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**“Improving The Role of Agricultural and Biosystem Engineering
Toward Food & Energy Self-sufficiency and Sustainable Agriculture”**

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E-mail : tep_ftp@ugm.ac.id

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Non Destructive Measurement of Catechin Content in Gambir (*Uncaria gambir Roxb.*) Using NIR Spectroscopy

Andasuryani¹ Y.A. Purwanto² I W. Budiastira² K. Syamsu³ and Lady C.E.Ch.Lengkey¹

¹PhD student at Department of Agricultural Engineering, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia, Email: andasuryani@gmail.com.

²Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia.

³Department of Agro industrial Technology, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, Bogor, Indonesia.

Abstract

Gambir is one of Indonesian export commodities. Catechin content is main determinant of gambir quality. In traditional market, determining of the gambir catechin content is conducted qualitatively based on experience of the assessor which led to subjective measurement. Chemical method to determine catechin content of gambir quantitatively. This method is not efficient since it requires expensive chemical reagents, takes a long time, and destructive. Near infrared (NIR) spectroscopy is one of the non destructive techniques which can inform gambir quality. The objective of this study was to demonstrate of NIR spectroscopy to measure of catechin content in gambir. Partial Least Square (PLS) method by combination pre-treatment between normalization between 0 and 1 (n01), and first derivative Savitzky-Golay 9 points (dgl) was used to develop calibration model. Value of consistency and V-Set PRESS was used to determine the optimum number of PLS factors. The result showed that calibration model with 6 PLS factors was the best predictive models for catechin content since it provided a high accuracy as well as precise models. Model for catechin content showed the bias value = 0.10 %, SEC = 3.56 %, SEP = 3.27 %, correlation coefficient (r) = 0.95, CV = 4.86 %, and RPD = 3.60. The result demonstrated that NIR spectroscopy might be applied to measure catechin content in gambir accurately.

Keywords: gambir, catechin, NIR spectroscopy, non destructive measurement, PLS.

Introduction

Gambir is an extracted product from the leaves and young twigs of gambir (*Uncaria gambier Roxb.*) plant. Gambir is one of the Indonesian export commodities. It contributes to around 80% of the gambir trading in the world (Gumbira-Sa'id, 2009). Some studies related the presence of catechin in gambir revealed that it is usually the most abundant bioactive compound (Taniguchi et al., 2007a; Apea-Bah et al., 2009; Anggraini et al., 2011). It is potential as raw material in various industries, particularly pharmaceutical and cosmetic industries. Catechin content in gambir is used as one of the quality parameters of gambir in accordance with the Indonesia gambir trading standard, SNI 01-3391-2000. Traditionally, determining of the gambir catechin content is conducted qualitatively based on experience of the assessor which led to subjective measurement. Meanwhile, there is chemical method to determine catechin content of gambir quantitatively. However, this method is not efficient since it requires expensive chemical reagents and takes a long time. In addition, this method

is destructive. Therefore, an efficient and non-destructive method is needed to determine gambir catechin content.

In recent years, Near Infra Red (NIR) spectroscopy is one of non-destructive techniques for measuring quality parameters of different commodities. NIR spectroscopy is particularly sensitive to the presence of molecules containing the C-H, O-H, and N-H groups (Abu-Khalaf, 2002). NIR spectroscopy has decisive advantages compared to traditional methods, whereby it analyses sample rapidly (a few seconds per sample) and no sample preparation (Guggenbitchler et al., 2006; Pissard et al., 2012; Saleh, 2012). In addition, it is a chemical-free (limited to the reagents required for reference analyses) and no waste is produced (Yan et al., 2009; Pissard et al., 2012) and can be carried out on-line (Saleh, 2012) and can be applied quickly, accurately and non-destructive which has grown rapidly and can replace the old chemical analysis (Chen *et al.*, 2009). NIR spectroscopy has been applied to determine bioactive compounds such as carotenoids, glucosinolates, phenolics, fatty acids (McGovern et al., 2010). The objective of this study was to demonstrate of NIR spectroscopy to measure of catechin content in gambir.

Materials and methods

Sample Preparation

The 162 raw gambir used in this study were randomly divided into two groups of samples: the first group was used to develop the calibration models (108 samples) and the other for predicting quality and model validation purposes (54 samples). The samples were obtained from Siguntur village, Koto 11 Tarusan subdistrict, Pesisir Selatan District, West Sumatera, Indonesia.

Methods

Chemical Analysis

Determining catechin content of gambir was conducted by referring to Indonesia National standard namely was SNI 01-3391-2000. Instrument of Spectrophotometer U-2010, Hitachi was used to measure gambir catechin content.

Spectra acquisition

This study used instrument Buchi NIRFlex N-500 Fiber optics solids which had resolution of 4 cm^{-1} and 8 scans, to collect sample spectra. The instrument was operated using software NIRWare 1.2 (Büchi Labortechnik AG, Flawil, Switzerland). Spectra data was collected from $10000\text{ to }4000\text{ cm}^{-1}$ ($1000\text{ to }2500\text{ nm}$) with interval of 4 cm^{-1} . Measurements of each sample were conducted three times at different positions in a room temperature of $25\text{ }^{\circ}\text{C}$. Spectrum which was produced by each samples, was averaged prior to build a calibration model.

Chemometrics

Chemometrics analysis in this study used NIRCals 5.2 (Büchi Labortechnik AG, Flawil, Switzerland) by using Partial Least Square (PLS) algorithm. This study did not only use the original spectra (original), but also combination of normalization between 0 and 1 (n01) with first derivative Savitzky-Golay 9 points (dg1). Selection the number of optimum PLS factors was done based on value of consistency (80-110%) and as smallest as

possible value of PredictedResidualSumSquareError(PRESS) of thevalidationset(V-set-PRESS). The number ofPLSfactorswas 1to 15.

Data analysis

Statisticalparametersusedtoevaluatethe resulted model were bias, standard errorofcalibrationset (SEC), standard errorofvalidationset (SEP), coefficient correlation (r),coefficientofvariation(CV) and ratio of prediction to deviation (RPD).

$$\text{Bias (\%)} = \frac{1}{N} \sum (x_n - y_n)$$

$$\text{SEC (\%)} = \sqrt{\frac{1}{N-1} \sum (x_n - y_n)^2}$$

$$\text{SEP (\%)} = \sqrt{\frac{1}{N-1} \sum (x_n - y_n - \text{Bias})^2}$$

$$r = \frac{\sum (x_n - \bar{x}_n)(y_n - \bar{y}_n)}{\sqrt{\sum (x_n - \bar{x}_n)^2 \sum (y_n - \bar{y}_n)^2}}$$

$$\text{CV (\%)} = \frac{\text{SEP}}{\bar{x}} \times 100$$

$$\text{RPD} = \frac{\text{SD}}{\text{SEP}}$$

$$\text{PRESS} = \sum (x_n - y_n)^2$$

$$\text{Consistency (\%)} = \frac{\text{SEC}}{\text{SEP}} \times 100$$

WhereNisthe number of samples; x_n is value of reference catechin; y_n isthe value ofNIRprediction catechin.

Results and discussion

Data of Gambir Catechin and Spectra

Table1 shows the statistics summary for all samples catechin content in the calibration set and validation set. Standard deviation between the calibration set and validation set showed insignificant differences. Therefore, the variance of data in the set calibration and validation set are equal.

Table 1. Statistical of value of gambir catechincontent (% w/w)

Sample Set	Number of sample	Minimum	Maximum	Average	Standard deviation
Calibration Set	108	40.71	84.79	65.65	11.88
Validation Set	54	41.54	83.91	67.21	11.76

The original reflectance spectra of some gambir samples reveal some valleys in the region of 1000-2500 nm (Figure 1). These valleys exist because molecule structures of catechin contain has many hydric groups. However, as NIR spectra in the region of 2222.22

- 2500 nm showed high noise, it was eliminated for analysis. Therefore spectra in the region 1000 - 2222.22 nm were used to develop the model.

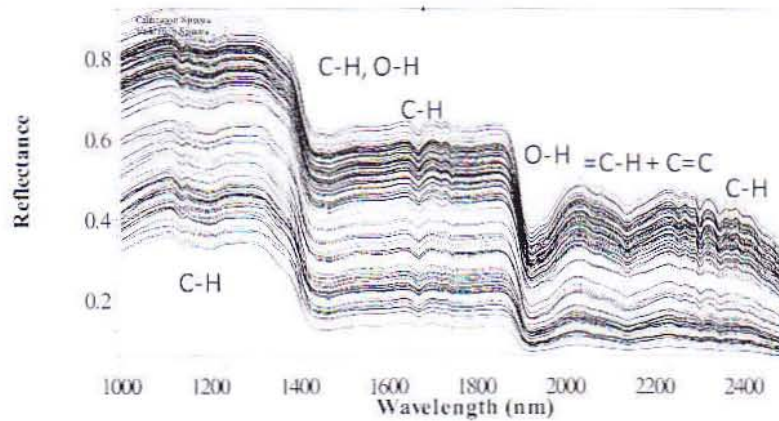


Figure 1. The original reflectance spectra of some gambir samples

The influence of different pre-processing methods and number of factors to accuracy of PLS Model

PLS is an effective dimension reduction method in near-infrared spectroscopy analysis. The spectrum information of sample components are showed the latent variables. Furthermore, the selection of number of PLS factors was very essential to reduce noise and use of spectral information fully (Chen et al., 2013). Figure 2 shows influence of the number of PLS factors to consistency and V-set PRESS value. If spectra pre-processing treatment was not applied, a minimum of validation error was observed in the 15 PLS factors. It provided consistency value 98.69 % and V-set PRESS value 948.51. Optimal number of factor obtained while using n01 in combination with dg1 pre-treatment method was 6. This number of factor provided consistency 108.96 % value and V-set PRESS value 566.226. It was found that if spectra pre-processing method was applied, the number of PLS-factors could be reduced. It could be explained that spectra pre-processing method of the derivative could overcome the overlapping spectra and throw other components except catechin which left factors that inform about catechin only.

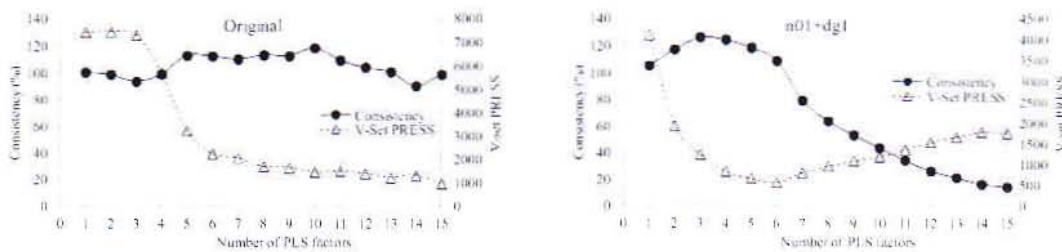


Figure 2. The influence of the number of PLS factors to consistency and V-Set-PRESS

Performance of parameters of the model using different pre-processing methods to predict catechin content of gambir are given in Table 2. Spectra pre-processing method of n01 in combination with dg1 increased correlation coefficient between catechin references and predicted from 0.94 to 0.95 whilst SEP value decreased from 4.14 % to 3.27 % and number

of PLS factors decreased from 15 to 6. It could be concluded that spectra pre-processing method and number of factor had effects on the prediction performance of model PLS.

Table 2. The result of calibration and validation of gambir catechin content

Pre-processing	PLS Factor	Consistency (%)	Calibration Set		Validation Set				
			SEC (%)	r	SEP (%)	r	CV (%)	Bias (%)	RPD
Original	15	98.70	4.09	0.94	4.14	0.94	6.16	-0.86	2.84
n01, dgl	6	108.96	3.56	0.95	3.27	0.96	4.86	0.10	3.60

Prediction of Catechin Content in Gambir

Figure 3 shows calibration model for predicting catechin content. It was $y = 0.91x + 5.90$. The model provided low value of SEC (3.56 %) and SEP (3.27 %). In addition, the SEP value was not greater than two times of SEC prevented over fitting (Hruschka, 1990). The values of SEC and SEP which were generated by the model indicated that the model has high precision. Bias values closed to zero indicated that the model has high accuracy (Williams and Norris, 1990). It means that the model will be maintained for the prediction of catechin content in gambir. Furthermore, the model also showed a good correlation between the reference catechin content with NIR prediction catechin, as indicated by the high value of the correlation coefficient ($r > 0.90$) (Williams and Norris, 1990).

The model obtained CV value $< 5\%$, which indicated that the resulted model was appropriate to predict catechin content of gambir in the new data set. RPD was measurement ability of NIR model to predict a component efficiently (Williams and Norris, 1990). Based on obtained RPD values (3.6), then the resulted model was excellent to predict the gambir catechin content. RPD value above 3.0 is very good for predicting (Mouazen et al., 2005). It could be concluded that model with application of pre-processing method of n01 in combination with dgl showed higher accuracy and precision than the original model. It indicated that pre-processing method was important prior to modeling as it would improve the accuracy and precision of calibration model (Schulz et al., 1999; Udelhoven et al., 2002; and Ouyang et al., 2012).

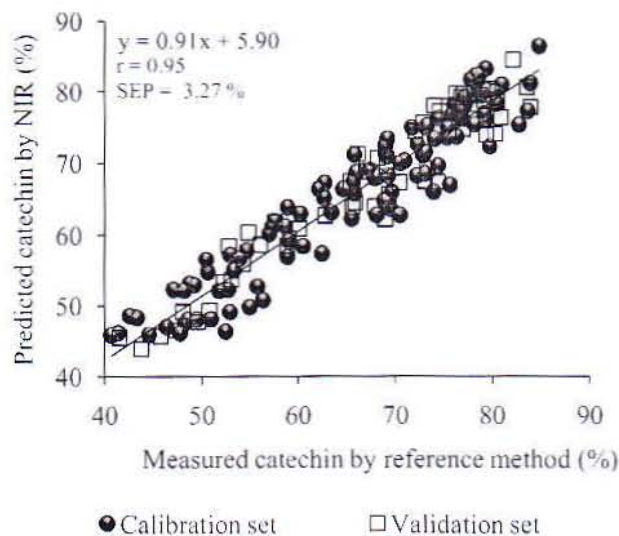


Figure 3. Scatter plot of measured versus predicted catechin content using n01 in combination with dg1 and 6 PLS factors

Conclusion

The prediction model was developed using the PLS algorithm to explain the relationship between NIR spectra data and catechin content. The model had a low SEC and SEP, a high r and a slightly difference between SEC and SEP values. These results were achieved when n01 in combination with dg1 pre-processing method and 6 PLS factors was applied. This study demonstrated the capability of NIR spectroscopy as a method which could replace traditional as well as chemical methods to determine catechin content of gambir. It is also shown that there is a possibility to develop a non-destructive method to measure gambir catechin content.

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