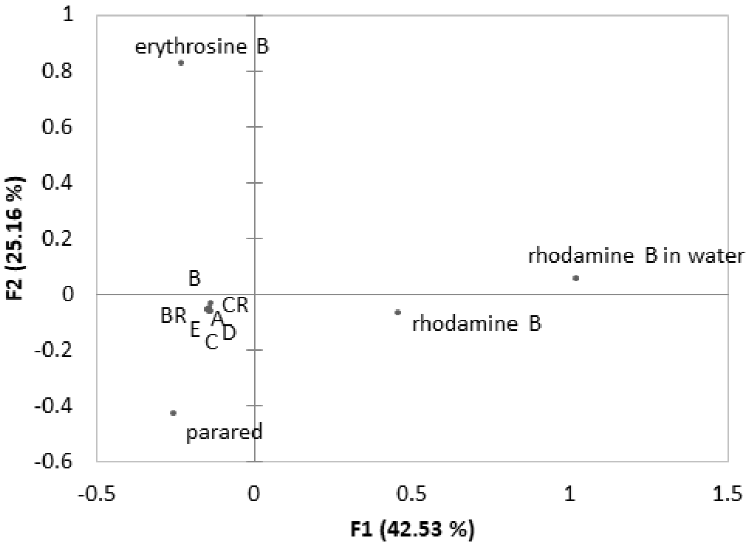


Fig. 5: PCA analysis of FTIR of commercial chili powder (A-E), big red (BR) pure chili powder, curly red (CR) pure chili powder, rhodamine B, erythrosine B, and parared obtained using the spectral region of 700 – 850, 1100 – 1700, and 2300 – 3500 cm^{-1}

The A-E sample has a physical similarity with pure chili powder. All samples are located in a different rhodamine B and erythrosine B quadrant. Furthermore, the samples were in the same quadrant as parared, indicating a physicochemical similarity to parared. Several similarities in functional groups between parared and capsaicin, the main compound in chili, attested to the chemical closeness. None of the tested samples were added to rhodamine B, erythrosine B, or parared. The result can be further confirmed by investigating the conditions in the wavelength region of 2000-3000 cm^{-1}.

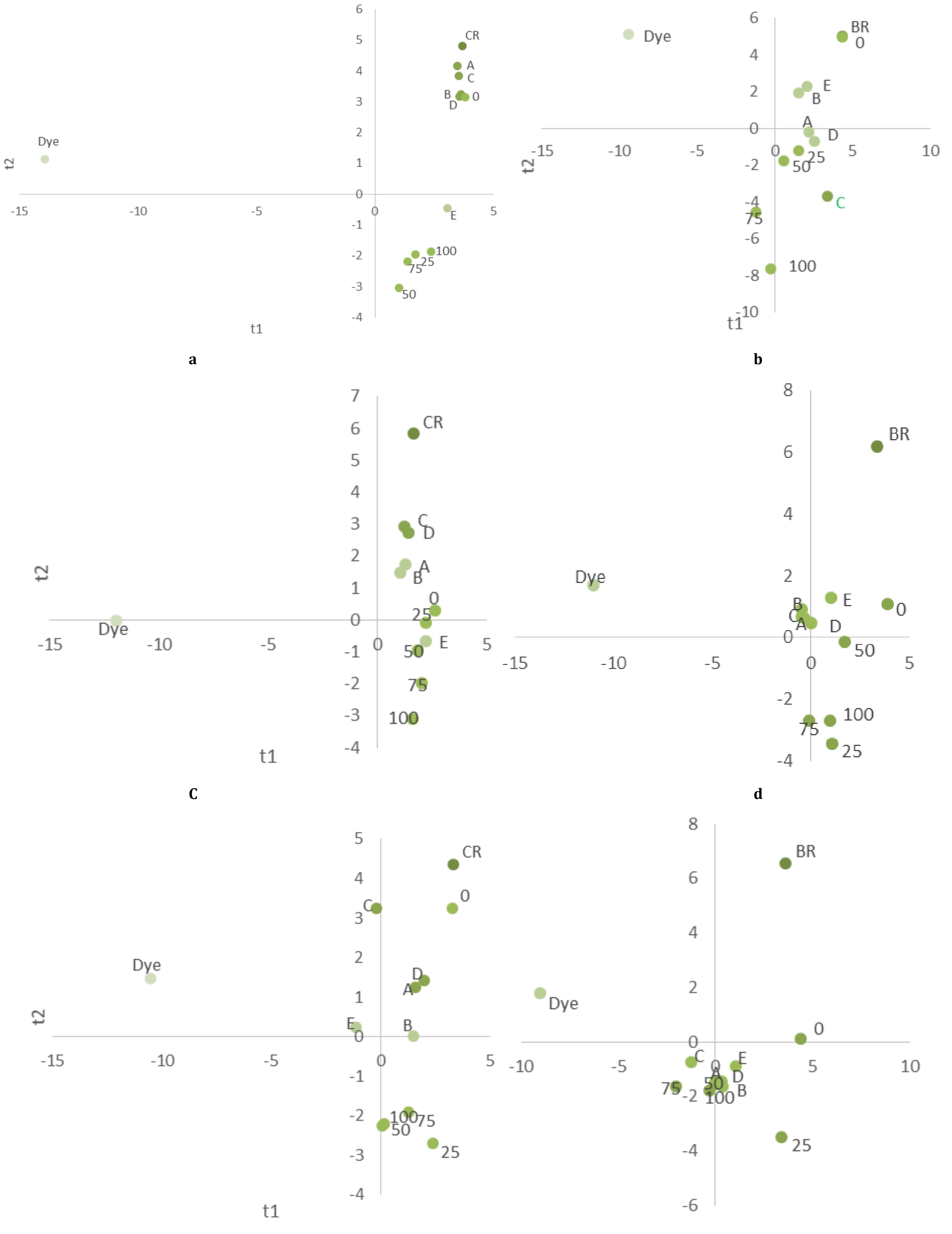
Partial least square (PLS) analysis

PLS was able to quantify dye counterfeiting in chili powder. PLS-DA and PLS-R were used to find the relationship between the X (predictive) matrix and the Y (response) matrix. The X matrix includes data resulting from the absorption measurement of pure chili powder by FTIR spectroscopy. The Y matrix contains data on the concentration of dyes in the mixture. The conditions were determined using a binary mixture of chili powder and dyes.

A set of standards containing a binary mixture of chili powder and dye were classified according to PLS-DA. PLS-DA is a supervised pattern recognition method used to find a rule for placing the object whose group is unknown into the correct or known group. The performance model was analyzed by model quality (table 1). The six mixtures were CR-rhodamine B, BR-rhodamine B, CR-erythrosine B, BR-erythrosine B, CR-parared, and BR-parared. Each set consists of six levels of dye concentration in the chili-dye powder mixture (100:0 = 0%); 25, 50, 75, and 100% (w/w). Validation was done using leave-one-out Jackknife cross-validation with randomization in selecting prediction data. Samples A, B, C, D, and E were placed in the prediction set to ensure predictability.

Table 1: Model quality for partial least squares-discriminant analysis (PLS-DA) by number of components in binary mixture of big red (BR) chili powder, curly red (CR) chili powder, and dye

Statistic	Index					
	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6
CR-rhodamine B						
Q^2 cum	-0.106	-0.095	0.746	0.871	0.880	1.000
R^2Y cum	0.451	0.768	0.989	0.999	1.000	1.000
R^2X cum	0.401	0.508	0.530	0.568	0.600	0.636
BR-rhodamine B						
Q^2 cum	-0.395	-0.131	-0.177	-0.168	-0.209	1.000
R^2Y cum	0.365	0.640	0.682	0.689	0.893	1.000
R^2X cum	0.269	0.549	0.635	0.725	0.736	0.758
CR-erythrosine B						
Q^2 cum	0.037	0.423	0.798	0.920	0.995	1.000
R^2Y cum	0.486	0.920	0.999	1.000	1.000	1.000
R^2X cum	0.286	0.379	0.445	0.511	0.552	0.586
BR-erythrosine B						
Q^2 cum	-0.125	0.178	0.574	0.737	0.786	1.000
R^2Y cum	0.425	0.871	0.990	0.997	0.999	1.000
R^2X cum	0.274	0.403	0.469	0.545	0.594	0.646
CR-parared						
Q^2 cum	-0.129	-0.138	-0.158	0.865	0.872	1.000
R^2Y cum	0.425	0.732	0.766	0.994	0.998	1.000
R^2X cum	0.244	0.339	0.459	0.472	0.496	0.525
BR-parared						
Q^2 cum	-0.214	0.095	0.133	0.881	0.954	1.000
R^2Y cum	0.337	0.849	0.917	0.995	0.999	1.000
R^2X cum	0.246	0.377	0.554	0.629	0.675	0.697



a

b

C

d

e

f

Fig. 6: Scores plot of PCA model obtained using the spectral region of 700–850, 1100–1700, and 2300–3500 cm^{-1}, demonstrating the separation of curly red (CR) chili powder, binary mixture CR-rhodamine B, and samples A-E (a); big red (BR) chili powder, binary mixture BR-rhodamine B, and samples A-E (b); CR, binary mixture CR-erythrosine B, and samples A-E (c); BR, binary mixture BR-erythrosine B, and samples A-E (d); CR, binary mixture CR-parared, and samples A-E (e); and BR, binary mixture BR-parared, and samples A-E (f)

According to the Q^2 model quality parameters, chili powder has 100% variability with six components in each binary compound (Q^2 cum = 1). As a result, the model's goodness of fit and predictive quality was acceptable. In comparison, the R^2Y cum parameter of CR-rhodamine B was 98.9 with only three components, indicating that the three components are capable of adequately summarizing the Y variable. However, the X variable can only capture 63.60% of the information in the predictor variation and the dependent variable in the six components. Therefore, the variation of 36.40% in the model is a consequence of the deviation of variable X due to external factors.

The confusion matrix indicates that all samples are classified correctly according to their respective categories (% correct = 100%). The value of the confusion matrix shows that all samples are classified correctly according to their categories (% correct = 100%). As a result, the model is capable of distinguishing between observations and achieving an accuracy of 100% or with no misclassifications.

The PLS-DA score plots of the six binary mixtures are shown in fig. 6. All samples were in different positions from the binary mixture containing rhodamine B and erythrosine B. Some samples were in the same quadrant as the binary mixture but not adjacent to the points in the binary mixture. Therefore, samples (A-E) did not contain rhodamine B and erythrosine B. Adulteration of chili powder with parared can be further defined in CR, but not BR. Accordingly, adulteration with parared can be further confirmed by investigating at a wavelength of 2000-3000 cm^{-1} or using other instruments such as chromatography.

The dye content of the sample can be determined using PLS-R. PLS-R was chosen as a chemometric method to resolve the complexity of the spectral data and to build a model for predicting the proportion of counterfeiters found in binary mixtures. PLS can provide spectral information over a wide area and relate changes in the spectrum to the concentration of components simultaneously by calculating the contribution of other spectral interferences. The root-mean-square errors *of* calibration (RMSEC) and the coefficient of determination (R^2) were used as calibration validity criteria [42]. The R^2 indicates the ability of a method to produce analytical numbers proportional to the sample concentration at certain concentration intervals. An R^2 value represents a linear relationship between the independent and dependent variables. PLS models with a low RMSE value were considered well-performing [29]. R^2 values and RMSECV of 1 and 0, respectively, were obtained for all binary mixtures. Furthermore, the observation value or sum of weights of all binary mixtures was 7.000, while the DF, standard deviation, and MSE were all 0.000. According to these results, the relationship between the actual and predicted values is good, as is the accuracy of the prediction. Chili powder dye content can be detected using the developed method. As stated in the PCA data, all samples analyzed did not contain rhodamine B, erythrosine B, or parared dyes.

CONCLUSION

In conclusion, the adulteration of red chili powder with rhodamine B, erythrosine B, and parared can be monitored using the FTIR spectroscopy instrument with the ATR sampling technique. Red chili powder and its counterfeiters can be discriminated and classified quickly and simply using PCA and PLS.

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AUTHORS CONTRIBUTIONS

STA has executed the experiments; WRS has been involved in results analysis and data validation; PIU contributed significantly to the data analysis and documented the results; ADD has designed the work plan, supervised the execution and results analysis, written draft, writing-review, and editing.

CONFLICTS OF INTERESTS

All authors have none to declare

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