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Determination of α-guaiene and azulene chemical content in patchouli aromatic oil (*Pogostemon cablin* Benth.) from Indonesia by Near-infrared spectroscopy

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This study evaluated the α-guaiene and azulene chemical composition of patchouli aromatic oil from Indonesia by Near-infrared Spectroscopy (NIRS) combined with chemometric treatments. 84 oil samples collected around Indonesia were used, Konawe, Kolaka, Bogor, Garut, Aceh, Jambi, and Masamba. The spectral data were obtained using Fourier Transformed Near-infrared (FT-NIR) spectrometer in 1000 to 2500 nm. Several pretreatments processed the resulted transflectance spectra, and then partial least squares regression (PLSR) was applied. The data obtained from the spectra were evaluated based on the physical and chemical properties of the oil. α-guaiene and azulene chemical content were measured by liquid chromatography-mass spectrometry as a reference method. The best calibration for α-guaiene and azulene was second derivative with normalization mean centre, as data pretreatments. Both models had a high correlation coefficient (r>0.90), a low coefficient of variation (CV<2.98%), and an elevated ratio of standard error of prediction to standard deviation (RPD >3), which proved the applicability of NIRS to quality evaluation of patchouli oil.

Keywords: Azulene, FT-NIR, Non-destructive, Patchouli oil, *Pogostemon cablin*, α-guaiene.

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Introduction

Patchouli oil has a unique aroma and is indispensable in many fragrances used for the human body. It has been mentioned that this oil is one of the most valuable materials available for the perfume industry[1]. Approximately 90% of the current global production, around 1200 to 1300 metric tonnes per annum, is realized in Indonesia[1]. Patchouli oil is rich in sesquiterpenes such patchoulenes, guaiene, seychellene, and other few hydrocarbons that characterize its aroma[2]. There are other minor compounds like caryophyllene, pogostol, α-, β-, γ- and δ-patchoulene, cycloseychellene, α- and β-bulnesene, and norpatchoulenol, which also determine the aromatic properties of patchouli[3]. The chemical composition of patchouli oil varies among samples collected from different geographic locations[2,3]. Significant effects are generated by different habitats, collection periods, and processing methods on the volatile oil yield and its main constituents [2,3].

In previous research, a gas chromatographic mass spectrometry (GC/MS) study of Indonesian patchouli oil indicated the presence of the following compounds,α pinene, δ-patchoulene, β-pinene, aciphyllene, limonene, δ-guaiene, δ-elemene, 7-epi-α selinene, α-copaene, norpatchoulenol, α-patchoulene, 1,10-epoxy-11-bulnesene, β-elemene, caryophyllene oxide, cycloseychellene, nortetrapatchoulol, β-caryophyllene, patchouli alcohol, α-guaiene, patchoulenone, seychellene, 9-oxopatchoulol, α-humulene, pogostol, αpatchoulene, isopatchoulenone, γ-gurjunene, and germacrene[2,4]. According to this research, Indonesian patchouli oil has a vast reputation as the best quality produced to be used. Nevertheless, continually it suffers irregularities due to the sources of its raw materials, like leaves contaminated by weeds, or willful manipulation during the distillation process for the oil extraction due to lack of cleaning[3,5,6]. Therefore, these situations make necessary the study of quality assessment of oil production. Several destructive methods have been applied to determine the quality of patchouli oil, such as gas chromatographic (GC) [6,7,8]. The problem with these destructive methods is that they are time-consuming and complicated. Based on this current issue associated with patchouli oil, it is crucial to develop an appropriate tool to assess its quality.

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Although some non-destructive methods as Raman spectroscopy have been used to analyze the quality of several essential oils[6,9,10], due to the characteristics of its light dispersion analysis, the assessment of a result in a short time with high accuracy still is complicated. In this context, vibrational spectroscopic methods such as NIRS (Near Infrared Spectroscopy) and chemometric treatments can be successfully introduced as a rapid analysis in this field. Previous research evaluated the quality of patchouli oil from Indonesia to determine its origin and chemical composition based on its NIR spectra characteristics and the calibration of a mathematical model[6]. In these studies, GC results, as a retrieve method, exposed that the main chemical compounds of patchouli oil are patchouli alcohol, α- guaiene, azulene, and seychellene. Besides, another experiment demonstrated that the primary chemical compound responsible for the aromatics properties is patchouli alcohol[2]; however, secondary sesquiterpenes compounds suchα-guaiene and azulene also give an aroma characteristic proper of the oil[2]. Therefore, the importance of determining the composition of those as a quality parameter in patchouli oil.

This study aims to evaluate the quality of Indonesian patchouli oil by NIRS developing a calibration model to predict α-guaiene and azulene chemical compounds, as essential components that determine its main aromatic characteristics.

Materials and Methods

Oil collected and NIRS machine characteristics

Eighty-four oil samples (50 mL per sample) of patchouli were collected from seven places around Indonesia; Konawe, Kolaka, and Masamba from Sulawesi island, Bogor, and Garut from west java, and Aceh and Jambi from Sumatra[6]. The machine used was a FT-NIR SPECTROMETER (Fourier Transform Type) NIRFlex N-500, which provides reliable analytical results for quality control, research, development, chemical, food, drinks, and feeding, in the pharmaceutical industry. The NIRFlex N-500 offers various modules of sample measurement and accessories for ultimate performance[6].

Measuring and data processing of NIR spectra

Transflectance spectra were used and analyzed[6,11] being this an innovative procedure. The processed signal was in function of the transmittance and reflectance of the spectrum[6,11,12]. The oil was arranged in a small bottle of 3 cm in height with 1.5 cm in diameter. In there, the gun of the NIR machine was introduced to scan the oil three times at different deepness inside the bottle. The wavelength detected was set between 1000 and 2500 nm for the subsequence analysis.

Reference method of analysis

The α-guaiene ($C_{15}H_{24}$) and azulene ($C_{10}H_8$) chemical compounds were measured by liquid chromatography-mass spectrometry (LC/MS)[6,13,14]. The oil samples suffered a mixture process of separation in individual components where each component in a sample was identified (qualitatively) and measured (quantitatively). Results were organized according to the percentage of concentration of patchouli alcohol per sample[6].

NIR spectra treatment and calibration parameters

Partial Least Square Regression (PLSR) analysis was carried out with the resulting transflectance spectra of every sample based on its origin for a linear model correlation between the NIR uptake value and the chemical dataused[6]. Due to the spectra' physical and chemical characteristics, and because of the method elaborated in this experiment, the spectra were not transformed to absorbance for the respective calibrations; even though absorbance spectra graphics were evaluated to determine wavelength chemical concentrations of the components[6,15,16]. Before carried out the PLSR, the spectra were analyzed using data pretreatments such as a normalization mean centre, first and second derivatives (Polynomial order of 2, 3 smoothing points, left and right sight point of 1)[6,17]. Because of the liquid properties, there were no effects like uneven surface, scattering and particle size, and multicollinearity changes, which can cause large variations in the final spectrum[6].

The main statistical parameters used to test the performance of the built calibration model by PLSR were the correlation coefficient (r), root mean square error of prediction (RMSEP) (Equation 1), coefficient of variation (CV) (Equation 2), and the ratio of the standard error of prediction to standard deviation (RPD) (Equation 3). The mathematical expressions are as follow:

$$R_{MSE}=\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_{NIRS}-Y\right)^2} \qquad \ldots(1)$$

$$C_v = \left(\frac{R_{MSEP}}{\bar{Y}}\right)\times 100\% \qquad \ldots(2)$$

$$R_{PD} = \frac{S_{Dp}}{R_{MSEP}} \quad \text{...(3)}$$

where n is equal to the number of samples, Y_{NIRS} is the result of the prediction by NIRS, Y is the current valour of the property analyzed, $\overline{Y}$ is the average valour of the analyzed property, and S_{Dp} is equal to the standard deviation of the prediction.

Results

Gas chromatography results and analysis

Table 1 shows the valour of α-guaiene ($C_{15}H_{24}$) and azulene ($C_{10}H_8$) in the composition percentage. Values of α-guaiene and azulene among each place had a significant difference. Bogor and Masamba displayed the highest percentage in α-guaiene; also, Bogor's azulene got the best value regarding the constitution. α-guaiene in Jambi, and azulene in Jambi and Masamba, exposed the lowest values. It shows that the patchouli oil from Bogor has the best constitution for the secondary metabolites and that the product origin location could highly influence physical and chemical properties related to the oil aroma and colour.

Spectra and model calibration

The wavelength used for the model development was between 2220 to 1800 nm. It has been reported that this range highly represents the main chemical compounds of the patchouli oil, like patchouli alcohol, α, β-guaiene, azulene, α, β–patchoulene, seychellene[12]. There were other peaks in the wavelength of 1790-1660, 1660-1600, and 1440-1400 nm, where it was detected the existence of aromatic properties.

Fig. 1 shows the absorbance wavelength behaviour for α-guaiene and azulene. It is possible to find the oil's main chemical composition in the combination region of a typical electromagnetic wave[12,18]. The wavelength of 2200 nm is established as the group –CHO (C-H stretch/C==O, stretch combination), which is highly related to the chemical constitution of α-guaiene ($C_{15}H_{24}$) and azulene ($C_{10}H_8$). Generally, it is possible to observe in many oils their chemical characteristics from the wavelength of 2310 nm[12,18]. Nevertheless, because of the characteristic of the aromatic oil manipulated and after analyzing the spectra graphic behaviour, it was determined that the most significant variance and reference points to be examined should be between 2220 until 1800 nm for the chemical components studied in this experiment.

Table 1 — LC/MS results of α-guaiene ($C_{15}H_{24}$) and azulene ($C_{10}H_8$) concentration of each production site.
SD: Standard Deviation.

Samples	α-Guaiene (%)	SD	Azulene (%)	SD
Jambi	13.86	0.5	17.11	0.78
Bogor	19.28	0.43	22.705	0.01
Kolaka	18.16	0.24	21.73	0.93
Garut	14.36	0.33	19.07	0.56
Masamba	19.37	0.87	17.53	0.41
Konawe	17.77	0.08	20.24	0.32
Aceh	17.18	0.65	21.52	0.81

Table 2 exposes the results of the model's calibration by PLSR. The best calibration model in the case of α-guaiene was the one with the second derivative and normalization mean centre as a data pretreatment; this model applied five factors inside the calibration process. In the case of azulene, also the model with the second derivative and normalization mean centre pretreatment obtained the best calibration results; four factors were used during this calibration. Indeed, these results are profoundly related to the physical and chemical properties of the spectra exhibited in this research.

Finally, Fig. 2 and Fig. 3 shows the calibration model developed. In the case of the α-guaiene model (Fig. 2) were used 210 data spectra, the same number of data for the azulene model (Fig. 3). The CV of the model was the lowest possible to establish a better result in the function of the standard deviation offered by the reference method.

Discussion

Gas chromatography results and analysis

It has been determined that differences in oil chemical composition are influenced by the extraction method in the field and the seed's quality, which explains why some oils from the same area could have a different percentage of the same chemical component[6,19,20]. The side reaction between the carbon double bond and oxygen or the hydroxyl ion transforms the chemical species into another form to reduce its content. This change increases some other components with the same number of carbon atom[20,21].

When there is a long extraction time, higher patchouli alcohol content can be obtained. It was reported that the best quality of patchouli oil is detected in ten hours, where the most optimal chemical constitution can be observed during the

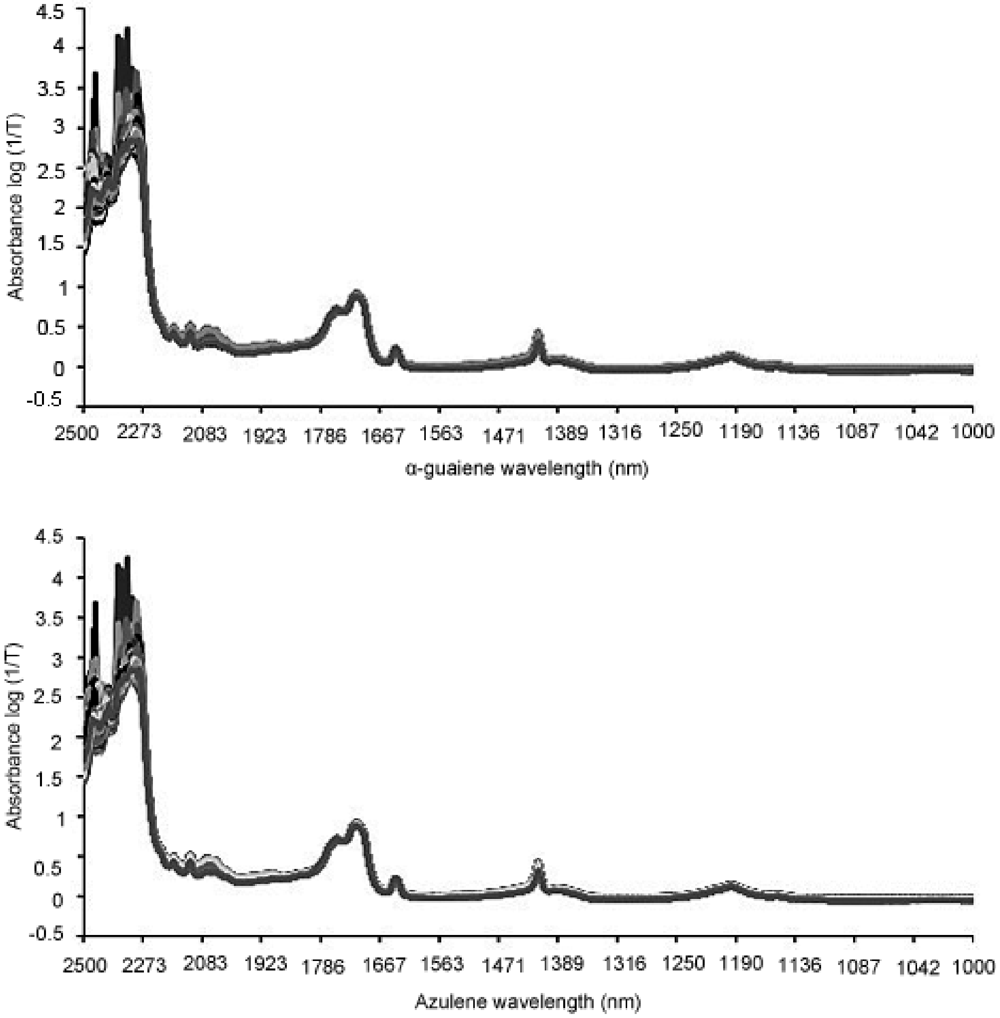

Fig. 1 — Absorbance spectra of patchouli oil respectively showing the behaviour of the wavelength between 2220 to 1800 nm.

Table 2 —The result of the calibration of the model by PLSR analysis for α-guaiene and azulene. D: 2nd derivative, N: Normalization mean centre, and Original: Transflectance spectra.

				α-guaiene ($C_{15}H_{24}$)			
Treatment	Factor	r	SEC (%)	SEP (%)	CV (%)	RPD	Consistency
original	4	0.97	0.47	0.63	2.76	4.06	73.82
D	4	0.97	0.47	0.53	2.78	4.14	89.84
N	5	0.98	0.42	0.50	2.46	4.69	83.22
D+N	5	0.98	0.43	0.48	2.50	4.60	88.97
				Azulene ($C_{10}H_8$)			
Treatment	Factor	r	SEC (%)	SEP (%)	CV (%)	RPD	Consistency
original	6	0.94	0.73	0.77	3.71	2.94	94.97
D	4	0.95	0.69	0.65	3.49	3.13	106.60
N	6	0.93	0.81	0.82	4.08	2.67	98.91
D+N	7	0.96	0.56	0.58	2.85	3.83	97.61

steam distillation[20,21]. Some samples could give a higher percentage than the others. Therefore, the extraction time and method placed a role in the oil constitution, impacting its properties physically and chemically. There were samples brighter than others during the experiment analysis; also, the aroma was related to the colour. It was determined that the darker the samples' colour, the more robust aroma it spread compared to the brighter ones. This situation indicated that samples from Garut and Masamba

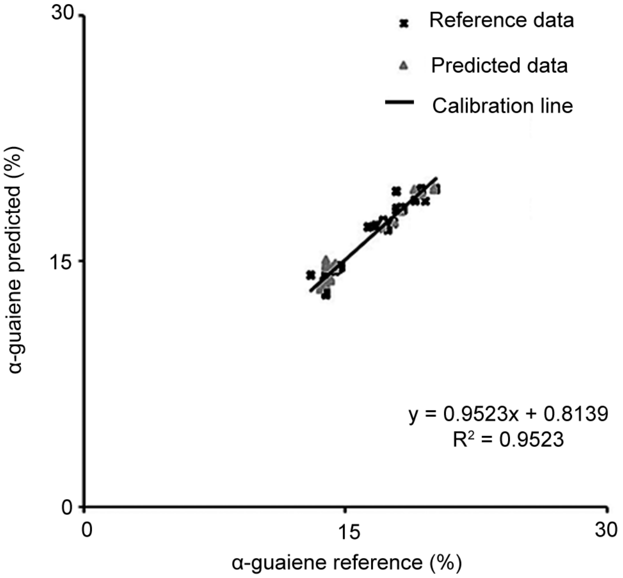

Fig. 2 — Plot of the concentration (%) of the reference against predicted α-guaiene data.

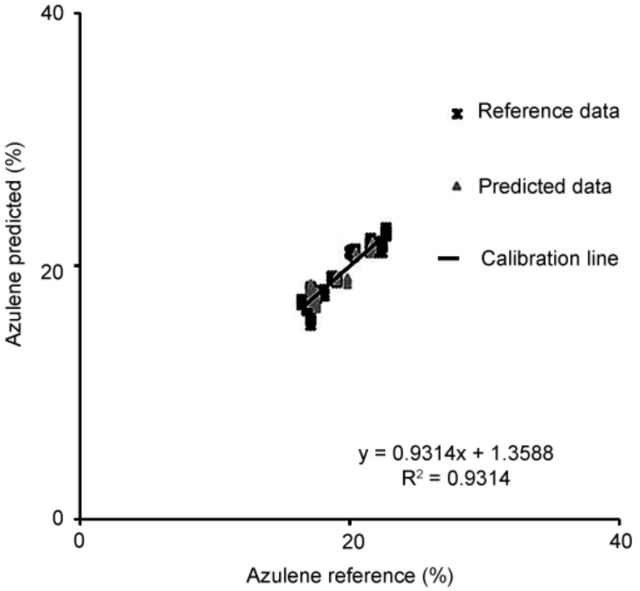

Fig. 3 — Plot of the concentration (%) of the reference against predicted azulene data.

could have an appropriate percentage combination of chemical compounds characteristic of patchouli oil like patchouli alcohol, α and β-guaiene, azulene, seychellene, creating this physical particularity[22].

Moreover, as the samples were directly received in the laboratory, the harvest plant period, time, and method of extraction were unknown; nevertheless, the previous information and outcomes suggest that those parameters differed from every oil production place, generating the results displayed in this study.

It has been described that the most important secondary metabolites present in patchouli are essential oil (up to 5%) and tanitos (1.7%)[23,24]. Besides, a comparison of the essential oil compositions was created between *Pogostemon cablin* and *Agatache rugosa*, founding that the main components of the patchouli essential oil were Seychellene, Bulnesene, α, β-guaiene, patchouli alcohol, β-longifolene, thujopsene, and β-patchoulene[25-27]. Moreover, research about the chemical composition of patchouli oil from Vietnam was realized; ten compounds, like β-bulnesene and α, β-guaiene were the common ones identified[24,28]. Furthermore, essential oil of patchouli plants collected from China (Gaoyao County, Guangdong Province) and their volatile chemical compositions were analyzed by GC/MS. The study revealed the presence of pogostone (30.99% in stems, 21.31% in leaves), patchouli alcohol (10.26% in stems, 37.53% in leaves), trans-caryophyllene (4.92% in stems, 6.75% in leaves), α-guaiene (2.27% in stems, 6.18% in leaves) and seychellene (1.56% in stems, 1.99% in leaves) as the main constituents[25,29,30]. Nevertheless, it is vital to notice that the percentage of composition of every chemical component that existed in patchouli oil concerning the industry is regulated by the producer country according to the law and national standards, which gives a characteristic proper to the oil extracted and application products of patchouli[6].

Spectra and model calibration

If an absorbance spectra graphic of an agricultural product is examined, it is possible to observe that every peak and valley that exists has a meaning and influence. The wavelength of 1680 and 2230 nm are reference values; water is located in 1940 nm, lignin is 2270 nm, while 2336 nm is associated with cellulose, 2180 nm to protein and carbohydrates are found in 2100 nm. For the oil, literature reported its main characteristics in the combination and overtone region (2310 nm to oil in general) of NIR spectrum[6,12,15,16].

Some pretreatments in the transflectance spectra data were carried out. The parameters for determining a correct calibration of the model improved after applying them. Thereafter, the calibration model was evaluated by PLSR statistical analysis. Furthermore, to emphasise more the previous method statements, the parameters used for calibration and validation were, among others r, standard error calibration (SEC), standard error perdition (SEP), CV, RPD, and consistency[26]. An optimal predicted model has an r close to 1, a low CV, consistency between 80 and 110%, and RMSEP, SEC and SEP value close to 0(ref. 19,31,32). Besides, a small difference between the SEC and SEP values generated an excellent model; otherwise, a significant difference indicates that the calibration set does not represent the validation set[27,28,31]. Also, an ideal prediction model has an RPD value greater than 2(ref. 19, 32, 33).

Derivate pretreatments are applied to resolve two fundamental problems in a NIR spectrum: the overlapping peaks, and the second is the large baseline variations[18,32,33]. Due to the graphics spectra results of this research, the second derivate in the model for both chemical components could decrease the baseline variations, permitting a better calibration based on the characteristics represented on the peak analyzed.

Furthermore, it has been determined that the water content of the samples makes an incidence in the spectra graphics results. Commonly, agricultural products have considerable water content, which creates almost similar spectral features in some cases. The O==H vibrations of water exhibit a large absorption in the NIR region and should be quickly and accurately quantifiable. The NIRS calibration error can increase as the ratios of pure water to bound or trapped water vary[12,18,34]. Derivative treatments can separate that shape behaviour and calibrate a model in function of the chemical composition studied. In conclusion, the oil samples studied had considerable water content, making the necessary application of derivate treatments to improve the goal model's accuracy.

On the other hand, the normalization spreads the gap between the wavelengths of every sample and corrects the baseline shape[12,18]. In the peak between 2220 to 1800 nm, there were some baseline spectra with a small gap; therefore, that baseline was spread, permitting a better calibration. Besides, this process allowed the study of chemical composition that those baselines could represent and how much percentage of the original spectra constituted in detail every one of the wavelengths analyzed[12,18,32]. In general, the chemical compounds of the spectra were similar; therefore, the need to evaluate those to have an exact representation of the composition.

Conclusion

The NIRS results exposed that by this technology was possible to determine the quality of the patchouli aromatic oil quantitatively. PLSR analysis allowed a calibration that determined the α-guaiene and azulene as essential chemical compounds of the patchouli oil. The best calibrations in α-guaiene and azulene model were the ones with second derivate and normalization mean center as pretreatments (α-guaiene: r= 0.98, SEC (%) = 0.43, SEP (%) = 0.48, CV (%) = 2.50, RPD = 4.60 and consistency (%) = 88.97; azulene: | r= 0.96, SEC (%) = 0.56, SEP (%) = 0.58, CV (%) = 2.85, RPD = 3.83 and consistency (%) = 97.61).

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Conflict of interest

The authors want to declare that they do not have any competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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