# Near Infrared Spectroscopy (NIRS): Fast and Non-destructive Metohd to Determination of Chemical Compositions of Modified Cassava Flour (Mocaf)

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# ABSTRACT

Mocaf is one of the local food commodities which is expected to succeed in the food diversification program to reduce dependence on wheat flour in Indonesia. Mocaf's chemical composition can be identified by time-consuming and destructive laboratory testing. Near-infrared spectoscopy (NIRS) is a new method that predicts the chemical compositions of Mocaf, since this method is fast, and non-destructive. This study aims to determine the best spectrum data processing and multivariate calibration methods (partial least squares regression (PLSR) method and principal component regression (PCR) method) from the NIRS method which can be used to determine the chemical compositions (water content, ash content, pH, and amylose content) of Mocaf in a non-destructive and fast. The wavelength ranges to predicting Mocaf chemical compositions, from 1000 to 2500 nm. Data treatment on the reflectance and absorbance spectrum curve estimation of Mocaf chemical compositions, among others: smooth average 3 points, second derivative Savitzky-Golay 9 points, and combination both of them. A number of 70 Mocaf were used as samples. Samples were divided into two phases: 2/3 of total samples for developing calibration equation and 1/3 of total samples for performing validation. NIRS data analysis result shows that PLSR method with NIRS reflectance data and the smooth average 3 points is the best method of calibration to predicting water content of Mocaf. The best Prediction of ash content, pH, and amylose content of Mocaf obtained with the PLSR method, the NIRS absorbance data, and combination data treatment. The standard error of prediction (SEP) and coefficient of variability (CV) respectively were 0.49% and 4.2% for moisture contents; 0.02% and 2.8% for ash contents; 0.02% and 0.4% for pH contents; and 0.39% and 1.3% for amylose contents. This study shows that the NIRS method can be used to determine the chemical compositions of Mocaf.

Keywords: NIRS, Mocaf, PLSR, PCR

### **INTRODUCTION**

Modified cassava flour (Mocaf) is a type of flour which is a derivative product of cassava flour that uses the principle of modification of cassava cells by fermentation. One of the locally produced commodities that is anticipated to succeed in Indonesia's food diversification program and lessen the country's reliance on wheat flour is Mocaf. Mocaf processing is fairly straightforward and comparable to processing standard cassava flour, but it also includes a 12 to 72 hour fermentation process (14). The use of Mocaf as a substitute for wheat flour has been widely used in various types of food, such as: pastries (7), noodles (11), cassava donuts (6), Pizza (3) and coconut butter cookies (12).

In general, conventional methods (laboratory chemical analysis) are used to determine the chemical composition of materials, but these processes are generally difficult, time-consuming, expensive, and labor-intensive, requiring a big laboratory space. At this time a number of methods with instrumentation techniques have been developed to determine the chemical composition with a fast, precise, accurate and non-destructive process. Near-infrared (NIR) measurement is one of these methods. Developed the first NIR technique by using transmittance spectroscopy to determine the water content of seeds and plant seeds. Near-infrared spectroscopy (NIRS) is a nondestructive method that is fast and easy to use (10).

Near-infrared spectroscopy has the ability to assess multiple quality parameters

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at once, which is one of its benefits. Applied the NIR method to estimate the moisture content and total carotene in oil palm fresh fruit bunches quickly and simultaneously (8). The relationship between reflectance and NIR absorbance with data quality parameters in this study was studied by calibration using Partial Least Square Regression (PLSR) method. The same calibration method has also been applied to quickly predict the yield index of "Crystal" guava (1). PLSR method is a multivariate calibration method that can extract chemical information from the resulting data and estimate a series of dependent variables from a very large number of independent variables. In addition, there is another multivariate calibration method, namely the Principal Component Regression (PCR) method. In order to estimate the fat content of whole cocoa beansused NIRS technology and PCR method (16). PCR method to predict the moisture content of dry grain (13) and PCR method to predict the levels of macronutrients in agricultural soils in the Blang Bintang area and its surroundings, Aceh Besar District, Aceh Province, quickly and simultaneously (4). The best calibration model generated by the pls method detection of chilling injury symptoms of salak pondoh fruit during cold storage (9). The PLSR method produces more accurate prediction results than the PCR method, on two multivariate calibration methods, PLSR method and PCR method in NIRS (16, 13, 4 & 9). This study aims to determine the best spectrum data processing and multivariate calibration methods from the NIRS method which can be used to determine the chemical compositions (water content, ash content, pH, and amylose content) of Mocaf.

#### MATERIAL AND METHODS

#### **Materials and Equipment**

The material used in this study was modified cassava flour (Mocaf) obtained from distributor PT. Tiga Pilar Sejahtera Agro, Tebet, South Jakarta, but produced by Loh Jinawi Trenggalek cooperative, East Java. 70 Mocaf samples were examined, and they were split into two stages: the calibration stage and the validation stage. The equipment used for data acquisition of the NIR spectrum is the NIRFlex *Fiber Optic Solids* N-500 Spectrometer with a wavelength of 1000-2500nm. MOCAF samples used in the estimation of chemical composition by the NIR method can be seen in Figure 1.



Figure 1. MOCAF Samples Which Will be Used in the Determination of Chemical Composition with NIRFlexFiber Optic Solids N-500.Procedures

#### Measurement of NIR Spectrum Reflection

Mocaf samples were taken in 1 sack and divided randomly into 70 samples (A total of  $\pm$  45 samples (2/3 of the total sample) were used for the calibration phase while the validation phase used  $\pm$  25 samples (1/3 of the total sample). The sample is put in transparent plastic so that it is easy to shoot infrared light from NIRFlex Fiber Optic Solids N-500. After that 70 Mocaf samples were shot with infrared light from the tool.

Sample measurement Mocaf by measuring the NIRFlex *Fiber Optic Solids* N-500 requires an estimated time of 8 - 9 seconds for each sample (Figure 2). NIRWare Operators software then supports the sample estimation procedure. Each sample was carried out 3 (three) repetitions with different estimation points. The NIR spectrum is in the wavelength range of

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1000-2500 nm with an interval of 0.4 nm, thus each sample has 1500 NIR reflectance and absorbance data.



Figure 2. The Process of Measuring the Reflection of the Mocaf Spectrum with NIRFlex Fiber Optic Solids N-500

#### **Measurement of Chemical Parameters**

Destructive measurement of quality parameters of Mocaf samples consisted of water content, ash content, pH, and amylose content. According to the SNI 01-3751-2006 procedure (5), the thermogravitymetric method is the standard analysis for estimating the water content. Determination of ash content requires an ashing process in an electric furnace according to the SNI 01-3751-2006 procedure (5). Determination of pH and amylose content was based on the procedure of Apriyantono et al. (2). The mean value of Mocaf samples is two repeats. NIRWare The software Management Console was used to enter the average Mocaf chemical analysis data.

#### **Data Treatment**

NIRFlex Fiber Optic Solids N-500 will generate measurement data in the form of reflectance (R) data. The absorbance data (A) was obtained by using the log (1/R) transformation of the reflectance data. NIR reflectance and absorbance curves require data treatment, such as: smoothing average 3 points, the second derivative of Savitzky-Golay 9 points, and the combination of the two treatments.

Smoothing serves to reduce noise and reduce errors that occur during NIRS measurements and laboratory chemical analysis without losing the existing spectrum information (15). The base effect of the increased absorbance process is reduced by the second derivative of Savitzky-Golay, which also solves the issue of the base slope of the regression equation (15). The combination of smoothing and the second derivative of Savitzky-Golay can be applied and obtain the optimum form and model of the calibration regression equation (15).

#### **Multivariate Calibration Method**

In order to avoid overfitting in the calibration phase, without losing one or useful information, chemical more information from the data generated by a chemical experiment needs to be extracted and large data sizes need to be reduced. The method to extract chemical information and reduce the large data is carried out through analysis of multivariate calibration methods. Principal component regression (PCR) method and partial least squares regression (PLSR) method are the multivariate calibration methods applied, and the software used is NIRCal 5. Data from measurements of NIR spectrum reflection, measurements of chemical parameter, and data treatment are calibrated and validated.

#### **NIRS** Calibration

The calibration stage is the stage to build a calibration regression equation from the estimation of chemical compositions of material. More samples must be collected for calibration than for validation. The performance of the estimation of chemical compositions of calibration phase can be known through the value of the coefficient of determination ( $\mathbb{R}^2$ ) and calibration standard error (SEC). SEC is determined by equation 1, as follows:

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$$SEC = \sqrt{\frac{\sum (Y_{C NIRS} - Y_C)^2}{n_C}}$$
(1)

description:  $Y_{CNIRS}$  is the estimated chemical composition of the NIRS for calibration,  $Y_C$  is the actual chemical composition for calibration,  $n_C$  is the number of calibration samples.

#### **NIRS** Validation

The validation stage is completed using the remaining data after the calibration regression equation model has been obtained. The data of different samples were entered into the calibration regression equation, in order to obtain data on the chemical composition of Mocaf of the suspected NIRS method.

Validation aims to test the accuracy of NIRS in estimating the chemical compositions (water content, ash content, pH, and amylose content) of Mocaf with a calibration regression equation that has been built. In the NIRS method, the validation stage graph does not need to be displayed. The parameters for the success of NIRS validation are seen from the value of the validation standard error (SEP) and the coefficient of diversity (CV). SEP and CV were determined by equations 2 and 3, as follows:

$$SEP = \sqrt{\frac{\sum (Y_{P \text{ NIRS}} - Y_{P})^{2}}{n_{P}}}$$
(2)

description:  $Y_{PNIRS}$  is the estimated chemical composition of the NIRS for validation,  $Y_P$  is the actual chemical composition for validation,  $n_P$  is the number of validation samples.

$$CV = \left(\frac{SEP}{Y}\right) \times 100\% \tag{3}$$

description: Y is the average of all samples of the actual chemical composition.

# RESULT

#### **Chemical Parameter Data**

The average value of Mocaf's chemical composition, as determined by destructive analysis, included among other things: moisture of 11.73%, ash content of 0.75%, while the average pH and amylose content were, respectively, 4.80 and 28.98%. The maximum and minimum values of the four Mocaf chemical compositions analyzed by laboratory chemistry have *range* a fairly high. The level of water content is 10 -13%, ash content is 0.6 – 1.0%, pH is 4.6 – 5.0, and amylose content is 27 - 32%.

#### NIRS Reflectance Data from Mocaf

The NIRFlex *Fiber Optic Solids* N-500 device will generate reflectance (R) data for NIR radiation. Figure 3 shows that different NIRS reflectance data shows that each Mocaf has a different chemical composition, such as water content, ash content, pH, and amylose content.



Figure 3. NIRS Reflectance Spectrum Curve on Mocaf

Data treatment is given on the reflectance and absorbance curve of NIRS. Data treatment on NIRS reflectance curve with a combination of smoothing average 3 points and the second derivative of Savitzky-Golay 9 points given to the

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estimation of water content, ash, pH, and amylose Mocaf using the PCR method and the estimation of the ash content, pH, and amylose Mocaf using the PLSR method. Then in the estimation of the water content of Mocaf with the PLSR method, data treatment was given, namely smoothing average 3 points

#### NIRS Absorbance Data from Mocaf

The log (1/R) transformation of reflectance data can be transformed into absorbance data, because it has a linear relationship with the chemical composition that is absorbed. The chemical composition certain materials experience of will absorption (absorbance) of light, this can be shown by the presence of wave peaks on the NIRS absorbance spectrum curve, the greater the chemical compositions of a material, the greater the near infrared absorption and the higher the wave peaks (Figure 4).



Figure 4. NIRS absorbance spectrum curve on Mocaf

The peaks of NIRS absorbance waves from Mocaf occur at wavelengths, including: 1200 nm, 1460 nm, 1700 – 1780 nm, 1940 nm, 2100 nm, 2280 nm, 2310 nm, 2360 nm, and 2460 nm. The chemical compositions of Mocaf absorbs NIRS waves at wavelengths, including: 1940 nm and 1200 nm for water content absorption, 1000 nm and 1180 nm for ash content absorption, 1000 nm and 1180 nm for pH absorption, and

2280 – 2320 nm for absorption of amylose content. Data treatment on the NIRS absorbance spectrum curve of Mocaf with the second derivative Savitzky-Golay 9 points, is given to the estimation of water content and ash content of Mocaf by PCR method. Estimation of Mocaf amylose content by PCR method was given a smoothing average 3 points. Meanwhile, the estimation of water content, ash content, pH, and amylose content of Mocaf using the PLSR method and the estimation of pH Mocaf using the PCR method were given a combination of the two data treatments.

#### **Determination of Water Content**

PCR and PLSR methods were used estimate the water content. The to calibration regression for equation estimating the water content of reflectance and absorbance data with multivariate calibration methods was given various data treatments. The most accurate calibration method and data treatment for calculating Mocaf water content is the PLSR approach with NIRS reflectance data and data treatment, specifically smoothing average 3 points. The highest calibration results for water content is show in figure 5.



Figure 5. The Highest Calibration Results for Water Content

The coefficient of determination  $(R^2)$ at the calibration process is 97,85%, whereas the values for SEC and CV

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calibration are 0,53% and 4,50%, respectively. SEP of 0,49% and CV of 4,20% are the results of the validation stage. Since the value is inside the acceptable ideal interval, the estimation is practical to use, as shown by the coefficient of determination  $R^2$  (>95%), SEP (1.00%), and CV (5.00%). Table 1 show the reflectance spectrum data using PLSR method on water content.

# Table 1. Reflectance Spectrum Data usingPLSR Method on Water Content

Sta	itistics	Smooth (%)	Second Derivative (%)	Combination (%)
С	$\mathbb{R}^2$	97,85	87,55	95,32
	SEC	0,53	0,65	0,58
	CV	4,50	5,80	4,90
V	SEP	0,49	0,65	0,58
	CV	4,30	5,30	4,60

#### **Determination of Ash Content**

The calibration regression equation for the estimation of ash content of reflectance and absorbance data using multivariate calibration methods was given with various data treatments. The best method of calibration and data treatment for determining Mocaf ash content is the PLSR method with NIRS absorbance data and data treatment, specifically a combination of smoothing average 3 points and the second derivative of Savitzky-Golay 9 points. Figure 6 show the highest calibration results for ash content.



#### Figure 6. The Highest Calibration Results for Ash Content

The calibration stage produces a coefficient of determination  $(R^2)$  which is close to 1 (one), namely 98.95%, standard error calibration (SEC) is 0.03% and coefficient of diversity (CV) is 3.30%. Then the validation stage produces an SEP is 0.02% and a CV is 2.80%. While both standard error levels are close to zero (1.0%), SEP evaluation is lower than SEC. Since both the two values of the coefficient of diversity are below the permitted optimum interval of 5% and the evaluation of the validation CV is lower than the calibration CV. Based on these results, the calibration regression equation model yields accurate estimation results. The absorbance spectrum data using PLSR method on ash content is show in Table 2.

# Table 2. Absorbance Spectrum Data usingPLSR Method on Ash Content

Statistics		Smooth	Second	Combination
		(70)	(%)	(70)
С	$\mathbb{R}^2$	96,84	99,17	98,95
	SEC	0,04	0,03	0,03
	CV	3,50	3,30	3,30
V	SEP	0,04	0,03	0,02
	CV	3,50	3,00	2,80

#### **Determination of pH**

Mocaf pH estimation was carried out using PCR method and PLSR method. multivariate calibration When using methods to determine the pH of reflectance and absorbance data, a variety of data processing methods were applied to the calibration regression equation. PLSR method with NIRS absorbance data and data namely a combination treatment, of smoothing average 3 points and the second derivative of Savitzky-Golay each 9 points is the best calibration method and data treatment for Mocaf pH estimation (Figure 7).

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Figure 7. The Highest Calibration Results for pH

At the calibration the stage, coefficient of determination ( $R^2$  is 96.16%. SEC and SEP, the values are 0.03 and 0.02, respectively. The SEP evaluation is lower than the SEC and both standard error are close to zero (<1.0%). While values the CV calibration and CV validation, the are below the allowable ideal values interval (<5.00%), the values of the two coefficients of variation are 0.50% and 0.40%, respectively. Table 3 show the absorbance spectrum data using PLSR method on pH.

Table 3. Absorbance Spectrum Data usingPLSR Method on pH

Statistics		Smooth	Second	Combination
		(%)	Derivative	(%)
			(%)	
С	$\mathbb{R}^2$	82,79	87,25	96,16
	SEC	0,05	0,02	0,03
	CV	0.60	0,50	0,50
V	SEP	0,04	0,02	0,02
	CV	0,50	0,50	0,40

#### **Determination of Amylose Content**

Amylose content was estimated using PCR and PLSR methods. The best method of calibration and data treatment for estimating Mocaf amylose content is the PLSR method with NIR absorbance data and data treatment, specifically a combination of smoothing average 3 points and the second derivative of Savitzky-Golay 9 points. The highest calibration results for amylose content is show in figure 8.



Figure 8. The Highest Calibration Results for Amylose Content

The coefficient of determination  $(R^2)$ at the calibration stage was 98.89%, which is a result quite near to 1. While the calibration standard error (SEC) is 0.40% and the coefficient of diversity (CV) is 1.40%. Then the validation stage resulted in an SEP is 0.39% and a CV is 1.30%. SEP evaluation is lower than SEC and both standard error values are close to zero (<1.0%). The evaluation of the validation CV is lower than the calibration CV and also the two values of the coefficient of variation are below the allowable ideal interval of 5%. Because the value is inside the acceptable ideal interval, it is clear that estimation using the the calibration regression equation can be used. The absorbance spectrum data using PLSR method on amylose content is show in Table 4.

# Table 4. Absorbance Spectrum Data usingPLSR Method on Amylose Content

Sta	tistics	Smooth (%)	Second Derivative (%)	Combination (%)
С	$\mathbb{R}^2$	96,84	99,17	98,95
	SEC	0,04	0,03	0,03

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	CV	3,50	3,30	3,30
V	SEP	0,04	0,03	0,02
	CV	3,50	3,00	2,80

# DISCUSSION

calibration The overall and prediction results are sufficient to prove that Mocaf's water content, ash content, pH, and amylose content can be promptly and nondestructively predicted using near infrared spectroscopy (NIRS). Prediction of the chemical compositions with NIRS used two multivariate calibration methods, that are PLSR method and PCR method. From the results of accuracy and reliability, it can be seen that PLSR method gives better prediction results than PCR method. This can be seen from the value of the coefficient of determination  $(R^2)$ , the standard error of validation (SEP), and the coefficient of diversity (CV) which is in accordance with the allowable ideal interval. Because the PLSR method directly combines the Y variable, meaning water content, ash content, pH, and amylose content in the model calibration process, the findings of the two methods differ from each other. Whereas PCR method, which only uses the X variable (data from the infrared spectrum), when data is extracted it becomes a fixed variable.

The results of this study are in accordance with the results of previous studies from Zulfahrizal et al. (16) and Devianti et al. (4) which states that NIRS is able to predict the fat content of cocoa beans and macronutrient levels in agricultural soil quickly and non-destructive. In addition, the most accurate and powerful multivariate calibration method in predicting the chemical composition of materials is the PLSR method. These findings are in agreement with those of Zulfahrizal et al. (16), Devianti et al. (4) and Mardjan et al. (9).

### CONCLUSION

The chemical compositions (water content, ash content, pH, and amylose concentration) of modified cassava flour can be promptly (Mocaf) and nondestructively predicted using near infrared spectroscopy (NIRS). The parameters of these four chemical compositions are obtained by building a predictive model with an appropriate multivariate calibration method approach. Estimation of water content was more accurate using the PLSR method with NIRS reflectance data, while the estimation of ash content, pH and amylose content was more accurate using the PLSR method with NIRS absorbance data. In order to acquire a calibration regression equation more than a wider range of chemical compositions of a material, additional modifications must be done by collecting samples from different Mocaf packages and utilizing a petri dish to prevent potential reflections (noise) due to entry of outside light. Further research needs to be done with additional estimation of the chemical composition of MOCAF, such as: protein content, fat content, and starch content.

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