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Feasibility Analysis of Using NIR Spectroscopy to Predict Ripeness Parameters of Red Guava Fruit (*Psidium guajava L.*)

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Abstract

Red guava is highly preferred by Indonesians because of its delightful flavor, refreshing taste, and numerous advantages. It is a climacteric fruit, and the age at which red guava is picked affects the quality of the fruit. The estimation of the maturity parameters of red guava is physically destructive (damages the fruit). This study aimed to assess the NIR method to predict the maturity parameters of red guava fruit with wavelengths of 1000-2500 nm. Red guavas of four different picking ages were used in this study. The tested maturity parameters included firmness, moisture content, total soluble solids (TSS), acidity, and sugar-acid ratio at various harvest ages. The partial least squares (PLS) method was used for calibration and validation of NIRS data and reference data. The results of this study show that NIRS can estimate the ripeness of red guava fruit based on firmness, acidity, sugar-acid ratio, and water content. The best-estimated value for firmness parameters was obtained with pretreatment MSC factor PLS 14, resulting in r calibration of 0.94, SEP 7.20, CV 25.91%, and RPD 2.90; acidity without pretreatment factor PLS 13 obtained r calibration of 0.82, SEP 0.17, CV 24.42%, RPD 1.68; sugar acid ratio with pretreatment SNV factor PLS 10 obtained r calibration 0.75, SEP 4.03, CV 30.81%, RPD 1.51; and for moisture content using SNV pretreatment factor PLS 13, r calibration 0.88, SEP 0.95, CV 1.08%, RPD 1.95.

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1. Introduction

Guava fruit (Psidium guajava L.) is one of the most popular agricultural commodities among Indonesians because of its sweet and fresh flavor. According to Ochtavia et al., (2015), guava is a plant that produces fruit throughout the year and is widely grown in various regions of Indonesia. According to data from the Central Statistics Agency (BPS) from 2018 to 2022, guava fruit production has increased significantly. In 2018, production reached 230,697 tons, whereas in 2019, it increased to 239,407 tons. Production figures continue to grow until they reach 396,268 tons in 2020, 422,491 tons

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in 2021, and a peak in 2022, with a total production of 472,686 tons. This increase indicates promising market potential for farmers, as the demand for guava fruit increases annually.

Guavas have flesh variants with two main colors: white and red. Guavas with red flesh are known to have high vitamin C content and health benefits, such as anti-anemia, antioxidant, anti-inflammatory, digestion support, and maintenance of a healthy cardiovascular system (Naseer et al., 2018). Red guava fruits fall under the category of climacteric fruits, in which the ripening process is followed by a surge in ethylene production and an increased respiration rate, as noted by Karuniasari (2022) and Fauziah et al., (2021). Therefore, to prevent spoilage during storage and distribution, it is crucial not to pick red guava fruits when they are fully ripe. Harvesting them at the right stage ensures their quality and maintains their selling value.

Determining the level of fruit maturity is generally done in various ways, namely by observing physical changes in the fruit, calculating the age of the fruit since flower pollination, or using the specific gravity method (Muchtadi 2010). However, these methods are often inefficient, because they require a significant amount of time and precision. Observations of color changes also do not always yield accurate estimates of fruit ripeness, because they are affected by individual subjectivity. In addition, Azrita et al., (2020) revealed that when selecting the ripeness level of a fruit, consumers tend to press the fruit. If repeated, this action can injure the fruit and ultimately damage its quality. Physical measurement methods often used to evaluate fruit ripeness are destructive or can damage the fruit.

Near-infrared spectroscopy (NIRS) can accelerate and improve the estimation of red guava fruit ripeness. NIRS is a spectroscopic method that utilizes infrared light in the specific wavelength range of 780–2500 nm in the electromagnetic wave spectrum (Ahmad and Sabihah, 2018). NIRS has several advantages, including a simpler preparation process, rapid detection, non-destructiveness to the product, and environmental friendliness, as it requires no chemical additives. Moreover, this technology can assess multiple quality parameters at the same time (Nafisah, 2019).

Several studies have applied NIRS to assess fruit ripeness and chemical composition of organic matter with varying accuracy values for the various ripeness parameters tested; for example, Alang (2021) compared the accuracy of ripeness prediction using NIRS with UV fluorescence spectroscopy on star fruit and found that the NIRS method provided the best accuracy with an R2 (regression) value of 0.93 on the TSS parameter and an R2 (regression) value of only 0.72 on the hardness parameter. Ana et al., (2021) used portable NIRS to predict the harvest index of crystal guava, with the best correlation results between the hardness parameters of 0.88, 0.74, and 0.59, and the acid sugar ratio of 0.71. This study aimed to evaluate the feasibility of using NIRS to predict red guava fruit ripeness parameters by identifying the correlation between the results of nondestructive measurements using NIRS and destructively measured fruit ripeness parameters. The maturity parameters evaluated included

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hardness, moisture content, total soluble solids (TSS), total acidity, and acid-sugar ratio at different picking ages.

2. Materials and Methods

This study used several types of tools, including the NIRFlex N-500 fiber optic solid spectrometer to collect the emitted spectrum data, CR500 dx rheometer to measure the hardness of red guava fruit, refractometer Atago PAL-22S to measure total soluble solids (TSS), and refractometer Atago PAL BX/ACID F5 to measure total acid. An oven was used to measure the moisture content. While measuring the total acid and moisture content, the mass of the sample was also measured using digital scales and OHAUS scales in Microsoft Excel 2019 and Unscrambler X 10.4. Red guava cultivars were obtained from the orchard of Mrs. Nikade Astuti, Tajur Halang, West Java, consisting of samples with four different picking age groups, namely from picking age 80, 90, 100, and 110 days after blossom (DAA), each with 20 fruits.

2.1 NIRS Measurement and Destructive Measurement

Before beginning the measurement process, the samples were cleaned and labeled at each specific measurement point: the base, middle, and end. A NIRFlex N-500 fiber optic solid spectrometer with a wavelength range of 1000–2500 nm was fired at three designated measurement points for each sample, and destructive measurements were taken after the completion of NIRS measurements. First, hardness was measured, followed by measurements of total soluble solids (TSS), total acid, and moisture content. The sugar-acid ratio was calculated based on the ratio of TSS and total acid values, expressed as °Brix/%Acid. The moisture content was measured over a period of 20 h or until the weight of the sample reached a constant point at 105°C.

2.2 Pre-Treatment Data NIRS

The use of NIRS for non-destructive measurements produces data in the form of reflected waves (reflectance). The basic principle of the NIR spectroscopy theory is absorption. Therefore, reflectance waves were transformed into absorbance data (Karlinasari et al., 2012). The reflectance waves were transformed into absorbance waves using Equation 1.

$$A = log(1/R) \tag{1}$$

where A is the absorbance and R is the reflectance.

The spectra generated from NIRS measurements are often affected by noise and artifacts caused by interference in the measurement or natural variability in the sample. Therefore, absorbance waves must be corrected using several pre-treatments to reduce the influence of noise and obtain accurate results. In addition, the data collected from destructive and non-destructive measurements were

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organized using Microsoft Excel, and outlier data were cleaned using the interquartile range method. The data were processed using Unscrambler X 10.4 software.

In this study, four (4) types of NIR spectroscopic data pretreatments were applied: normalization (N), multiplicative scatter correction (MSC), standard normal variate (SNV), and de-trending. Normalization is a pre-treatment to change the scale of spectra to be more uniform, which is between 0-1 (Lengkey et al., 2013). SNV is a correction method that focuses on averaging each spectrum individually to reduce the effects of scattering caused by material components that affect the spectra (Kurniasari et al., 2017). De-trending is a pre-treatment method designed to eliminate nonlinear trends from spectroscopic data (Masdar et al., 2016). MSC is an approach to reduce the effects of amplification and offset in NIR spectra. MSC operates by adjusting the spectrum according to the difference between the average spectrum data and the reference spectrum (Ramadhan et al., 2016).

2.3 Evaluation of Calibration and Validation Result

The data obtained from the measurements were processed and analyzed using Microsoft Excel, NIRWare Management Console, which is built-in software of the BUCHI brand NIRFlex N-500, and Unscrambler X 10.4. Both destructive and non-destructive measurement data were compiled in Microsoft Excel and then processed using Unscrambler X 10.4 to produce outputs in the form of graphs, estimated data, and calibration regression between destructive and non-destructive measurement data. Calibration was performed to determine the relationship between destructive measurements (hardness, TSS, moisture content, total acid, and acid-sugar ratio) and non-destructive measurements (NIRS). Calibration was performed using the partial least squares (PLS) method. Validation is the final step in testing the predictive accuracy of the obtained calibration equation.

The calibration and validation processes were performed by dividing the sample data into two parts: 2/3 of the part was used for the calibration process and 1/3 of the part was used for validation. A total of 240 data points were collected for each measured parameter in this study. However, some measured parameters contained outliers, so the data was cleaned to remove these outliers before beginning the calibration and validation processes. Calibration and validation models were developed based on the absorbance spectra using the partial least squares (PLS) method, where the PLS factors varied from one to 20 factors. The calibration and validation results were evaluated using correlation coefficient (r), standard error of calibration (SEC), standard error of prediction (SEP), coefficient of variation (CV), residual predictive deviation (RPD), and consistency.

3. Result and Discussion

3.1 Red Guava Destructive Measurement Result

The results of destructive measurements of red guava fruits are presented in Table 1.

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Table 1. Destructive Measurement Result

Picking Age (DAA)	Number of Samples	Hardness (N)	TSS (°Brix)	Total Acid (%)	Sugar to Acid Ratio (°Brix/%*Acid*)	Water Content (%)
80	20	52,23±9,21	7,75±0,53	1,03±0,29	8,14±2,26	85,37±2,62
90	20	44,95±11,76	7,76±0,49	0,77±0,22	11,08±4,23	86,56±1,19
100	20	12,87±4,48	7,89±1,01	0,46±0,13	18,89±6,98	89,02±1,09
110	20	6,58±2,35	7,45±1,16	0,47±0,12	17,19±5,68	89,25±1,13

Changes in hardness, TSS, total acid, acid-sugar ratio, and moisture content were observed concerning different picking ages (Table 1). Red guava fruits picked at the optimum ripeness (110 DAA) had a relatively higher moisture content. An increase in water content causes an increase in turgor pressure in fruit tissue, leading to a decrease in fruit hardness (Waluyo et al., 2006). The decrease in fruit hardness can also be caused by the breakdown of protopectin, which is initially insoluble in water, into pectin (Pantastico 1975). In addition, there was a relationship between the increase in TSS value and the decrease in total acid content at each picking age of red guava fruit, as described by Ana et al. (2021). The older the fruit picking age, the higher the TSS value, while the total acid content tended to decrease. The sugar-acid ratio, which was influenced by TSS and total acid values in the sample, also showed a similar trend. The older the fruit picking age, the higher the acid-sugar ratio value tends to increase.

Red guava fruits with a picking age of 100 DAA reached their maximum TSS value and minimum total acid content. At a certain limit, the fruit experiences a decrease in TSS, because the carbohydrates and sucrose contained in the fruit are used as energy sources (Arti et al., 2020). In this case, the decrease in the TSS was due to the use of sugar as a substrate for respiration to produce energy. Total acid is the total concentration of acid contained in the material (Ana et al., 2021), including citric acid, malic acid, and oxalic acid produced from the Krebs cycle (Kamaluddin 2018). This confirms the relationship between total dissolved solids (TSS) and total acid content, where a decrease in TSS is usually accompanied by an increase in the total acid content.

3.2 Absorbance Analysis of Red Guava

The absorbance value in the NIR spectrum is influenced by the chemical composition of the fruit. There is a linear correlation between the absorbance value produced in the spectra and the substance content in each sample, and infrared radiation is selectively absorbed by various chemical components at different wavelengths. This was reflected in the NIR absorbance spectrum of the red guava fruit, with peaks and valleys in the spectrum image, as shown in Figure 1.

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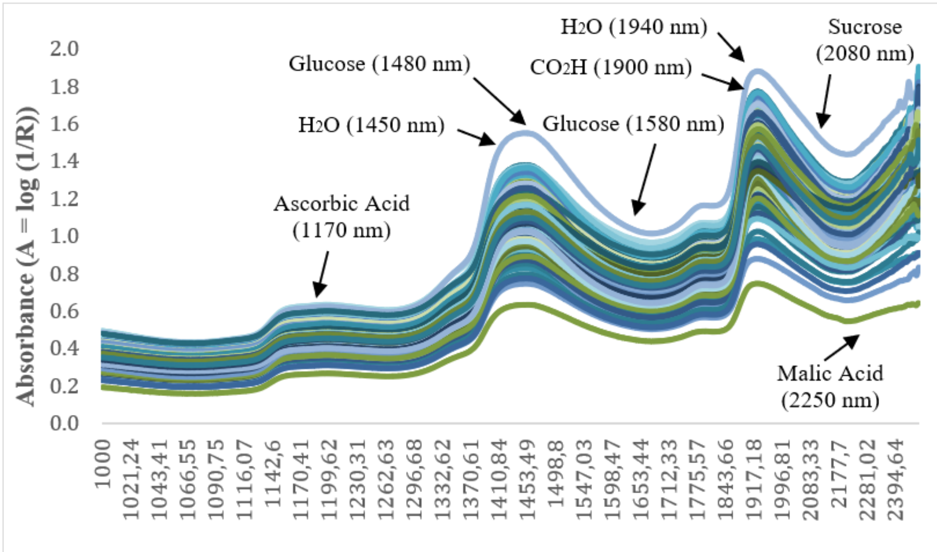

Figure 1. Original NIR absorbance spectrum of red guava fruit

The NIR absorbance spectrum in Figure 1 contains information about the components of the red guava fruit tissue. Osborne et al. (1993) reported that glucose is absorbed at wavelengths of 1480 nm and 1580 nm, while sucrose is absorbed at 1430 nm and 2080 nm. The glucose and sucrose content can be used to estimate the ripeness of red guava fruit based on the total soluble solids (TSS) value. The hardness of red guava fruit is indicated by the CO2H chemical bond, found in pectin, at a wavelength of 1900 nm. Ali et al. (2010) explained that fruit softening results from damage to the cell structure, involving the biochemical degradation of insoluble pectin into water-soluble pectin, which decreases cell wall adhesion. Water molecules, which have O-H bonds, absorb energy at wavelengths of 1450 nm and 1940 nm. According to the research results of Kusumiyati et al. (2020), wavelengths of 1170 nm and 2250 nm indicate the content of acids such as ascorbic acid, citric acid, and malic acid.

3.3 Calibration and Validation

Table 2 presents the data used in the calibration and validation processes to predict the ripeness parameters of red guava fruit. Calibration and validation models built on absorbance spectra were processed using the partial least squares (PLS) method. Calibration requires more data than validation so that the resulting model accurately represents the real situation. Validation data were selected within the calibration data range to test the accuracy of the measurements.

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Table 2. Division of Calibration and Validation Data

Parameter	Testing Stage	Number of Samples	Average	Standard Deviation	Maximum	Minimum
Hardness (N)	Calibration	160	29,47	21,45	74,75	2,94
	Validation	80	27,79	19,70	63,27	3,24
TSS (°Brix)	Calibration	147	7,69	0,67	9,00	6,10
	Validation	74	7,71	0,47	8,90	6,70
Total Acid (%)	Calibration	157	0,65	0,29	1,52	0,18
	Validation	79	0,69	0,28	1,42	0,26
Sugar to Acid Ratio (°Brix/%*Acid*)	Calibration	158	13,73	6,40	30,00	4,00
	Validation	79	13,07	5,43	28,97	6,06
Water Content (%)	Calibration	155	87,78	1,99	91,84	82,45
	Validation	77	87,86	1,52	90,94	84,67

Table 3 displays the calibration and validation results for the ripeness parameters of red guava fruit. The best calibration model was determined using certain statistical parameters, including r value, SEP, SEC, CV, and RPD.

3.4. Estimation of Read Guava Fruit Maturity Parameters

As shown in Table 3, the multiplicative scatter correction (MSC) data pretreatment method on the 14th PLS factor produced the best estimation for the hardness parameter, with a calibration correlation coefficient (rk) of 0.94. A correlation value close to 1 indicates a strong relationship between the variables x (reference data) and y (predicted data). The consistency was 105.15% and the RPD value was 2.90. Williams and Norris (2018) stated that a range of RPD values between 2 and 3 indicates a good level of fit for rough estimation. The SEC and SEP values were 7.57 N and 7.20 N, respectively. Low SEC and SEP values indicate good calibration model formation, indicating that the calibration dataset represents the validation dataset. A plot of the calibration and validation results for red guava fruit hardness obtained using the best model is shown in Figure 2a.

For the prediction of TSS parameters, calibration and validation of the original data (without pretreatment) using a PLS factor of 20 yielded the best results. The plot of the calibration and validation results of the red guava fruit TSS parameters with the best model is shown in Figure 2b. Destructive TSS measurements at four different picking ages showed almost similar TSS values, so the data distribution formed rows at certain TSS values. The correlation coefficient obtained was 0.70 for calibration and 0.43 for validation. The SEC and SEP values were 0.47°Brix and 0.47°Brix, respectively. However, the RPD value obtained from the TSS parameter was less than 1.5, which was

1.29, indicating that the model cannot be considered an accurate estimation. The coefficient of variability (CV) has a relatively small value of 6.13%, with a consistency rate of 100.50%.

Table 3. Calibration and Validation Results of Red Guava Maturity Parameters

Pretreatment	PLS	Calibration Set		Validation Set		Consistency	RPD
	Factor	r	SEC	SEP	CV%	(%)	
Violence (N)							
Original	15	0,93	7,61	7,25	26,09	104,95	2,88
Normalize	15	0,94	7,13	7,37	26,53	96,76	2,83
SNV	15	0,95	6,95	7,52	27,07	92,35	2,77
Detrending (PO = 1)	16	0,94	7,02	8,01	28,83	87,67	2,6
MSC	14	0,94	7,57	7,2	25,91	105,15	2,9
Total Soluble Solids (°Brix)							
Original	20	0,7	0,47	0,47	6,13	100,5	1,29
Normalize	18	0,67	0,49	0,48	6,22	102,83	1,27
SNV	17	0,68	0,49	0,49	6,32	100,77	1,25
Detrending (PO = 2)	18	0,7	0,48	0,46	5,99	103,56	1,32
MSC	16	0,66	0,5	0,47	6,13	106,19	1,29
Total Acid (%)							
Original	13	0,82	0,16	0,17	24,42	96,98	1,68
Normalize	9	0,79	0,17	0,17	24,94	100,89	1,64
SNV	10	0,8	0,17	0,17	24,74	98,95	1,66
Detrending (PO = 2)	10	0,8	0,17	0,17	25,14	97,44	1,63
MSC	11	0,81	0,17	0,17	24,93	95,96	1,64
Sugar to Acid Ratio (°Brix/%Acid)							
Original	18	0,83	3,58	4,37	33,44	82,01	1,39
Normalize	12	0,76	4,15	4,17	31,9	99,43	1,46
SNV	10	0,75	4,24	4,03	30,81	105,24	1,51
Detrending (PO = 2)	16	0,82	3,68	4,38	33,54	83,87	1,39
MSC	10	0,75	4,24	4,08	31,2	104	1,49
Water Content (%)							
Original	18	0,9	0,88	1	1,14	87,58	1,83
Normalize	12	0,87	0,98	0,95	1,08	102,61	1,93
SNV	13	0,88	0,95	0,95	1,08	100,89	1,95
Detrending (PO = 1)	19	0,91	0,83	0,98	1,12	84,98	1,88
MSC	13	0,88	0,95	0,96	1,09	99,74	1,92

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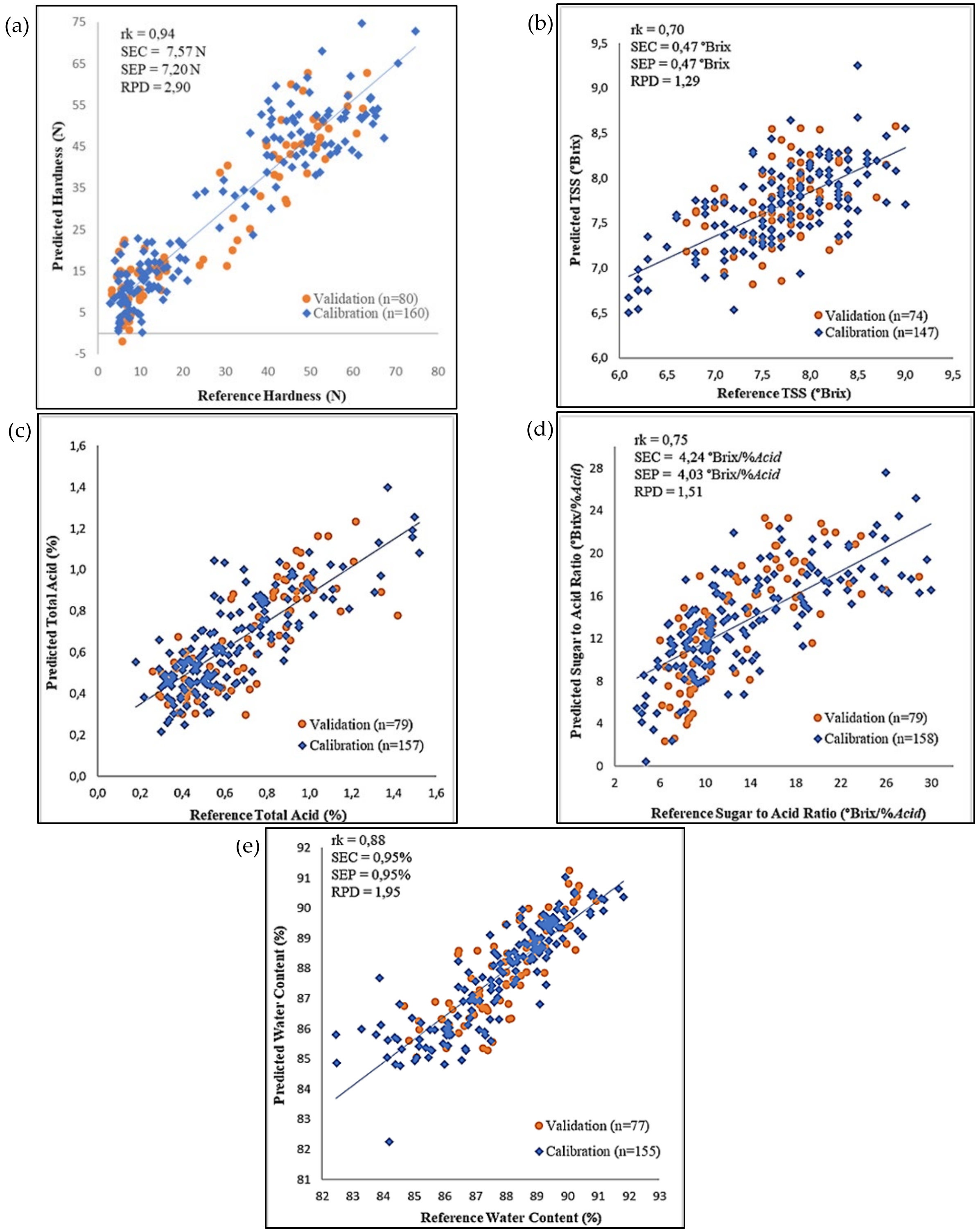

Figure 2. Calibration and validation plots of (a) hardness with MSC data pretreatment and (b) TSS with the original data. (c) Total acid with original data, (d) acid-sugar ratio with SNV data pretreatment, and (e) moisture content with SNV data pretreatment.

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For the total acid parameter, the best estimation was obtained without using data pre-treatment, namely, with the original data. Using 13 PLS factors, a rk value of 0.82 was achieved with a consistency of 96.98%. This value indicates that the calibration and validation results can be used to estimate the maturity parameters of red guava fruit. The obtained RPD value was greater than 1.5, that is, 1.68. The RPD value was an approximate estimate. Figure 2c displays a plot of the calibration and validation results for the total acid content of red guava fruit, derived from the best model.

The optimal estimation of the acid-sugar ratio parameter was accomplished through standard normal variate (SNV) data pretreatment, yielding a calibration correlation coefficient (rk) of 0.75 with a PLS factor of 10. The RPD value obtained was 1.51, with a consistency level of 105.24. Figure 2d displays the best model plot of the calibration and validation results for the acid sugar ratio parameter using the SNV pre-treatment.

The SNV data pretreatment method produced the best estimation of the moisture content. A calibration correlation coefficient (rk) of 0.88 was obtained using a PLS factor of 13. The SEC and SEP values are 0.95% and 0.95%, respectively. The RPD and consistency values obtained were 1.95 and 100.89, respectively. A plot of the calibration and validation results for the moisture content parameter using the best model is shown in Figure 2e.

The performance of the model in predicting red guava fruit ripeness was evaluated based on several factors including the correlation coefficient in the calibration and validation stages, Standard Error of Calibration (SEC), Standard Error of Prediction (SEP) value, coefficient of variability (CV), consistency, and Ratio of Performance to Deviation (RPD). Hardness, total acid content, acid sugar ratio, and moisture content are considered suitable for predicting the ripeness of red guava fruit. However, the TSS parameter did not meet the required criteria as the RPD value obtained did not exceed 1.5. Therefore, the TSS parameter is not accurate in predicting the ripeness of red guava fruit. To improve the prediction accuracy for each parameter, it is advised to increase both the number of samples and the data diversity at each picking age.

4. Conclusion

The best result for hardness parameter prediction was obtained using MSC data pre-treatment with a correlation value (R) of 0.94 and RPD of 2.90. Meanwhile, the best prediction results for the TSS parameters used the original data, with a correlation of 0.70 and an RPD of 1.29. Similarly, for the total acid parameter, the most optimal correlation value was achieved when using the original data, with a value of 0.82 and an RPD of 1.68. The most accurate prediction for the acid-sugar ratio parameter was obtained using SNV pre-treatment, yielding a correlation of 0.75 and an RPD of 1.51. For the moisture content parameter, SNV pre-treatment also provided the best prediction, with a correlation of 0.88 and an RPD of 1.95. Therefore, the hardness and moisture content of red guava fruit can be

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accurately predicted using NIR spectroscopy. However, the NIR method could not accurately predict the TSS, total acid, and acid-sugar ratio parameters.

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