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Original Article

Near infrared spectroscopy for the quality control of rice bran

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Abstrak

Tujuan: Penelitian ini bertujuan untuk mengkaji ketepatan penentuan kandungan nutrisi dedak padi sebagai bahan pakan secara cepat menggunakan teknologi Near Infrared (NIR) dibandingkan dengan analisa proksimat.
Metode: Dedak padi digunakan sebagai sampel pakan untuk membandingkan hasil analisis data menggunakan kedua metode tersebut. Pada penelitian ini Fourier Transform Near-infrared menggunakan infra merah dengan panjang gelombang 12500-4000 cm -1. Perangkat lunak OPUS 7.8 terintegrasi dengan NIRS. Model dibuat dengan menggunakan analisis regresi Partial Least Square untuk mengkorelasikan hasil spektrum dengan hasil metode analisis proksimat.
Hasil: Kandungan nutrisi dalam dedak padi yang tersedia di pasaran berupa Protein kasar (PK), Lemak kasar (LK), dan Serat kasar (SK), cukup bervariasi sehingga kurva prediksi yang muncul menjadi lebih luas dan tidak menutupi seluruh permukaan garis kurva. Akibatnya dua titik terluar (*outliers*) harus dihilangkan. Penghilangan *outliers* tersebut dapat meningkatkan nilai akurasi dalam pengukuran kualitas dedak padi yang ditunjukkan dengan peningkatan koefisien determinasi atau R^2, *Root Mean Square Error for Cross Validation* (RMSECV), dan *Standard Error* (SE).
Kesimpulan: NIR adalah alat yang berguna untuk memperkirakan komposisi nutrisi dedak padi yang tersedia di pasaran terutama untuk PK, LK, dan SK dengan menghilangkan dua *outliers*. Kurva Prediksi/True menjadi tidak melebar setelah dua outliers dihilangkan, serta dapat meningkatkan nilai R^2, RMSECV, dan SE.

Kata Kunci: Analisis proksimat; Dedak padi; NIRS; Nutrisi Ternak; Pakan

Abstract

Objective: The objective of this study was to examine the accuracy of estimating the nutrient content in rice bran as a feed ingredient by using Near Infrared (NIR) technology compared to proximate analysis method.
Methods: Rice bran was used as feed sample to compare the results of data analysis using these two methods. In this study the Fourier Transform Near-infrared used infrared with wavelength of 12500-4000 cm^{-1}. The OPUS 7.8 software was integrated with NIRS. The model was made by using Partial Least Square regression analysis to correlate the result of the spectrum and the result of proximate analysis method.

Results: In this study, the nutrient content in rice bran available in markets, which commonly are Crude Protein (CP), ether extract (EE), and Crude Fiber (CF), are quite varied and wide in range, so that the curve prediction/true showed up wider and did not cover the entire surface of the line. In consequence two outliers were removed so that the accuracy value could be increased in measuring the quality of animal feed as indicated by the improvement of the coefficient of determination or R^2, Root Mean Square Error for Cross Validation (RMSECV), and Standard Error (SE).

Conclusions: NIR is a useful tool to estimate nutrient composition of rice bran available in the market especially for CP, EE, and CF by removing two outliers. The Prediction / True curve does not widen after removing two outliers, and can improve R^2, RMSECV, and SE values.

Keywords: Proximate analysis; Rice bran; NIRS; Animal Nutrition; Feed

INTRODUCTION

Rice bran is a secondary product derived from rice milling [1]. Livestock and Animal Health Statistics notes that Indonesia as one of rice producing areas can produce as much as 81,382,451 tons per year [2]. This quantity of grains can produce by-products up to 35% (28,483,857.8 tons/year) which includes 25% husk and 10% rice bran [3]. It shows that the production of rice bran in Indonesia is relatively high with low demand when it comes to human consumptions. Considering that, rice bran is a potential source of animal feed. Furthermore, as stated by Stein et all. [4] that it is high in amino acids, starch, fat, vitamins, and certain minerals.

The microbial populations in the fore stomach (rumen) of ruminants allow rice bran to be used effectively compared to monogastric livestock [5]. Given in a right amount, rice bran can be used as a low cost poultry feeding. As what Sanchez et all. [6] stated that it is recommended to add up to 10%-20% rice bran in broilers diets because it contains trypsin inhibitors and is high in fibers.

The problem however, the amount of nutrition in rice bran available in Indonesia or in this case Jember Regency is varied [2]. It is due to different amount of the mixture of bran and husk in each brand. Rice bran contains organic matter (OM) = 88.9%, crude protein (CP) = 10.93%, and crude fiber (CF) = 15.07% [7]. While Nurcahyani et all. [8] stated that the nutrient content of crude bran is 90.68% OM, 5.95% CP, and 32.45% CF". The considerable best variety of rice bran is IR-64 which contains the lowest CF of 14.62% and CP of 11.1%.

This varied nutrition facts found in rice bran causes confusion between farmers to directly feed their animals farm with it. Thus, analysis toward nutrient content is required. However, this process can take approximately a week. NIRS technology is developed as a non-destructive method, is able to analyze at high speed, does not cause pollution, with simple sample preparations and does not require any chemicals [9]. Near-infra red spectroscopy (NIRS), an alternative to standard chemical analytical techniques, is a quick non-invasive method to assess physical and chemical composition, reducing the cost of routine analysis [10]. Calibration and validation are needed to be confirmed in early stage before using electromagnetic spectrum of NIRS continually. According to Ingle et all. [11] NIRS is a secondary method of analysis that provides quantitative predictions by the correlation of NIR absorption features to component concentrations.

Lacking number of studies about this method, in this case for animal feed, there are minimum data around characteristic spectrum of near infrared reflectance (NIR). In this study, an analysis toward rice bran is done by comparing the calibration and validation from proximate data analysis. The objective of this study is to examine the accuracy of determining nutrient content in rice bran as a feed ingredient by using NIR technology compared to proximate analysis [12].

MATERIALS AND METHODS

Sample collection and chemical composition analysis

A total of 15 rice bran samples were collected from all animal feed distributors in

Table 1. Nutrient content of rice bran

No	Kind of rice bran samples	Nutrients				
		DM	CP	EE	CF	Ash
		%				
1	Coarse, jember A	92.7	6.5	3.4	23.7	14.5
2	Coarse, jember B	93.1	6.1	4.7	22.1	17.0
3	Fine / *bekatul*, sumbersari	91.2	13.1	15.2	3.4	7.7
4	Fine / *bekatul* wirolegi	91.3	13.2	16.9	5.4	7.1
5	Fine / *bekatul*, ambulu	92.2	14.3	10.6	3.4	7.2
6	Dedak kasar/ *grantek* ambulu	92.1	3.7	2.0	36.5	17.8
7	Fine / *bekatul* sumber baru	90.6	13.5	15.0	3.5	6.9
8	PK sumber baru*	89.8	10.5	14.6	4.6	7.2
9	Coarse / *grantek* ambulu	92.0	6.6	3.0	27.2	17.8
10	Coarse, wuluhan	90.8	8.5	8.4	17.2	12.1
11	Coarse, wirolegi	93.8	6.5	3.5	28.4	14.2
12	Coarse / *grantek* pakusari	95.9	6.2	4.2	32.2	17.4
13	Fine/ *bekatul*, jelbuk	93.4	12.1	16.4	12.1	9.3
14	PK, kaliwates*	92.2	11.8	10.8	5.7	5.4
15	Fine/ *bekatul*, arjasa	91.9	12.9	14.9	2.3	6.9
	Average	92.2	9.7	9.6	15.2	11.2

*PK= rice bran which has a quality between coarse bran and fine bran; DM=dry matter; CP=crude protein; EE=ekstrak eter; CF=crude fiber.

Jember Regency and its surroundings by buying all kinds of rice bran (5 kg/sample) at the location of the animal feed distributor. Different types of rice bran were indicated by differences in quality and price. Every animal feed distributor did not always sell a kind of rice bran. As Ambulu subdistrick sold 3 kinds of rice bran with different prices, Sumber Baru subdistrick there were 2 kinds of rice bran, and Wirolegi subdistrick sold 2 kinds of rice bran (Table 1). The types of bran on the market from the best to the worst are: fine bran (bran), PK, and coarse (grantek). Each sample was then subjected to chemical composition analysis, i.e., Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF) and ash content [12].

NIRS (Near Infrared Reflectance Spectroscopy) spectra collection

The sample spectra collection was carried out using approximately 25 g of rice bran samples (a moisture content of 5-10%). The sample was placed on a petri dish then placed on a rotating sample, arranged as evenly as possible. Samples were irradiated using infrared short wavelength 12500-4000 cm^{-1} [13] at a temperature of 16-20^{o}C. The irradiation was done in duplo to reduce bias. The sample spectrum value was calculated in R where R was the reflectant value.

Calibration and validation of NIRS

Rice bran samples were used to make modeling using OPUS software 7.8 which was integrated with NIRS. The tool used is the Bruker brand from the United States with the Tango model (complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50). Modeling was made using partial least square (PLS) regression analysis to correlate between the spectrum results and laboratory data (proximate chemical analysis results). The analysis using NIRS was divided into calibration and validation.

Calibration was done by making NIRS modeling. Validation to evaluate and validate the accuracy of the model. The statistical test in the form of the coefficient of determination (R^2) showed the ability of the model to explain the diversity of the dependent variable values. The greater the R^2 value, the more the model was able to explain the behavior of the dependent variable. If R^2> 80-95%, the analysis could be stated as good [14].

$$R^2 = \left| \frac{n\sum XY - \sum X \sum Y}{\sqrt{[n\sum X^2 - (\sum X)^2][n\sum Y^2 - (\sum Y)^2]}} \right|$$

Note:

X = nutrient composition using proximate analysis

Y = estimated value using FT NIRS
n = number of samples
A good result would have a high residual predictive deviation (RPD) value [14]. The RPD equation was:

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

Note:
SD = Standard Deviation
SEP = Standard Error Prediction
An adequate validation of the calibration models is a crucial step to determine the suitability of the model to predict new samples (chemical composition). The expected standard error (SE) values is close to the standard deviation of the chemical data reference values [14].

RESULTS

Nutritional content of rice bran

The average nutrient content in rice bran circulating in the market were: for dry matter (DM): 92.2%; crude protein (CP): 9.7%; ether extract (EE): 9.6%, crude fiber (CF): 15.2% and ash: 11.2% (Table 1). According to the Indonesian National Standard in 2013 the quality of rice bran is shown in Table 2.

Table 2. Quality of rice bran according to SNI 2013

Parameter	Unit	Requirements Quality I	Quality II	Quality III
Dry matter (min.)	%	87.0	87.0	87.0
Crude protein (min.)	%	12.0	10.0	8.0
Crude fiber (max.)	%	12.0	15.0	18.0
Ash	%	11.0	13.0	13.0
Husk content	%	5.0	10.0	15.0

*SNI 2013 (Standar Nasional Indonesia) = Indonesian National Standard 2013

Dry matter (DM)

The Prediction vs. True / DM (%) / Cross Validation graph is a comparison of the DM content estimated by NIR to the results of proximate analysis of rice bran (Figure 1). Without eliminating the outliers, it appears that the results of statistical analysis are quite good, namely high R^2 of 82.01 and RMSECV close to zero, namely 0.61 (Table 3).

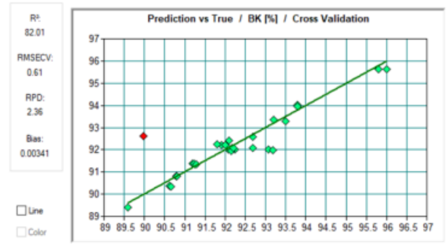


Figure 1. The graph of prediction vs True / DM (BK) (%) / Cross validation

The result showed that the laboratory value and predictive value had value of R^2 = 82.0. In predicting this DM, there was one outlier point. The RMSECV value was quite good, showed by the results obtained close to zero which was 0.539. The outer removal was not necessary because the DM of the rice bran samples were relatively homogeneous. It appeared on the data that did not spread and the SE was quite low, close to zero (0.06) (Figure 1, Table 3).

Table 3. Statistical analysis for rice bran DM

Statiistic analysis	Early: Outliers are not removed	End: Removed 2 outliers
R^2	82,01	Not done
RMSECV	0,61	Not done
RPD	2.36	Not done
SE	0,06	Not done

*R^2= Coefficient of determination; RMSECV= Root mean square error for cross validation; RPD= Residual predictive deviation; SE= Standard error

Crude protein (CP), ether extract (EE), and crude fiber (CF)

Figure 2 is prediction graphs of CP composition estimated by NIR from the result of proximate analysis before removing the outlier (a) and the CP composition after removing two outliers (b). The results show that the estimated value of CP has low R^2 value of 75.08 and the RMSEV value is 1.7, and

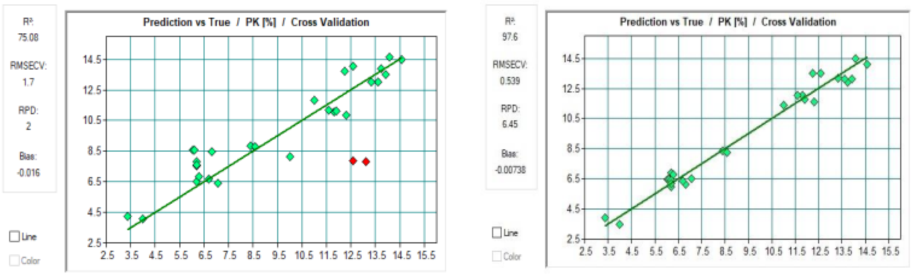


Figure 2. The graph of prediction vs True / CP (PK) (%) / Cross validation before removing the outer (a) and after removing the outer (b)

the curve appears to be wide (Figure 2a). The Prediction / True curve covers the entire surface of the line and does not widen after removing two points of outliers. R^2 increases to 97.6, and RMSEV is close to zero (0.539), and the SE composition is smaller, range from 0.22 to 0.12 (Figure 2 and Table 4).

Table 4. Statistical analysis for rice bran CP

Statiistic analysis	Early	End
	Outliers were not removed	Removed 2 outliers
R^2	75,08	97,60
RMSECV	1,70	0,54
RPD	2,00	6,45
SE	0,22	0,12

*R^2= Coefficient of determination; RMSECV= Root mean square error for cross validation; RPD = Residual predictive deviation; SE= Standard error

As in protein, the improvement of the graphical curve and statistical value after removing the 2 outliers also occurred in predicting fat and crude fiber as indicated by the prediction/true curve covering the entire surface of the line, where before removing the outliers the shape of the curve widened (Figures 3 and 4). Statistically, it was indicated by the improvement in the values of R^2, RMSECV, RPD, and SE (Tables 5 and 6).

Ash content

Figure 5 is a graph of comparison of ash content of NIR estimated from the results of proximate analysis of rice bran. The graph shows the prediction / true curve on the ash content is quite wide, with a low R^2 value of 69.29 and a high RMSECV value of 2.54, even though the SE is close to zero (Table 7).

DISCUSSION

The average nutrient content in rice bran in the market is classified to be quality III. Low quality rice bran or quality III contains water with maximum amount of 13%, minimum CP = 8%, maximum ash = 15.0%, and maximum CF = 18% [15], Table 2). The average value samples analyzed were not suitable to be used as a reference, this due to wide range of nutrient content. Those are CP which ranged from 3.7% to 13.5%, and CF ranged from 3.4% to 36,5% (Table 1). The different nutrient content existed because the rice bran taken from the market varies in quality.

There are several terms for rice bran with different quality and prices. The best quality and the most expensive one is called fine bran (*bekatul*) which has CP composition of at least 12%, this bran is mainly used for poultry feed. The lowest quality is coarse bran or *grantek* which contains a lot of husk mixture (> 15%). It shows in Table 1 that the CP composition of grantek is quite low, around 3.7% to 8.5%, and the crude fiber composition is quite high, around 22.1% to 32.2%. This type of bran is not good for poultry feed and should only be used as addition to ruminant feed which can be given by fermenting it first, so that its nutrient digested can be increased [8]. The fermenting process before giving it to ruminants is needed because the husk mixture in *grantek* is quite high, causing high percentage of CF, as a consequence it becomes difficult to digest. Husk also contains antinutrients, including tannins, alkaloids, phenols and phytates [16]. Fermentation using microbial inoculums can reduce the amount of NDF and ADF cell walls and alkaloid compounds of feed ingredients

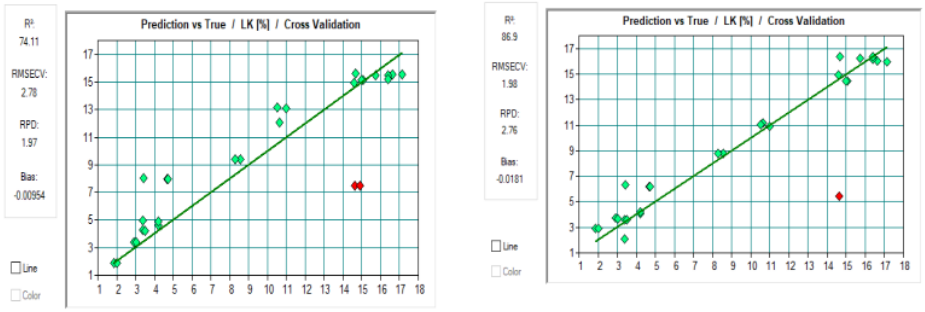


Figure 3. The graph of prediction true / EE (LK) (%) / Cross validation before removing outliers (a) and after removing outliers (b)

[17]. PK bran which is better than grantek but has lower quality compared to refined bran can be used for ruminants and as an addition to poultry feed.

The NIRS estimate for the DM value is related to the absorption of the –OH group in the sample [18]. The higher the coefficient of determination and the lower standard error, the better the equations are formed, meaning that the calibration model is able to estimate the DM composition from laboratory analysis results accurately. In this study, the calibration and validation R^2 of rice bran DM showed suitable results to be used as prediction. The value of R^2 calibration and validation in this study was 0.82 (82%). This value is lower than the results previously obtained by [18] which were 99% and 96% and by [19] which was as much as 99%. The lower value was the result of the existence of outliers that was probably coming from grantek Pakusari. Grantek Pakusari's condition was very dry compared to the others, which consists of 95.9% DM. Whilst the DM composition in other samples was 92.2% on average. However, the calibration R^2 was quite good. The high value of R (0.95) indicates a good correlation between the predicted and the reference values for calibration and validation model [14]. This condition could also be seen from the RMSECV value which was close to zero (0.61). The smaller the number, the better the RMSECV is. It indicates the accuracy of the measurement from the sample data to the calibration [9]. The result model is considered to be good if R^2 value is high and the RMSECV value is low [19].

The result of statistical testing showed that the SE value was quite small at 0.308. Small SE value indicated the accuracy of the measurement value [20], in this case the prediction value from NIRS. Overall, it could be said that the result of calibration model in estimating DM in samples was good, so the removal of the outliers was not necessary (Table 3). This condition occurred because in general, the samples of rice bran as animals feed contain relatively homogeneous DM composition which was more than 85%.

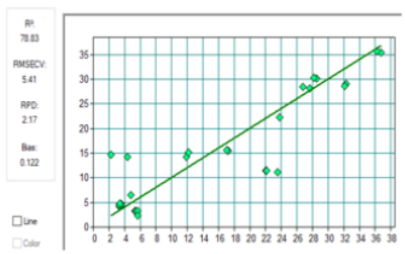
Table 5. Statistical analysis for rice bran EE

Statiistic analysis	Early	End
	Outliers were not removed	Removed 2 outliers
R^2	74,11	86,90
RMSECV	2,78	1,98
RPD	1,97	2,76
SE	0,33	0,19

*R^2= Coefficient of determination; RMSECV= Root mean square error for cross validation; RPD= Residual predictive deviation; SE= Standard error

The graphs show standard curve to predict EE has low R^2= 74.11 while the RMSECV value = 2.78. After removing two outliers, the R^2 yields to 86.9 and the RMSECV value drops slightly to 1.98. SE also changes from 0.33 to 0.19 (Figure 3 and Table 5).

Figure 4 is a graph of the comparison of CF composition estimated by NIR from the result of proximate analysis before removing outliers (a) and CF level after removing two outliers (b). The figure shows that the standard curve for predicting the CF value of



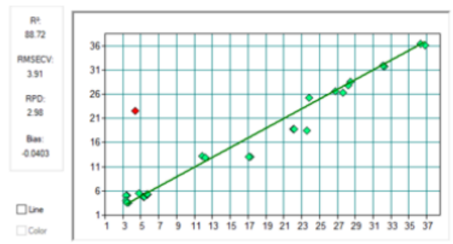


Figure 4. The graph of prediction vs True / CF (%) / Cross validation before removing the outer (a) and after removing the outer (b)

rice bran has the same pattern as CP and EE (Figure 4a). The Prediction / True curve does not widen after removing two outliers (Figure 4b). R^2, RMSECV, and SE also improves (Figure 4 and Table 6).

Table 6. Statistical analysis for rice bran CF

Statiistic analysis	Early	End
	Outliers were not removed	Removed 2 outliers
R^2	78,83	88,72
RMSECV	5,41	3,91
RPD	2,17	2,98
SE	0,70	0,41

*R^2= Coefficient of determination; RMSECV= Root mean square error for cross validation; RPD= Residual predictive deviation; SE= Standard error

The nutrient content of rice bran circulating in the market, especially CP, EE, and CF are quite varied and has fairly wide range. This condition affected standard or prediction / true curve that appeared to scatter and did not cover the entire surface of the line (Figures 2a, 3a, and 4a). In this research, in order to get better the curve results, two outliers that were farthest from the curve line were removed. As a result there was an increase in R^2 value and a decrease in RMSECV value; 1) CP had an initial R^2 value of 75.08 then turned to 97.6, while RMSECV moved from 1.7 to 0.539; 2) The initial EE value of R^2 which was 74.11 increased to 86.9, and the initial RMSECV which was 2.78 turned to 1.98; and 3) CF with the initial R^2 value of 78.3 turned to 88.72, while the RMSECV value turned from 5.41 to 3.91 (Tables 3, 4, and 5). If $R^2 > 80\text{-}95\%$, the model were able to explain the behavior of dependent variables (as results of CP, EE, and CF analysis) well [14]. The standard error reflects the accuracy of the sample we choose from its population. The smaller the standard error value, the more it indicates that the sampling is sufficiently representative of the population being studied and vice versa [21]. The removal of the two outliers also showed that the resulting SE value was smaller so that the estimation of CP, EE, and CF content was more accurate.

Regarding with the results of statistical tests, especially around R^2, RMSECV, and SE, recent studies have showed that in measuring nutrient content consisted of CP, EE, and CF in animals feed samples, in this research rice bran, NIRS is indeed a useful tool. Rapid measurement using NIRS is essential in controlling the quality of ingredients before the feed processing is carried out, so that the quality of the feed produced can be maintained.

To improve the results of predicting the feed nutrient, the RPD value of EE and CF curves can be improved by increasing the number of samples analyzed to be used for NIRS calibration and validation. The increased number of samples will increasingly represent the population [20]. The RPD value between 1.5-1.9 indicates that the prediction is still rough and needs improvement in the calibration. RPD values between 2-2.5 indicate a fairly good predictive model. Meanwhile, RPD values between 2.5-3 or more indicate a good to very good accuracy prediction model [22].

Infrared on NIRS vibrates indicating there are functional groups which are organic components in the sample. The limited results for ash can relate to the absence of energy absorption in the near-infrared region of the inorganic substances as the minerals. [18]. The results of this study showed that the R^2 value of the calibration obtained in the measurement of rice bran was quite low (69.29) (Figure 5).

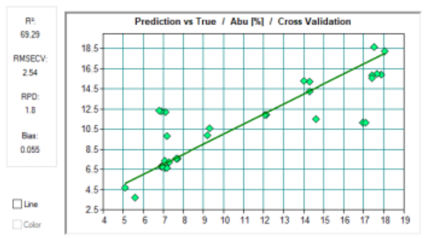


Figure 5. The graph of prediction vs True / Ash (Abu) (%) / Cross validation of rice bran

Near infrared (NIR) is a technique that includes vibration spectroscopy. Its radiation (750 to 2500nm) interacts with organic matter, and its absorption spectrum is rich in chemical and physical information from organic molecules [23]. The three main atoms in all biomass materials, including rice bran, are C, H, and O. Near infrared spectrum consists of vibrational bonds of chemical structures such as C-H, C-H-O, N-H, O-H, R-O-H, and C-C. These bonds are vibrated as certain wavelength in near infrared region when biological sample is irradiated by incident light [24]. The use of NIRS can effectively meet this need by providing a fast and accurate analysis, at a much lower cost [9].

Table 7. Statistical analysis for Ash content of rice bran

Statiistic analysis	Early	End
	Outliers were not removed	Removed 2 outliers
R^2	69,29	Not done
RMSECV	2,54	Not done
RPD	1,8	Not done
SE	0,308	Not done

* R^2= Coefficient of determination; RMSECV= Root mean square error for cross validation; RPD= Residual predictive deviation; SE= Standard error

The value of the ash despite of an inorganic component could still be detected using the NIR tool with SE of 0.308 (Table 6). It was possible because ash is bound to the organic material components so that NIRS is able to predict the ash content. The main mineral component found in rice husks is silica which consists up to 87% - 97% [25]. These results were in line with previous studies which suggested not to measure the ash content by using NIRS due to the absence of spectrum absorption in NIR [9]. For this reason, the improvement of the model for predicting the ash content was not carried out (Table 7).

CONCLUSION

NIR is a useful tool to estimate nutrient composition and is as accurate as chemical analysis method toward the nutrient content of rice bran available in the market especially for CP, EE, and CF, which are quite varied and wide in range, by removing two outliers. The Prediction / True curve does not widen after removing two outliers, and can improve R^2, RMSECV, and SE values. Additionally, this study has shown that NIRS is not able to examine the composition of ash content accurately because infrared on NIRS would only vibrate indicating the existence of organic components, while minerals are inorganic components.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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