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Rapid Prediction of Moisture and Ash Content in Sungkai Leaves Herbal Tea (*Peronema canescens* Jack.) using NIR Spectroscopy

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Abstract

It is imperative to measure the chemical composition of Sungkai leaf herbal tea in order to produce high-quality goods that promote human health. The moisture and ash content of Sungkai leaf herbal tea are critical parameters for assessing the quality of herbal tea. This study aimed to evaluate an NIR spectroscopy method for quickly determining the moisture and ash content of Sungkai leaf herbal tea. Sungkai leaf herbal tea has a moisture content between 3.93% and 7.59%, and an ash content between 3.94% and 5.51%. We developed a calibration model using partial least squares (PLS) with several pretreatment methods. We split the data into calibration and prediction sets and performed an internal random cross-validation. A PLS calibration model with R_p^2 = 0.86, a root means square error of prediction (RMSEP) of 0.30 (%), and a residual predictive deviation (RPD) of 2.76, performed exceptionally well at predicting the moisture content when the standard normal variate (SNV) pre-treatment was applied to the NIR spectra. The Savitzky-Golay derivative (a 9-point smoothing window, second-order polynomial, dg2) pre-treatment method also generated the best PLS calibration model for ash content determination, with R_p^2 = 0.70, RMSEP = 0.16 (%), and RPD = 1.86. NIR spectroscopy can quickly determine the moisture and ash content of Sungkai leaf herbal tea, as suggested by these findings.

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1. Introduction

The Sungkai plant (*Peronema canescens Jack*), which belongs to the Verbenaceae family, has several other names such as Jati Sabrang, Ki Sabrang, Sungkai Kurus, and Sekai (Yani & Putranto, 2014). The Sungkai plants originate in Southeast Asia, especially Indonesia, Malaysia, and the Philippines. Sungkai leaves have numerous health benefits and are commonly used in traditional medicine (jamu) to treat various conditions. These include fever, influenza virus infections, antimalarial worms (ringworms), postpartum bath ingredients for women, and mouthwash to prevent toothaches (Ibrahim & Kuncoro, 2012).

Sungkai leaves are being processed into simplicia or as raw materials for herbal teas because of the growing interest in their health benefits. Sungkai leaves were prepared as raw materials for

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Sungkai herbal teas. Quality control during the production process must be considered to ensure that the final product meets specified quality standards. Moisture content is an important parameter for assessing the quality and simplicity of herbal tea. Moisture content that is too high can damage the quality of taste and aroma and support mold and bacterial growth, whereas too low a moisture content can cause a bitter taste. In addition, Castillo et al. (2020) stated that poor quality control makes dried simplicia products susceptible to fungal contamination, producing microbial toxins and aflatoxins, which are known for their carcinogenic activity. Ash content is another essential parameter to be considered when assessing the quality or simplification of herbal tea. Very high or low ash content indicates a problem with plant growth or postharvest handling. Ash content meeting standards reflects a well-executed production process. Based on the dry tea quality standard SNI 01-3836-2013, the maximum moisture and ash content were 8% each.

The moisture and ash content of tea can be determined using standard analysis methods related to ISO 1573 and ISO 1575, respectively. These methods take a lot of time. Near-infrared (NIR) spectroscopy-based methods can be considered as a substitute for traditional analysis. NIR spectroscopy is an instrumental method that can characterize the responses of the C-H, OH–, and N-H groups in organic compounds (Shen et al., 2022). It is quick, easy to prepare, does not harm the product, and is non-invasive (Zhu et al., 2019). Analysis of dried herbs using NIR spectroscopy demonstrated both qualitative and quantitative potential. Examples of these analyses include the classification of dried herbs (Dankowska et al., 2022; Carvalho et al., 2020; Mahgoub et al., 2020) and quantification of the constituents of dried herbs (Jintao et al., 2021; Tian et al., 2022). Therefore, this study aimed to evaluate an NIR spectroscopy method for quickly determining the moisture and ash content of Sungkai leaf herbal tea.

2. Materials and Methods

2.1 Samples

Sungkai leaf samples were obtained from Sungkai Village, Pauh District, Padang City, West Sumatra, Indonesia. A rack-type dryer with an LPG gas energy source was used to dry the fresh Sungkai leaves. The drying was performed at temperatures below 60°C. The dried Sungkai leaves were ground using a grinder, filtered through a 40-mesh sieve, and packaged in LDPE plastic packaging. Sixty Sungkai leaf herbal tea samples, each weighing approximately 8 g, were prepared.

2.2 NIR Spectrum Collection

A Buchi NIRFlex N-500 solid-state spectrophotometer (Büchi AG, Switzerland) was used to obtain the NIR spectrum of the Sungkai leaf herbal tea. Measurements were carried out by filling a 10 cm

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diameter petri dish with each sample of Sungkai leaf herbal tea (± 8 g), and scanning was carried out on a scale range of 10000–4000 cm^{-1} (1000–2500 nm), producing 1501 variables.

2.3 Reference Analysis for Moisture and Ash Content

The reference analysis for measuring the moisture content and ash follows the ISO 1573 and ISO 1575 standards, respectively (Prawira Atmaja et al., 2021).

2.4 Calibration and Validation Analysis

This study used partial least squares (PLS) to create a calibration model for the moisture and ash contents of Sungkai leaf herbal tea. By lowering the dimensions of the dataset, PLS is a statistical technique that facilitates the exploration of the link between independent and dependent variables. This makes it useful for creating calibration models that use data from the infrared spectra (Zhang et al., 2021). Identifying outliers is crucial in creating a calibration model. An outlier indicates the retention of a significant amount of data. This can occur for several reasons, including mistakes made with instruments, in the lab, or with objects belonging to different populations (Valderrama et al., 2007). According to Zhu et al. (2021), the steps in the outlier identification procedure used in this study started with principal component analysis (PCA), which was used to identify and remove spectra that were thought to be outliers and would interfere with creating a model. The next step involved integrating F-residues with Hotelling's T-squared statistics (T^2) to identify deviant samples for each variable Y. T^2 indicates how well the model characterizes the sample and F indicates how close the sample is to the model. A 95% significance level was used to identify the sample as an outlier if the F and T^2 values were higher than the threshold. Next, we used the Kennard-Stone technique to separate the samples into calibration and prediction sets. The model was constructed using a calibration set and assessed using the prediction set. The samples were divided in a 2:1 ratio. This study used the K-fold approach to perform random internal cross-validation with ten segments. To calculate the number of samples for each segment (denoted as n), we divided the total samples by ten. If the total number of samples is not divisible by ten, then some segments will have n samples, and others will have n+1.

Some parameters used to assess calibration model performance were RMSEC = root mean square error of calibration, R_c^2 = coefficient of determination of calibration, RMSECV = root mean square of cross-validation, R_{cv}^2 = coefficient of determination of cross-validation, R_p^2 = coefficient of determination of prediction, RMSEP = root mean square error of prediction, and RPD = residual predictive deviation. If a model has an RPD> 2, then the model has excellent predictive ability; if the RPD is between 1.8 and 2.0, then the model has a favorable effect and can be used for the quantitative analysis of samples; if the RPD is between 1.4 and 1.8, then the model can be used for rough predictions and to evaluate sample correlation; and if the RPD is <1.4, then the model has poor

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influence and cannot be applied well (Shen et al., 2022). Furthermore, a model with a value of $0.82 < R^2 < 0.90$ indicates a good and acceptable model (Cheng and Sun, 2017), and R^2 above 0.91 indicates an excellent model (Mouazen et al., 2005). High R2 and low RMSE values indicate a good model (Huang et al., 2021).

In addition, this study used a maximum of 15 latent variables (LVs) to select the optimal number of LVs based on the smallest RMSECV value (Malvandi et al., 2022). The consistency value determines whether overfitting or underfitting occurs in a calibration model. Good models are characterized by consistency values of 80–110%. A consistency value below 80% indicated the possibility of overfitting in the calibration model. Conversely, if the consistency value exceeded 110%, this indicated the possibility of underfitting the calibration model.

2.5 Pre-treatment Methods

Before building the calibration model, researchers used pretreatment techniques to eliminate or reduce undesirable influences. Tian et al. (2022) stated that aspects such as noise and baselines appearing in the detector, background, or physical differences in the structure between samples are considered useless information in the raw spectrum. In this study, various pretreatment methods were applied to build the calibration model, including the Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), Savitzky-Golay derivative (9-point smoothing window, first-order polynomial, dg1), Savitzky-Golay derivative (9-point smoothing window, second-order polynomial, dg2), and combinations of SNV+dg1, MSC+dg1, SNV+dg2, and MSC+dg2. We used Unscrambler X 10.3 software (CAMO Inc., Oslo, Norway) for the analysis.

3. Results and Discussions

3.1 Spectral Features

The raw NIR spectrum of the Sungkai leaf herbal tea sample displayed multiple absorption peaks in the 1000–2500 nm wavelength region (Figure 1). The absorption peak at 1225 nm was due to C-H str. s overtone of the CH structure, an absorption peak at a wavelength of 1450 nm occurred due to O-H str. first overtone of starch and H_2O, the absorption peak at a wavelength of 1725 nm occurred because of C-H str. first overtone of CH_2, the absorption peak at a wavelength of 1939 (near 1940 nm) occurred owing to O-H str. +O-H def. from the H_2O structure, and the absorption peak at wavelength 2310 nm was due to C-H str. +C-H def. from the CH_2 structure (Osborne et al., 1993). Because free moisture in dry food items is linked to the absorption peak at approximately 1430 nm, as Büning-Pfaue (2003) explained, these molecular linkages in the NIR spectrum indicate the presence of moisture. Furthermore, other absorption peaks in the NIR spectrum indicated the presence of other chemical components in Sungkai leaf herbal tea. Transformation techniques using various

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pretreatment methods can overcome the NIR spectrum problems, including spectrum variations and overlapping spectra.

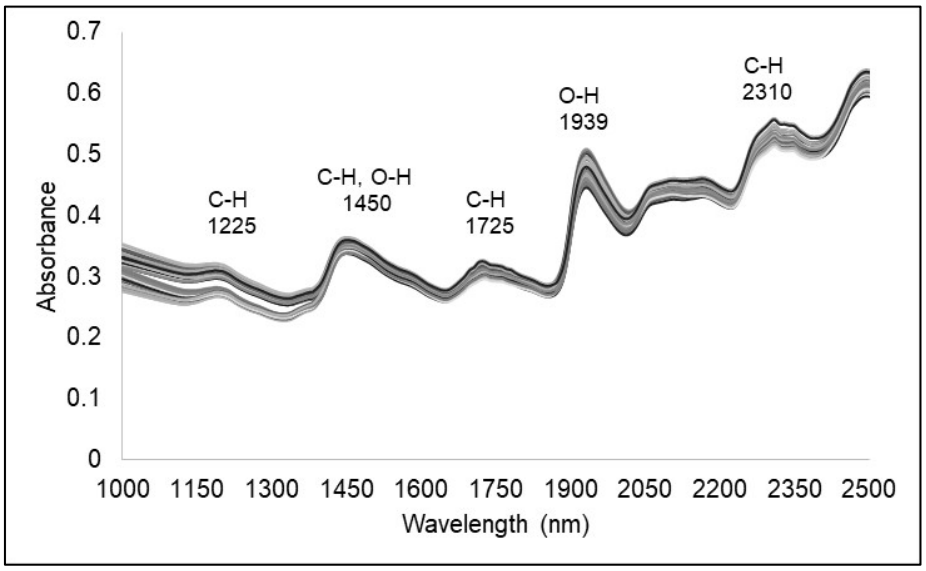

Figure 1. Raw specturm of Sungkai leaves herbal tea

3.2 Outlier Sample Elimination

In Figure 2a, which displays the PCA score plot of all the original spectra, no spectra are found to be outliers. Then, using the 60 original spectra, PLS moisture and ash content models were constructed, linking each spectrum to a corresponding Y value (reference data). Hotelling's T-squared statistics (T^2) and the F-residual plot were the basis for outlier detection in the two PLS models. Because none of the samples was outside the rectangular range, none were recognized as outliers in the PLS model for moisture content (Figure 2b). However, for the PLS model of ash content, we found and removed one outlier sample (Figure 2c).

Using two-thirds of the sample size, we constructed the model and utilized the remaining one-third to evaluate the robustness of the model. We also examined the potential use of Y-variance and residual sample calibration variance to identify outliers in the PLS model of the ash content used in this study. Three samples with a significant Y-variance were excluded (Figure 3). To create PLS models of moisture and ash content, we kept 60 and 56 samples, respectively. The statistical parameters of the PLS model for moisture and ash content for the calibration and validation datasets are listed in Table 1. As the lowest and highest values were found in the calibration set, the data in the validation set were used to evaluate the calibration model.

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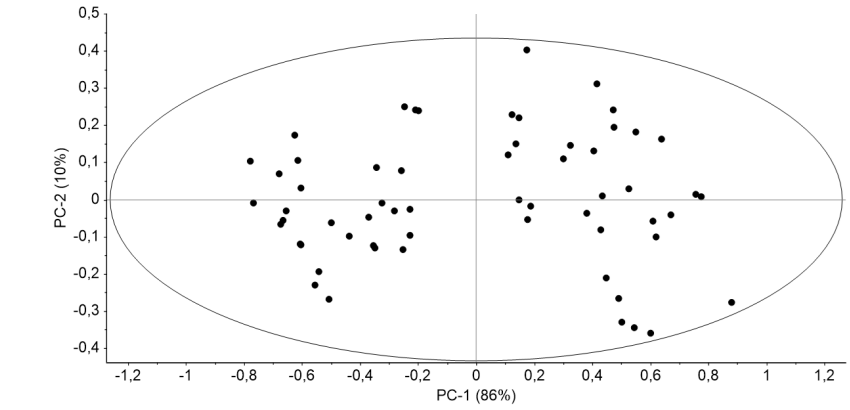

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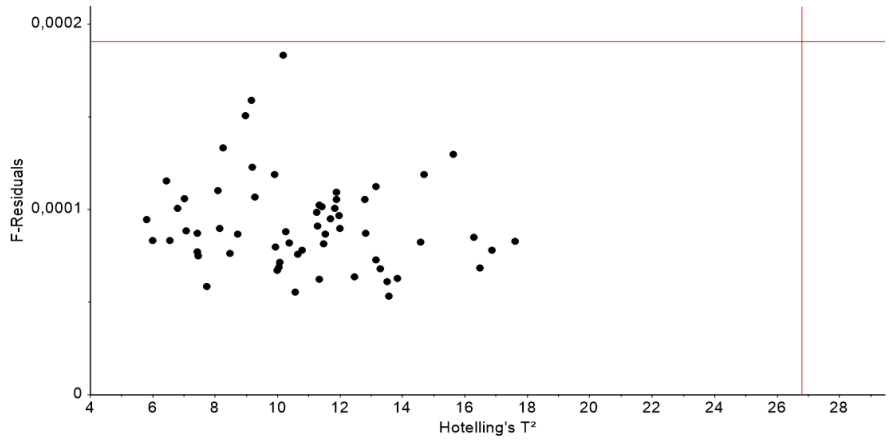

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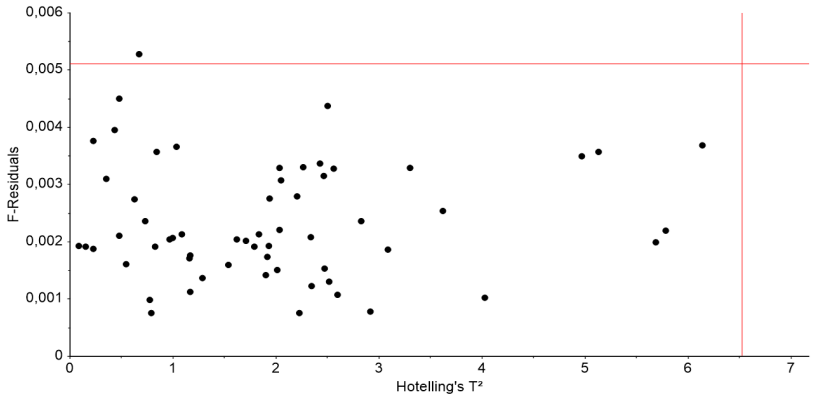

c

Figure 2. Score plot of principal component analysis (PCA) (a); Residues and influence plot with Hotelling's T^2 and F-residuals for moisture content (b) and ash content (c)

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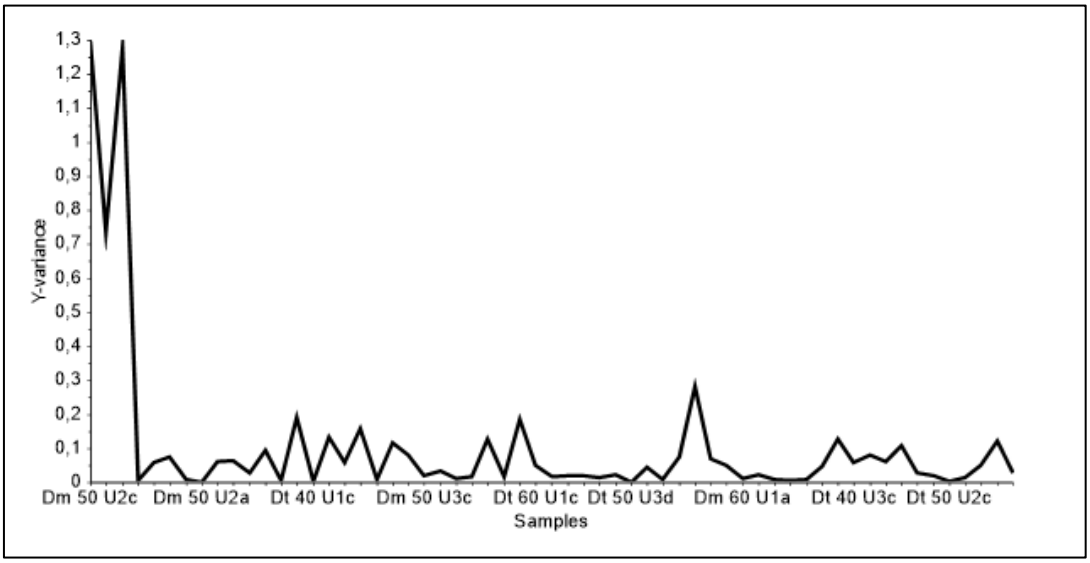

Figure 3. Residual sample calibration variance

Table 1. Statistical moisture and ash content parameters in Sungkai leaves herbal tea for calibration and validation sets

Content	Sets	Number of samples	Min	Max	Mean	SD
Moisture (%)	Calibration	40	3.93	7.59	5.57	0.87
	Prediction	20	4.36	7.50	5.58	0.83
Ash (%)	Calibration	38	3.94	5.51	4.59	0.37
	Prediction	18	4.13	5.19	4.64	0.31

Min = minimum, Max = maximum, SD = standard deviation

3.3 PLS Model

The PLS model utilizes the NIR spectra from 1000 to 2500 nm to predict the moisture and ash content of Sungkai leaf herbal tea. Applying a variety of pretreatment techniques to the NIR spectrum and selecting the ideal number of LVs created the best model for estimating the moisture and ash content of Sungkai leaf herbal tea. Zhu et al. (202)1 employed different pretreatment techniques before chemometric analysis to address variations in sample properties and mitigate the additive or multiplicative effects resulting from light scattering, NIR spectrum baseline deviations, and signal noise.

Based on the R_p^2, RMSEP, RMSEC, SEC, SEP, RPD and Consistency values, we assessed the most effective pretreatment techniques. Table 2 shows that the calibration model with SNV pretreatment had an R_p^2 value of 0.86, exceeding the original model, which only reached 0.74. Meanwhile, the other models showed lower R_p^2 values than the original model.

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Table 2. Statistical variables of PLS analysis on spectra NIR of tea herbal leaves Sungkai samples with different pre-treatments

Pre-treatment method	LVs	Calibration set			Cross Validation		Validation set			RPD	Consistency (%)
		R_c^2	RMSEC (%)	SEC (%)	R_{cv}^2	SECV (%)	R_p^2	RMSEP (%)	SEP (%)		
Moisture											
Raw	13	0.96	0.17	0.17	0.76	0.45	0.74	0.44	0.45	1.89	39.04
SNV	**5**	**0.86**	**0.32**	**0.33**	**0.76**	**0.43**	**0.86**	**0.30**	**0.31**	**2.76**	**106.71**
MSC	10	0.93	0.22	0.22	0.75	0.44	0.74	0.47	0.47	1.78	47.51
dg1	9	0.94	0.21	0.22	0.76	0.43	0.56	0.59	0.58	1.41	37.28
dg2	6	0.94	0.22	0.22	0.70	0.48	0.55	0.58	0.57	1.42	38.85
SNV+dg1	6	0.86	0.33	0.33	0.74	0.45	0.65	0.52	0.52	1.60	63.74
MSC+dg1	10	0.97	0.15	0.15	0.73	0.46	0.71	0.48	0.47	1.74	32.71
SNV+dg2	3	0.88	0.30	0.31	0.45	0.65	0.33	0.69	0.71	1.20	43.01
MSC+dg2	8	0.98	0.14	0.14	0.60	0.55	0.58	0.56	0.55	1.49	25.15
Ash											
Raw	5	0.80	0.16	0.17	0.69	0.21	0.67	0.17	0.18	1.75	93.39
SNV	8	0.86	0.14	0.14	0.70	0.21	0.60	0.20	0.20	1.55	69.59
MSC	9	0.92	0.10	0.11	0.75	0.20	0.62	0.19	0.19	1.64	55.95
dg1	3	0.78	0.17	0.17	0.72	0.20	0.80	0.14	0.14	2.24	127.61
dg2	**3**	**0.82**	**0.15**	**0.16**	**0.75**	**0.19**	**0.70**	**0.16**	**0.17**	**1.86**	**92.82**
SNV+dg1	5	0.86	0.14	0.15	0.66	0.22	0.54	0.21	0.21	1.46	69.42
MSC+dg1	5	0.86	0.14	0.14	0.70	0.21	0.54	0.21	0.21	1.46	65.73
SNV+dg2	7	0.97	0.06	0.06	0.74	0.19	0.73	0.16	0.16	1.96	39.75
MSC+dg2	7	0.97	0.07	0.07	0.75	0.19	0.74	0.15	0.16	1.98	41.81

It is important to note that calibration models with R_p^2 between 0.82 and 0.90, as stated by Mouazen et al. (2005) and Cheng and Sun (2017), have good predictive ability. The same applies to the RPD values. The calibration model involving SNV pretreatment had an RPD value of 2.76, exceeding the original model, which only reached 1.89. In contrast, the other models showed lower RPD values than the original model. Calibration models with RPD >2 showed excellent predictive ability (Shen et al., 2022). A calibration model with a high RPD indicates a strong and accurate model (Malvandi et al. 2022). The calibration model with pretreatment SNV had the smallest difference in SEC and SEP values compared to other models, and it can also be seen that the SEP value was no more than twice the SEC value, so there was no overfitting (Hruschka, 1990). The number of LVs used to build the calibration model influences the overfitting or underfitting problem. Despite using the smallest RMSECV value to select the optimal LVs, the calibration model still experienced overfitting or

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underfitting. Therefore, the consistency value can be used to determine whether the calibration model is underfitted or overfitted. In the calibration model for predicting the moisture content of Sungkai leaf herbal tea, it can be seen that the calibration model with SNV pretreatment does not show underfitting or overfitting because it has a consistency value between 80 and 110%, while the other calibration models show a tendency to overfit because they have a consistency value below 80%. Therefore, we selected the calibration model involving SNV pretreatment as the best model for estimating the moisture content of Sungkai leaf herbal teas. This model increased the R_p^2 value from 0.74 to 0.86, the RPD value increases from 1.89 to 2.76, and the RMSEP value from 0.44% to 0.30%.

From Table 2, it is evident that the calibration model, which includes pre-treatment dg1, dg2, SNV+dg2, and MSC+dg2, exhibits higher R_p^2 and RPD values than the original model in predicting ash content in Sungkai leaf herbal tea. The other models had lower R_p^2 and RPD values than the original model. However, the calibration model involving pre-treatment dg1, SNV+dg2, and MSC+dg2 showed greater differences in SEC and SEP values than the original model, as well as consistency values, which indicated underfitting in the calibration model with pre-treatment dg1 and overfitting in the calibration model with SNV+dg2 and MSC+dg2 pre-treatment. Therefore, the results of this study indicate that the best calibration model for predicting ash content in Sungkai leaf herbal tea is the calibration model with dg2 pretreatment. The R_p^2 value increases from 0.67 to 0.70, which can be categorized as a model close to quantitative predictions. In addition, the RPD value increased from 1.75 to 1.86, indicating that the model was suitable for quantitative sample analysis, whereas the RMSEP value decreased from 0.17 0.16. The PLS model with SNV and dg2 pretreatment showed the best prediction performance for moisture and ash content in Sungkai leaf herbal teas. A graphical representation of this process is shown in Figure 4.

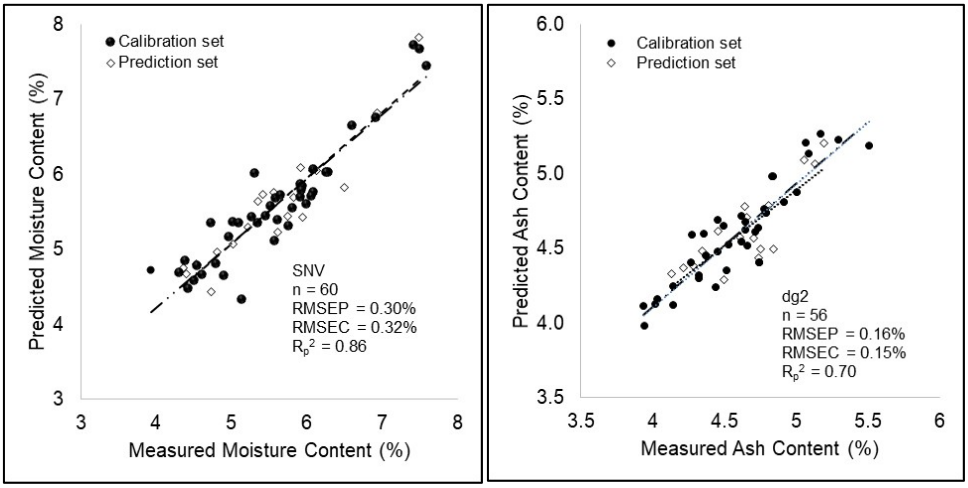

Figure 4. Scatter plot of measured vs. NIR predicted moisture and ash content in Sungkai leaves herbal tea

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This study also reports that the effects of some applied pre-treatment methods can vary, positively and negatively affect model performance. Several previous studies have also highlighted the improvement in model performance after applying pretreatment compared with the original model. For example, applying dg2 pretreatment to the caffeine prediction model in Gayo Arabica coffee beans improved its performance compared with that of the original model (Rosita et al., 2016). Researchers have also found an improvement in the performance of the caffeine and chlorogenic acid prediction model using dg2 pretreatment in research related to Bondowoso Green Arabica coffee beans. At the same time, trigonelines increased with dg2+MSC pretreatment (Madi et al., 2018). Furthermore, Frizon et al. (2015) demonstrated that the MSC+1st derivative can enhance the performance of models in predicting the total phenolic compounds of yerba mate (*Ilex paraguariensis*). The increase in PLS model performance after applying pre-treatment is due to the ability of pre-treatment to separate signals related to the measured components. This helps improve the model's ability to predict the dependent variable. In addition, pretreatment is also effective in reducing or eliminating noise in the spectra, which contributes to improving the accuracy of the PLS model.

However, some studies have also shown that applying pretreatment can reduce model performance compared to the original model. For example, applying SNV pretreatment to predict caffeine in S. arabica coffee beans caused a decrease in R_p^2 and RPD values. In contrast, the SEP and RMSEP values increase (Yuwita et al., 2023). A similar phenomenon also occurred in predicting the acidity level of green coffee beans using MSC pretreatment, which caused a decrease in the R_p^2 and RPD values and increased the SEP and RMSEP values (Araújo et al., 2020). The decrease in the performance of the PLS model after applying pre-treatment can be caused by some pre-treatment, making the PLS model overfit to the training data, thereby hindering the model's performance on new data. Additionally, some pretreatment methods may cause loss of valuable information in the spectra, thereby reducing the ability of the model to capture important variations in the data.

4. Conclusion

This study shows that NIR spectroscopy combined with chemometrics can determine the mosirure and ash content of Sungkai leaves herbal tea. Different pre-treatment methods on the NIR spectrum have a different performance of the predictive model. Applying the SNV pre-treatment method to NIR spectra produced the best PLS calibration model for determining moisture content (R_p^2 = 0.86, RMSEP = 0.30 (%), and RPD = 2.76). On the other hand, the dg2 pre-treatment method was proven to be the best PLS calibration model for determining ash content (R_p^2 = 0.70, RMSEP = 0.16 (%), and RPD = 1.86). These results indicate that NIR spectroscopy has the potential for rapid measurement of moisture and ash content in Sungkai leaves herbal tea.

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