Estimation of main chemical content of nutmeg oleoresin by using near infrared spectroscopy

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Abstract. Determination of the main chemical content of nutmeg oleoresin is usually carried out by the GCMS method that time-consuming and expensive. It is required a faster and cheaper method for the determination of chemical contents of nutmeg oleoresin. The purpose of this study was to estimate the main chemical composition of Banda nutmeg oleoresin by NIR Spectroscopy. Transflectance of nutmeg oleoresin were measured by the NIR spectrometer. The samples were then subjected to composition analysis using GCMS. The transflectance spectra were processed using some data pretreatments, and the calibration between transflectance and chemical composition were carried out by using Partial Least Square (PLS) and Principle Component Regression (PCR) method. The best estimation for myristicin by NIRS was obtained using original spectra and 3 factors of PLS ( r=0.95, SEC=1.56%, SEP=1.71%, CV=10.18%, RPD = 2.60, and consistency of 91%). The best estimation by NIRS for myristic acid was also obtained using original spectra and 5 factors of PLS ( r=0.95, SEC=3.94%, SEP=3.82%, CV=32.47%, RPD = 2.75, and consistency of 103%). Whereas for safrole was obtained by using data pre-treatment of de-trending and 7 factors of PLS ( r = 0.98, SEC=0.08%, SEP = 0.10%, CV =10.87%, RPD = 3.82, and consistency of 82%).

1. Introduction

Nutmeg oleoresin is an extractive form of nutmeg in which the main components of flavouring are contained in the form of volatile substances (essential oils) and non-volatiles (resin and gum), each of which plays a role in determining the aroma and taste. The nutmeg oleoresin has been widely used in various fields of food as a flavouring agent as well as in health and beauty fields such as aromatherapy, perfume, toothpaste, soap, and traditional medicine [1]. The use of nutmeg oleoresin has several advantages compared to the use of whole nutmeg seeds, which are easier to use, have the aroma and taste like the original fruit, are more hygienic, free from microbial contamination, and has a longer shelf life. The quality of nutmeg oleoresin is determined by the main chemical components such as myristicin, myristic acid, and safrole.

Commonly, the chemical contents of nutmeg oleoresin are determined based on the chemical method using GC-MS (Gas Chromatography-Mass Spectroscopy). Testing using the GC-MS method requires quite a long time and expensive. This problem causes the quality assurance process in the essential oil and oleoresin oil trading industry to become inefficient. Therefore, the faster and cheaper measurement methods are needed, one of which is the method of near infrared spectroscopy (NIRS). NIR spectroscopy has been successful in determining chemical content in patchouli oil [2], caffeine content [3], classification, and analysis of citrus oil [4], classification of patchouli oil [5], and classification of arabica coffee based on their origin [6]. The purpose of this study was to estimate the three main chemical compositions of Banda nutmeg oleoresin by NIR Spectroscopy.
2. Material and Method

2.1. Material and Apparatus
The material used in this research was Banda nutmeg oleoresin which is produced from the extraction process of seeds and mace. Twenty samples of oleoresin were prepared for NIR measurement and chemical content analysis. The instrument used to measure the transfectance of nutmeg oleoresin was a unit of NIRFlex N-500 type NIR spectrometer (Buchi, Switzerland) that was connected to a computer, and petri dish with 10 mm diameter for sample container of oleoresin. The tools used to determine the chemical content of nutmeg oleoresin include Shimadzu GC-6890, HP-5MS capillary column (0.25 mm x 30 m x 0.25 μm film thickness), injectors, ovens, and desiccators.

2.2. Method

2.2.1. Measurement of transfectance of oleoresin. Oleoresin of 20 ml was placed on a petri dish. Transfectance of nutmeg oleoresin were measured by the NIR instrument of NIRFlex500 at wavelengths of 1000–2500 nm. Each sample was measured three times, so a total of 60 transfectance spectra were obtained.

2.2.2. Measurement of chemical contents of oleoresin. Right after transfectance measurement, the chemical analysis using GCMS is carried out to determine the chemical contents of nutmeg oleoresin. Instrument control parameters used in the GC-MS test are as follows; GC-MS analysis of essential oils performed with a GC 6890 type tool that uses an HP- capillary column 5MS (0.25 mm x 30 m x 0.25 μm film thickness). The oven is set at 100 °C up to a maximum temperature of 350 °C for 25 minutes. Front inlet used is split mode with a split speed of 194.5 mL/min and a split ratio of 200: 1. The temperature of the injector used is 250 °C. Helium gas flows with pressure admission of 10.17 psi with a flow rate of 1.0 mL/min. The type of pressure at the outlet used is the vacuum type. After obtaining possible compounds (based on the NIST Library), then each compound is grouped based on the similarity value of each compound in the chromatogram. The compounds identified in this study have a quality of more than or equal to 70% of the similarity. If there is a compound that has a quality value below 70%, but which often appears in nutmeg oleoresin, the compound will be identified. The amount of compound is expressed as a percent (%) of the area to the total area of chromatogram. This data can be used as a reference to find out the dominant volatile component in the sample and can represent the amounts of volatile compounds in oleoresin.

2.3. Spectral pretreatment, calibration, and validation
The transfectance spectra were firstly transformed to absorbance values (log 1/R) and then processed using six spectral pretreatments such as normalization, the first derivative of Savitzky-Golay (dg1, five data points), the second derivative of Savitzky-Golay (dg2, five data points), standard normal variate (SNV), de-trending, and multiple scatter correction (MSC). The calibration and validation between these processed NIR spectra and chemical composition were carried out by using Partial Least Square (PLS) and Principle Component Regression (PCR) method. The accuracy of prediction of chemical contents of nutmeg oleoresin is determined by the correlation coefficient (r>0.9), low standard error of prediction, low coefficient of variation (CV), high RPD (>1.5) and in the range from 80% to 110% of consistency [7][8].

3. Results and Discussion

Table 1 shows the results of calibration and validation using original and six spectral pretreatments and the PLS calibration method for the prediction of myristicin. The best prediction is obtained by the original spectra (log 1/R), 3 factors of PLS with r of 0.95, SEC of 1.56%, CV of 10.18%, RPD of 2.60 and consistency of 91%. The derivative spectral pretreatment could not improve the accuracy of prediction since there is no spectral overlapping. Variation in spectra may be caused by unnormalized spectra values, so the normalization and SNV data pretreatment combined with PLS calibration gives the acceptable accuracy of prediction of myristicin. Other spectral pretreatments and PLS calibration could not be used to predict myristicin accurately since r<0.9 or consistency <80%.
Table 1. Results of calibration and validation using PLS for myristicin

| Pre-treatment | Factor/Point | Calibration (n=39) | Validation (n=21) | Consistency (%) |
|---------------|--------------|--------------------|-------------------|-----------------|
|               |              | R²     | r | SEC (%) | SEP (%) | CV (%) | RPD  |                |
| Original      | 3            | 0.91   | 0.95 | 1.56    | 1.71    | 10.18  | 2.60  | 91.23          |
| Normalization | 4            | 0.80   | 0.90 | 2.18    | 2.40    | 14.35  | 1.68  | 90.77          |
| 1st Derivative| 2/5          | 0.78   | 0.89 | 2.37    | 13.19   | 78.73  | 0.31  | 17.95          |
| 2nd Derivative| 3/5          | 0.73   | 0.85 | 2.68    | 3.13    | 18.66  | 1.29  | 85.83          |
| SNV           | 3            | 0.86   | 0.93 | 1.96    | 2.37    | 13.35  | 1.81  | 87.45          |
| De-trending   | 6            | 0.89   | 0.94 | 1.57    | 2.04    | 11.93  | 1.95  | 76.95          |
| MSC           | 6            | 0.85   | 0.92 | 1.86    | 2.47    | 14.54  | 1.60  | 75.29          |

Similar results are also shown for myristic acid (table 2). The original spectra combined with PLS calibration also give the best prediction of myristic acid with r of 0.95, SEC of 3.94%, CV of 32.47%, RPD of 2.75, and consistency of 103%. The spectral pretreatments and PLS could not be used to predict myristic acid accurately since r<0.9 or consistency <80%. All spectral pretreatments and PLS deteriorated the prediction performance of myristic acid. Results showed that all treatments would enhance the noise or lose the information for the quantitative estimation of myristic acid. More data might be needed to increase the accuracy of the prediction of myristic acid.

Table 2. Results of calibration and validation using PLS for myristic acid

| Pre-treatment | Factor/Point | Calibration (n=39) | Validation (n=21) | Consistency (%) |
|---------------|--------------|--------------------|-------------------|-----------------|
|               |              | R²     | r | SEC (%) | SEP (%) | CV (%) | RPD  |                |
| Original      | 2            | 0.90   | 0.95 | 3.94    | 3.82    | 32.47  | 2.75  | 103.14         |
| Normalization | 5            | 0.83   | 0.91 | 5.24    | 8.17    | 69.41  | 1.29  | 64.15          |
| 1st Derivative| 1/5          | 0.69   | 0.83 | 6.93    | 6.97    | 59.17  | 1.51  | 99.42          |
| 2nd Derivative| 2/5          | 0.53   | 0.73 | 8.60    | 17.56   | 149.14 | 0.60  | 48.98          |
| SNV           | 4            | 0.80   | 0.89 | 5.61    | 10.73   | 91.13  | 0.98  | 52.29          |
| De-trending   | 4            | 0.92   | 0.96 | 3.56    | 7.43    | 63.14  | 1.41  | 47.83          |
| MSC           | 1            | 0.68   | 0.82 | 7.14    | 9.16    | 77.76  | 1.15  | 77.95          |

For safrole, the best and accurate prediction is obtained by de-trending spectral pretreatment combined with 7 factors of PLS with r of 0.98, SEC of 0.08%, CV of 10.87%, RPD of 3.82 and consistency of 82% (table 3). De-trending spectral pretreatment can improve the accuracy of prediction for safrole since it can eliminate spectral trends. The original spectra and PLS calibration also give an excellent prediction for safrole (r of 0.98, SEC of 0.10%, CV of 11.54%, RPD of 3.60, and consistency of 92%). Normalization gives a good prediction for safrole. Derivative, SNV, and MSC spectral pretreatments could not improve to acceptable accuracy since r<0.9 and consistency <80%.
Table 3. Results of calibration and validation using PLS for safrole

| Pretreatment       | Factor/point | Calibration (n=39) | Validation (n=21) | consistency (%) |
|-------------------|--------------|-------------------|-------------------|-----------------|
|                   |              | $R^2$  | r     | SEC (%) | SEP (%) | CV (%) | RPD   |              |
| Original          | 4            | 0.95  | 0.98  | 0.10   | 0.11   | 11.54  | 3.60  | 91.87        |
| Normalization     | 4            | 0.86  | 0.93  | 0.17   | 0.21   | 22.53  | 1.84  | 83.51        |
| 1st Derivative    | 1/5          | 0.71  | 0.84  | 0.23   | 0.51   | 56.54  | 0.73  | 47.31        |
| 2nd Derivative    | 3/5          | 0.86  | 0.93  | 0.17   | 0.22   | 24.28  | 1.71  | 75.43        |
| SNV               | 2            | 0.80  | 0.89  | 0.20   | 0.28   | 30.35  | 1.37  | 73.39        |
| De-trending       | 7            | 0.97  | 0.98  | 0.08   | 0.10   | 10.87  | 3.82  | 81.80        |
| MSC               | 3            | 0.95  | 0.98  | 0.10   | 0.11   | 11.54  | 3.60  | 56.06        |

Table 4 shows the result of calibration and validation using PCR. Similar to PLS results, the original spectra give the best prediction for myristicin ($r$ of 0.95, SEC of 1.54%, CV of 10.73 %, RPD of 2.47, and consistency of 86%). Normalization and SNV with PCR calibration can also be used to predict myristicin but with lower accuracy than original spectra. Other data pretreatments gave no satisfactory prediction of myristicin using PCR calibration.

A similar result is also shown for myristic acid (table 5). The original spectra and PCR calibration give the best prediction for myristic acid ($r$ of 0.95, SEC of 3.78%, CV of 32.17 %, RPD of 2.73, and consistency of 108%). Normalization data pretreatment combined with PCR calibration may be used to predict myristic acid, but with lower accuracy. Other data pretreatments gave no convincing prediction of myristic acid using PCR calibration.

For safrole, the best prediction is obtained by de-trending data pretreatment and PCR calibration with high accuracy ($r$ of 0.98, SEC of 0.09%, CV of 11.92 %, RPD of 3.48 and consistency of 82%) (table 6). The original spectra and PCR calibration also gives the excellent prediction for safrole.

Figure 1-3 shows the best prediction for myristicin, myristic acid, and safrole using PLS, respectively. For myristicin and myristic acid, the best prediction using original spectra, as for safrole, the best prediction is obtained using de-trending data pretreatment. The high SEC and CV may be reduced by the increment of the number of samples.

Figure 4-6 show the best prediction for myristicin, myristic acid, and safrole using PCR respectively. Similar to PLS results, the best prediction for myristicin and myristic acid are obtained by original spectra, as for safrole is obtained by de-trending data pretreatment. Overall, the accuracy of the prediction of three main components of nutmeg oleoresin using PCR is lower than PLS.

Table 4. Results of calibration and validation using PCR for myristicin

| Pre-treatment  | Factor/point | Calibration (n=39) | Validation (n=21) | Consistency (%) |
|----------------|--------------|-------------------|-------------------|-----------------|
|                |              | $R^2$  | r     | SEC (%) | SEP (%) | CV (%) | RPD   | (%)  |
| Original       | 6            | 0.91  | 0.95  | 1.54   | 1.80   | 10.73  | 2.47  | 85.90|
| Normalization  | 6            | 0.79  | 0.89  | 2.26   | 2.54   | 14.92  | 1.56  | 88.96|
| 1st Derivative | 6/5          | 0.60  | 0.77  | 2.73   | 4.88   | 28.76  | 0.81  | 55.83|
| 2nd Derivative | 4/5          | 0.28  | 0.53  | 3.51   | 12.31  | 72.43  | 0.32  | 28.49|
| SNV            | 2            | 0.73  | 0.85  | 1.95   | 2.09   | 12.29  | 1.77  | 93.25|
| De-trending    | 7            | 0.88  | 0.94  | 1.63   | 2.18   | 12.80  | 1.82  | 74.86|
| MSC            | 6            | 0.85  | 0.92  | 1.86   | 2.47   | 14.54  | 1.60  | 75.29|
Table 5. Results of calibration and validation using PCR for myristic acid

| Pre-treatment       | Factor/ point | Calibration (n=39) | Validation (n=21) | Consistency (%) |
|---------------------|---------------|--------------------|-------------------|-----------------|
|                     |               | R²     | r      | SEC (%) | SEP (%) | CV (%) | RPD |               |
| Original            | 4             | 0.91   | 0.95   | 3.78    | 3.51    | 32.17  | 2.73 | 107.69        |
| Normalization       | 4             | 0.74   | 0.86   | 6.42    | 6.79    | 57.66  | 1.55 | 94.61         |
| 1st Derivative      | 1/5           | 0.53   | 0.73   | 8.55    | 9.97    | 84.64  | 1.05 | 85.80         |
| 2nd Derivative      | 1/5           | 0.20   | 0.44   | 11.22   | 28.28   | 240.14 | 0.37 | 39.68         |
| SNV                 | 5             | 0.78   | 0.88   | 5.88    | 9.42    | 79.96  | 1.12 | 62.43         |
| De-trending         | 7             | 0.93   | 0.97   | 3.23    | 5.08    | 43.16  | 2.07 | 63.60         |
| MSC                 | 4             | 0.72   | 0.85   | 6.66    | 9.87    | 83.84  | 1.06 | 67.45         |

Table 6. Results of calibration and validation using PCR for safrole

| Pre-treatment       | Factor/ point | Calibration (n=39) | Validation (n=21) | Consistency (%) |
|---------------------|---------------|--------------------|-------------------|-----------------|
|                     |               | R²     | r      | SEC (%) | SEP (%) | CV (%) | RPD |               |
| Original            | 5             | 0.95   | 0.97   | 0.10    | 0.11    | 12.11  | 3.43 | 92.42         |
| Normalization       | 6             | 0.84   | 0.92   | 0.18    | 0.29    | 31.44  | 1.32 | 62.80         |
| 1st Derivative      | 3/5           | 0.81   | 0.90   | 0.19    | 0.50    | 54.89  | 0.76 | 38.83         |
| 2nd Derivative      | 4/5           | 0.75   | 0.87   | 0.22    | 0.90    | 98.54  | 0.42 | 25.00         |
| SNV                 | 2             | 0.77   | 0.88   | 0.22    | 0.26    | 28.36  | 1.46 | 83.24         |
| De-trending         | 8             | 0.96   | 0.98   | 0.09    | 0.11    | 11.92  | 3.48 | 82.17         |
| MSC                 | 6             | 0.85   | 0.92   | 0.17    | 0.33    | 36.12  | 1.15 | 52.56         |

Figure 1. Plot of reference myristicin vs. myristicin predicted by NIR using PLS

Figure 2. Plot of reference myristic acid vs. myristic acid predicted by NIR using PLS
Figure 3. Plot of reference Safrole vs. predicted safrole by NIR using PLS

Figure 4. Plot of reference myristicin vs. predicted myristicin by NIR using PCR

Figure 5. Plot of reference myristic acid vs. predicted myristic acid by NIR using PCR

Figure 6. Plot of Safrole vs. predicted safrole by NIR using PCR

4. Conclusion
The best estimation for myristicin by NIRS was obtained using original spectra and 3 factors of PLS (\(r=0.95, \text{ SEC}=1.56\%, \text{ SEP}=1.71\%, \text{ CV}=10.18\%, \text{ RPD} = 2.60\), and consistency of 90%). The best estimation by NIRS for myristic acid was also obtained using original spectra and 5 factors of PLS (\(r=0.95, \text{ SEC}=3.94\%, \text{ SEP}=3.82\%, \text{ CV}=32.47\%, \text{ RPD} = 2.75\), and consistency of 103%). Whereas for safrole was obtained by using spectral pretreatment of detrending and 7 factors of PLS (\(r = 0.98, \text{ SEC}=0.08\%, \text{ SEP} = 0.10\%, \text{ CV} = 13.8\%, \text{ RPD} = 3.48\), and consistency of 82%). The model performance provided by the PCR calibration method was inferior to that build from the PLS method. NIR spectroscopy can be used to estimate three main chemical contents of nutmeg oleoresin.
5. References

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