Research Article

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Machine learning-based prediction of total phenolic and flavonoid in horticultural products

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Abstract: The purpose of this study was to predict the total phenolic content (TPC) and total flavonoid content (TFC) in several horticultural commodities using near-infrared spectroscopy (NIRS) combined with machine learning. Although models are typically developed for a single product, expanding the coverage of the model can improve efficiency. In this study, 700 samples were used, including varieties of shallot, cayenne pepper, and red chili. The results showed that the TPC model developed yielded R^2cal, root mean squares error in the calibration set, R^2pred, root mean squares error in prediction set, and ratio of performance to deviation values of 0.79, 123.33, 0.78, 124.20, and 2.13, respectively. Meanwhile, the TFC model produced values of 0.71, 44.52, 0.72, 42.10, and 1.87, respectively. The wavelengths 912, 939, and 942 nm are closely related to phenolic compounds and flavonoids. The accuracy of the model in this study produced satisfactory results. Therefore, the application of NIRS and machine learning to horticultural products has a high potential of replacing conventional laboratory analysis TPC and TFC.

Keywords: chemometrics, multivariate data analysis, near-infrared spectroscopy, nutritional compounds, rapid measurement

1 Introduction

Natural antioxidants are produced from plants with secondary metabolites such as phenols and flavonoids. Phenolic compounds are the largest group of compounds that act as natural antioxidants. Furthermore, polyphenols are the most common types of natural phenolic compounds from which ether, ester, or glycoside compounds, such as flavonoids, tannins, tocopherols, coumarins, lignins, cinnamic acid derivatives, and polyfunctional organic acids, are produced. These phenolic compounds influence the sensory properties of food, with tannins contributing significantly to food astringency. The ability of antioxidants to reduce free radicals increases as the total phenolic and flavonoid levels rise [1].

Chemical analysis in the laboratory is generally used to determine quality attributes of horticultural products, which requires a significant amount of time and money. It also generates chemical waste, which can harm the environment [2]. Therefore, a technique for measuring the quality attributes of horticultural commodities without the need for chemical analysis in the laboratory is required.

In recent decades, various techniques have been developed as alternatives to chemical analysis, including hyperspectral imaging [3], visible/near-infrared spectroscopy [4–6], acoustic vibration [7,8], nuclear magnetic resonance [9], and electronic nose [10]. Near-infrared spectroscopy (NIRS) has several advantages over other techniques, including the ability to predict organic samples in solid, liquid, and gaseous forms. Various studies have shown that the accuracy of NIRS, when used on agricultural products, is generally high. Nieto-Ortega et al. [11] used NIRS to predict the non-polysaccharide content in monogastric cereal feed ingredients and obtained an average accuracy value (R^2) of 0.90. According to Digman and Runge [12], NIRS performed excellently (R^2 = 0.86) in predicting the maturity of green peas. Reis [13] also concluded that NIRS showed satisfactory performance in predicting meat attributes. Furthermore, the NIRS tool is easy to use, allowing beginners to use it with ease.

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The agricultural industry has begun implementing automation in areas such as harvesting, watering, fertilizing, and pesticide application. Machine learning is a branch of artificial intelligence that allows a machine to learn from data. It is also the application of computers and mathematical algorithms to generate future predictions through data-driven learning [14]. In this case, machine learning was combined with NIRS to predict quality attributes and classify agricultural commodities without damaging them. The application of NIRS and machine learning allows for automation in grading agricultural commodities based on prediction results of desired quality attributes.

Most NIRS studies only develop models for specific products, such as apples [15,16], peaches [17], pears [18], and lemons [19,20]. Developing a model that predicts the quality attributes of various agricultural commodities can increase its efficiency. Therefore, this study aimed to predict quality attributes, such as total phenolic and flavonoid contents using NIRS combined with machine learning on several horticultural commodities. This study also tested several spectra preprocessing methods for reducing noise in the spectral data.

2 Materials and methods

2.1 Sample preparation

The samples used in this study were shallot (var. Batu Ijo, var. Bima, var. Trisula, and var. Sumenep), cayenne pepper (var. Domba, var. Manik, and var. Ratuni UNPAD), and red chili (var. UNPAD CB2, var. Lingga, var. Tanjung, and var. Tanjung 2). Planting took place at an altitude of 829 m above sea level (masl), with average daily temperatures ranging from 20 to 30°C. The harvested samples were in good condition and free of pests and diseases before transferring them to the Laboratory of Horticulture, Faculty of Agriculture, Universitas Padjadjaran, for further analysis. The sample was thinly sliced, dried in an oven at 60°C for 24 h, and ground with a mortar until smooth [21]. Furthermore, powdered samples were prepared for spectral data collection.

2.2 Spectra data acquisition

NirVana AG410 (Integrated Spectronics Pty, Ltd., North Ryde, Australia) with a wavelength of 702–1,065 nm was used for data collection of spectra samples. The sample was placed in a Petri dish with a black cardboard base. Each sample was then scanned four times before obtaining the spectra data from the mean value of the measurements. Spectral data in this study were collected in diffuse reflectance mode and then converted into absorbance values.

2.3 Phenolic and flavonoid analysis

Total phenolic content (TPC) and total flavonoid content (TFC) measurements were carried out using a UV–Vis spectrophotometer (Shimadzu, UV mini-1240, Tokyo, Japan). The Folin–Ciocalteu method, developed by Lim and Murtijaya, was used to measure TPC [22]. The extracted filtrate was mixed with 2.5 mL of Folin–Ciocalteu reagent, followed by 2 mL of sodium carbonate. After incubation for 1 h, the filtrate was measured at a wavelength of 765 nm. Sytar et al. developed a procedure for measuring TFC [23]. The extracted filtrate was mixed with 2 mL of methanol and 0.1 mL of $AlCl_3$. Subsequently, 1 M sodium acetate and 2.3 mL of water were added, before incubating for 30 min and measuring at 432 nm.

2.4 Multivariate data analysis

Preprocessed of the sample absorbance spectra was performed using multiplicative scatter correction (MSC), standard normal variate (SNV), moving average (MA), baseline correction, de-trending, first derivative (dg1), and second derivative Savitzky–Golay (dg2). The spectra preprocessing was done to reduce the variety of spectra produced by light interference, temperature differences during data collection, and background information [24,25]. Furthermore, spectra preprocessing was expected to improve the predictive ability of the developed model [26]. Partial least squares (PLS) regression was used in model development for predicting phenolic (TPC), and flavonoid content (TFC), while data analysis and model development were carried out using the Unscrambler X 10.4 software (Camo AS, Oslo, Norway).

3 Results and discussion

3.1 Measured data analysis

Table 1 shows the TPC and TFC of various horticultural commodities. Several factors influence physiological

properties in agricultural products, including genetics, growing environment, plant cultivation techniques, post-harvest handling, and analytical methods. Cayenne pepper var. Ratuni UNPAD had values ranging from 1136.14 to 1982.53 mg/100 g dry weight (DW), with the highest mean TPC value being 1453.87 mg/100 g DW. Meanwhile, shallot var. Sumenep had the lowest mean TPC value of 455.79 mg/100 g DW, with values ranging from 276.86 to 734.64 mg/100 g DW. Shallot var. Trisula showed the highest mean TFC value of 310.33 mg/100 g DW, with values ranging from 57.02 to 587.80 mg/100 g DW, while shallot var. Batu Ijo had the lowest total flavonoid of 45.28 mg/100 g DW, with values ranging from 18.09 to 73.87 mg/100 g DW. The difference in TPC and TFC values for each commodity and variety is due to differences in chemical composition. The range of TPC and TFC for powdered samples in this study has values similar to that reported in previous studies [27,28]. However, the analytical method for determining TPC and TFC affects the measurement results. In this study, phenolic compounds are sensitive to high temperatures, but the samples used were powdered samples that had been oven-dried. Therefore, the findings in this study are lower than previous reports on TPC and TPF in fresh samples [29,30].

3.2 Spectra characteristics

The absorbance data reflect the characteristic spectra displayed by the sample. Differences in commodity, variety, sample surface, and temperature affect the spectral data obtained. In this study, spectral data with a wavelength of 702–1,065 nm covering the near-infrared region for model development were used. These areas detect various quality attributes in agricultural commodities, including water content, starch, sugar, and other physicochemical properties [31–33]. Moreover, several other studies reported using NIRS to monitor adulteration in agricultural commodities, fruit pesticide residues, and early disease detection in potatoes [34–37]. One of the challenges in analyzing NIRS data is the amount of noise in the sample spectra. Therefore, special handling is sometimes required to reduce the noise. The sample spectra data were analyzed using various preprocessing methods before the model development stage. Based on the results of the spectral preprocessing shown in Figure 1, the peak was not visible in the 702–1,065 nm region in the original spectra, as well as in the MSC and MA spectra. Furthermore, the SNV and MSC methods are similar in that they normalize data by reducing the effect of multiplicative and light scattering on the original spectra. In several other studies, the application of MSC and SNV showed similar spectral patterns, but this was not the case in this study. MA works by averaging adjacent points and using the value as a new point. Although the detrending method is generally used to process grain samples [38], it was used in processing powder samples in this study. Several high peaks were seen in the baseline, SNV, detrending, dg1, and dg2 spectra at 840, 900, 940, and 1,025 nm. The peak was clearly visible, particularly in the detrending spectra, dg1 and dg2. The Savitzky–Golay derivative method (dg1 and dg2) is typically used to detect compounds in small concentrations. Therefore, the peaks in the spectral data are more clearly observed with this method. It is necessary to have the right strategy in determining the spectra preprocessing method since the Savitzky–Golay derivative method sometimes increases the error value in the resulting model. Despite preprocessing, this study was

Table 1: Wet chemistry data of total phenolic (TPC) and flavonoid content (TFC) derived from several horticultural products

Commodity	Variety	TPC (mg/100 g DW)		TFC (mg/100 g DW)	
		Range	Mean	Range	Mean
Shallot	Batu Ijo	270.65–669.70	526.18	18.09–73.87	45.28
	Bima	614.18–1612.53	944.64	65.78–475.59	212.66
	Trisula	717.36–1531.78	1045.32	57.02–587.80	310.33
	Sumenep	276.86–734.64	455.79	48.02–461.12	174.93
Cayenne pepper	Domba	1129.29–1358.46	1221.30	162.69–210.31	187.03
	Manik	1025.49–1279.43	1153.44	169.75–232.72	204.98
	Ratuni UNPAD	1136.14–1982.53	1453.87	30.83–625.56	117.50
Red chili	UNPAD CB2	784.16–1418.33	1078.20	76.88–324.29	224.60
	Lingga	893.42–1242.37	1065.22	112.98–282.14	173.19
	Tanjung	606.47–1571.25	945.63	112.34–406.96	243.87
	Tanjung 2	968.61–1209.78	1082.45	167.46–419.49	262.03

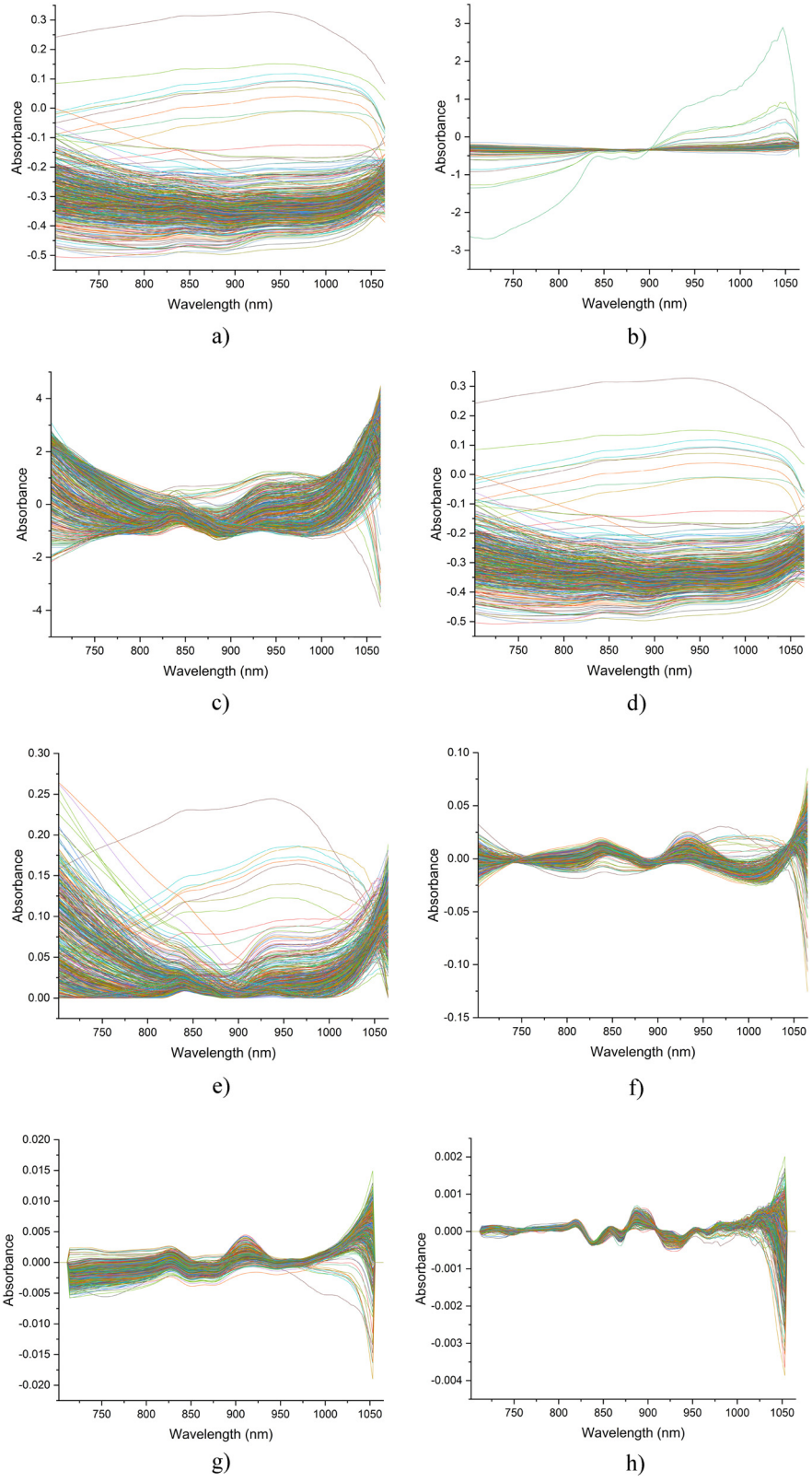

Figure 1: Original and preprocessed spectra of samples. (a) Original, (b) MSC, (c) SNV, (d) MA, (e) baseline, (f) detrending, (g) dg1, and (h) dg2.

still unable to determine the best spectra preprocessing method for this dataset. This can be determined after modeling the results of each preprocessing spectra.

3.3 Model development

Regression modeling involves spectral and wet chemistry data. As shown in Table 2, 525 samples were assigned to develop a regression model using PLS, while 175 samples were used to test the reliability of the developed model. The regression model was then validated using *K*-fold cross-validation, and the samples were divided into 20 segments, each segment containing 26–27 samples. This cross-validation aimed to determine the optimum principal components and avoid overfitting, a condition in which the model fails to predict an unknown sample. A good model is expected to have a high R^2 and a low error in the calibration and prediction set [39]. Therefore, to improve the accuracy of the developed model, various spectra preprocessing methods were tested. The determination of the best model was based on the coefficient of determination in the calibration set (R^2cal), root mean squares error in the calibration set (RMSEC), coefficient of determination in the prediction set (R^2pred), root mean squares error in prediction set (RMSEP), and the ratio of performance to deviation (RPD). However, when compared to other results, the original spectra (without preprocessing) presented the best model in this study. R^2cal, RMSEC, R^2pred, RMSEP, and RPD values for the TPC model were 0.79, 123.33, 0.78, 124.20, and 2.13, while the TFC model had values of 0.71, 44.52, 0.72, 42.10, and 1.87, respectively. This phenomenon sometimes occurs in NIRS data analysis, where preprocessed spectra do not improve model accuracy. It is possibly due to the high variability of the dataset in this study. Spectra preprocessing reduces the variability or diversity in data spectra samples. Therefore, the original spectra had the best performance on both quality attributes. Similar results were obtained in a study conducted by Zhao et al. [40], who found that the original spectra outperformed the MSC and SNV preprocessing results. Rubini et al. [41] reported that the model developed from the SNV spectra was less accurate than the original spectra in predicting levopimaric acid and turpentine in maritime pine.

Figure 2 shows scatter plots for TPC and TFC obtained from the best model, which is the original spectra. The red data distribution is the result of the analysis of the calibration set, while the yellow one is the prediction set. The closer the regression line data distribution, the better the resulting model. PLS is the most commonly used regression method for NIRS data analysis. The advantage of PLS is that it can reduce correlated independent variables (spectral data) and convert them into new, uncorrelated variables. PLS also involves the dependent variable (wet chemistry data) in forming these new variables. Therefore, the new variable contains information from the dependent variable, affecting good model accuracy. Kusumiyati et al. [42,43] conducted a study using NIRS

Table 2: PLS regression results for prediction of total phenolic (TPC) and flavonoid content (TFC) in horticultural products

Trait	Preprocessing	Calibration (n = 525)		Prediction (n = 175)		RPD
		R^2cal	RMSEC	R^2pred	RMSEP	
TPC	**Original**	**0.79**	**123.33**	**0.78**	**124.20**	**2.13**
	MSC	0.53	185.16	0.50	187.87	1.41
	SNV	0.75	136.12	0.70	143.87	1.84
	MA	0.77	128.61	0.75	131.78	2.01
	Baseline	0.77	128.07	0.75	130.89	2.02
	Detrending	0.77	128.88	0.75	130.64	2.02
	dg1	0.75	135.10	0.70	143.53	1.84
	dg2	0.74	138.00	0.70	145.04	1.82
TFC	**Original**	**0.71**	**44.52**	**0.72**	**42.10**	**1.87**
	MSC	0.49	58.88	0.57	52.70	1.53
	SNV	0.66	47.85	0.69	44.95	1.80
	MA	0.70	44.88	0.72	43.05	1.88
	Baseline	0.70	45.37	0.67	46.22	1.75
	De-trending	0.65	48.65	0.63	48.68	1.66
	dg1	0.67	47.26	0.67	45.84	1.76
	dg2	0.65	48.66	0.67	46.02	1.75

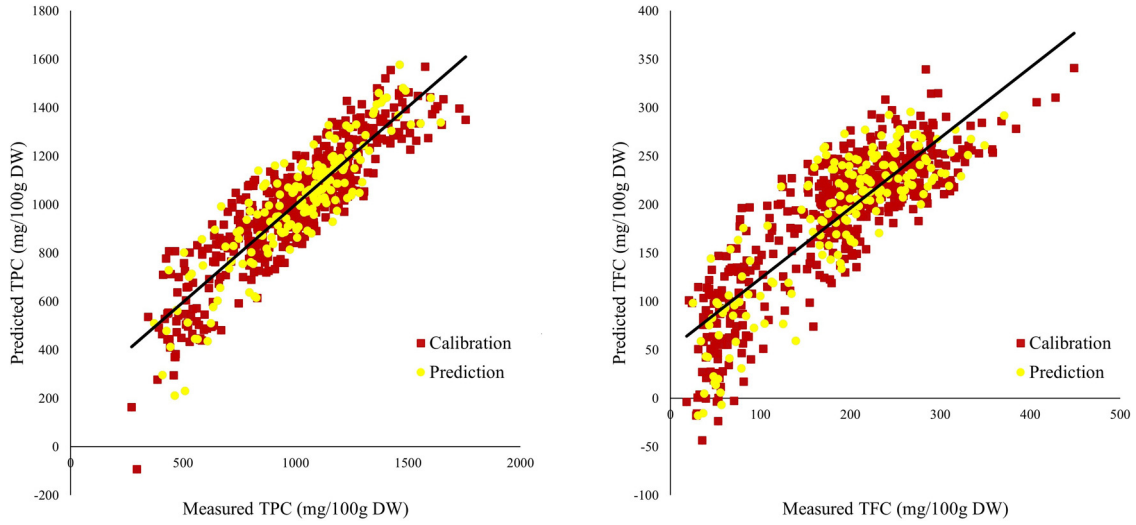

Figure 2: Scatter plot for calibration and prediction of total phenolic (TPC) and flavonoid content (TFC) in horticultural products.

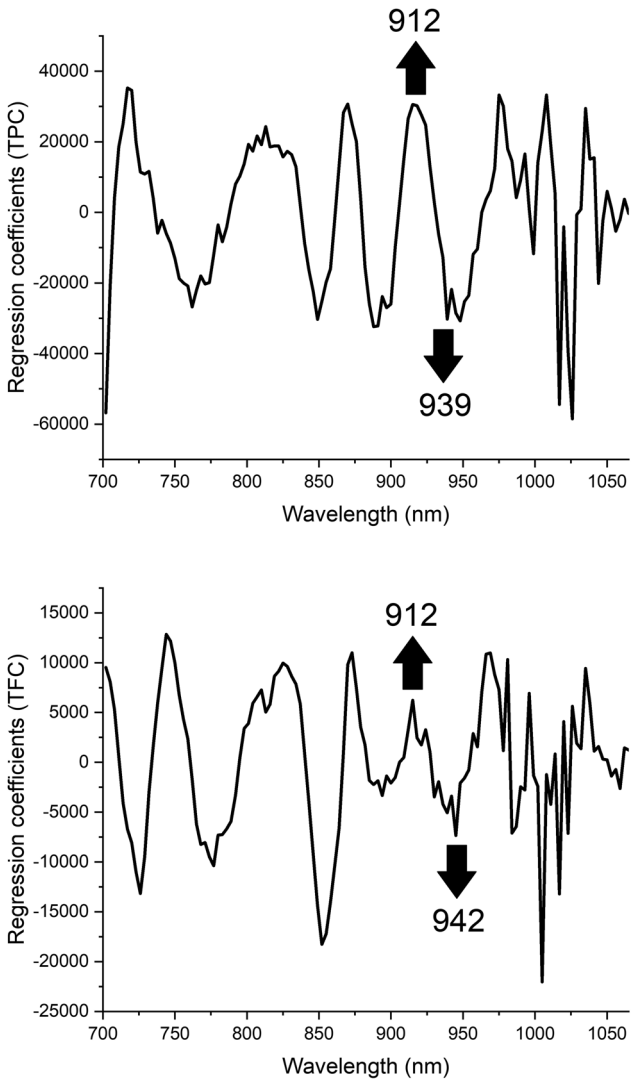

Figure 3: The regression coefficient of a machine learning model for predicting total phenolic (TPC) and flavonoid content (TFC).

combined with PLS regression to predict SSC in sapodilla and mango. The results revealed that NIRS combined with PLS is effective for SSC prediction. Furthermore, accuracy in model development is influenced by various factors, including data variability, modeling techniques, and instruments [44].

Agricultural commodities, particularly horticulture, have a complex chemical composition that comprises a large set of overtones and combination bands. In this research, PLS regression employed NIRS spectra and chemical composition, specifically TPC and TFC. The regression coefficient is utilized for understanding the calibration model resulting from PLS regression. As shown in Figure 3, regression coefficients indicate which wavelength contributes the most to the modeling of each quality attribute, and these important wavelengths are characterized by peaks and valleys. The regression coefficients were analyzed from the best model on both quality attributes, namely the model developed using the original spectra. Furthermore, peaks at wavelengths 912, 939, and 942 nm are associated with the third CH overtone and the second overtone of OH vibrations. These are also associated with the detection of antioxidants and phenol groups [45].

4 Conclusion

Based on the result of this study, NIRS can be combined with machine learning to predict TPC and TFC in horticultural products. The results show that the developed model has a fairly good performance with $R^2 > 0.7$ and that spectra preprocessing did not increase model accuracy. It could be because of the huge variance of the input data. Spectra preprocessing generally operates by minimizing the variability of data. Hence, the models generated from the preprocessed spectra performed no better than the original spectra. Furthermore, the wavelength of 900–950 nm is essential in developing TPC and TFC models. Therefore, the application of NIRS and machine learning is reliable enough to replace conventional measurement methods for TPC and TFC.

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