Spectral observation of agarwood by infrared spectroscopy: The differences of infected and normal *Aquilaria microcarpa*

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Abstract. *Adi DS, Hwang SW, Pramasari DA, Amin Y, Widyaningrum BA, Darmawan T, Septiana E, Dwianto W, Sugiyama J. 2020. Spectral observation of agarwood by infrared spectroscopy: The differences of infected and normal* Aquilaria microcarpa*. Biodiversitas 21: 2893-2899.* This study was conducted to evaluate and to determine the potential spectral band assignments that influenced the differentiation of normal and infected agarwood of *Aquilaria microcarpa* using Fourier Transform Infrared Spectroscopy (FTIR) and Fourier Transform Near-Infrared Spectroscopy (FTNIR). The results showed that the differences in band intensity on FTIR were identified as C=O stretching (lignin), COO-stretching (hemicellulose), aromatic skeletal vibration (lignin), and C-H bending vibration. The increasing absorbances of infected agarwood were supposed as the change on the wood tissues due to the releasing resinous compound. The C-O bond (aromatic alkane) and stretching (ether), C-C stretching (aromatic alkane), and C-H bond (aromatic ring) which related to the scented fragrance of agarwood have appeared on the FTIR spectra. Multivariate analysis with principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) of the second derivative NIR spectra at the wavenumber 8,000-4,000 cm^{-1} showed that normal and infected agarwood was successful to be separated. Discriminant model one-on-one classification exhibited good performances as the R^2 performance (R^2P) values was 0.99. There were eight major wood components which contributed to the separation based on NIR spectra, where lignin, hemicellulose, and xylans were the most valuable chemical compound.

Keywords: FTIR, FTNIR, agarwood, PCA, PLS-DA

INTRODUCTION

Agarwood is a valuable non-wood forest product even the trading is measured by weight. On the other hand, agarwood contained a unique resin for some purposes such as medicinal and fragrant (Wang et al. 2018; Azren et al. 2019). This resin formation is supposed as defense reaction following the internal injury or infection of the agarwood tree (López-Sampson and Page 2018). The agarwood is originated especially from the genera *Aquilaria* and *Gyrinops* of family Thymelaeaceae (Azren et al. 2019). *Aquilaria malaccensis, A. microcarpa, A. filaria, A. beccariana,* and *Gyrinops versteegii* are the high quality of agarwood species (Pasaribu et al. 2015a).

The healthy or normal tree of *Aquilaria* or *Gyrinops* has no valuable meaning. Liu et al. (2013) described that the normal wood of *Aquilaria* was white, soft, and without scented resins. In addition, Dwianto et al. (2019) explained that *Gyrinops* and *Aquilaria* had a low density (0.31-0.35 g/cm^3) and consequently they were not suitable for structural material. Otherwise, the perspective will be different when the valuable resin with an aromatic fragrance is developed and accumulated in the stem and branches of the tree both those genera after a process of injury, such by physical force, insect or fungal infection

(Novriyanti et al. 2010; Liu et al. 2013; Hoque et al. 2019). In fact, the occurring rate of agarwood from especially *A. sinensis* in wild forest is relatively low (7-10%) (Wang et al. 2018).

Healthy and infected agarwood can be recognized by their color and odor. By the visual inspection, the color of agarwood stems is changed due to the resin existence. Moreover, infected stem has specific aromatic when it was burned. According to the National Standardization Agency of Indonesia (*Badan Standardisasi Nasional* 2011), the resin color of agarwood was black and gradually decreased to the white, which corresponded to the decreasing of their class quality. The chemical analysis of the resin content in various agarwood quality usually measured by Gas Chromatography-Mass Spectrometry (GCMS). Aromatic constituent, sesquiterpenoids, and chromone are the most chemical compound, which is found in the agarwood product (Pasaribu et al. 2015a; Yin et al. 2016; Wang et al. 2018; Putro et al. 2019; Wulansari et al. 2019). In contrast, healthy agarwood contains abundant fatty acids and alkanes (Chen et al. 2011).

In this present study, the analysis of chemical compound of healthy and infected *A. microcarpa* was used by Infrared (IR) spectroscopy. Organic materials consist of various functional groups, whose fundamental vibration

can be observed at IR range (Tsuchikawa and Kobori 2015). IR spectroscopy is a rapid method for small wood characterization and its major component (Faix and Binhoff 1988). NIR spectroscopy analysis is well-known as a rapid and powerful tool for non-destructive characterization of organic materials (Ito et al. 2019). In addition, the application of Fourier Transform Infrared spectroscopy (FTIR) and Fourier Transform Near-Infrared spectroscopy (FTNIR) has been successful to distinguish between the difference band assigned to the chemical content in wood species, both original and modification (Gebreselassie et al. 2017; Popescu et al. 2018; Traoré et al. 2018; Ito et al. 2019). Thus, IR analysis has several advantages include minimal sample preparation and rapid acquisition times to identify several functional group bands of agarwood resin from different points of view with an existing method. This study was conducted to evaluate and to determine the potential band assignment of the spectra that influenced the identification of normal and infected agarwood from *A. microcarpa*.

MATERIALS AND METHODS

Materials

The samples used in this study were normal and infected woods of *A. microcarpa* which were collected from Balikpapan Botanical Garden, Samarinda, East Kalimantan, Indonesia. Five standing trees were selected for the normal wood. Whilst three standing trees were selected for the naturally infected or injured wood samples, which having discrepancies in color for indicated the infected spot of wood. The sampling used a knife to get flakes or chips samples. The infected agarwood was classified as a "*gaharu kemedangan*" according to private statement from a local expert. The samples were dried in the ambient temperature to constant weight. Then, the samples were powdered to make homogeneous material (Sandak et al. 2016). Its size was around 80-100 mesh. The sample powder was used for FTIR analysis. In addition, the sample powder was prepared by using hand-pressed equipment with diameter of around 0.7 cm to obtain tablet samples with density of 1 g/cm^3 for FTNIR analysis. In total, thirty spectral were collected from normal as well as infected agarwood.

Procedures
FTIR and FTNIR analysis

The identification of the active functional groups present in agarwood powder samples was characterized by Perkin-Elmer Spectrum two FTIR. The investigation was obtained using the Universal Attenuated Total Reflectance (UATR) method and was recorded with an average of 16 scans at a resolution of 4 cm^{-1} within wavelength ranging from 4,000-400 cm^{-1}.

Tablet samples were scanned using a FTNIR machine (PerkinElmer Spectrum 100N) to collect the spectrum at the wavenumber 10,000-4,000 cm^{-1}. In addition, the process of collecting spectra used spectral resolution of 16 cm^{-1} and 32 scans per record process (Horikawa et al. 2015;

Hwang et al. 2016). On average, thirty numbers of spectra were measured from normal as well as infected agarwood. For multivariate data analysis, the original spectra were then processed into second derivative by using a method of Savitzky-Golay (Savitzky and Golay, 1964). In addition, the spectra were also smoothed at 9 points and fifth-order polynomial.

Multivariate data analysis

Partial least square-discriminant analysis (PLS-DA) was used to analyze the NIR spectra of the agarwood samples. These analyses were performed using Unscrambler software (CAMO software, Inc., Woodbridge, NJ) at the spectral range 8,000-4,000 cm^{-1}, because there was no significant information at the spectral region over than 8,000 cm^{-1}. PCA was done to decompose the data and variability into first few PCs (Hori and Sugiyama, 2003). PLS-DA was conducted to determine and to separate the normal and infected agarwood samples. Leave-one-out cross-validation was applied to construct the models. In addition, the separation was based on the one-to-one classification. The coefficient of determination for calibration (R^{2}C) and the root mean square error of calibration (RMESC) were used to evaluate the calibration performance, while the determination for prediction (R^{2}P) and root mean square error of calibration prediction (RMSEP) were used to assess the model (Horikawa et al. 2015; Hwang et al. 2016). For discriminant model development of PLS-DA, the class value was set from number (1) and (2), thus the reference value was set to 1.5. Furthermore, a histogram of reference value was made to confirm the accuracy of training and validation of each group.

RESULTS AND DISCUSSION

Absorbance characteristic of the agarwood spectra

Figure 1 shows the illustration of infected (A) and normal (B) *A. microcarpa* agarwood. It showed that the resin color was brown dark, and the resin was located as a spot in the stem. It was common that the darker the resin, the higher the quality was. Furthermore, the higher the resin contents, the higher the quality of agarwood was. In contrast, the normal agarwood had a white color and no resin constituent in their stem.

The FTIR spectra of normal and infected *A. microcarpa* are shown in Figure 2. From those, both spectra did not show significant changes of the band's shape, but there was difference of the band intensity at the several wavenumbers. The increased absorbance intensity of infected agarwood may be caused by changes in wood tissue due to infections that result in agarwood releasing resinous compounds (Faizal et al. 2017).

The main characteristic absorption band appeared at 3,350 cm^{-1} assigned to the presence of hydroxyl group (-OH). The presence of methyl was observed at 2,924 cm^{-1}, the band at 1,729 cm^{-1} showed C=O stretching in carboxyl acid and acetyl groups (hemicellulose), and 1,650 cm^{-1} was due to C=O stretching (lignin). The presence of COO-

stretching (hemicellulose) at 1,600 cm⁻¹, 1,508 cm⁻¹ for the presence of aromatic skeletal vibration (lignin), and the band at 1,370 cm⁻¹ showed the presence of C-H bending vibration. The band at 1226 cm⁻¹ and 891 cm⁻¹ assigned to the presence of C-O stretching and C-C stretching, respectively (Horikawa et al. 2019). According to the spectra in Figure 2, the presence of C-O bond (aromatic alkane), stretching (ether), C-C stretching (aromatic alkane), and C-H bond (aromatic ring) related to sweet to pleasant fragrance of agarwood (Nasardin et al. 2018). In addition, the differences in intensity were founded on lignin, hemicellulose, and C-H bending vibration. Previous study revealed that 2-(2-phenylethyl)-4H-chromen-4-one derivatives and sesquiterpenes were the main chemical constituent of the agarwood from the genus *Aquilaria*, which contributed to the fragrant smell (Wang et al., 2018). On the other report, aromadendrene compounds were found at the various quality of agarwood, which was also proposed as the effective chemical distinguisher for agarwood (Pasaribu et al. 2015a). All these compounds were not found at the healthy *Aquilaria* because healthy wood has only consisted of fatty acids and alkanes. However, the recognition name or specific chemical compounds of agarwood could not be done by using FTIR.

Figure 3 shows the original NIR spectra of normal and infected *A. microcarpa* at the wavenumber 10,000-4,000 cm⁻¹. First glance, there was no significant difference and important information, which could be extracted from the band at any specific wavenumber. There were also limited numbers of the bands on the original spectra. The absorbance of the infected wood was higher than that of the normal wood. However, this was only showed the difference of the spectra baseline (Gierlinger et al. 2004). Therefore, analysis based on the original spectra was difficult to obtain the crucial wavenumber for determining the normal and infected agarwood. In contrast, second derivative transformations helped visualize differences in the spectra (Sandak et al. 2016), thus it would be used in further multivariate analysis.

Figure 1. The infected (A) and normal (B) agarwood (*Aquilaria microcarpa*). Yellow arrow showed the dark resin on the stem. Photographs by ES
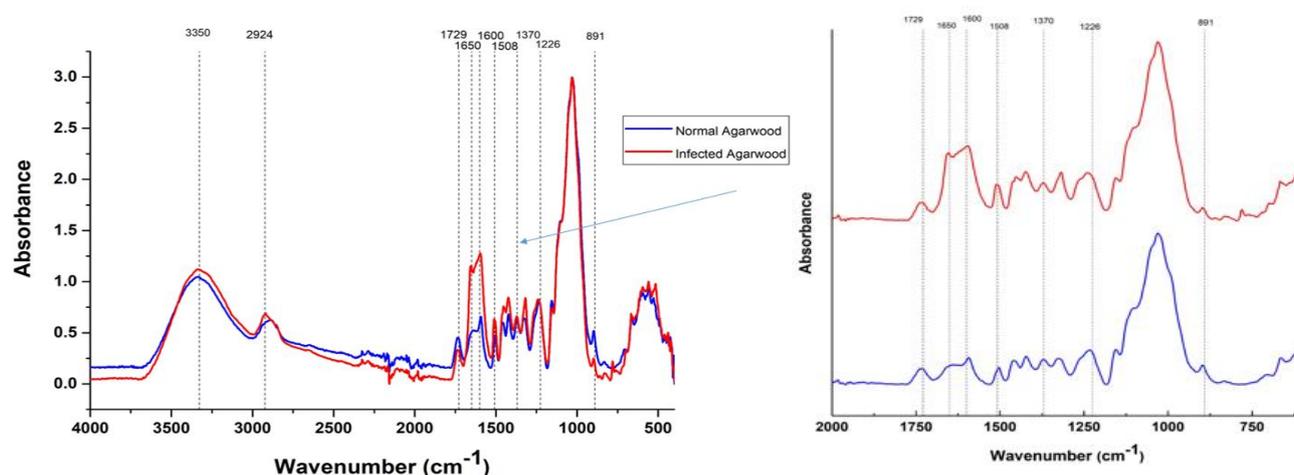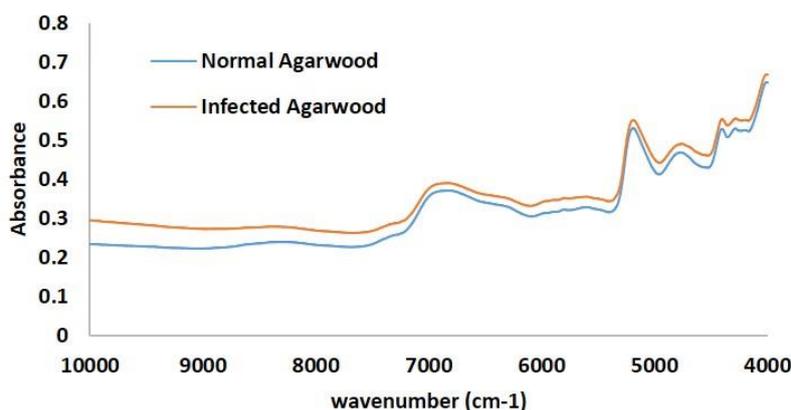

Figure 2. FTIR spectra of normal and infected agarwood (*Aquilaria microcarpa*) at the wave number 4,000-400 cm⁻¹

Multivariate data analysis

PCA was used to decompose the data, while PLS-DA was used to identify the differences between normal and infected agarwood. Those analyses provided a cluster through score plots and some information about the significant band assignment of the spectra which influenced to determination of the sample. Figure 4 presents the PCA's score plot (A) and PLS-DA's score plot (B). It was shown that normal and infected agarwood clustered in different PCs and Factors. In the PCA's score plot (Figure 4.A), PC1 was contained 83% of explained variance, while PC2 was 5%. PCA is a tool to decompose a data matrix into few principal components, then clustered the set of data based on the unique similarities of the spectra characteristics (Hori and Sugiyama 2003; Sandak et al. 2016). In addition, the PCA's score plot indicated the different clusters, negative of PC1 for infected agarwood,

and positive PC1 for normal agarwood. These assignments provide conclusion that the two samples of agarwood have discrepancies.

PLS-DA's score plot (Figure 4.B) indicated that healthy and infected agarwood was well classified. It means that normal and infected agarwood was different from each other. Factor 1 was contained 95% information of explained variance and Factor 2 was 4%. The contrary result from PCA's score plot was found on the PLS-DA's plot, which normal agarwood was in the positive, while infected agarwood was in the negative axis. Thus, it required a further investigation to determine the factor which gives impact to the clustering. The relationship between second derivative spectra and loading PC (PCA score plot) and Factor (PLS-DA score plot) than were used to know the specific wavenumber or chemical content that have impact on the score plot.

Figure 3. The average original FTNIR spectra of normal and infected agarwood (*Aquilaria microcarpa*) at the wavenumber 10,000-4,000 cm^{-1}

A

B
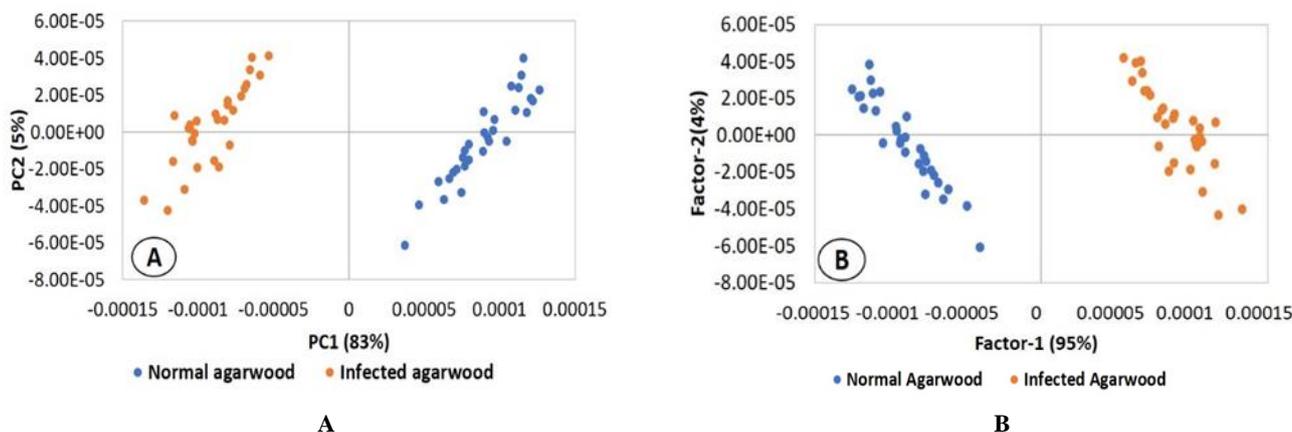
Figure 4. PCA's score plot (A) and PLS-DA's score plot (B) at the spectral range 8,000-4,000 cm^{-1}
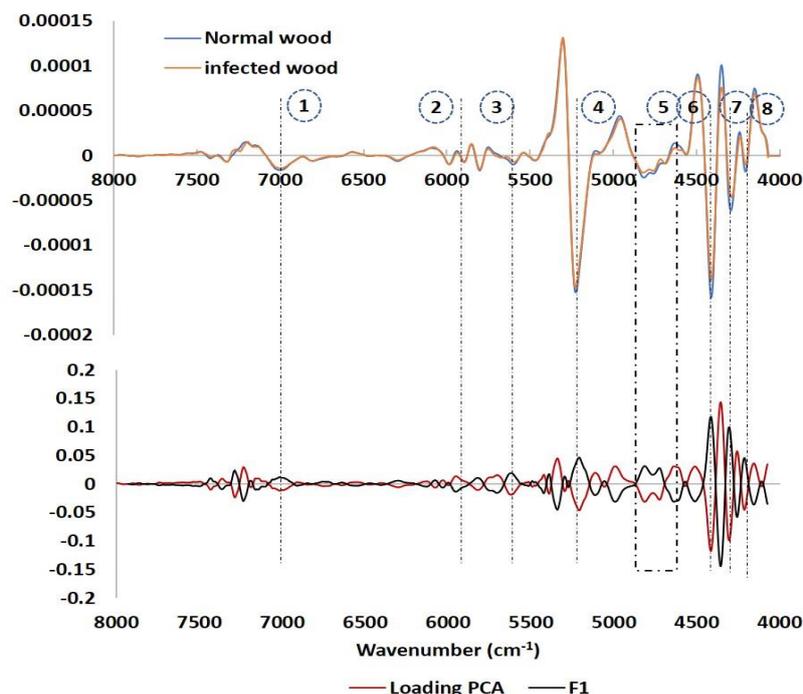
Figure 5. Relationship between second derivative spectra, loading (PCA), and factor (PLS) at the spectral range 8,000-4,000 cm⁻¹

Figure 5 represents eight absorption bands representing the spectral range 8,000-4,000 cm⁻¹ that contributed to the data plotting. Loading PC1 and Factor 1 contained the most valuable information for clustering data in the score plots. Figure 5 showed that band of loading PC1 and Factor 1 were in the opposite direction. This result was related to their clustered plot position in Figure 4. Although there were different bands direction, the chemical components, which affected the score plots, was identical. For instance, normal agarwood was plotted in positive axis of PCA, in other hands, the PLS-DA's plotted in negative axis. It means that negative band of loading PC1 was the main contributor for clustering in PCA, whilst positive value of Factor 1 was the major contribution in PLS-DA. However, the number of absorption bands of loading PC1 and Factor 1 was similar.

In this study, we used band assignments proposed by Schwanninger et al. (2011) to determine the absorption bands on the second derivative spectra. Figure 6 shows the different of the specific absorption bands, which indicated an important chemical component for separation between normal and infected agarwood. The chemical components were (1) 7,000 cm⁻¹ amorphous cellulose; (2) 5,800 cm⁻¹ furanose/pyranose due to hemicellulose; (3) 5,594 cm⁻¹ semi-crystalline or crystalline of cellulose; (4) 5,220 cm⁻¹ water; (5) 4,890-4,620 cm⁻¹ cellulose, lignin and extractive; (6) 4,411 cm⁻¹ lignin; (7) 4,296-4,288 cm⁻¹ hemicellulose and xylans; (8) 4,202 cm⁻¹ holocellulose. In addition, loading PC1 and Factor 1 (Figure 5) showed that the

highest bands were at number six and seven, which were identified as lignin and hemicellulose or xylans. It showed that the bands intensity of those two wood components were different between normal and infected agarwood. This result corresponded to the other previous studies that lignin, α-cellulose, and hemicellulose have tendency to change in the producing process of agarwood in *A. malaccensis* (Lamk.) (Dwianto et al. 2019; Herawati et al. 2009). However, the NIR result could not precisely provide the specific name of the chemical compound. On the other hand by using GCMS method, *kemedangan* quality of *A. microcarpa* contained guaiene, 2.5 furandione, 3-dodecenyl and agorospirol for *Aquilaria sp.* (Pasaribu et al. 2015b; Wulansari et al. 2019).

The best model for PLS-DA was up to Factor 2. However, Factor 1 had R^2P more than 0.9. The RMSEP, RMSEC, R^2P, and R^2C at the best model were 0.049, 0.053, 0.99, and 0.99, respectively. In general, the discriminant model for all samples was powerful and the R square prediction was high. This indicated that the model could distinguish the sample very well. The histogram of class values has also confirmed the result. It was used to know the correct misclassification between training and validation set. Figure 7 shows the histogram of class value between normal and infected agarwood. The histogram showed that all samples were not passed the reference value line (1.5). It indicated that the recognition and the classification of the agarwood sample were successful.
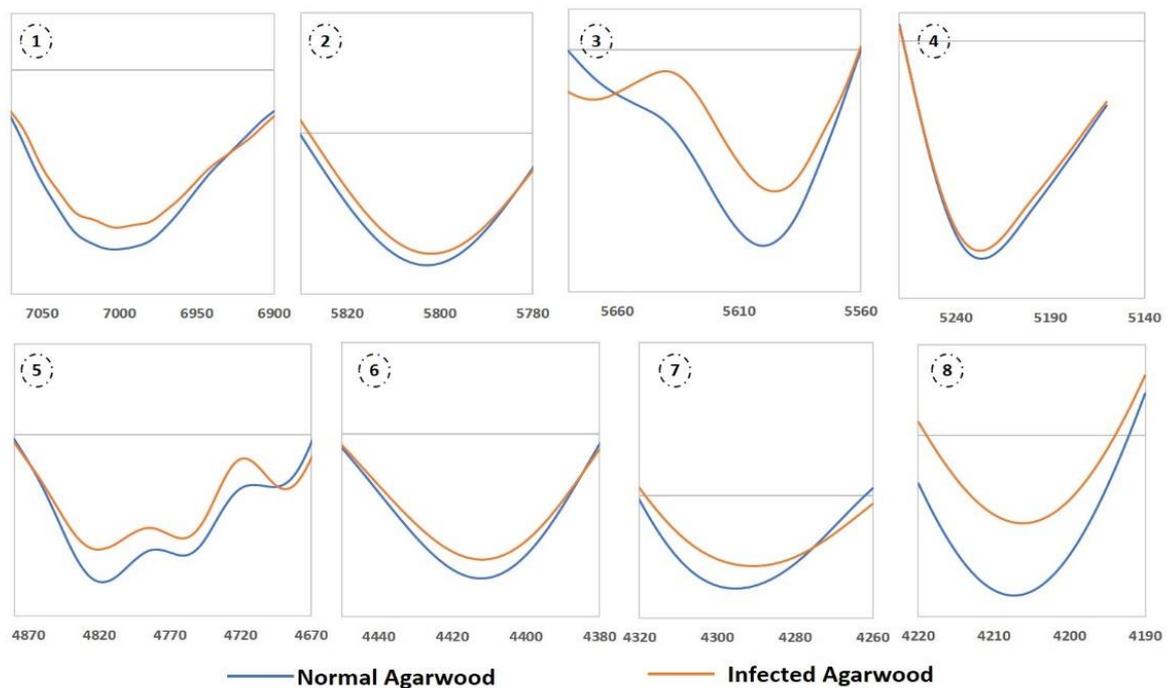
Figure 6. NIR spectral bands assigned to the major wood components: (1) 7,000 cm^{-1} amorphous cellulose; (2) 5,800 cm^{-1} furanose/pyranose due to hemicellulose; (3) 5,594 cm^{-1} semi-crystalline or crystalline of cellulose; (4) 5,220 cm^{-1} water; (5) 4,890-4,620 cm^{-1} cellulose, lignin and extractive; (6) 4,411 cm^{-1} lignin; (7) 4,296-4,288 cm^{-1} hemicellulose and xylan; (8) 4,202 cm^{-1} holocellulose
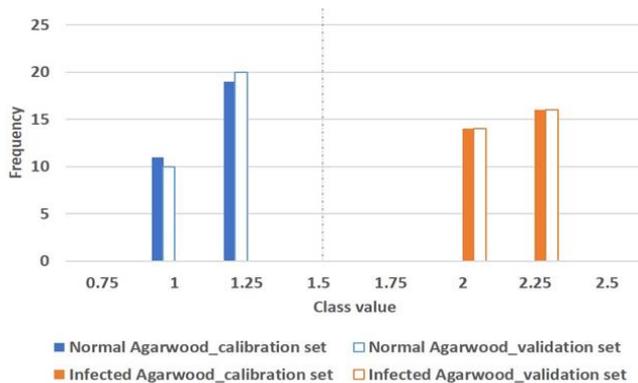
Figure 7. Histogram of class value computed by PLS-DA in the second derivative spectra at the region 8,000-4,000 cm^{-1}

In conclusion, the evaluation of the wood component change in normal and infected *Aquilaria microcarpa* using FTIR and FTNIR spectroscopy and chemometric analysis was successful in this study. The differences of intensity on FTIR were identified as C=O stretching (lignin) at 1,650 cm^{-1}, COO-stretching (hemicellulose) at 1,600 cm^{-1}, aromatic skeletal vibration (lignin) at 1,508 cm^{-1}, and C-H bending vibration at 1,370 cm^{-1}. The model has high accuracy to separate between the normal and infected agarwood based on their NIR spectra. There were eight major wood components that contributed to the separation, which lignin (4,411 cm^{-1}) and hemicellulose or xylans (4,296-4,288 cm^{-1}) were supposed as the most valuable

chemical component. Although NIR method could not obtain the specific chemical compound, in comparison with the general method (using GCMS), the application of this method was promising for determining the agarwood because this method is fast, nondestructive, and labor effortless.

ACKNOWLEDGEMENTS

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