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RESEARCH ARTICLE

INVESTIGATION OF FACTORS AFFECTING THE RAPID DETECTION OF AGARWOOD FORMATION IN *Aquilaria crassna* BY NEAR-INFRARED SPECTROSCOPY

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ABSTRACT

Agarwood is a highly valued fragrant resin produced inside a few tree species belonging to the family Thymalaeaceae as a self-defense response to plant stress. The amount of resin developed inside the tree cannot be estimated by outside inspection. Consequently, harvesting trees before they reach their potential yield is a severe drawback to the agarwood industry. Therefore, developing effective techniques for detecting agarwood resin status inside the tree species has become a critically important task for the agarwood industry to increase productivity. The present study evaluates the factors affecting Near-Infrared Spectroscopy (NIRS) models when predicting agarwood formation inside *A. crassna* trunks using NIR spectroscopy. The research used 110 wood specimens obtained from well-grown agarwood trees in a commercial plantation in Nawimana GS Division, Matara District, Sri Lanka. NIR meter FQA-NIR Gun (588-1100nm) with a custom-made probe was used to acquire NIR reflectance spectra without outside light interference. SIMCA models were built to identify the agar resin-developed wood log areas from the normal wood areas in the tree trunk. SIMCA prediction models were built to investigate three influencing factors, namely present or absent outside tree bark, surface roughness and wood thickness agarwood prediction. Better prediction results were obtained from the bark-removed samples (at the accuracy rates of 97%) to the bark present (85%), smooth wood surfaces (98%) to the rough surface (90%) and 2mm thickness (98%) to the other thickness. The most effective wavelength for the separation of agarwood present and absent samples was located at 978 nm of NIR. The study has demonstrated the potential possibility of using NIR spectroscopy to identify the agarwood formation in *A. crassna* in non-destructive and rapid mode.

Keywords: Agarwood, *A. crassna*, Rapid detection, NIR spectroscopy, SIMCA

INTRODUCTION

Agarwood, a resinous heartwood, is produced in species Thymelaeaceae family species as a self-defense mechanism (Azren *et al.* 2019; Xiang *et al.* 2017; Yin *et al.* 2016). In some cases, it is categorized as a pathological product (Tajuddin *et al.* 2016). This oleoresin is available in viscous semisolid and solid form within trees (Rasool & Mohamed 2016). Recognized as the most luxurious and expensive forest and plant-based aromatic product globally (Kanazawa 2017; López-Sampson and Page 2018) the value arises after the formation of this fragrant resin inside the stem, branches, and even roots due to its self-defense mechanism (Subasinghe & Hettiarachchi 2013, 2015, 2016).

Various synonyms exist for this resinous wood, including Agarwood, Eaglewood, Aloeswood, and Gaharu. However, different regions and eras attribute different names with diverse meanings (Chowdhury *et al.* 2016; López-Sampson & Page 2018; Zich & Compton 2001). In some countries, this is referred to as “The wood of the god” (Persoon & van Beek 2008) while in Sri Lanka, it is known as Walla-Patta (Dharmadasa *et al.* 2020).

Modern agarwood identification methods are limited. Mostly at the experimental level, leading to the continued use of traditional

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methods in the industry. Misidentification remains a common issue, as external visual characteristics for agarwood identification are not always apparent (Karlinasari & Nandika 2016; Lee & Mohamed 2016). Traditional knowledge involves cutting down the trees and chopping them into pieces to search inside the trunk for the resinous wood (Zich & Compton 2001), with variations in techniques and indicators across countries (Mohamed & Lee 2016).

Recent experimental methods for agarwood identification include Magnetic Induction Tomography (MIT) and Acoustic-based nondestructive technologies are the most recent and experimental methods that are being used for agarwood identification (Karlinasari & Nandika 2016; Zakaria *et al.* 2013). Additionally, thermogravimetry (TG) gas chromatography-mass spectrometry (GC-MS) Mass Spectrometry (MS) and Infrared spectroscopy (IRS) are suggested technical methods that can be used for identifying agarwood associated chemical compounds (Yin *et al.* 2016).

Spectroscopic methods, recognized for their non-destructive and rapid nature, offer a potential solution for analyzing wood samples across various industries (Hori & Sugiyama 2003). NIR spectroscopy, in particular is a well-established nondestructive method for evaluating organic materials, finding applications in pharmaceuticals, foods, agriculture, and wood texture analysis (Abe *et al.* 1995; Faix & Beinhoff 1988; Ito *et al.* 2019). Acknowledging the benefits and necessity of a nondestructive identification method for the agarwood industry, this study aims to identify the effects of influencing factors, such as bark availability, surface roughness, and wood thickness, when identifying normal healthy wood from agarwood-formed resinous wood in *A. crassna* using NIR spectroscopy.

MATERIALS AND METHODS

Sample Collection

Freshly harvested wood logs obtained from 10 *Aquilaria crassna* trees belonging to a commercial agarwood plantation in Nawimana GS division, Matara district, Sri Lanka were used in the study. The wood logs were approximately 1.5m in length and approximate diameter between 15 to 18 cm. The wood logs were chosen by inspecting cross sections with higher volumes of agarwood formation. The harvested logs were approximately 7-year-old trees having fungal inoculation (FI) as an artificial agarwood-inducing method after 5 years of transplanting.

Sample Preparation and Data Acquisition

Experiment-1 Assess the Bark Influence

The obtained wood logs from 10 agarwood trees were cut into small 20 cm lengths representative 5 wood log specimens from each tree. Accordingly, there were 50 logs representing 5 replicates from each tree. Cross-sections of representative log specimens were observed and dark color resin present areas and light color resin-free areas were selected for the spectra observation. Spectra from both resin-present areas and resin-free areas were obtained once with bark and then without bark for the comparison of the results. The same procedure was followed for all the wood logs been tested.

NIR Spectrometer FQA-NIR Gun (Shizuoka Shibuya Seiki, Hamamatsu, Japan) with a custom-made probe extension was used to acquire reflectance NIR spectra from 588 nm up to 1100 nm. First, the NIR spectra were recorded when the logs including the bark *A. crassna* wood specimens. Thirty-five wood logs obtained from seven trees with five replicates (7trees *5log replicates*5 spectra/ log =175) were used to get 175 spectra and feed in the Soft Independent Modeling of Class Analogy (SIMCA) algorithm for model building and another 75 spectra obtained from Three independent trees (3 trees* 5 log replicates * 5 spectra/ log =75 spectra) were used in the prediction sets for the model validation. Accordingly, there were 250 spectra were used in the performance evaluation for wood logs with bark present conditions. The NIR exposure integration time was set to 65 milliseconds for the bark present specimens.

After the data were recorded, the bark layers of those log specimens were removed carefully without any damage to the internal wood during the pealing. Then, another 250 spectra were recorded following the same procedure for agarwood present and absent areas of bark removed wood samples. It was found that 130 milliseconds of integration time was better for the bark-removed specimens.

Experiment-2 Assess the Wood Surface Roughness

Ten representative samples from 10 *Aquilaria crassna* trees with three replicates per tree were used in the experiment. Samples for coarse wood surfaces were made by sawing the wood log and made 1mm and 2mm wood thickness up to the agarwood-formed area in *A. crassna*. After the spectra were taken from the coarse surfaces, the same sample surfaces were changed into smooth surfaces with the help of an electric grinder with 120-gauge sandpaper. Both coarse and smooth samples were prepared from the agarwood present and absent areas. NIR Spectrometer FQA-NIR Gun with 140 milliseconds of integration time was applied for the spectra recording.

250 spectra were recorded for each coarse and smooth surface separately by maintaining 1 mm and 2mm depth thickness up to the agarwood formation. Prepared healthy agarwood absent specimens were also tested by getting 250 spectra for each smooth surface and coarse surface. These spectral data were used for SIMCA model development and another 50 spectra per category were used as the SIMCA prediction set.

Experiment-3 Assess the Wood Thickness on Agarwood Prediction

A. crassna 10 wood logs with agarwood formed area located more than 6mm thickness deep inside from the bark were selected. Then the bark was removed and white color healthy wood thickness was removed from each specimen to prepare 6mm, 5mm, 4mm, 3mm, 2mm, and 1mm thickness. The same number of samples without agarwood was also prepared. A hand grinder was used to remove the wood layers after spectral data acquisition at each higher thickness levels to maintain specific wood thickness up to dark color resinous wood. NIR Spectrometer FQA-NIR Gun (Shizuoka Shibuya Seiki, Hamamatsu, Japan) with a custom-made probe was used with an integration time of 130 milliseconds. A total number of 200 spectra were taken from each wood thickness (6mm, 5mm, 4mm, 3mm, 2mm, 1mm) that were maintained up to the agarwood formation. The same number of 200 spectra were recorded without agarwood formation areas by getting 50 spectra for one side and then four sides from each log that was tested.

Data Analysis

The acquired spectra were first evaluated by Principal Component Analysis (PCA) and removed the noise carrying abnormal outlier spectra. Then, the informative spectra were applied into the discriminate analysis algorithm of the Soft Independent Modeling of Class Analogy (SIMCA) calibration model for the agarwood present and absent class identification. The reference class status of agarwood present or absent were input to the SIMCA algorithm based on the visual observation of the dissected surface of the wood specimens. SIMCA models were developed to predict the agarwood formation in bark present and absent zones. All the data analysis were performed using Pirouette software 4.5 (Infometrix, Inc ®) Woodinville, WA, USA). The model parameters of the number of factors, best data pretreatment methods and data transformation for the best chemo-metric model configurations were optimized to get the best accurate prediction for the agarwood status inside the *A. crassna* trunk. The class separability was assessed as the statistical distances between the classes presented in the results multi plots of the SIMCA output algorithm. After developing the best model to determine the agarwood formation. The model performance was assessed in terms of the number of correct classifications out of the total prediction set spectra.

RESULTS AND DISCUSSION

Influence of Bark on Agarwood Prediction

SIMCA Prediction models were prepared to assess the potential impact of *Aquilaria crassna* tree bark on the non-destructive prediction of agarwood status within wood logs because if there are no clear impacts it will ensure agarwood status prediction of the tree as it is in the field having the bark (Fig. 1).

Upon examination of the prediction outcomes, it becomes evident that the models developed for bark-removed samples outperformed those for bark-present samples., In the case of bark-removed specimens, the identification accuracy for agarwood availability reached an impressive 96%, with an equally notable accuracy of 97% for predicting agarwood-absent areas. In contrast, the bark-present sample group exhibited lower accuracy rates, predicting agarwood presence and absence at rates of 95% and 75%, respectively (Table 1). These results suggest a notable improvement in prediction precision when the bark is removed, emphasizing the significance of bark influence in accurate agarwood status assessments.

Similarly, the class projections for bark-removed specimens have better separation (Fig. 2) than the bark-present specimens class projections.

Interclass distance serves as a crucial indicator of the separability of class groups in chemometric modeling. In our study, the SIMCA classification algorithm was employed prepared to assess the separability of agarwood present and absent areas of wood logs once using the logs with their bark and then after removing their bark. It was observed that the models prepared with the logs having the bark gave a higher interclass distance between agarwood present and

Figure 1: Bark present and absent samples

Table 1: SIMCA prediction rates of agarwood for bark present and absent samples

	Agarwood present			Agarwood absent			
	Number of spectra	Correct classifica-tions	Predic-tion rate	Number of spectra	Correct classifica-tions	Predic-tion rate	Overall
With bark	75	71	95%	75	56	75%	85%
Without bark	75	72	96%	75	73	97%	97%

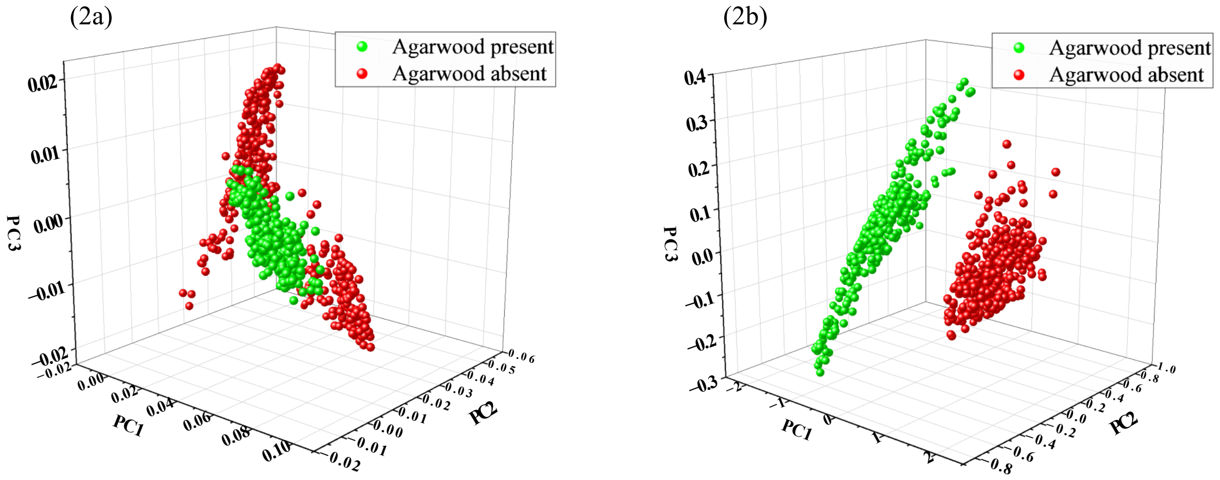

Figure 2: SIMCA class projections for samples (2a) with bark and (2b) without bark

absent samples (Fig. 3). This observation implies that the agarwood present and absent samples be better separated when the spectra were taken from wood logs having the bark.

In the bark present sample's class projections, 3 separations can be identified (Fig. 2-a). When observing the bark present samples, the bark has many variations. This scenario is a common thing in every tree trunk. The environmental conditions and many other factors would cause this to occur (Richardson *et al.* 2015). In this situation, lichen growth can be clearly identified in the obtained barks. The obtained logs were also harvested from a commercial agarwood plantation in the wet zone

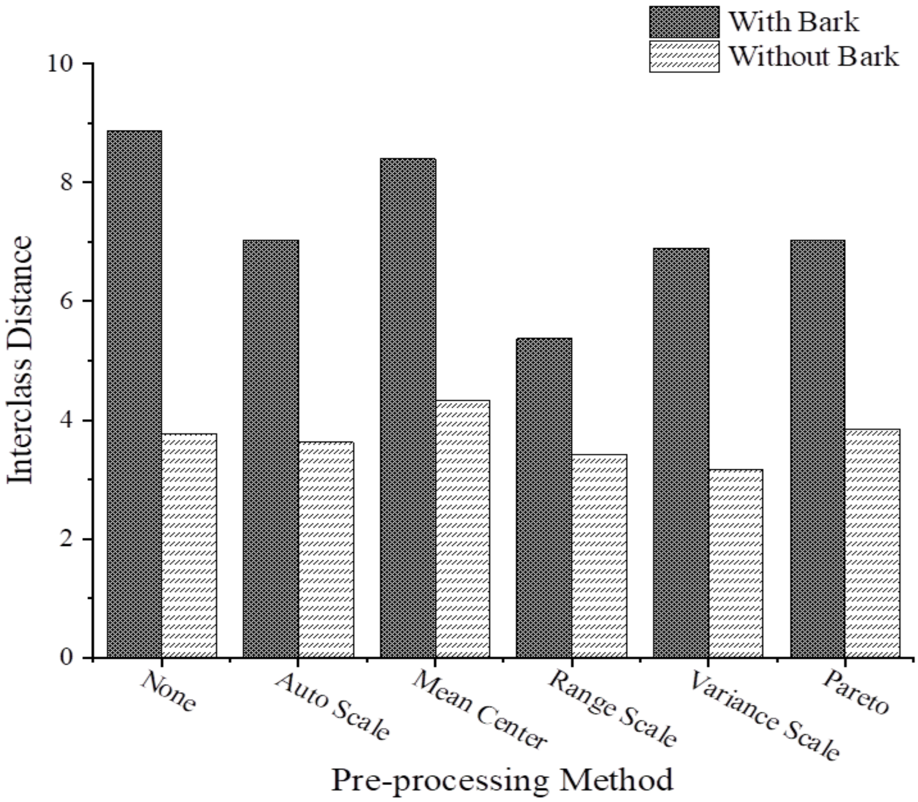

Figure 3: Preprocessing methods vs. Interclass distances for bark present and absent samples

in Sri Lanka. The agarwood-producing trees are also recommended for the wet zone and intermediate zone in Sri Lanka (Subasinghe & Hettiarachchi 2015). So, there is a high possibility to influence the lichens in these *Aquilaria* and *Gyrinops* species. Because lichens are frequently found on tree trunks, branches, and twigs, the bark provides a stable environment for the lichens to collect necessary sunlight, rainwater, and materials from the air. They grow on healthy, stressed, or unhealthy trees (Nimis *et al*. 2002). These variations in the bark reduce the accuracy of predicting agarwood by the NIRS. Because spectral data for bark present samples include reflected properties of these variations. Therefore, the SIMCA prediction results for bark present samples resulted in lower prediction rates.

When considering the model optimization, the highest interclass distance always resulted in 1st derivative math transformation for class separation in agarwood present and absent zones with and without bark specimens (Table 2).

The results, show a quick detection of agarwood formation by NIRS, bark removal, and spectra acquisition is better than NIR spectral acquisition from bark present samples. The fragrance agarwood resin is not extracted from the bark. It is only available in stems, branches, and roots (Subasinghe & Hettiarachchi 2015, 2016). So, removing the bark does not influence the harvest of the tree. Also, the entire bark of the tree does not need to be removed for spectral data acquisition. This study suggests a simple new tool to remove the tree bark (Fig. 4) so that even in-situ spectra acquisition is also possible conveniently.

Table 2: Best pre-treatments for bark present and absent samples

Bark availability	Math transformation	Preprocessing method					
		None	Auto scale	Mean center	Range scale	Variance scale	Pareto
Present	1st Derivative	8.8611	7.0356	8.3977	5.3745	6.8941	7.0283
	2nd Derivative	4.8281	5.2875	4.7462	5.1607	5.3213	4.7632
Absent	1st Derivative	6.1756	6.0370	7.3547	6.0426	4.8129	7.5449
	2nd Derivative	3.7675	3.6328	4.3372	3.4231	3.1754	3.8423

Figure 4: New tool for removing small patch of the bark for in-situ spectra acquisition

For the used FQA-NIR Gun, the direct probe is also possible for that task. A small area of bark removal does not highly impact the tree. There is an agarwood-inducing method by removing the outer bark (Liu *et al*. 2013). So, this research recommends to remove bark and record spectral data for agarwood prediction by NIRS. Class Distances for bark removed samples by Pareto preprocessing method and 1st derivative math transformation show a clear separation between agarwood present and absent areas of the considered log. Overall, the detection of agarwood formation in *A. crassna* is possible by NIRS. Furthermore, when data acquired after removing the bark gives better prediction results.

Influence of Surface Roughness on Agarwood Prediction

Once the agarwood logs are harvested, the agarwood-formed areas will be separated from the normal softwoods. This task is strenuous and demands much labour and if the agarwood present areas were identified, the rest could be easily sorted out from the agarwood separation. The surface roughness is an important factor in this regard as the rough surface just after chopping the wood can be used to get the spectra and otherwise, we may need to finish the surface before to get the spectra. Normally just after sawing or chopping, the wood surface of the *A. crassna*, is usually having with a rough surface. Because sawing does not give a perfect finish for this wood because this species is under the softwood category (Adi *et al*. 2020). Also, *A. crassna* has fibrous wood. So it would be better to use a tool that gives a smooth wood surface. This was proven by the SIMCA prediction rates.

The results have shown that the highest prediction rate was accounting when the smooth surface wood samples were used in the spectra acquisition. The impact of surface roughness was investigated with two sample thicknesses as 1mm and 2mm and there is a positive results tendency when the 2mm thick logs were used in the spectra acquisition (Table 3).

Figure 5: Preparation of smooth surface for spectra acquisition

Table 3: SIMCA prediction rates for different surface roughness

Surface roughness	Wood thick-ness	Agarwood present			Agarwood absent			Overall
		Number of spectra	Correct classifi-cations	Predic-tion rate	Number of spectra	Correct classifi-cations	Predic-tion rate	
Coarse	1mm	50	38	76%	50	38	76%	76%
	2mm	50	45	90%	50	45	90%	90%
Smooth	1mm	50	45	90%	50	45	90%	90%
	2mm	50	50	100%	50	48	96%	98%

Table 3 shows that for the smooth surface samples, 2mm wood thickness gives the best prediction rate as high as 100% for the agarwood present samples. It was reduced to 90% when the 1mm thick wood specimens were used. When the coarse surface samples were used the maximum prediction rate could be reached was 90% at the 2mm samples and it was as low as 76% when using the 1mm thickness. In other way, in overall performance, using the 1mm samples thickness the maximum prediction rates could be reached was only up to 90% even they were smoothed and when it is coarse the prediction rate is as low as 76%.

This study shows that smooth samples are better than the coarse surface sample for agarwood predicted by NIR spectroscopic tool for both 1mm and 2mm samples. Similarly, 2mm samples with both smooth and coarse surfaces get higher prediction rates than the 1mm thickness.

When the SIMCA class projections for 2mm coarse surface samples were observed, a clear separation was not seen (Fig. 6a). However, SIMCA Class projections for 2mm smooth surface samples, clear class separation can be observed with higher interclass distance resulting Auto scale data preprocessing (Fig. 6b). According to these results, it can be concluded that higher interclass distance or the better class separations can be seen for both for agarwood present and absent samples when the specimens having smooth wood surface and 2mm wood thickness.

When the influence of data pre-treatments were investigated, a consistent results to the above discussed prediction result were observed. As shown in the Table 4, the smooth surface samples were giving the highest statistical distance between the agarwood present and absent areas of the wood logs in terms of interclass distances. This was consistent for all the data pre-treatment options available in the Pirouette software algorithm for SIMCA discriminating models. However, for 1mm thickness, the interclass distances were higher than the 2mm thick wood samples for both coarse and smooth samples. This is again proving the concept that only the higher interclass distance will not ensure the higher perdition rate (Raghuraj & Samavedham 2007).

Assess the Influence of Wood Thickness on Agarwood Prediction

According to the investigation for the surface roughness, better prediction results were observed for 2mm wood thickness (98% overall). However, it was observed to become low with 1mm samples as low as 76% and 90% for coarse and smooth samples, respectively. These observations motivate to assess the influence of wood thickness on the prediction results and accordingly, the experiments were carried out and separate SIMCA models were prepared for 6 thickness

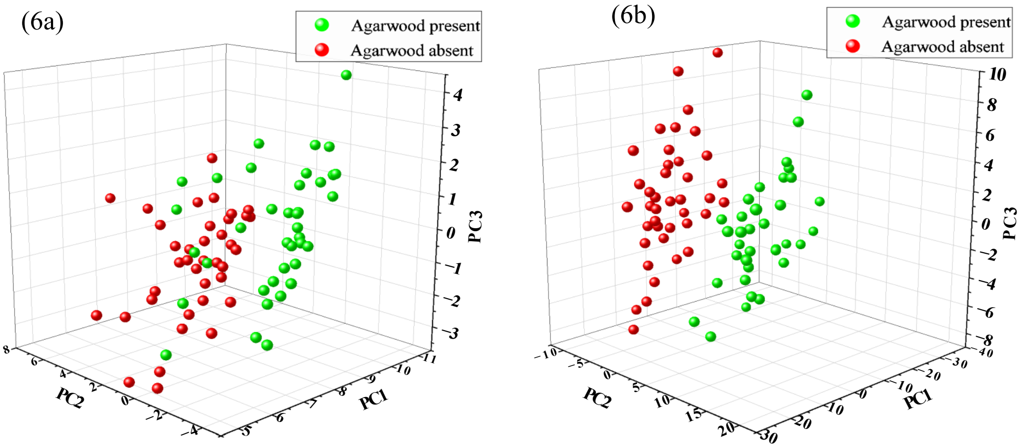

Figure 6: SIMCA class projections for 2mm (6a) coarse surface samples (6b) smooth samples

Table 4: Interclass distances for 1mm/2mm with smooth and coarse surface samples

Thickness	Surface status	Preprocessing method					
		None	Auto scale	Mean center	Range scale	Variance scale	Pareto
1mm	Coarse	8.9457	6.8495	7.9387	6.3578	8.1522	7.6779
	Smooth	10.9960	13.4757	12.2146	17.7779	13.8187	15.1784
2mm	Coarse	2.9302	3.0876	3.3223	3.6787	2.9670	3.3163
	Smooth	3.3854	3.9056	3.4822	3.8420	3.5812	3.8972

levels from 1mm up to 6mm. The results in the Table 5 shows that the overall lowest prediction rate was recorded for the 1mm and 6mm wood thickness samples as low as 92% overall and in between this range a gradual increment and then the decrement was happen. As shown both 2mm and 3mm wood thicknesses were giving equally better results.

The study recommends to detect the agar-resinous wood inside the *A. crassna* species maintaining with 2to 3 mm thicknesses however it is even 6mm thickness is also giving more than 90% of prediction rate which is a very good accuracy rate that could be used to avoid significant number of wood logs unnecessarily be processing for seeking agarwood inside. Moreover, the Near-Infrared radiation can only be penetrated a few millimeters beneath the surface of a sample before being diffusely reflected in wood (Schwanninger *et al*. 2011). So this NIRS is more suitable for detecting the agarwood formation that is located near the outer bark.

In this study, all the investigations were carried out based on the wood specimens obtained from commercial agarwood plantations that are being practiced artificial inoculation. There, they apply the drilling and inoculum-applying method (Chowdhury *et al.* 2016). There is a new method called Trunk Surface agarwood-Inducing Technique (Agar-Sit) as an alternative artificial inducing method. In this method, the agarwood resin formation is concentrated on the surface of the tree trunk (Chen *et al*. 2018). As such, this research would suggest to use such method for *A. crassna* trees that can be effectively used for the identification process.

Table 5: SIMCA prediction rates for different wood thicknesses

Wood thickness	Agarwood present			Agarwood absent			Overall
	Number of spectra	Correct classifica-tions	Predic-tion rate	Number of spectra	Correct classifica-tions	Predic-tion rate	
1mm	50	46	92%	50	46	92%	92%
2mm	50	50	100%	50	48	96%	98%
3mm	50	50	100%	50	48	96%	98%
4mm	50	48	96%	50	46	96%	96%
5mm	50	48	96%	50	46	92%	94%
6mm	50	46	92%	50	46	92%	92%

Table 6: Interclass distances for 1st derivative with 15 points

Preprocessing method	Interclass distances					
	1mm	2mm	3mm	4mm	5mm	6mm
None	28.548486	36.628613	48.825817	45.702915	45.903011	37.77581
Auto scale	13.253919	17.170635	23.648571	25.040705	25.714413	24.298838
Mean center	28.537571	36.298885	46.332706	43.452209	43.345062	35.951225
Range scale	25.760725	30.275227	36.071964	36.375469	36.574593	31.279947
Variance scale	13.611231	18.442251	26.391226	28.470581	29.327913	27.856339
Pareto	26.256252	31.715387	41.749928	38.687222	41.542408	37.097672

The Class Group Separability Under Different Wood Thickness

Similarly as discussed in the smooth and rough surface investigation, the class separability can be illustrated by the interclass distance. The results indicates that the interclass distance multi-plots in the SIMCA discriminate algorithm is consistent with the prediction results. As the best prediction rates were observed from 2mm and 3mm thickness the interclass distances were also have followed the same pattern (Table 5 and Fig. 7).

Important Wavelength for Agarwood Identification

The SIMCA model multi-plot allows the user to observe important wavelengths involving in the discriminating models for parameter classification in terms of discrimination power

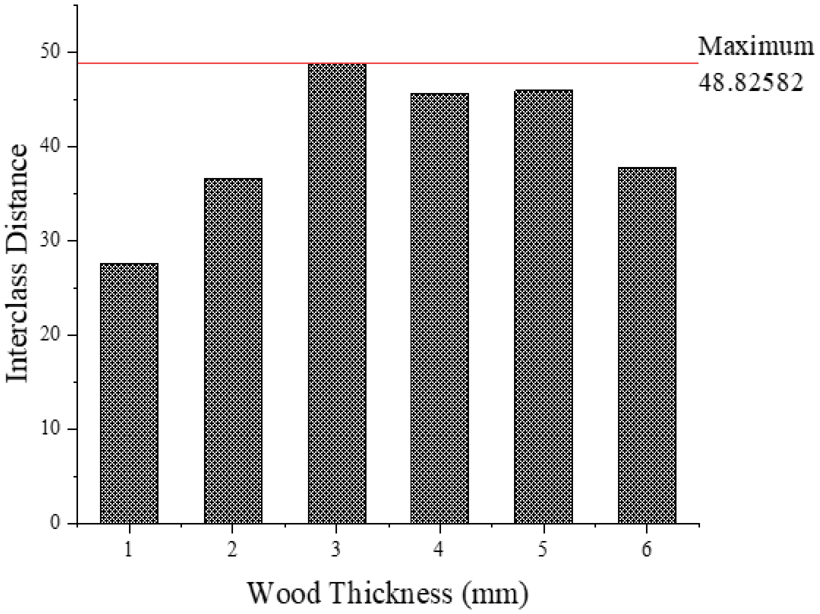

Figure 7: Effect of wood thickness on interclass distances (1st Derivative (15))

graph. The discriminating power plot obtained from the best model configurations i.e. 2mm wood thickness smooth surface and bark removed samples is shown in the Figure 7. According to the figure the wavelength having with higher discriminating power were 635nm, 718nm, 752 nm, 795nm, 844nm, 894nm, 975nm and 1012nm were having the higher discriminating power. As apeared in Fig.7, the most highly contributed wavelength is 975nm. The range of wavelength 970 to 976nm is a frequently sighted wavelength for plant canopy base spectroscopic modeling (Suzuki 2002). Penuelas *et al.* once in 1993 and again in 1998 observed appearing a clear response of plant at 975nm at plant canopy water stress.

The behaviour of water under various perturbed conditions has been extensively discussed in the recent history and has formed as a new discipline aquaphotomics (Tsenkova *et at.* 2009). Kuroki *et al.* (2019) have demonstrated that novel aquaphotomics spectral analysis, that dynamic regulation of water molecular structure during dehydration of plants. They observed drastic decrease of free water molecules, increase of water molecules with 4 hydrogen bonds, and a massive accumulation of water dimers in the full desiccation stage. They suggest that the changes in major metabolites changes the water structure which together constitute a robust defense system to tolarate the dehidration stree of the plants. As the 975 nm clear wavelength pleak we observed in this study is direlctely related to water and other subseequant peaks has alwo in varieng degree of relation to the behavious of water in various aquvious systems. The appeard other low peak wavelengths in the discriminating power profile and possible band assignment of the reference are given in the Table 6.

Jinendra *et al.* (2010), have consistently reported that the shifting of wavelength 976nm towards the shorter wavelength direction at plant water stress due to mosaic virus infection in soybean. In the particular study, it was reported that only the healthy plant spectra profile showed a clear peak at 976 nm and the virus-infected diseased plant

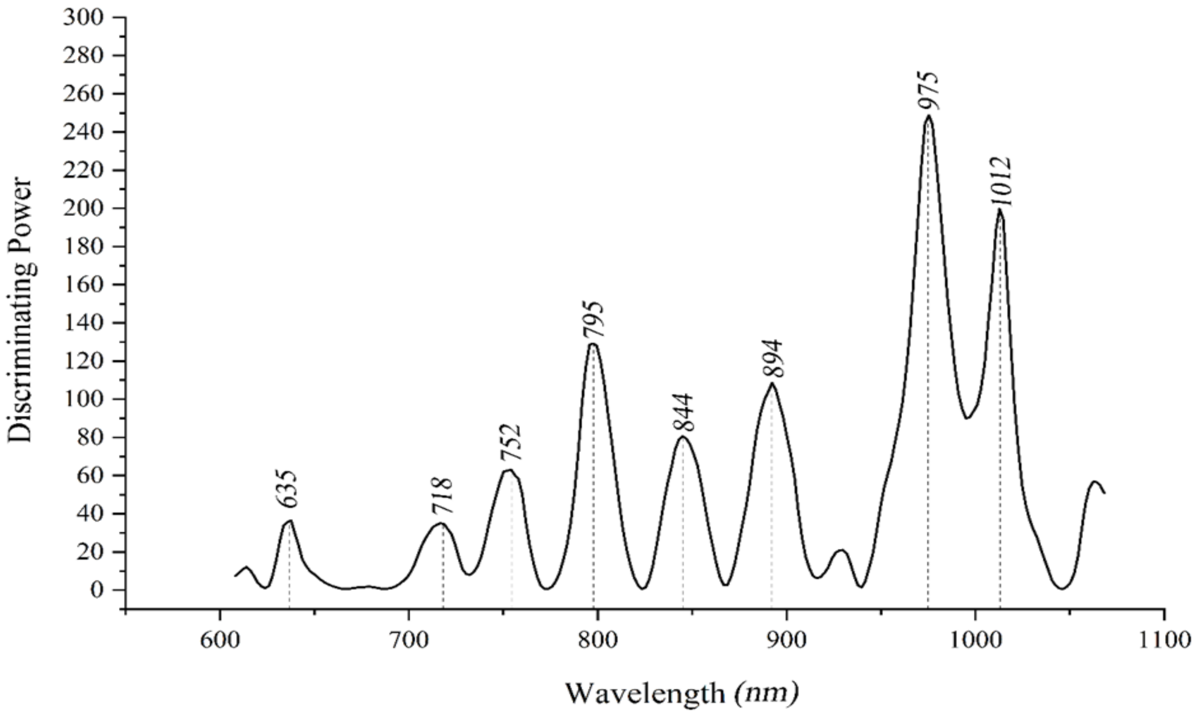

Figure 8: Important wavelength for agarwood identification

Table 7: Important wavelength and possible band assignment for agarwood identification

Wavelength	Potential band assignment	References
752 nm	NaOH-$Ti_3C_2T_x$	Yoon *et al.* 2023
795 nm	1st overtone H_2O(5%)– 2*v2+v3	Clayton, 1978
844 nm	Water (O_2-$(H_2O)_4$)	Tsenkova, 2009
894 nm	3rd overtone superoxide tetrahydrate O_2-$(H_2O)_3$	Weber *et al.* 2000
975 nm	Water S_2	Penuelas *et al.* 1993,1998
1012 nm	2nd overtone superoxide tetra hydrate O_2-$(H_2O)_4$	Weber *et al.* 2000

spectra had been shifted the spectral profile peak 6 nm to the short wavelength direction.

As Penuelas *et al*. (1993) previously reported, this blue shift can be attributed to a shortage of moisture due to the impact of the virus on the infected plants. Some other studies have described this shift in relation to the hydration potential of solutes in water (Buning-Pfaue 2003), which seems to indicate the existence of different hydrogen bonding activities of water molecules only appearing in the infected plants. The present study also consistently showed higher discriminating power at 976 nm possibly because only the agarwood fungal inoculated infected wood log sample spectra shifting towards the short wavelength direction and the healthy was not doing it and these differences allowed the assigned class to be separated in the model. These observations have clearly indicated that the wavelength observed in this study has strong consistency with the studies that are related to the investigation related to the class group separation for disease-infected and non-infected sample group identification.

CONCLUSION

The study has evaluated the three most important factors, i.e. the presence of tree bark, wood surface roughness and wood thickness affecting the accuracy of the Near Infrared Spectroscopic models to identify the agarwood present areas in the *A. crassna* trunks in rapid and non-destructive mode. From the investigations, it could be concluded that the better prediction results were reported from the bark-removed samples at the accuracy rates of (97%) to the bark present (85%) and smooth wood surfaces (98%) to the rough surface (90%) and 2mm thickness (98%) to the other thickness.

The SIMCA discriminating power graphics have revealed that the most effective wavelength for the agarwood present and absent sample classification is located at 978 nm wavelength which is consistently observed in a number of previous studies that are based on class separation targeted in disease-infected and non-infected class groups.

The research has also presented a simple new tool that can be used to acquire the NIR spectra without bark from *A. crassna* trunks in the in-situ mode.

Therefore, this research has demonstrated the potential possibility of using NIR spectroscopy for the identification of agarwood formation in *A. crassna* in non-destructive and rapid mode.

AUTHOR CONTRIBUTION

Herath HMWAI; Field NIR data acquisition, conducting experiments, data analysis and writing the original draft. Jinendra B.M.S. conceptualization, field NIR data acquisition, coordinating experiments and manuscript editing.

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