

Determination of the Characteristics and Classification of Near-Infrared Spectra of Patchouli Oil (*Pogostemon Cablin Benth.*) from Different Origin

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Abstract. Research related to the non-destructive method of near-infrared (NIR) spectroscopy in aromatic oil is still in development in Indonesia. The objectives of the study were to determine the characteristics of the near-infrared spectra of patchouli oil and classify it based on its origin. The samples were selected from seven different places in Indonesia (Bogor and Garut from West Java, Aceh, and Jambi from Sumatra and Konawe, Masamba and Kolaka from Sulawesi Island). The spectral data of patchouli oil was obtained by FT-NIR spectrometer at the wavelength of 1000-2500 nm, and after that, the samples were subjected to composition analysis using Gas Chromatography-Mass Spectrometry. The transmittance and absorbance spectra were analyzed and then principal component analysis (PCA) was carried out. Discriminant analysis (DA) of the principal component was developed to classify patchouli oil based on its origin. The result shows that the data of both spectra (transmittance and absorbance spectra) by the PC analysis give a similar result for discriminating the seven types of patchouli oil due to their distribution and behavior. The DA of the three principal component in both data processed spectra could classify patchouli oil accurately. This result exposed that NIR spectroscopy can be successfully used as a correct method to classify patchouli oil based on its origin.

1. Introduction

Indonesia is a rich country of aromatic plant species with around 40 kinds produced. They are easy to plant, maintain and harvesting, easy to process and transport making them preferable for small farmers and remote communities.

Patchouli (*Pogostemon cablin* Benth.) is a plant from *Lamiaceae* family that is well known for its medicinal and aromatic properties. Patchouli is cultivated mainly for its essential oil and especially notable how this extracted oil is internationally important and valuable, principally for the aromatherapy, perfumery, cosmetics, incense stick production and food flavoring industries [1].

Indonesia shares around 80% of all the production of Patchouli plant in the world market, meanwhile the other 20% comes from countries like Malaysia, Philippine, China, India, and Brazil. Nevertheless, despite its reputation in the industry, continually it suffers irregularities due to the sources of its productions [2]; Doing necessary the study of the origin of the plant for the classification and assessment of the quality of patchouli production.

Several destructive methods have been applied for determining the quality of patchouli oil such as gas chromatography [3] [4] [5] and gas chromatography-sniffing [6] [3]. The problem with



destructive methods like gas chromatography is that they are time-consuming and complex. Given the current issues associated with aromatic plants, it is necessary to develop an appropriate tool to assess the quality of the patchouli production. Despite some non-destructive methods as a Raman spectroscopy has been used to analyze the quality of several essential oils [7] [8], due to the characteristics of its light dispersion analysis, the assessment of a result in short time with high accuracy still is complicated. In this context, vibrational spectroscopic methods such as NIRS (Near Infra-Red Spectroscopy) together with chemometrics methods can be successfully introduced as a rapid analysis for a non-destructive determination of metabolites occurring in the aromatic plants including patchouli.

The purpose of this study is to determine the characteristics and classifying the NIR spectra samples of patchouli oil from different origin. The research tries to expose that there is a significant difference in the NIRS spectra patterns between each place, despite it is complicated to determine visually and explaining how this makes an important incidence in the quality presentation of the patchouli oil and its assessment process.

2. Material and method

2.1. Samples and device

In this study were selected 84 oil samples of Patchouli aromatic plant from seven different places around Indonesia; Konawe, Kolaka and Masamba from Sulawesi island, Bogor, and Garut from west java and Aceh and Jambi from Sumatra. In total it was around 50 ml per samples in recipient based on every field production.

The instrument used for transmittance measurement was an FT-NIR Spectrometer (NIRFlex N-500, manufactured by BÜCHI Labortechnik AG, Switzerland). The methodology applied for taking the spectrum of the samples is known as a transreflectance [9], being this innovative procedure. The device was adapted to measuring liquid samples taking advantage of its characteristics. According to this, the signal processed was in function of the transmittance and reflectance of the spectrum before the respective data analysis.

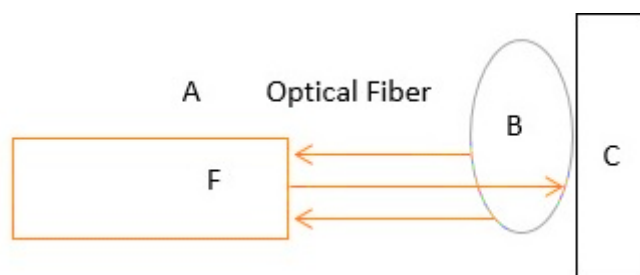


Figure 1. The figure of the explanation of the way of NIRS machine use in the laboratory for taking the transreflectance data. A: Gun with the NIR signal and spectrometer detector, B: Oil sample, C: White background, F: Transmittance Sensor. The NIR radiation coming from the optical fiber (gun) goes through the liquid sample, it reflects in the white background surface making contact with the sensor. Then, it backs through the liquid sample, heading to the spectrometer detector.

This methodology has not been well documented because normally the NIRS investigation results focus more on the analysis and calibration of the model and only special name arrangements in the machine if those are required.

2.2. Patchouli spectra acquisition

The transmittance of samples was measured by scanning the samples 3 times at 3 different points, taking the data in there and setting the gun inside the recipient of the oil during the process. After that, the spectra was transformed to absorbance for the subsequent analysis. The wavelength interval used was 1000-2500 nm.

2.3. Chemical analysis

The chemical analysis was assessed to get the chemical content of patchouli oil. The proximate analysis was estimated according to INS (Indonesian National Standard). The main chemical compound of patchouli oil (Patchouli-Alcohol - $C_{15}H_{26}O$) was measured by a liquid chromatography-mass spectrometry (LC-MS) method in every sample studied [10] [11]. The results were assorted based on the percentage of concentration of the patchouli alcohol per sample.

2.4. Spectra processing

The transmittance and absorbance spectra were processed with several spectra data pre-treatments such a Smoothing Savitzky-Golay [12] and first and second derivatives (Polynomial order of 2, 3 smoothing points, left and right sight point of 1) [13] [14]. That method was applied to the standard spectrum with the aim of sharpening their profile and eliminating the interference which is caused by baseline shifts and noise disturbances. Due to the liquid characteristics of the samples, there were not uneven surface effects, multiplication effects like scattering, particle size and multi-colinearity changes, which can cause large variations in the transmittance spectrum [15]. Spectrum data processing was carried out using Unscrambler software X 10.5.

Principal Component Analysis (PCA) was used to cluster the different kinds of patchouli oil based on principal components of processed spectra data. Display of the plot spectra scores of various peaks at the specific wavelengths of spectrum analysis results by PCA was plotted and could become a characteristic or fingerprint of the oil generating a basic in grouping the patchouli cultivars [16] [17]. Discriminant analysis of the result of qualitative analysis by PCA was constructed and applied for classification of the seven kinds of patchouli oil. Discriminant analysis creates functions based on a linear combination of predictor variables to produce the best discrimination between groups. The discriminant function is generated from the sample where the group identity of the subject has been known. The input data were the results of PC1, PC2, and PC3.

3. Results and discussion

3.1. The original spectra of patchouli oil

Some differences are exposed by results of the chemical analysis (Table 1), which indicates the valor of major chemical component (Patchouli-Alcohol - $C_{15}H_{26}O$ among the patchouli oil types). It is important to notice that there are other chemical components like Seychellene and Alpha Bulnesene which are mainly in the patchouli oil. The idea of showing this analysis is to expose a descriptive different between the seven kinds of oil and its behavior based on its constitution.

Figure 2 shows the transmittance spectra as an example, of the different patchouli oil with several peaks and valleys in a specific wavelength for each origin sample. The valley existed related to electromagnetic wave and its responses to the molecular bonds of O-H, C-H, C-O, and C-C (figure 3). The result is subjected to vibrational energy, both of stretch and bent vibration [18].

Despite the spectra are likely similar, if it is possible to review in detail that some interesting data are shown. The patchouli alcohol content in every type of oil has a significant difference. Jambi has the highest content (32.34%) followed by Aceh (30.91%), and Garut (30.98%) and the lowest are Bogor together with Masamba, which is 24.18% and 24.72 % respectively.

Table 1. Chemical characteristic of the patchouli oils based on the main chemical component (Patchouli-Alcohol $C_{15}H_{26}O$). *sd: Standard deviation

Patchouli oil	
Patchouli alcohol $C_{15}H_{26}O$	
Sample	% of the component
Jambi	32.34
Kolaka	26.45
Aceh	30.91
Konawe	29.81
Masamba	24.72
Garut	30.98
Bogor	24.18
Average	28.48
sd*	3.31

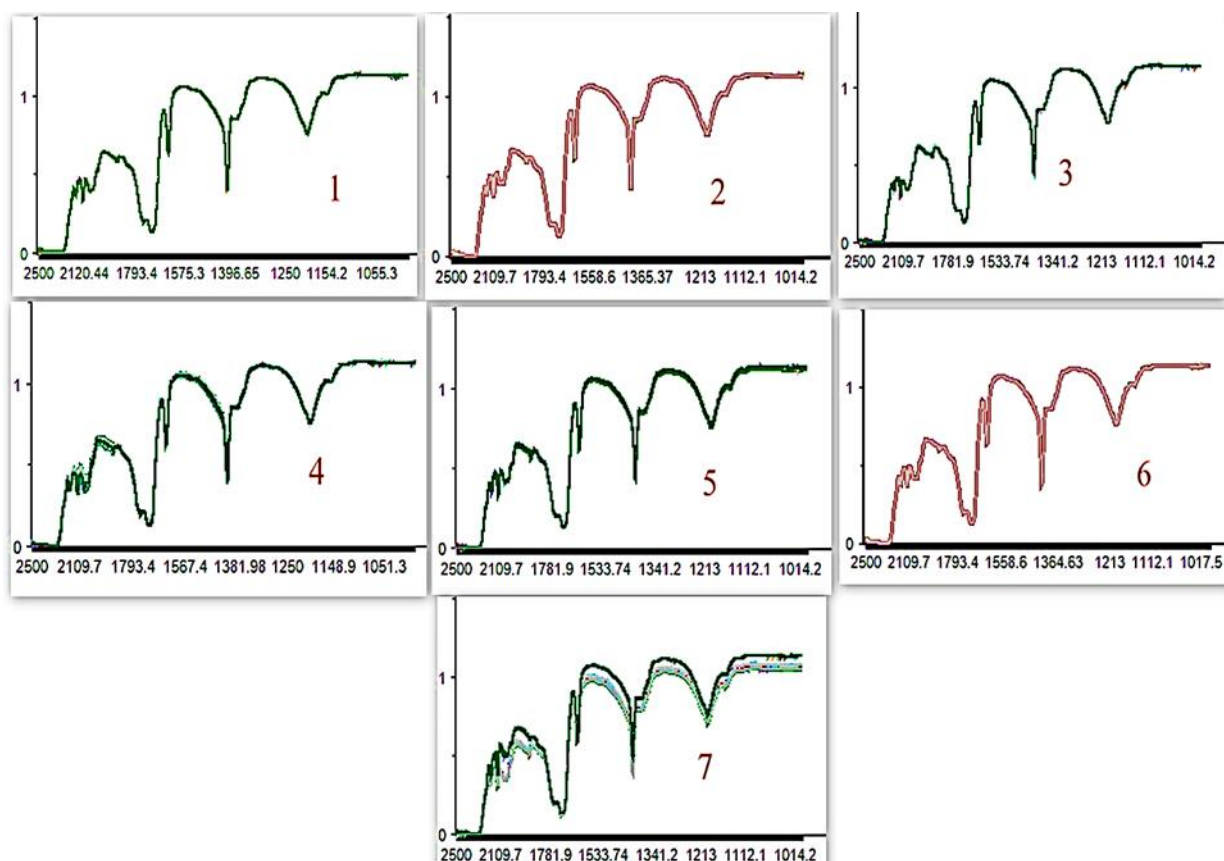


Figure 2. Transmittance spectra of different types of patchouli oil tested. Axis X: Wavelength (nm), Axis Y: Transmittance. 1: Aceh, 2: Bogor, 3: Garut, 4: Jambi, 5: Kolaka, 6: Konawe and 7: Masamba.

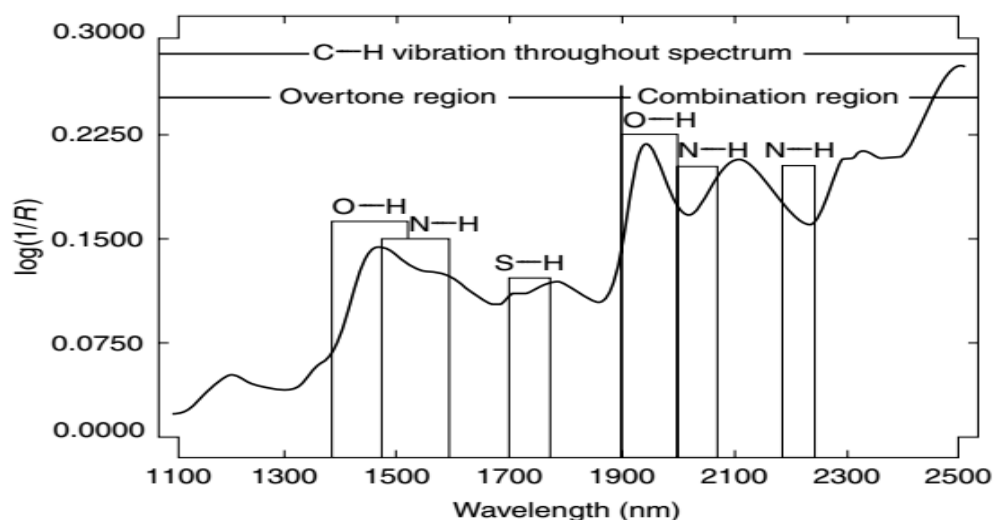


Figure 3. General representation of the valley related to electromagnetic wave and its responses to the molecular bonds.

If it is analyzed the absorbance spectra graphic of the patchouli oil, which contains the spectra of the seven origin places (figure 4), there are several peaks and valleys that exist in there. Based on [19], the wavelength of 1680 and 2230 nm are reference values, 1940 nm to water, 2270 nm to lignin, 2336 nm to cellulose, 2180 nm to protein, 2100 nm to carbohydrate and finally 2310 nm to oil in general, in the case of the combination region of the vibration throughout the spectrum. In the case of the oil, the literature exposed that it is possible to find its main characteristics in the combination region and overtone region of NIR spectrum [20] [21].

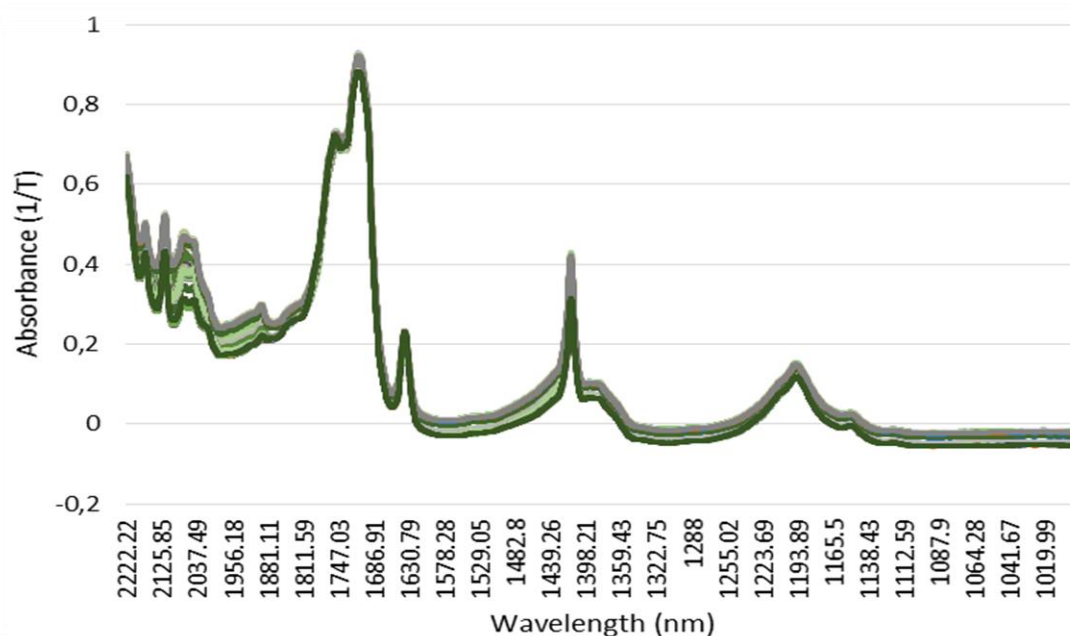


Figure 4. Absorbance spectrum between 2222 – 1019.99 nm, which contain all the spectra of the seven types of oil. Axis X: Wavelength (nm), axis Y: Absorbance (log 1/T). There is representative peak in the range between 1800 to 1600 nm, which can provide information about the chemical composition of the samples.

3.2. Clustering analysis using PCA of spectra data pretreatment

Figure 5 shows the loading transmittance spectra of first and second principal component. The first and second principal components (PC1 & PC2) represent most variation in original spectra. Furthermore, the principal component was emphasized in the spectra signal that was centered on the wavelength of 1780-1680 nm, which allegedly shows the aromatic characteristics of patchouli oil and its components according to [19]. For example, the Patchouli alcohol ($C_{15}H_{26}O$) tested before, is considered the main chemical compound because this gives the basic aromatic properties of patchouli oil [22] [23].

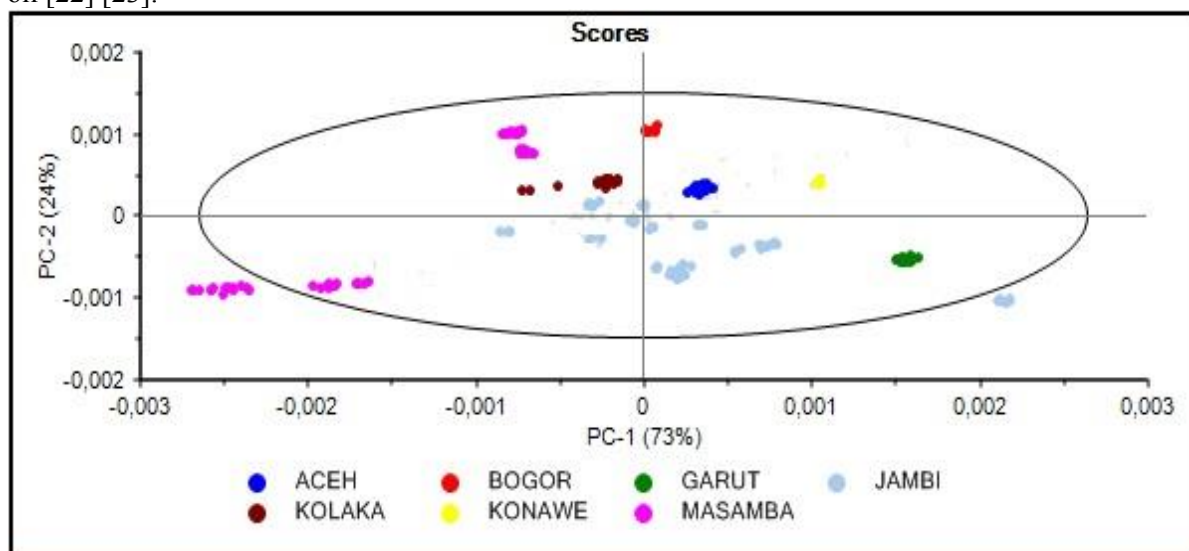


Figure 5. Cluster score graphic of the principal component analysis of the transmittance spectra based on the wavelength of 1780-1680 nm for the seven kinds of patchouli oil tested.

The result of clustering analysis using PCA from the transmittance spectra data after applying the respective pretreatment (first and second derivative Savitzky-Golay due to the region where the PCA was applied) are exposed in figure 5. It is possible to conclude that there is a clear grouping of the data and moreover, in many of them there is no a high correlation, which means that by this analysis it is possible to determine how is different between the spectra of the one place to others. In the general, the variance of all the samples is very significant. The results of the PCA (PC1 and PC2) explain around 97% of the variance spectrum analyzed with 1% of the PC3. There are some samples in data spectra of Jambi and Masamba that are outside of the variance explained, also it can be exposed in the residual plot where around 7 samples of Masamba and 5 of Jambi are identified as outliers (figure 7). it could be related to the range of the chemical data manipulated (highest and lowest values), which are reproduced in the spectra taken it.

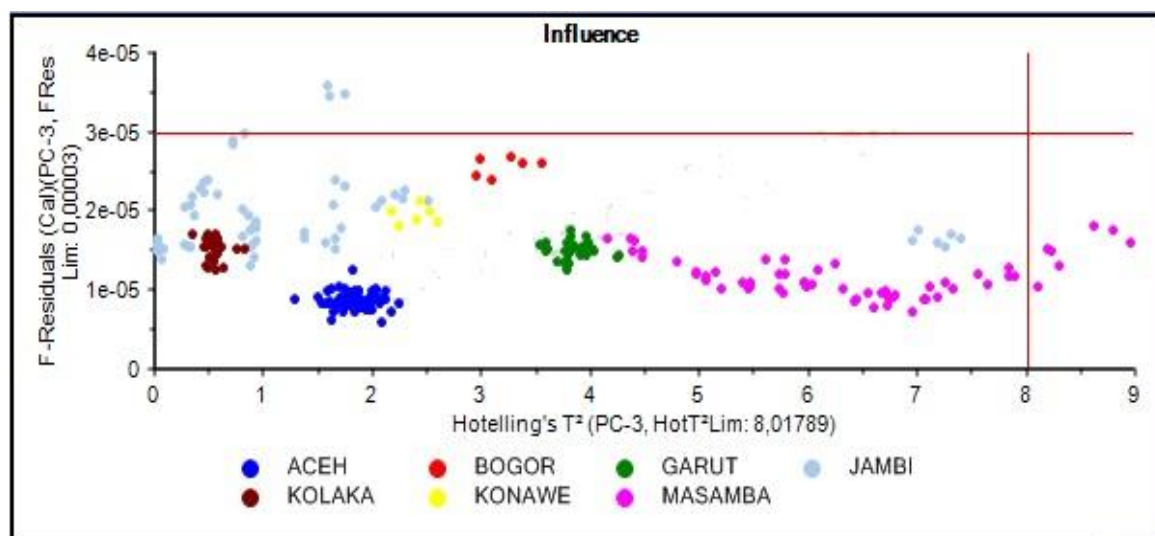


Figure 6. Residual plot of the PCA analysis of the samples for the transmittance spectra

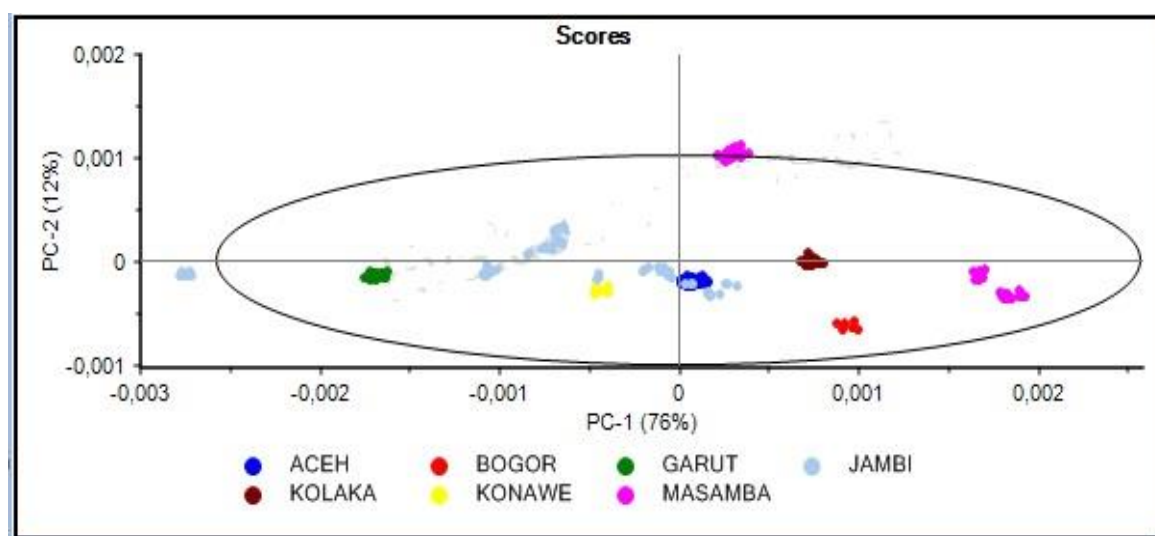


Figure 7. Cluster score graphic of the principal component analysis of the absorbance spectra based on the wavelength of 1780-1680 nm for the seven kinds of patchouli oil tested.

In this case, the result of clustering of PCA also exposed that there is a clear grouping of the data and in the same way of the transmittance, many of them are not highly correlated, which means that by this analysis it is also possible to determine how is the difference between each the spectra. The results of the PCA (PC1 and PC2) explain around 86% of the variance spectrum analyzed with 7% of the PC3.

By this graphic is show that the covariance of the date is a little bit higher compared with the transmittance results and at the same time the correlation of some data increase, which is the case of Aceh, Masamba and Konawe samples. The stated purpose before, open the possibility to determine that transmittance spectra in some way can be a better way to classify the spectrum of the patchouli oil nevertheless. There are some samples outliers in the absorbance spectra, in this case only for Jambi with similar behavior to the PCA results of the transmittance spectra.

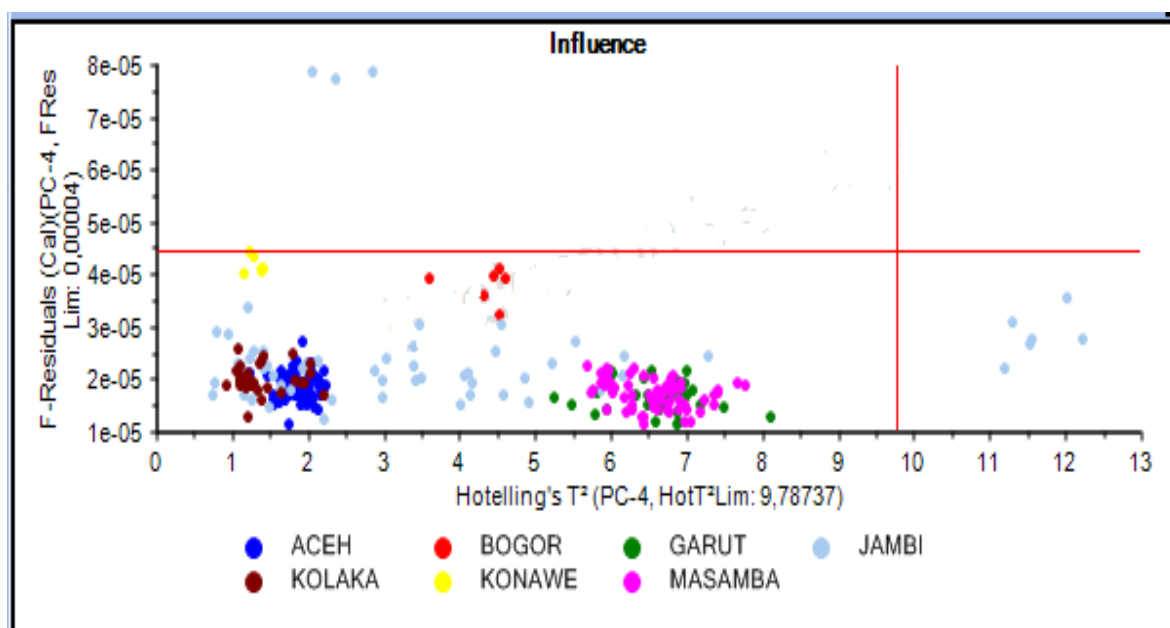


Figure 8. Residual plot of the PCA analysis of the samples for the absorbance spectra

3.3. Discriminant analysis

The discriminant analysis was applied focus in the wavelength close to the peak of 1780-1680 nm in where was carried out the PCA analysis, according to the information of [19]. The result of classification using discriminant analysis for the transmittance spectra showed that the overall prediction values were the highest, which a probability of classification of 100% for all the spectra sample. The discriminant analysis was applied to the PC data. The results exposed that it is possible to classify the spectra of the oil based on its origin due to the variance of the data exposed in the clustering beforehand.

The percentage of the prediction shown in table 3 explains that all the spectra samples can be discriminate in function of the others with the highest probability of success. The samples of Jambi, Kolaka, Garut, Aceh, Masamba, Bogor and Konawe have all perfect predicted values, which means that its data spectrum can be identified as a its specific place of origin.

In the absorbance case, the results are similar to the transmittance case. However, it is not possible to say which spectroscopy is better, because despite transmittance spectra has better PCA results and lower colinearity in the clustering, the absorbance spectra expose a better-explained variance with less outliers and moreover, the results of the discriminant analysis are similar for both of them.

Table 2. Table of classification of each sample for transmittance spectra based on its origin applied to the data spectra in the range wavelength of 1780-1680 nm.

	Aceh	Bogor	Garut	Jambi	Kolaka	Konawe	Masamba	
Aceh	60	0	0	0	0	0	0	60
Bogor	0	6	0	0	0	0	0	6
Garut	0	0	30	0	0	0	0	30
Jambi	0	0	0	60	0	0	0	60
Kolaka	0	0	0	0	30	0	0	30
Konawe	0	0	0	0	0	6	0	6
Masamba	0	0	0	0	0	0	60	60
	60	6	30	60	30	6	60	252

Table 3. Summary table with the percentage of discrimination between each sample for transmittance spectra

	Correct	Total	% correct	% missed
Aceh	60	60	100	0
Bogor	6	6	100	0
Garut	30	30	100	0
Jambi	60	60	100	0
Kolaka	30	30	100	0
Konawe	6	6	100	0
Masamba	60	60	100	0
	252	252	100	0

Table 4. Table of classification of each sample for absorbance spectra based on its origin applied to the data spectra in the range wavelength of 1780-1680 nm.

	Aceh	Bogor	Garut	Jambi	Kolaka	Konawe	Masamba	
Aceh	60	0	0	0	0	0	0	60
Bogor	0	6	0	0	0	0	0	6
Garut	0	0	30	0	0	0	0	30
Jambi	0	0	0	60	0	0	0	60
Kolaka	0	0	0	0	30	0	0	30
Konawe	0	0	0	0	0	6	0	6
Masamba	0	0	0	0	0	0	60	60
	60	6	30	60	30	6	60	252

Table 5. Summary table with the percentage of discrimination between each sample for absorbance spectra

	Correct	Total	% correct	% missed
Aceh	60	60	100	0
Bogor	6	6	100	0
Garut	30	30	100	0
Jambi	60	60	100	0
Kolaka	30	30	100	0
Konawe	6	6	100	0
Masamba	60	60	100	0
	252	252	100	0

4. Conclusions

The final results showed that it is possible to classify the oil samples based on its origin which a high accuracy using NIR spectroscopy. The results of the PCA and the discriminant analysis suggest that there is different between each grouping sample which is related to the existence of a significant difference between the data of the chemical composition.

Some of the results got it in this research could be due to the liquid characteristics of the samples. There were no effects of scattering, particle size and multi-colinearity, which could change in each

sample, this produces a spectrum very similar in every case. Therefore, it makes a huge influence in the moment of analyzing the data.

Transmittance and absorbance spectra give a similar response in the moment of classifying the data; both of them give the same values in the discriminate analysis. This behavior does not permit to make a clear preference in the moment of selecting which one have a better accuracy for the objective purpose in this experience.

The variance was decent, therefore, the result achieved in the PCA which many data not highly correlated. On the other hand, the data pre-treatment carry out here were the best that the literature suggests.

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