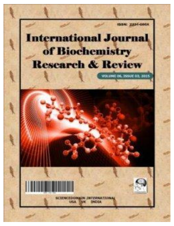

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Nondestructive Analysis of Litchi Fruit Quality Using FT-NIR Spectroscopy

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Authors' contributions

This work was carried out in collaboration between both authors. Author NSR designed the study, supervised the work, managed the analyses of the study and wrote the protocol and the first draft of the manuscript. Author BKY managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

A non-destructive optical method based on near-infrared spectroscopy has been used for the evaluation of litchi fruit quality. Diffuse reflectance measurements (12500–3600 cm^{-1}), physical, and biochemical measurements were performed individually on 100 litchi fruits of cv. Shahi cultivar harvested at different ripening stages. Relationships between spectral wavelengths and quality attributes were evaluated by application of chemometric techniques based on partial least squares (PLS) regression. The fruit set was divided into two groups: 60 fruits for calibration and 39 for validation. Good prediction performance was obtained for pH, soluble solids, and titratable acidity with correlation coefficients of 0.96, 0.91 and 0.94 respectively and root mean square errors of prediction of 0.009, 0.291ºBrix and 0.011% malic acid respectively. For the other quality traits such as vitamin C and color the prediction models were not satisfactorily accurate due to the high error of calibration and prediction.

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Keywords: FT-NIR technique; soluble solids content (SSC); pH, titratable acidity (TA); vitamin C; litchi; partial least square (PLS); non-destructive.

1. INTRODUCTION

Litchi (*Lychee chinensis* Sonn.), is a popular subtropical to tropical fruit belonging to the family *Sapindaceae*, originated in China, with high commercial value in a global trade. And due to its delicious, juicy, refreshing taste and nutritive value litchi consumption has increased significantly in recent years. India is the second largest producer of litchi and its productivity is higher as compared to China [1]. India and China account for 91 percent of the world litchi production but it is mainly marketed locally. In recent years, the market potential of litchi is recognized internationally by other countries. However, the highly perishable nature of the litchi fruit causes difficult to withstand the long-distance shipments. Therefore, it is more essential for the higher quality and more consistent fresh litchi at the origin country, in order to meet the quality standards upon arrival at the end location.

Quality is an important feature in determining the consumer acceptance of any product, including fruits [2]. The quality traits of a fresh litchi include appearance (size, shape, color, gloss, and free from defects and decay) and internal quality (firmness, titratable acidity, soluble solids content, pH, and vitamins). While external appearance can be evaluated by modern imaging or computer vision technology [3,4,5,6,7], whereas internal quality aspects are normally measured by traditional analytical methods. Among the internal quality attributes of a lychee, soluble solids content (SSC) and acidity are the properties most likely to match the consumer's perception and probably the most important internal quality indicator of fruit maturity and postharvest quality of a lychee. Most instrumental methods used to measure these properties are based on complex preparation of samples, using expensive chemicals and involving a considerable amount of work. Furthermore, these methods are also destructive. Therefore, there is a demand for the development of nondestructive measurements of these quality attributes to meet the quality requirements and to improve the competitiveness of the fruit production industry to ascertain fast evaluation.

Near-infrared (NIR) technique is a fast and nondestructive technique, which can obtain internal information of a product by measuring the amount of light absorbed by functional groups over the NIR range without or with little sample preparation. Optical VIS/NIR spectroscopy (400-2500 nm) or only near-infrared spectroscopy (800-2500 nm) have been tested for nondestructive evaluation of soluble solids, acidity and other physiological properties of many fruits and vegetables such as apple [8,9,10], tomato [11], pear [12], grape [13,14], pineapple [15], kiwifruit [16], sweet cherry [17], watermelon [18], avocado [19], mandarin [20] etc., showing the capability of spectroscopic technique for the prediction of fruit internal quality characteristics. In litchi, however, there had been very limited literature on the use of the spectroscopic technique for quality estimation. [21] conducted a hyperspectral investigation for soluble solids and pH prediction in litchi in the visible/long-wave near infrared range (1000-2500 nm). The main advantage of NIR spectroscopy is that, once best PLS models are developed, it allows a non-destructive and individual characterisation of fruits, with the simultaneous prediction of several quality attributes. It offers the feasibility of 'on-line' screening of the fruits and estimation of fruit quality which open new objective of market segmentation and fruit valorisation in a fresh or processed market [22].

The objective of this study was to investigate the potential of near-infrared spectroscopy in diffuse reflectance mode for predicting litchi quality traits such as soluble solids, titratable acidity, pH, and vitamin C contents through the comparison with standard techniques. Samples harvested at different stages of ripening were used as a calibration set. The prediction models for each quality parameters were developed with partial least square (PLS) technique.

2. MATERIALS AND METHODS

2.1 Litchi fruit samples

Fresh litchi fruits (*Litchi chinensis* Sonn. “Shahi”) were obtained from the commercial orchard in Coorg, India, from December to January 2016. Fruits were manually picked during commercial off-season period, transported immediately to the laboratory.

2.1.1 <u>Preparation of samples for spectral study</u>

A total of 99 litchi were selected for the study. Before the spectral analysis of the samples, all litchi fruits were manually classified into three groups based on visually rated pericarp colour for representing immature, mature and browning stages with each groups consisting of 33 lychee fruits such that to distribute equally into calibration and prediction easily. The fruits containing equal or more than 30% green area was classified as immature fruits. The fruits which are having pericarp in brown color are considered as browning sample. The group was randomly selected for spectral analysis and the tested fruits in each maturity stage were divided into two subgroups. Subgroup 1 consisted of 60 fruits with 20 samples for each maturity stages, which were used as a training set for developing PLS calibration models. Subgroup 2 consisted of 39 fruits with 13 samples for each maturity stages and were used to verify the prediction power of the calibration models. Spectroscopic measurements were performed the day of picking and quality measurements were carried out after a day upon refrigerated storage.

2.2 FT-NIR Measurements

The spectral data were recorded on a Fourier Transform Near Infra-Red (FT-NIR) spectrometer (Bruker Optics, MATRIX-I, Germany) equipped with an integrating sphere to provide diffuse reflectance measurements and a lead sulphide (PbS) detector. The MATRIX-I was completely software-controlled by the OPUS software Version 7.2 which was provided by Bruker Optics. The NIR spectrum of each sample was obtained by taking the average of 64 scans. It was acquired between 12500 and 3600 cm^{-1} at 8 cm^{-1} spectral resolution, with scanner velocity of 10 kHz and a background of 64 scans. The time required to achieve a spectral measurement was 30 s. Litchi samples were placed steadily upon the fruit holder, with their stem–calyx axis horizontal. On each fruit, a diffuse reflectance spectrum was measured on two opposite sides. Before sample spectra collection, the standard reference spectrum was obtained by placing a Teflon block on the fruit holder and measuring the intensity of reflected light.

2.3 Determination of Quality Traits Using Reference Analyses

After acquiring the spectral measurements, each fruit was peeled and the pulp portion was juiced to determine quality attributes such as pH, SSC, etc., at room temperature of 25°C. The pH of the lychee juice was determined with a pH meter, and soluble solids content was determined using a digital refractometer, expressed in °Brix at 20°C. Titratable acidity was determined by a titration method with 0.1 N NaOH and expressed in % malic acid. Vitamin C content was measured by titration method by 2, 6 Dichlorophenol Indophenol. And the pericarp colour was determined using a Hunter color lab and expressed in L*, a*, b* values.

2.4 Developing Model to Predict Internal Quality of Fruit

2.4.1 Preprocessing of data

Three different data pre-processing techniques were considered: SNV (Vector Normalization), First Derivative (FD), and first derivative plus vector normalization (FD+SNV). These data techniques are normally used to eliminate the irrelevant information from spectra due to unknown sources such as surface irregularities, distance variation of sample and detector [17].

2.4.2 Developing PLS regression model

The PLS regression method was used to develop the models for predicting the composition of litchi fruits. The wavenumber ranges between 12500 and 3600 cm^{-1} were analysed to find the optimal sub- wavenumber ranges that would yield the best correlations between the spectral data and physical parameters. The non-informative regions were tentatively purged and the resulting performances were estimated.

The performance of the calibration models were evaluated by the root mean square error of cross validation (RMSECV), the root mean square error of prediction (RMSEP) and the coefficient of determination R^2 between the predicted and the measured parameters. Acceptable models should have low RMSECV and RMSEP, high R^2 and small differences between RMSECV and RMSEP. All absorbance spectra (abs=log [1/R], R: reflectance) were analysed using QUANT software (version 7.2, Bruker optics, Germany), which performs partial least square regression (PLSR) technique for developing models.

2.4.3 Selection of optimal wavenumber range for developing PLS modelling

The wavenumber region selection is critical in developing a robust calibration model. Wavenumbers where the data were noisy and provided little predictive ability were eliminated prior to selection of regressions. And the selection of the optimum wavenumber range for the best calibration model was done by PLS analysis in OPUS software v7.2 on the basis of containing spectral peaks to be utilized for prediction of quality parameters.

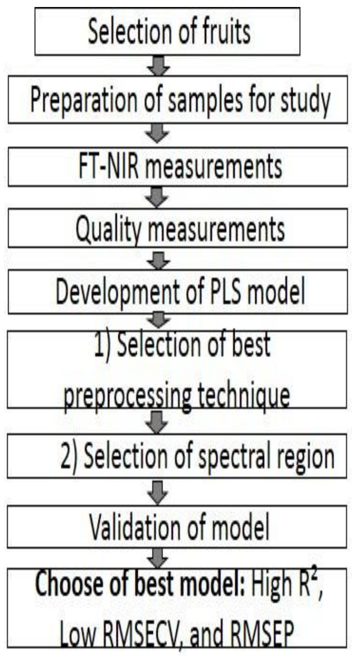

Fig. 1. Flow chart of design of experiment

2.4.4 <u>Design of Experiment</u>

The below flow chart represents the process of present whole study that starts from collection of fruit samples to the final predictive model. The model performance depends on selection of wavenumber region and preprocessing technique. In this study NIR spectra is independent whereas reference values are dependent.

3. RESULTS AND DISCUSSION

The general profile of the absorption spectra for litchi measured with FT-NIR spectrometer between 12500 and 3600 cm^{-1} is presented in Fig. 2. This full range known to contain important carbohydrate, sugar, and water absorbance. It is evident from the figure that the absorbance values is higher with lower value of wave number, i.e., the highest at 3600 cm^{-1}.

In the resulted spectra of litchi, the absorption bands around 6996 cm^{-1} and 10,244 cm^{-1} are related to the –O-H first and second overtones of water, respectively and bands at 5586.59 cm^{-1} and 8403.36 cm^{-1} are related to the C-H first and second overtones, respectively. The absorbance at 5154.64 cm^{-1} is related to the –O-H combination band of water. It was similar to that of other plant materials like, apple [23] and mandarin [20]. A synthetic view of the observed quality traits is presented below (Table 1).

3.1 Prediction of Soluble Solids Content (SSC, °Brix)

The values obtained for litchi were ranging from a minimum of 10.4 to a maximum of 17.2 °Brix. While the values obtained from FT-NIR spectroscopy were in the range of 10.2 to 16.55°Brix shown in Table 1. The correlation between NIR measurement and SSC of litchi was good with coefficient of determination (R^2) equal to 0.91 and root mean square error of cross validation (RMSECV) of 0.352°Brix. When the model was applied to predict 39 other litchi fruits, the prediction results

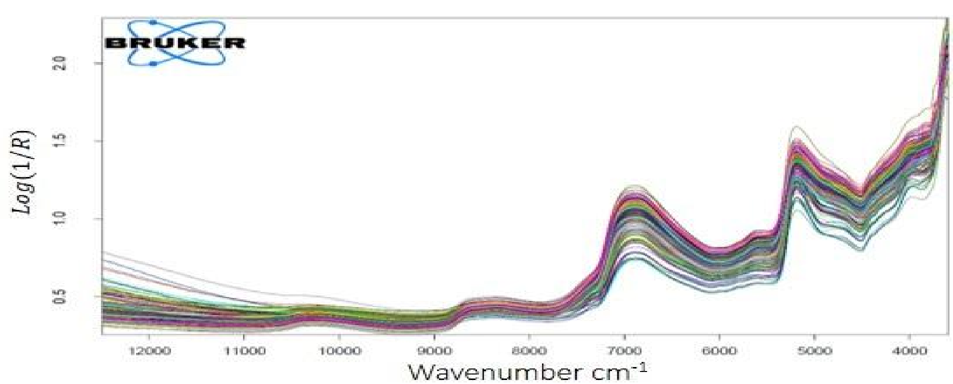

Fig. 2. Typical original spectra (log [1/R]) for litchi samples

Table 1. Range, mean, and standard deviation (SD) of the litchi quality traits in both calibration and prediction sample sets

Quality parameters	Calibration (60)			Prediction (39)		
	Range	Mean	SD	Range	Mean	SD
SSC (°Brix)	10.4-17.2	14.42	1.64	12.55-16.6	14.55	0.96
pH	3.59-3.87	3.73	0.07	3.61-3.87	3.68	0.08
TA (% malic acid)	0.54-0.83	0.69	0.09	0.66-0.82	0.73	0.03
Vitamin C (mg 100 ml^{-1} Juice)	19.31-27.59	23.63	2.93	21-27.3	25.04	0.87
L*	21.66-27.8	24.62	1.98	22.71-27.56	25.17	0.97
a*	10.3-11.09	10.68	0.24	10.3-11.09	10.89	0.21
b*	13.67-16.35	15.17	0.85	12.8-16.35	15.11	0.73
Weight(g)	13.8-20.2	16.76	1.72	14.26-19.74	17.04	1.30

were similar with R^2 of 0.91 and root mean square error of prediction (RMSEP) of 0.291°Brix (Table 2). The best PLS model for measuring the SSC was obtained in the wave number range of 8269.7 – 7498.3 cm^{-1} and 6102 – 5446.3 cm^{-1} by following vector normalization (SNV) of the spectra that has given as maximum as coefficient of determination (R^2) of 0.91. The linear regression plot between the experimentally estimated values and the values obtained by FT-NIR spectroscopy is given in Fig. 3(a). The linear equation was in the form of ‘y = 0.9165x + 1.2154’ for the developed model.

The regression value obtained for the validation in this work was similar to those obtained on other fruits. Results found on ‘Satsuma’ mandarin with R^2= 0.96 [23], on ‘Fuji’ apple with R^2 = 0.97 [23] and on prune with R^2 = 0.98 [24]. In these quoted references, RMSEP from 0.16 to 0.52 °Brix were obtained.

3.2 Prediction of Acidity (pH) Content

The pH values obtained for litchi during conventional analysis were ranging from a minimum of 3.59 to a maximum of 3.87. While, the pH values predicted by FT-NIR spectroscopy were in the range of 3.617 to 3.839. A high correlation of calibration was found between the NIR spectra and acidity (pH) values with a coefficient of determination (R^2) of 0.97 and a root mean square error of cross validation (RMSECV) of 0.008 (Table 3). When the model was applied to predict 39 other litchi fruits, the prediction results were closer with R^2 of 0.96 and root mean square error of prediction (RMSEP) of 0.009. The PLS model appeared to be robust used in the calibration model for litchi. The best PLS model for measuring the acidity (pH) content was obtained by following FD+SNV preprocessing in the wavenumber range of 9141.4 – 8316 cm^{-1}. The linear regression plot between true values and predicted values of pH is given in Fig. 3(b). The regression equation is of the form of ‘y = 0.9705x + 0.1076’ for the developed model.

The performance of the models was evaluated by leave-one-out cross validation that is, the minimum RMSECV and maximum correlation Coefficient of regression (R^2). The calibration results of pH by thePLS model is shown in Fig.3 (b). Similarly, [25] used FT-NIR reflectance

Table 2. Calibration and prediction results of SSC of litchi using FT-NIR spectroscopy

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
SNV	0.91	0.352	0.91	0.291
FD+SNV	0.88	0.417	0.82	0.406
FD	0.85	0.466	0.82	0.408
No preprocessing	0.81	0.525	0.73	0.50

FD: first derivative; SNV: vector normalization; FD+SNV: first derivative plus vector normalization; R^2: coefficient of determination; RMSECV: root mean square error of cross validation; RMSEP: root mean square error of prediction

Table 3. Calibration and prediction results of pH of litchi using FT-NIR spectroscopy

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
FD+ SNV	0.97	0.008	0.96	0.009
SNV	0.95	0.01	0.94	0.011
FD	0.94	0.011	0.93	0.012
No preprocessing	0.94	0.121	0.93	0.012

spectroscopy in the wavelength range of 928-2331 nm to measure pH in peach fruits and obtained a result with r_p of 0.95 and SEP of 0.13. On 'Fuji' apples, the best model obtained with (SEP) of 0.068, and R^2 of 0.83 [23]. Fu et al., 2009 investigated the acidity (pH) of intact loquats using NIR spectroscopy, obtained the best results with R^2 of 0.60 and RMSEP of 0.194.

3.3 Prediction of Titratable Acidity, (TA, % Malic Acid)

The titratable acidity of litchi obtained during conventional analysis was in the range from a minimum of 0.54-0.83% malic acid. Whereas titratable acidity predicted by FT-NIR spectroscopy was in the range of 0.52-0.83% malic acid. The correlation between NIR measurement and TA for litchi was very good with R^2 equal to 0.95 and RMSECV of 0.011 % malic acid (Fig. 3(c)). And the prediction results obtained were (R^2, RMSEP) of (0.94, 0.011%) respectively. Few works have been made on the prediction of the titratable acidity by using NIR spectroscopy. The linear regression plot between titratable acidity estimated experimentally using the standard method and nondestructive method by using FT-NIR spectroscopy is given in Fig. 3(c). The linear equation is of the form 'y = 0.9545x + 0.0326' for the developed model.

The result is given as, the RMSECV decreased with an increasing coefficient of determination (R^2) until it reaches to a maximum correlation between spectra measurement and titratable acidity. On 'Fuji' apples, [23] obtained a prediction model r = 0.72 and RMSEP = 0.0043 g 100 g^{-1} expressed in % malic acid. On apricot, Bureau et al., 2009 was obtained a good prediction performance for TA with R^2 = 0.89 and RMSEP = 3.62 meq 100 g-1 FW. The difficulty concerns NIR spectroscopy: the penetration of NIR radiation into fruit tissue decreases exponentially with the depth and the skin drastically reduces the light penetration [26].

3.4 Prediction of Vitamin C Content (mg 100 g^{-1} Juice)

Within the cultivar of litchi at different ripening stages used in this work, the mean content of vitamin C was 25.10 mg 100 g^{-1} juice. Concerning the relationships between NIR measurement and vitamin C content in litchi, R^2 is equal to 0.85 and RMSECV is equal to 0.38 mg 100 g^{-1} juice (Table 5).

Table 4. Calibration and prediction results of TA of litchi using FT-NIR spectroscopy

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
SNV	0.95	0.011	0.94	0.011
FD+ SNV	0.94	0.012	0.93	0.012
No Preprocessing	0.94	0.012	0.92	0.013
FD	0.94	0.013	0.92	0.013

Table 5. Calibration and prediction results of vitamin C of litchi using FT-NIRS

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
SNV	0.85	0.381	0.82	0.364
FD+ SNV	0.84	0.398	0.82	0.368
FD	0.83	0.406	0.81	0.377
No preprocessing	0.82	0.417	0.80	0.386

Table 6. Overall results of NIR calibration and prediction performance of litchi for non-destructive quality assessment

Quality traits	Best preprocessing method	Best wavenumber range(cm^{-1})	Calibration		Prediction	
			R^2	RMSECV	R^2	RMSEP
SSC (° Brix)	SNV	8269.7– 7498.36102 – 5446.3	0.91	0.352	0.91	0.291
pH	FD + SNV	9141.4 – 8316	0.97	0.008	0.96	0.009
Titratable acidity (% malic acid)	SNV	9002.6 – 8223.4	0.95	0.011	0.94	0.011
		6464.6 – 6024.9				
Vitamin C(mg 100 ml^{-1} Juice)	SNV	5454 – 4242.9	0.85	0.381	0.82	0.364
L*	No preprocessing	4605.4 – 4420.3	0.85	0.343	0.81	0.355
a*	FD+ SNV	9002.6 – 7498.3	0.98	0.016	0.98	0.018
b*	FD	5454 – 4597.7	0.87	0.168	0.84	0.183

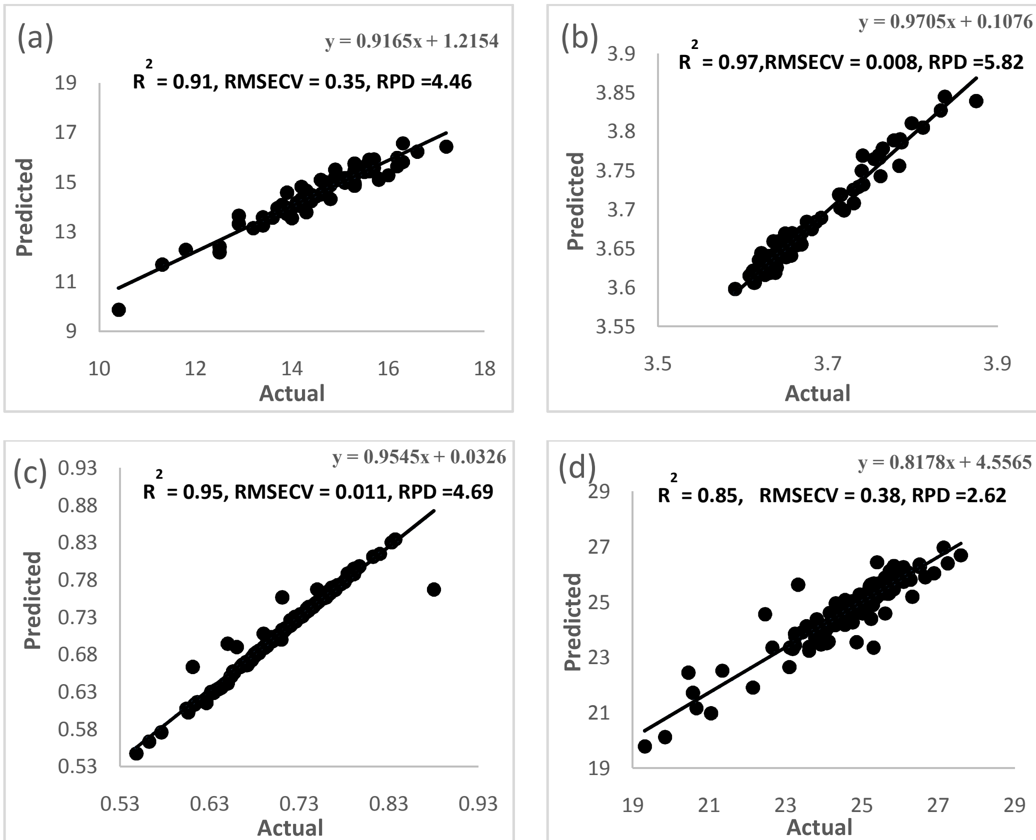

Fig. 3. Prediction models developed for (a) SSC, (b) pH, (c) TA, and (d) vitamin C in litchi samples

When the model was applied to predict litchi, the prediction results were varied with R^2 of 0.82 and RMSEP of 0.36 mg 100 g^{-1} juice. The best PLS model for measuring the vitamin C content was obtained by following SNV preprocessing technique that has given as maximum as coefficient of determination of 0.82 in the range of 5454 – 4242.9 cm^{-1}. The linear equation was in the form of 'y = 0.8178x + 4.5565' for the developed model.) is given in Fig. 3(d). Xia et al., 2007 (a, b) predicted vitamin C content in oranges using spectra in the 833-2500 nm wavelength range. The optimal waveband found for prediction was 1333-1835 nm, and a PLS model built with spectra in this waveband performed excellent, with *r*p of 0.96 and RMSECV of 0.039 mg/g, indicating the feasibility of NIR spectroscopy.

4. CONCLUSION

The obtained results showed that FT-NIR spectroscopy in the diffuse reflectance mode combined with the PLS regression has good potential for non-destructive determination of inner and superficial quality attributes. Performed quant tests for unknown samples. Analysis results found very satisfactory. The soluble solids content (SSC), titratable acidity (TA), and acidity (pH) can be predicted with root mean square errors (RMSEP) up to 0.291°Brix (R^2 = 0.91), 0.011% (R^2 = 0.94) and 0.009 (R^2 = 0.96) respectively. The preprocessing of the data influences the performance of the model. The high residual predictive deviation (RPD) values up to 4.46, 4.69 and 5.82 for soluble solids, titratable acidity and pH respectively. The limited prediction accuracy possibly due to the presence of uneven thickness and roughness of litchi pericarps. It can be concluded that FT-NIR measurements provided good estimates of the internal quality indices of litchi fruit and the predicted values were correlated with destructively measured values for pH, SSC and titratable acidity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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