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NON-DESTRUCTIVE QUALITY ASSESSMENT OF CITRUS FRUITS USING FT-NEAR-INFRARED SPECTROSCOPY

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Abstract: Nondestructive method of measuring the internal quality of citrus fruits was developed using fourier transform near-infrared (FT-NIR) spectroscopy. Also the models describing the relationship between quality attributes and the NIR spectra of the fruits were developed and evaluated. Diffuse reflectance measurements (12500–3600 cm^{-1}) and biochemical measurements were performed individually on each fruit purchased from a commercial supermarkets. Relationships were evaluated by the application of chemometric techniques based on partial least squares (PLS) regression on fruit set divided randomly into two groups: 60 fruits for calibration and 30 for validation. Spectra preprocessing options include: the vector normalization (SNV), first derivative (FD), and first derivative plus vector normalization (FD+SNV). Good prediction performance was obtained with coefficient of determination (R^2) of 0.911 and 0.897 for soluble solids content (SSC) and acidity (pH) respectively and root mean square errors of prediction (RMSEP) of 0.026% Brix and 0.055 respectively. The overall results demonstrate that FT-NIR spectroscopy can be used to evaluate the quality of lemons.
Keywords: FT-NIR technique, Soluble solids content (SSC), pH, Titratable acidity (TA), Vitamin C, Lemons, Partial least square (PLS), Non-destructive.

1. Introduction

Fruits are the main constituents of human diet providing abundant nutritional composition for human body. Therefore it is given great importance to the quality of fruits. Soluble solids content (SSC) and acidity (pH) are the properties most likely to match the consumer's perception of fruit internal quality. Determination of these properties are still destructive and involving a considerable amount of manual work. Hence, there is a demand for the development of rapid analytical technique to evaluate the internal quality of fruits.

Near-infrared spectroscopy (NIRS) has been used as a rapid and nondestructive technique for assessing the quality traits of citrus fruits (lemons) such as soluble solids, pH, titratable acidity, and vitamin C contents through the comparison with standard methods. In

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this technique, information about the internal quality of the products can be obtained by measuring the amount of near-infrared light absorbed by functional groups with or without little sample preparation (Banwell, 1983). One of the main advantages of NIR spectroscopy that it has the potential to evaluate several quality attributes at the same time. By now, many studies have shown that NIR spectroscopic methods can assess the internal qualities of fruits and vegetables nondestructively such as apple, (Fan, Zha, Du, & Gao, 2009) pear, (Fu, Ying, Lu, & Xu, 2007) kiwifruit, (Clark *et al*., 2004) peach, (Golic & Walsh, 2006) tomato, (Pedro & Ferreira, 2005; Shao *et al*., 2007). Xudong *et al*., 2009 developed a portable near infrared spectroscopy system for measuring the quality of Nanfeg mandarin fruit and the best results showed the correlation coefficient (r) and root mean square error of prediction (RMSEP) of (0.93, 0.65% Brix), (0.66, 0.09%), (0.81, 2.7 mg/100g) and (0.57, 0.81) for SSC, TA, vitamin C and color, respectively.

More recently, fourier transform NIR (FT-NIR) technique has been applied for non-invasive quality detection of fruits (Peirs *et al*., 2002; Ying *et al*., 2005). Liu *et al*., 2005 examined the feasibility of FT-NIR spectrometry in prediction capacity of the quality characteristics such as soluble solids contents (SSC), titratable acidity (TA) and acidity (pH) of 'Fuji' apple (*Malus domestica* Borkh. cv. Fuji) with a wavelength range of 812-2357 nm. The main advantage of NIR spectroscopy is that, once best PLS calibration models are developed, it allows to a non-destructive and individual characterisation of fruits, with simultaneous prediction of several quality attributes. Ying *et al*., 2005 has been developed PLS models, using FT-NIR spectroscopy to predict SSC of unknown kiwi fruit samples with correlation coefficient (R^2) of 0.9828 and RMSEP of 0.679% Brix.

The objective of this study was to investigate the potential of near-infrared spectroscopy in diffuse reflectance mode for predicting quality traits of lemons such as soluble solids, titratable acidity, pH, and vitamin C respectively. The prediction models for each quality parameters were developed with partial least square (PLS) technique.

2. Materials and methods

2.1. Lemon samples

A total of 90 samples for measuring internal parameters were purchased from three supermarkets in Coimbatore, Tamil Nadu, India. In that 60 fruits were used for the calibration, and 30 samples were used for prediction. Non-destructive measurements were performed on the day of purchase and conventional destructive measurements were carried out a day later on 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. Fruit 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 cut in to two halves and juiced to determine quality attributes such as SSC, pH, titratable acidity and vitamin C at room temperature of 25 °C. The pH of the lemon juice was determined with a pH meter, and soluble solids content was determined using a digital refractometer with a range of 0–53 °Brix and expressed in % Brix at 20°C. Titratable acidity was determined by titration method with 0.1 N NaOH and expressed in % citric acid and Vitamin C was measured by titration method by 2, 6 Dichlorophenol Indophenol.

2.4. Data processing and analysis

Three different data pre-processing techniques were considered: SNV (vector normalization), First Derivative (FD), and first derivative plus standard normal variate (FD+SNV) respectively. 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 (Lu, 2001). The PLS regression method was used to develop the models for predicting the composition of fruit samples. The wavenumber ranges between 12500 and 3600 cm^{-1} (Fig. 1) were analysed to find the optimal sub- wavenumber ranges that would yield the best correlations between the spectral data and quality attributes. 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.

3. Results and discussion

The general profile of the absorption spectra for lemon (Fig. 1) is very similar to that of other plant materials apple (Liu & Ying, 2005), mandarin (Gomezet al., 2006). 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. The water absorption bands should be eliminated in order to reduce the interference with the chemical structures of our component of interest (Tripathi and Mishra, 2009).

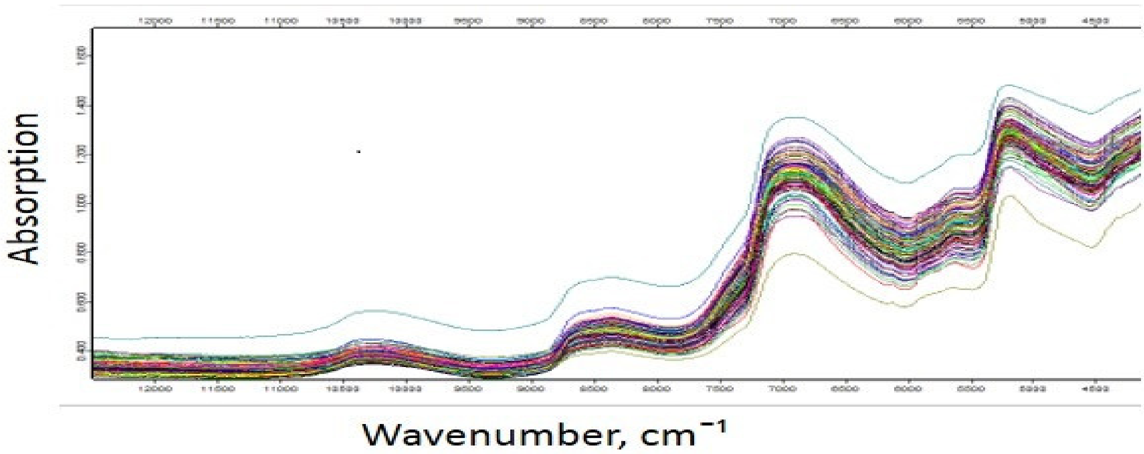


Fig. 1. Typical original diffuse reflectance spectra for lemon samples

A synthetic view of the observed quality traits is presented in below Table 1.

Table 1: Mean, standard deviation (SD) and range of lemon quality traits in both calibration and prediction sample sets

Quality parameters	Calibration(n=60)			Prediction(n=30)		
	Range	Mean	SD	Range	Mean	SD
SSC (% Brix)	7-7.50	7.23	0.09	7.1-7.30	7.20	0.08
pH	1.9 – 3.31	2.56	0.23	2.12-3.13	2.56	0.244
TA (% citric acid)	4.0-6.84	5.65	0.57	4.65-6.59	5.59	0.49
Vitamin C (mg 100 ml^{-1} Juice)	18.53-42	31.43	6.69	20.31-40.44	32.02	5.88

3.1. Prediction of soluble solids content (SSC, %Brix)

The values obtained for SSC from conventional analysis were ranging from a minimum of 7.0 to a maximum of 7.5% Brix. While the values obtained from FT-NIR spectroscopy were in the range of 7.02 to 7.46% Brix. The preprocessing of the diffuse reflectance spectra, including vector normalization (SNV), first derivative (FD), and first derivative plus vector normalization (FD+SNV) were taken into account and the results obtained were shown in table 2. The best calibration model was selected on the basis of higher R^2 value and minimum RMSECV.

Table 2: PLS model for determination of SSC of lemon using FT-NIR spectroscopy

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
FD	0.920	0.027	0.911	0.026
SNV	0.910	0.028	0.890	0.027
No preprocessing	0.908	0.028	0.875	0.029
FD + SNV	0.901	0.029	0.869	0.03

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

A high correlation of calibration was found between the NIR spectra and soluble solids content (SSC) values with a coefficient of determination (R^2) of 0.92 and a root mean square error of cross validation (RMSECV) of 0.027% Brix. Among all preprocessing techniques, the linear regression plot for the best PLS model obtained using the first derivative (FD) of the spectra in the wavenumber range of 9403.7-6094.3 cm^{-1} and 5454-4242.9 cm^{-1}. When the model was applied to predict 30 other unknown lemon fruits, the prediction results were closer with R^2 of 0.911 and root mean square error of prediction (RMSEP) of 0.026% Brix. The PLS model appeared to be robust used in the calibration model for lemons. Looking at the obtained RPD values (not lower than 4.0), according to Williams and Norris (2001) RPD values ranging 4.0-5.9 indicate that these models could be used for quality control. The regression (R^2) value obtained for the prediction in this work was similar to those obtained on other fruits with R^2=0.91 on tomato 'Heat wave' (Shao et al., 2007), R^2=0.92 on apricot (Bureau et al., 2009) and $R^2 \geq 0.92$ in kiwi (McGlone et al., 2002) have been observed.

3.2. Prediction of pH content

PLS model was developed using the spectra preprocessed with including SNV, FD and FD+SNV were taken into account and the results obtained is shown in table 3. The pH values obtained during conventional analysis were ranging from a minimum of 1.9 to a maximum of 3.31. While, the pH values predicted by FT-NIR spectroscopy was in the range of 2.12 to 3.13. The performance of the models was evaluated by leave-one-out cross validation that is, the minimum RMSECV and maximum coefficient of determination (R^2).

Table 3 PLS model for determination of pH of lemon using FT-NIR spectroscopy

Preprocessing method	**Calibration**		**Prediction**	
	R^2	**RMSECV**	**R^2**	**RMSEP**
No preprocessing	0.905	0.071	0.897	0.055
FD	0.902	0.077	0.867	0.076
FD + SNV	0.845	0.098	0.824	0.087
SNV	0.837	0.101	0.786	0.096

A good correlation of calibration was found between the NIR spectra and acidity (pH) values with a coefficient of determination (R^2) of 0.905 and a root mean square error of cross validation (RMSECV) of 0.071 without any preprocessing and in the wave number range of 7506–5446.3 cm^{-1}. The result is given as, the RMSECV decreased with an increasing coefficient of regression (R^2) until it reaches to a maximum correlation between spectra measurement and acidity (pH). When the model was applied to predict remaining lemons, the prediction results were closer with R^2 of 0.897 and root mean square error of prediction (RMSEP) of 0.05, which can be observed in above table 3. The RPD value obtained 4.47 indicating a good performance of the model, used for quality control. On 'Fuji' apples, Liu and Ying (2005) obtained the best model with a standard errors of prediction (SEP) of 0.068, and correlation coefficients of 0.831.

3.3. Prediction of titratable acidity, TA (% citric acid)

The titratable acidity obtained during conventional analysis was in the range from a minimum of 4.0-6.84 percent. And titratable acidity predicted by FT-NIR spectroscopy was in the range of 4.65-6.36 percent. The results obtained by preprocessing the spectra with vector normalization (SNV), first derivative, and FD+SNV is shown in (Table 4).

Table 4 PLS model for determination of TA of lemon using FT-NIR spectroscopy

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
No preprocessing	0.812	0.266	0.802	0.212
SNV	0.796	0.275	0.759	0.238
FD + SNV	0.504	0.419	0.471	0.353
FD	0.324	0.486	0.232	0.426

The most suitable spectral preprocessing was chosen in order to obtain the best statistical regression parameters (high coefficient of determination R^2 and low RMSECV). The above table shows that among all preprocessing techniques, the best PLS model for measuring the titratable acidity (TA) was obtained by with no-preprocessing the spectra in the wavenumber range of 9403.7-7498.3 cm^{-1} which gave the highest coefficient of determination of R^2 (0.812) minimum RMSECV of 0.266% and root mean square error of prediction (RMSEP) as 0.212%. Few works have been made on the prediction of the titratable acidity by using NIR spectroscopy. On apricot, Bureau et al., 2009 was obtained a good prediction performance for titratable acidity with R2 = 0.89 and RMSEP = 3.62 meq 100 g-1 FW. On ‘Fuji’ apples, Liu and Ying (2005) obtained a prediction model R^2 = 0.72 and RMSEP = 0.0043 g 100 g-1 expressed in % malic acid.

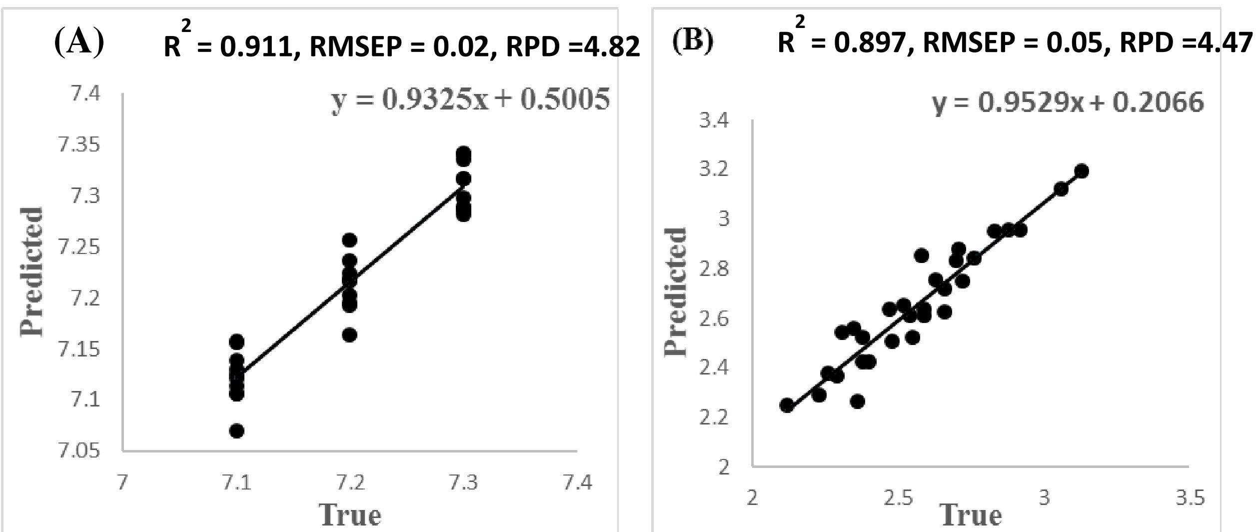
3.4. Prediction of Vitamin C content

The PLS model was developed using three spectral preprocessing methods inorder to evaluate the best model for determination of vitamin C content. The vitamin C content was determined by visual titration using 2,6-dichlorophenol-indophnol and values obtained were in the range of 18.53 to 42 mg 100 ml^{-1} juice. And values predicted by FT-NIR spectroscopy was in the range of 21.55-42.51 mg 100 ml^{-1} juice. The results obtained by preprocessing the spectra with vector normalization (SNV), first derivative, and FD+SNV is shown in Table 5.

Table 5 PLS model for determination of vitamin C content of lemon using FT-NIR spectroscopy

Preprocessing method	Calibration		Prediction	
	R^2	RMSECV	R^2	RMSEP
FD	0.798	3.16	0.746	3.57
SNV	0.741	3.63	0.601	4.23
FD + SNV	0.689	3.90	0.480	4.83
No preprocessing	0.642	4.18	0.451	4.96

Concerning the relationships between NIR measurement and vitamin C content in lemons, The results shows that, the suitable spectral preprocessing technique for determination was first derivative (FD) which gave the highest correlation coefficient of regression, R^2 (0.798) and minimum RMSECV of 3.16 mg 100 ml^{-1} juice. The optimal wavenumber was chosen in order to obtain the best statistical regression parameters (high R^2 and low RMSECV) which falls in the range of 6102-5446.3 cm^{-1}. When the model was applied to predict, the prediction results were varied with R^2 of respectively 0.746 and RMSEP of respectively 3.57 mg 100 ml-1 juice.



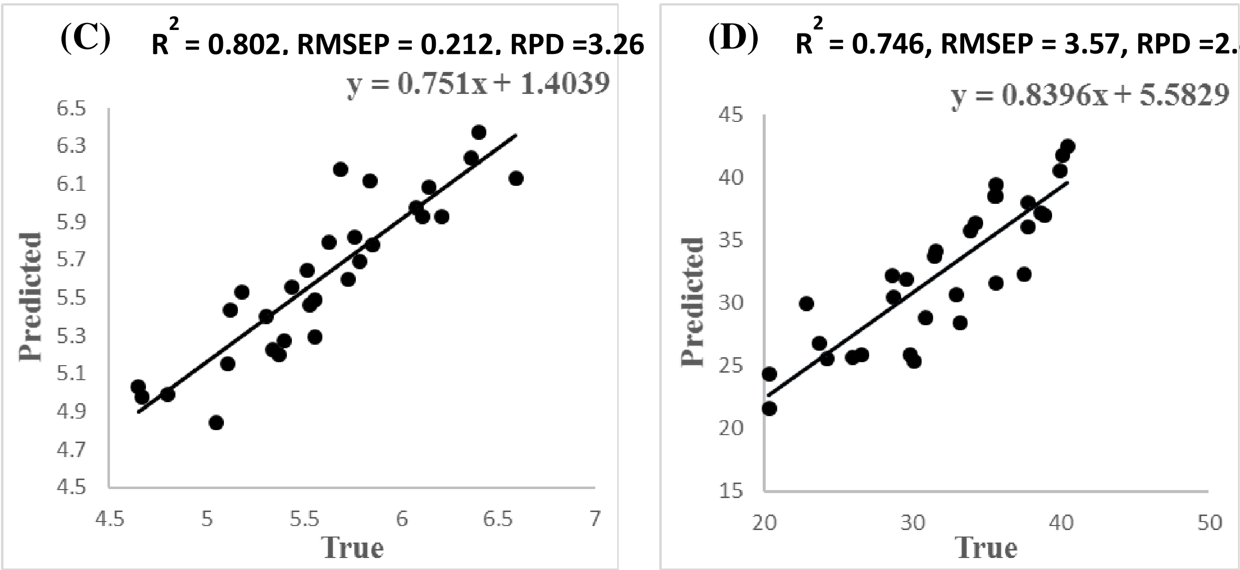


Fig. 2. Predictions of PLS by the FT-NIR system vs. laboratory measurements of lemon (A) TSS (% Brix)**, (B) pH, (C) TA** (% citric acid) **and (D) vitamin C**

The RMSECV of less than 1 is acceptable in the present model while the $\mathbf{R^2}$ value should be still improved in order to develop a reliable model. The overall results of NIR calibration and prediction performance for several quality traits of lemon: soluble solids content, titratable acidity, pH, and vitamin C content were presented in below table 6.

Table 6: Overall results of NIR calibration and Prediction performance of lemon for non-destructive quality assessment:

Quality traits	Best Preprocessing method	Best wavenumber range(cm^{-1})	Calibration		Prediction	
			R^2	RMSECV	R^2	RMSEP
TSS (% Brix)	First Derivative	9403.7 – 6094.3 5454 – 4242.9	0.920	0.027	0.911	0.026
pH	No pre-processing	7506 – 5446.3	0.905	0.071	0.897	0.055
Titratable acidity (% citric acid)	No pre-processing	9403.7 – 7498.3	0.812	0.266	0.802	0.212
Vitamin C (mg 100 ml^{-1} Juice)	First Derivative	6102 – 5446.3	0.798	3.16	0.746	3.57

4. Conclusion

The obtained results showed that NIR spectroscopy in the diffuse reflectance mode combined with the PLS regression is a good and reliable technique to measure the lemon quality traits. The soluble solids content (SSC), acidity (pH), and titratable acidity (TA) and vitamin C can be predicted with root mean square errors (RMSEP) upto 0.026% Brix (R^2 = 0.911), 0.055 (R^2 = 0.897), 0.212% (R^2 = 0.802) and 3.57 mg 100 ml^{-1} Juice (R^2 =0.746) respectively. The high residual predictive deviation (RPD) values (upto 4.82, 4.47, 3.26 and 2.46 for soluble solids content, pH, titratable acidity and vitamin C respectively) were obtained. In the future it is required to check the robustness of these established models on other lemon fruits, including different cultivars, harvested over different periods and cultivated in different orchards. The non-invasive FT-NIR measurements provided good estimates of the internal quality indices of lemons and the predicted values were correlated with destructively measured values for SSC, pH, titratable acidity and vitamin C respectively. According to its non-destructive and rapid characteristics, the near-infrared spectroscopy appeared already as a suitable technique for screening quality parameters of a large number of other fruits as well as quality control.

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