



Visible-Near Infrared Reflectance Spectroscopy for Rhodamine B Detection in Chili Paste Using Principal Component Analysis

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ABSTRACT

A destructive testing for identification the presence of non-food grade Rhodamine B (RhB) dyes in food products requires complex sample preparation and is not environmentally friendly because of chemical using. Even though, this testing for foodstuffs such as chili paste requires more simple, rapid testing and does not pollute the environment. Therefore, this study aims to develop a non-destructive method to detect RhB dye in chili paste using Visible-Near Infrared Reflectance (Vis-NIR) Spectroscopy. The sample spectra were acquired using light waves in the wavelength spectrum of 300 - 1000 nm. The acquired spectra were analysed using Savitzky-Golay's Derivative pre-treatment to reduce noise in the spectra. Principal Component Analysis (PCA) was used to classify chili paste sample spectra data into two groups, consisting of samples containing RhB and without RhB. The results showed that PCA combined with Savitzky-Golay derivative pre-treatment was able to distinguish samples with and without RhB better than PCA in the raw spectrum without pre-treatment. Samples without RhB clustered well with a success rate of 83.67% while samples containing RhB clustered with a success rate of 94.52%. Capsanthin and anthocyanins compounds as red pigments in fruits and vegetables were detected at wavelengths of 562 nm and 590 nm.

Keywords: PCA, Rhodamine B, Spectra, Visible-NIR.

1. INTRODUCTION

Rhodamine B (RhB) is a non-food grade synthetic substance that is used to dye processed products in the textile, paper, and paint industries [1]. It is often used by some traders illegally to produce a bright red color in food products [2]. This is because color plays a role in food perception, where the brighter the red color in food, the greater the appetite [3]. A previous study by Ripaldy et al. [4] showed that 4.68% of all samples of chili paste sold in several traditional markets in the Sleman Regency, Yogyakarta, Indonesia were positive for the harmful RhB dye. The use of RhB in various food products poses a significant health risk because it is carcinogenic [5]. In addition, these substances cause

negative effects on the body such as various irritations and respiratory tract disorders [6].

Commonly, RhB testing on food products is still using the destructive method by damaging the test sample to obtain the required test result information. One example of the destructive RhB testing method on chili paste uses the Thin Layer Chromatography or TLC method [7] which requires various additions of reagents in the testing process and the stages are quite complicated. These inefficiencies can be avoided by conducting non-destructive testing. In various studies, the non-destructive tests have been used to test the

quality of agricultural products because it is proven to be fast and accurate [8]. One of these technologies is the use of the Visible-Near Infrared Reflectance (Vis-NIR) Spectroscopy. Meanwhile, the Vis-NIR Spectroscopy is a spectroscopic method that does not require test sample preparation, offers non-invasive analysis, and provides reliable information on describing the distribution of constituents analysed. As the name implies, the light used in the Vis-NIR Spectroscopy is in the wavelength region between the visible light spectrum and infrared light with the electromagnetic wave spectrum region between 350-2500 nm [9]. Spectroscopic technology uses infrared light that has high potential in the food analysis process. It is an alternative to the test carried out in food technologies with advances in chemometric and computer programs [10]. Furthermore, Vis-NIR has been used successfully to detect the cherry bruise degree combined with multivariate analysis [11] and measure capsaicinoid levels in red chili powder with a good coefficient of determination (R^2) of 0.85 [12].

The study of detecting RhB in chili paste using Vis-NIR spectroscopy has not been conducted and studied. Therefore, this study was conducted to detect the harmful RhB dye in chili paste products using Vis-NIR Spectroscopy. The spectroscopic data analysis was combined with multivariate analysis using the Principal Component Analysis (PCA). PCA is able to distinguish certain data sets from other data by transforming all data into main components [13].

2. METHOD

2.1. Sample Preparations

Curly red chilies (*Capsicum annum L.*) were

purchased from local farmers in Pakem, Sleman, Yogyakarta, Indonesia. Good and uniform chilies quality is needed by sorting, including separating rotten chili and non-red chilies then the sorted chilies were washed with running water. The washed chilies were ground to a paste texture and divided into two types, 49 samples of chili paste without the addition of RhB and 146 samples of chili paste with the addition of RhB.

2.2. Vis-NIR Spectral Acquisition

Reflectance spectra of samples were measured using a self-arranged Vis-NIR instrument with several components such as Vis-NIR Miniature Spectrometer (Flame-T-VIS-NIR Ocean Optics, 350-1000 nm) with a tungsten halogen light of 360-2400 nm, HL-2000-HP-FHSA Ocean Optics, and a reflection probe (QR400-7-VIS-NIR Ocean Optics). The schematic of the Vis-NIR Spectroscopy is shown in Figure 1. All spectra were collected using OceanView 1.67 with 100 ms integration time, 100 scans to average, and 1 boxcar width. Before taking sample spectra, white and dark references were measured for spectrometer calibration purposes. In this study, the white reference was a ceramic diffuse reflectance standard (WS-1, Ocean Optics, USA), while the dark reference was measured when the light was minimal by blocking the light source. Subsequently, spectrometer calibration was conducted for every 5 samples taken. Calibration was carried out in order to obtain accurate spectral results and ensure that the condition of the instrument is always consistently in good condition. For each chili paste sample, 3 spectra with a wavelength range of 300-1000 nm were examined and averaged. By using 2 groups of samples, there were 195 spectra in total.

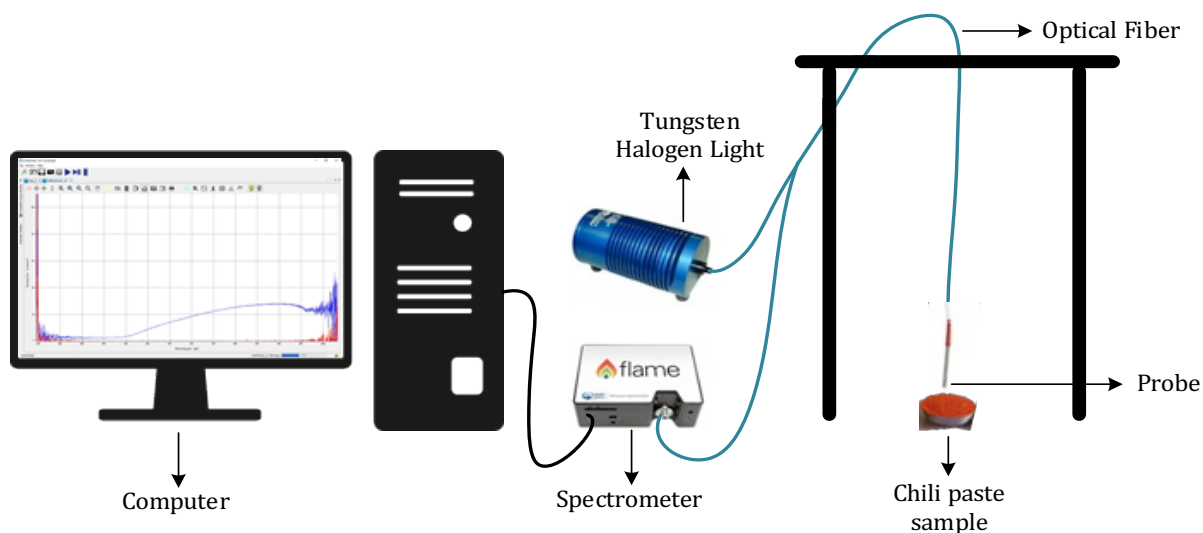


Figure 1 The schematic of the Vis-NIR spectroscopy

2.3. Chemometrics Analysis

All spectra measured were compiled using MS Excel and imported into the Unscrambler® X software (CAMO, Oslo, Norway). Spectra in the 450-950 nm range were used in chemometric analysis because it indicated the presence of information that can be analyzed in more detail. Pre-treatment was used to reduce noises in the spectra so that it does not affect the analysis results. The pre-treatment used in this research is Savitzky-Golay which has a working principle of eliminating the effect of interference on the signal and storing information in the data [14].

Chemometric analysis in the form of Principal Component Analysis (PCA) was applied to the spectra to show the results of the data mapping and to classify samples between samples containing RhB and samples without RhB. Spectra is complex because it has a lot of data information that can be analysed simultaneously. On the other hand, the spectra also contain noises that must be reduced or eliminated so as not to affect the analysis results. The principle of the PCA method is to reduce the dimensions of complex data sets [13]. Therefore, PCA, as an unsupervised method is used in this study. The validation method applied to PCA analysis is the full cross-validation (CV) method. PCA

performance was analyzed through the total percentage of Principal Component (PC) variance and the percentage of success in grouping between samples.

3. RESULTS AND DISCUSSION

3.1. Spectra

The spectrum of light that can be captured by the probe is forwarded to the spectrometer. In a spectrometer, light is reflected into a diffraction grating and then forwarded to a detector. The light that hits the pixels on the detector is transformed into information in the software so that the atoms and molecules of a compound in the material can be identified. The transformation results captured by the detector are then visualized on the monitor screen in the form of a series of spectra or wavelengths containing sample information. The spectra were then analysed based on the reflectance intensity.

Reflectance spectra of all chili paste samples are shown in Figure 2 and 3. Figure 2 shows all the raw spectra without any pre-treatment of both types of chili paste samples, samples without RhB are marked in blue, and samples with RhB are marked in red. The wavelength of light used for the analysis process in this

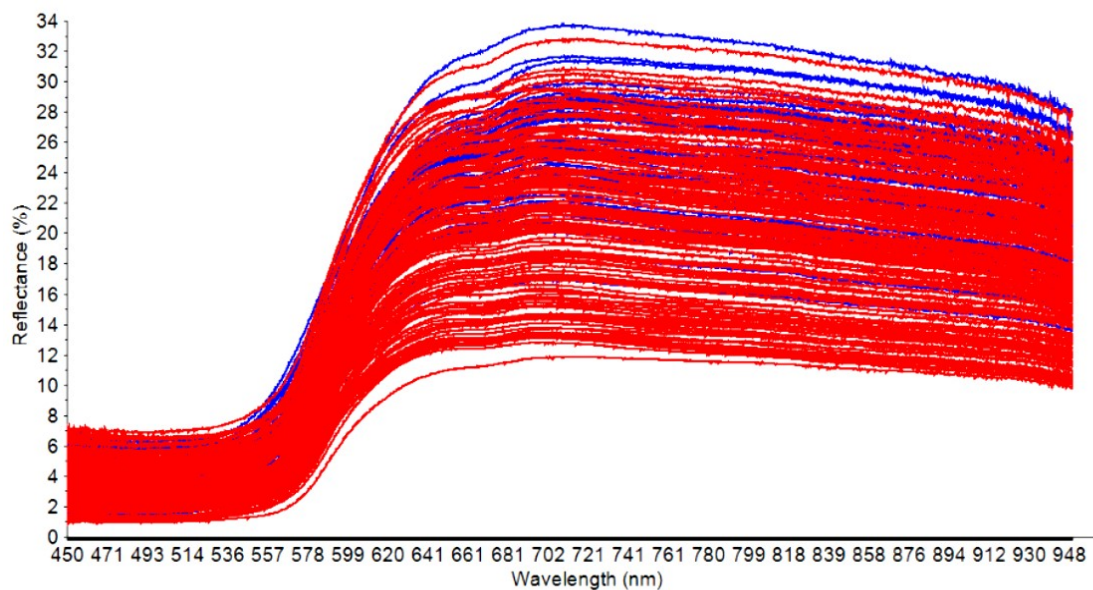


Figure 2 Chili paste raw spectra plot. (Legend: Blue: samples without RhB, Red: samples with RhB)

study covers the range of 450-950 nm because there are interactions in that range and the wavelength area outside that range produces noise that cannot be used as an object of analysis. The acquired spectra have a similar pattern with very low reflectance values at a wavelength of 450-560 nm, which was influenced by the absorbance of carotenoids [15].

The reflectance intensity in the NIR region around 800 nm tends to be constant, no peak is formed because the wavelength region is an absorption area for water compounds which causes a decrease in the reflectance intensity that can be read by the spectrometer. The water content in the sample causes scattering on the NIR instrument [16] so that the reflectance results do not

show the characteristics of a specific compound in the sample. This is due to the texture of the chili paste

sample which is in the form of a paste and contains high enough water.

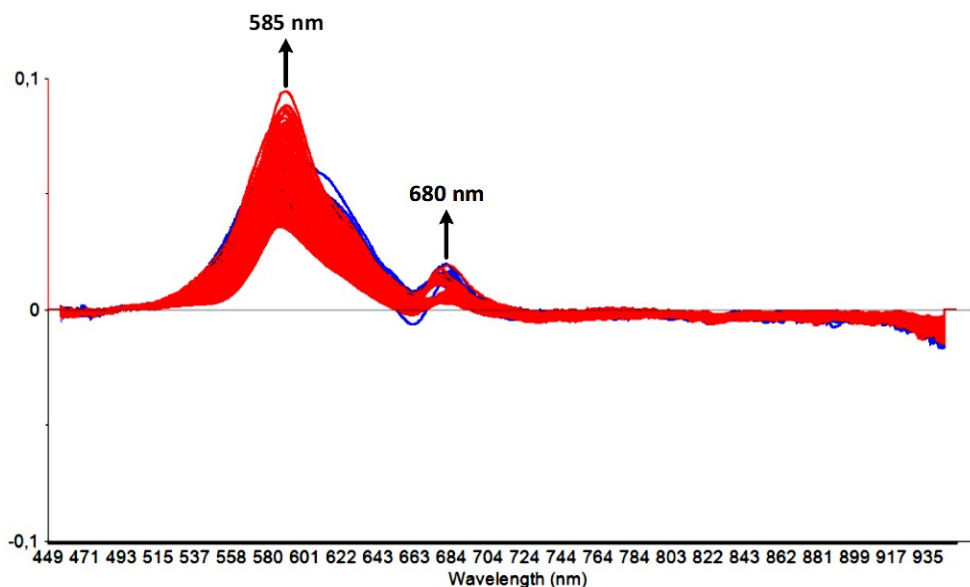


Figure 3 Savitzky-Golay's derivative pre-treatment spectra plot of chili paste. (Legend: Blue: samples without RhB, Red: samples with RhB)

Through the use of Savitzky-Golay's derivative pre-treatment spectra (Figure 3), two dominant peaks appeared at a wavelength of approximately 585 nm and 680 nm. Peaks at the 500-600 nm wavelength region correspond to capsanthin and anthocyanins [17], while spectra at 680 nm are associated with chlorophyll [18]. Moreover, capsanthin with the molecular formula of $C_{40}H_{56}O_3$ is a type of carotenoid, which is responsible for the red pigment in red chili [19] while anthocyanin ($C_{15}H_{11}O^+$) is responsible for red, purple, and blue color in vegetables and fruits [20].

3.2. Principal Component Analysis

In this study, the samples were divided into two

groups, namely with and without RhB. Meanwhile, the result of the PCA score plot of raw spectra is shown in Figure 4. The samples that contained RhB are marked in red, while those without RhB are in blue. The value of the variances described by PC-1 is 98% and PC-2 is 0%, making a total of 98%. This indicated that the total of variances described by the PC has exceeded the minimum target set by 70%, which can show good data visualization [21]. Furthermore, the percentage of success in grouping samples without RhB is 63.27% and for samples with RhB is 87.67%. analysis on raw spectra resulted in samples without RhB having a relatively low success rate while samples containing RhB could cluster well.

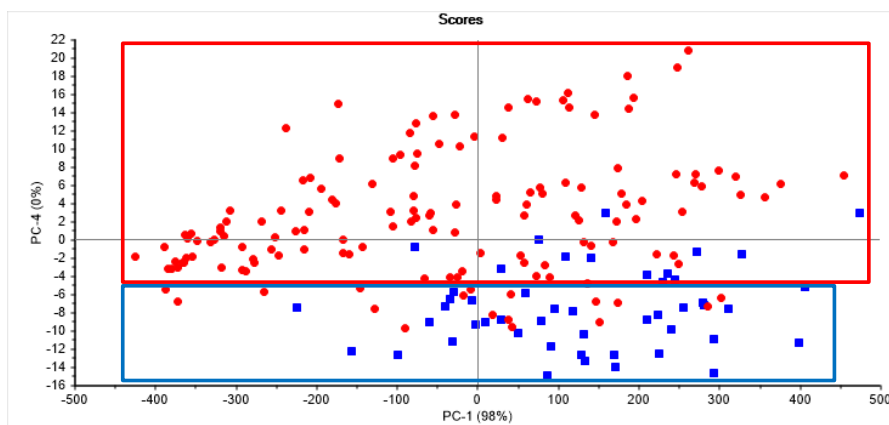


Figure 4 PCA score plot of raw spectra. (Legend: Blue: samples without RhB, Red: samples with RhB)

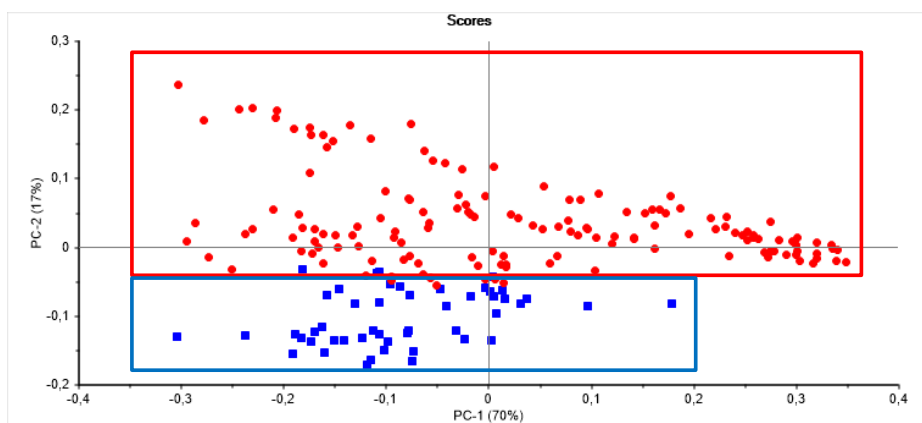


Figure 5 PCA score plot of Savitzky-Golay's derivative pre-treatment spectra. (Legend: Blue: samples without RhB, Red: samples with RhB)

The result of the PCA score plot of Savitzky-Golay's derivative pre-treatment spectra is shown in Figure 5. The value of the variances described by PC-1 is 70% and PC-2 is 17%, making a total of 87%. This indicated that the total of variances described by the PC has exceeded the minimum target set by 70%, which can show good data visualization [21]. Furthermore, the two types of samples were well grouped based on PC-2 with percentage of success in grouping samples without RhB are 83.67% and for samples with RhB are 94.52%. Visually, PCA analysis on pre-treated spectra showed better clustering for each type of sample when compared to analysis on raw spectra. The grouping of both samples without RhB and samples with RhB both had a higher percentage value than the results in the raw spectra so that each type of sample in the pre-treatment spectra was able to group well. The PCA analysis method in this study was able to classify the two types of chili paste samples between pure samples and containing RhB better when compared to similar studies

conducted by Rohaeti et al. [22] where samples of pure chili powder and containing RhB cannot be distinguished using only PCA analysis.

3.3. Loadings

In this study, the loadings plot shows the strength of the variables, namely reflectance values on the PC formation. The wavelength with the maximum loading value close to 1 or approximately -1 illustrates that it has a major influence on the PCs that can classify the data. The loadings plot of the chili paste spectra is shown in Figure 6. The wavelength at 562 nm and 590 nm that have high loadings values were affected by anthocyanins and/or capsanthin compounds [23]. These results strengthened the analysis of reflectance spectra, where the variables with high loadings values also occur at wavelengths below 700 nm which are in the visible spectrum and also show the interaction of capsanthin and anthocyanins.

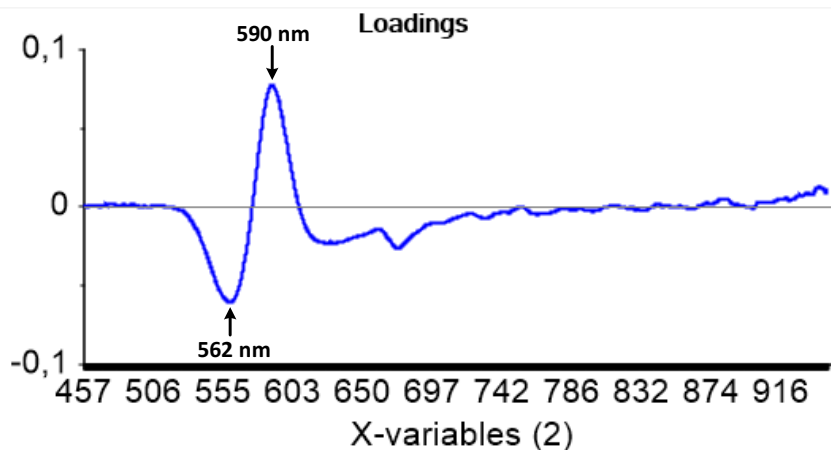


Figure 6 Loadings plot that shows the influence of the wavelength on the grouping samples.

4. CONCLUSION

The non-destructive test method for detecting the Rhodamine B (RhB) harmful dye was successfully developed on chili paste samples using Vis-NIR spectroscopy at a wavelength of 450 nm–950 nm. Based on the spectrum and loading plot, it can be seen that there is an interaction of compounds in the visible light spectrum which is correlated with capsanthin and anthocyanin compounds. The PCA method was able to distinguish samples with and without RhB in the Savitzky Golay pre-treatment spectrum compared to the raw spectrum. Therefore, Vis-NIR spectroscopy combined with the PCA method can be applied to chili paste samples to detect RhB dye effectively.

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