Near-infrared spectroscopy (NIRS) as an integrated approach for rapid classification and bioactive quality evaluation of intact *Feronia limoni*

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Abstract. *Feronia limoni* (Wood-apple) is one of the most researched plants containing bioactive compounds that have biological activity. So far, research on chemical compounds and fruit activity of F. limoni is often carried out using laboratory analysis, such as chromatographic analysis. This study aimed to assess the ability of NIRS to predict organic compounds contained in *F. limoni* based on its spectrum. Intact *F. limoni* samples were obtained from five different geographical origins in Aceh Province. Spectra data in form of absorbance spectrum were acquired using a portable sensing device NIRS instrument PSD FTNIRS i16 in wavenumbers 4000-10000 cm-1 or in the wavelength range from 1000 to 2500 nm with resolution windows of 0.02 nm and optical gain 4x. The results showed that NIRS was able to classify *F. limoni* samples based on geographical origins with the maximum explained variance of two PCs is 93%. Moreover, the respective wavelength related to this classification is 1090, 1270,1390, 1459, 1602,1713, 1912, and 2211 nm which corresponds to C-H, O-H, C-H-O, N-H stretching. It is related to acids, carbohydrates, and the fibre content of *F. limoni* samples from bent and stretched along the near-infrared region.

1. Introduction

Indonesia as a tropical country has biodiversity which is currently being developed to be utilized in the health sector [1]. Many studies have been developed to investigate the bioactive content and secondary metabolites of certain plants as well as to assess their potential as antimicrobial, antibacterial [2]-[6], antibiofilm [7], antioxidant [8], [9]. Therefore, chemical content analysis is needed in determining various biological activities of plants. The most important elements of bioactive compounds that are abundant in plants are alkaloids, flavonoids, terpenoids, steroids, tannins, and saponins [10]-[12].

Assessments of the content of medicinal plant compounds, both qualitative and quantitative, have been analyzed using several laboratory analysis methods. To determine the amount, type, and composition of a compound in various types of plants, chromatographic methods such as Gas Chromatography - Mass Spectrophotometry (GCMS) [13], [14] or Liquid Chromatography Mass Spectrophotometry (LCMS) [15] are generally used. These methods have many advantages such as being able to provide more accurate results, but in practice, this method requires a long time, uses many types of solvents in large quantities, and is also less healthy for the surrounding environment

Therefore, in the last three decades, alternative methods have been developed to predict the content of chemical compounds without destroying the sample (non-destructive), fast, effective, efficient, and environmentally friendly, by utilizing spectrum technology. Near infrared reflectance (NIRS) spectroscopy is a spectroscopic technique that works based on the interaction of NIR wave electromagnetic radiation at certain wavelengths against constituent compounds in organic materials such as carbohydrates, proteins, fats, and water [16]. NIRS spectrum detects vibrations in the near infrared region range from 800 to 2500 nm wavelength (12,500-4000 cm^{-1}) where absorption and reflectance occur at a certain intensity and a combination of vibration due to the presence of molecular bonds O-H, C-O, C-H and N-H in the near infrared region [17].

Several studies have used NIRS, such as for the analysis of flavonoids in medicinal plants [10], where the results showed the ability of NIRS to predict flavonoid concentrations in 20 samples of medicinal plant leaves. Meanwhile, Cozzolino [18] investigated the quantitative determination of antioxidants in agricultural, food, and plant products. Other studies include analysis of soil macro nutrients [19] determinations mango quality and vitamin content [2], coffee quality evaluation [3], nutmeg quality classification [6], and others.

Aside from the advantages proffered by NIRS, the complex spectra from the analysis result interpret to be difficult especially in the comparison purposes by various sample spectra. So, the use of a chemometric that is able to solve the complex data [20]-[23] is needed. Principal Component Analysis (PCA) is the common chemometrics method that is widely used for this purpose. This method can provide information about the similarity or the difference between identical samples [24], [25].

Feronia limoni (wood apple) rich in carbohydrates, protein, fat, and minerals [26], and has been researched many contents of flavonoids, phenols, terpenoids, saponins, fatty acids, and others [27], [28], also have activity as an antioxidant [29], anti-inflammatory, antipyretic and analgesic [30], anti-diabetic [31] so it has great potential as ethnomedicine. This fruit is classified in the Rutaceae family which grows in tropical areas such as Indonesia. It has a round and hard shell with a grayish gray color, the pulp is fibrous and has a lot of seeds and gives off a distinctive sour / citrus fragrance when it is ripe [32].

Based on literature studies, much research has been carried out on the content of chemical compounds and biological activity of *F. limoni* using laboratory analysis methods. Therefore, we tried to assess whether the NIRS method can be used to predict *F. limoni* compounds quickly, simultaneously, and non-destructively as an assessment of the quality parameters of *F. limoni* as a whole through the prediction model, so that we can determine whether there are differences in the chemical content of the peel and pulp of *F. limonia* from a different geographical area.

2. Materials and methods

2.1 *Samples*

F. limoni is collected from five different locations in Aceh which are from Lhokseumawe (Northern Aceh), Sigli, Batee, Laweung (Pidie), and Lubok (Aceh Besar). The sample collection was carried out in July 2020. Before spectra acquisition, samples were stored at room temperature 25^{o}C and relative humidity 86% to equilibrate.

2.2 *Spectra data acquisition*
Near infrared spectra data of intact *F. limoni* samples were acquired and measured in the wavelength range from 1000 to 2500 nm with optical gain 4x and resolution windows 0.02 nm. Spectra data were recorded as absorbance spectrum and saved as spa and csv extension files

2.3 *Classification*
Spectra data of *F. limoni* samples were used to classify data based on similarities and differences among samples. Spectra data were projected onto principal component analysis (PCA) followed with a cross validation method respectively. The maximum number of principal components (PCs) were set to 7 PCs and data were classified by looking at the PCA score plot. The relevant wavelength for PCA classification was observed by looking at the loading plot resulting from the PCA analysis and sample grouping.

2.4 *Spectrum Processing Methods*
Spectrum data are processed using the CAMO Unscrambler X 10.3 software and for spectrum data processing using Principal Component Analysis (PCA).

3. Results and discussion

3.1. *The Peel and Pulp Spectrum of F. limoni*
The NIRS spectrum of the *F. limoni* peels and pulp as shown in Figure 1, It is a known difference in the spectral characteristics of the peel and pulp. NIRS radiation on a small area of the peel is reflected to the outer surface and most of it is absorbed into the pulp

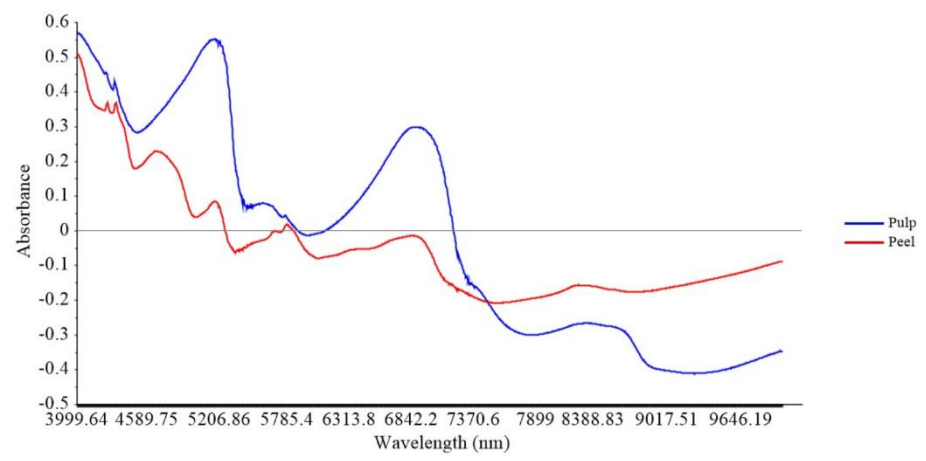

Figure 1. NIR irradiation of the peel and pulp of *F. limoni*

Based on Figure 1, it is known that the spectrum between the peel and pulp looks different (far apart). Light hitting the outer skin surface penetrates a depth of less than 1 cm. The tough peel and the response of the orientation of the fibres to the radiation direction of the waves cause a different absorbance spectrum between the peel and pulp. The thickness of the peeled fruit also greatly affects radiation and reflected scattering, causing energy reaching the pulp to experience reflection, absorption, dispersion, and light transmission [33].

The peel and pulp of the fruit receive near infrared waves at different wavelengths which cause vibrations and stretching of O-H, N-H, and C-H group bonds respectively. The water molecule which consists of O-H bonds in the peel is different in composition compared to the pulp, which can be seen based on the pattern of sharp peaks and valleys in the pulp which indicates the amount of water content

in the pulp but not too much on the peel. Several previous studies stated that some spectral differences were influenced by the amount of water [33]–[35].

The spectrum results were then analyzed using PCA (Principal Component Analysis) spectrum data processing, which showed the differences in the spectrum of the peel and pulp. This can be seen from the wide euclidian distance between the peel and pulp spectrum. Based on the PCA results, it is known that NIRS spectrum in the peel is not the same as the pulp, so it cannot predict the content or composition of F. limoni fruit through irradiation from the peel, as can be seen in Figure 2.

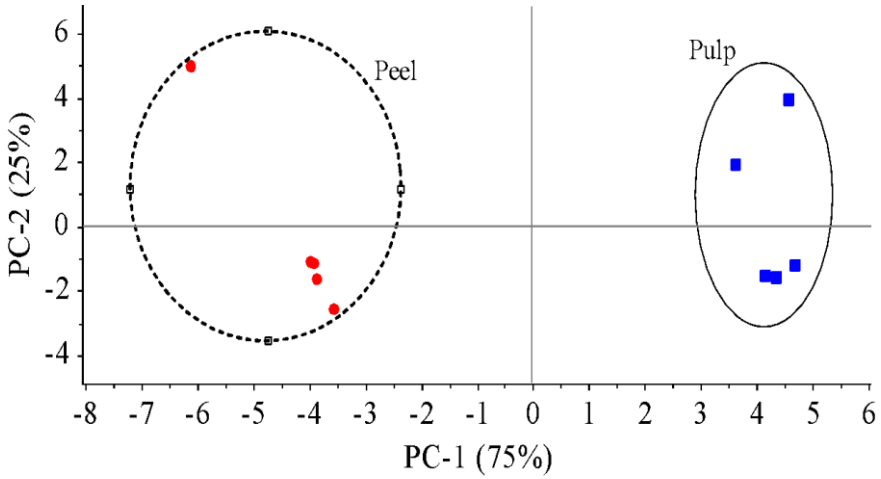

Figure 2. PCA analysis of the peel and pulp spectrum of *F. limoni*

Principal component analysis (PCA) shows a significant difference between the peel spectrum data (red) and pulp spectrum data (blue), so the peel spectrum obtained cannot describe the pulp spectrum.

3.2 *NIRS Spectrum from Five Geographical Locations*

The average spectral reflectance of the five combined areas of *F. limoni* fruit sampling can be seen in Figure 3. The resulting spectral pattern shows that each sample is classified, which means that the *F. limoni* fruits from the five regions have different compositions from one another.

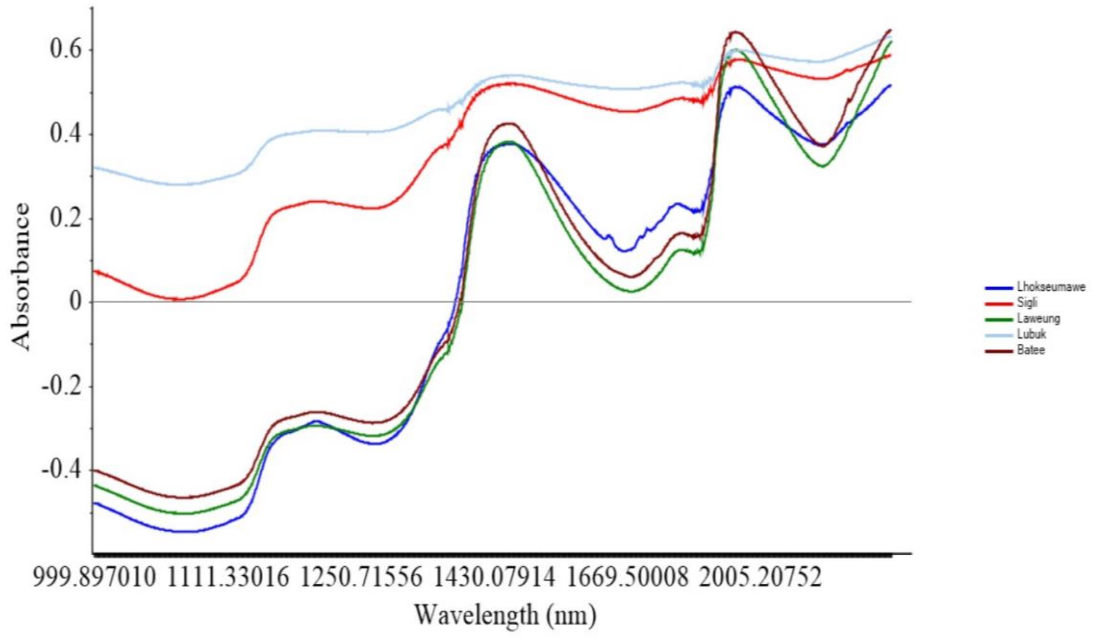

Figure 3. NIRS spectrum from five sampling areas

The optimum wavelength results from the five sampling areas of *F. limoni* fruit are shown in Figure 4, which can be used to see the relevant wavelengths of the sample classification results based on the region, then related to the chemical content of the sample based on these wavelengths.

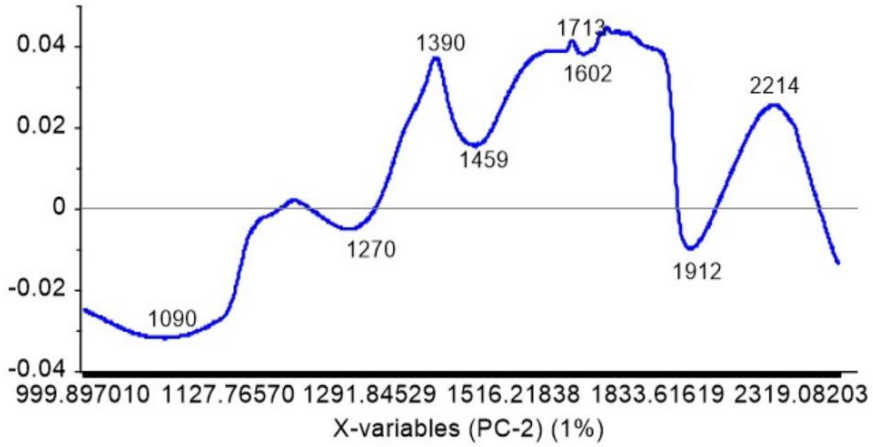

Figure 4. The optimum wavelength is based on five sample regions

It is estimated that there are hydrogen bonds in the form of H2O bonds identified at the wavelengths of 1459 nm and 1912 nm which are marked by fairly sharp valleys in the NIR reflectance spectrum. This result is in line with Workman and Weyer (2008) which states that water absorption occurs at wavelengths of 1420 - 1490 nm and 1900 - 1980 nm [36].

The CH, CH2, and CH3 bonds are probably at the peak of the 1390 nm and 1713 nm waves which are thought to be related to the presence of saturated and unsaturated fatty acids. Meanwhile, the sucrose,

fructose, and glucose content in the fruit have CHO bonds, and proteins that have NH bonds are estimated to be in the wave range of 2100 - 2300 nm, so the spectrum that forms peaks in this region indicates the possibility of finding quite a lot of carbohydrate and protein levels. Table 1 shows the distribution of F. limoni organic bonds based on the distribution of organic bonds in NIRS electromagnetic waves [37].

Table 1. The wavelengths of various type of bonds in chemical compounds of *F. limoni* (NIRS)

Wavelenghts (nm)	Type of Bond
1090	NH_2
1270	CH, CH_2, CH_3
1390	CH, CH_2,CH_3
1459	H_2O, ROH, Acid
1602	ArCH, CH_3, CH_2, CH
1713	CH, CH_2, CH_3
1912	H_2O, Acid
2214	CHO, NH_2, ArCH

The PCA analysis results of five fruit samples from different geographic areas are shown in Figure 5. Each sample is lit at three different points. The results obtained show that each sample from various regions is separated (classified) or forms clusters each with variations in its euclidean distance. This indicates that the five samples have different characteristics so that the chemical content of *F. limoni* fruit varies from one region to another.

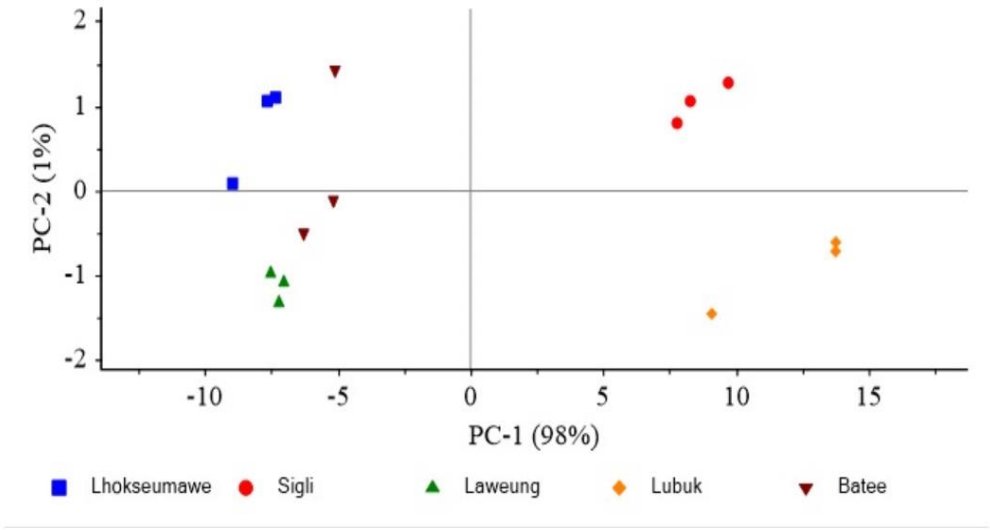

Figure 5. PCA spectrum analysis from 5 geographic areas

It can be seen that the *F. limoni* from the five regions is separated (classified) based on the great euclidean distance. Two main clusters were formed. The first cluster adjacent includes samples from Lhokseumawe- Batee-Laweung and the second cluster consists of samples from Sigli and Lubuk, so it is assumed that *F. limoni* from the Lhokseumawe, Batee, and Laweung regions has the same bioactive content, while *F. limoni* Sigli has a similar composition with samples from the Lubuk area.

4. Conclusions

Based on the results obtained, it can be concluded that NIRS can classify *F.limoni* samples based on geographic origin with a maximum explanation variant of the two PCs of 93%. Besides, the respective wavelengths associated with this classification are 1090, 1270, 1390, 14 59, 1602,1713, 1912, and 2214 nm which identify possible molecular bonds of C-H, O-H, C-H-O, and N-H as the main components of forming organic matter. This is related to differences in carbohydrate, acid, protein, or fibre content in *F. limoni* in five geographic areas.

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