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Application of spectroscopic method to predict sugar content of sugarcane internodes

(Penggunaan kaedah spektroskopi untuk meramal kandungan gula pada ruas tebu)

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Keywords: sugarcane quality, Brix, stalk, cross sectional scanning method (CSSM), skin scanning method (SSM)

Abstract

The aim of this study was to investigate the potential of near-infrared (NIR) reflectance spectroscopy for predicting sugar content of sugarcane from internode samples. NIR spectral data were measured using a full-range spectroradiometer (FRS) in the wavelength region between 350 and 2,500 nm based on cross sectional scanning method (CSSM) and skin scanning method (SSM). Statistical models were developed using the partial least square (PLS) to interpret the spectral data and develop calibration model for the sugar content (Brix) of sugarcane. Both CSSM and SSM had good prediction accuracies in predicting Brix values, with the corresponding correlation of determination (R^2) values of 0.92 and 0.82 and root mean square error of prediction (RMSEP) of 1.03 and 1.50 Brix respectively. These results showed that the FRS can be used to predict the sugar content from internode samples using CSSM or SSM. However, CSSM was found to give better prediction accuracy than SSM. These findings showed that spectroscopic methods have the potential to be applied for rapid determination of sugar content from stalk samples in the fields.

Introduction

Sugarcane (*Saccahrum* spp.) is an important crop in many countries including Australia. Commercial payment in the Australian sugarcane industry is based on yield and quality of the crop. Sugarcane quality is determined based on its sugar content, known as commercial cane sugar (CCS) which includes the soluble solids (Brix), sucrose (Pol) and estimated fibre contents. In recent years, near-infrared (NIR) spectroscopic methods have been increasingly implemented in the sugarcane industry to measure the quality components of the product. These measurements have been performed on different sample forms including fibrated samples (Berding et al. 1991a; Madsen et al. 2003), raw juices (Cadet and Offmann 1997), clarified juices (Berding et al. 1991b; Mehrotra and Seisler 2003; Valderrama et al. 2007), whole stalk samples (Nawi et al. 2012a) and internode samples (Nawi et al. 2012b; Nawi et al. 2013).

Recently, there is a growing interest within the industry to measure sugarcane quality in the fields which will be very useful for assessing the crops growth and

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development, harvesting management, adoption of precision agriculture and payment purposes to growers. However, the measurement of quality in the fields requires a suitable sample form which should be relatively easy to prepare prior to the measurement. Nawi et al. (2012a) applied NIR spectroscopic methods to measure the sugarcane quality using whole stalk samples. In their study, the authors used visible and shortwave near-infrared spectroradiometer (Vis/SWNIRS; 400 to 1,000 nm) and full-range spectroradiometer (FRS; 400 to 2,500 nm) to predict sugarcane quality based on cross sectional scanning method (CSSM). They found that the coefficient of determination (R^2) for Brix values as predicted by the Vis/SWNIRS and FRS were 0.68 and 0.76 respectively. Based on the results of their study, the authors suggested that the stalk samples could be the most suitable sample form for spectroscopic measurement in the fields since they do not require complicated sample preparation.

In addition, Nawi et al. (2012b) carried out a preliminary study using the Vis/SWNIRS to predict Brix values from sugarcane internode samples based on the CSSM and skin scanning method (SSM). The R^2 values obtained in this study for the CSSM and SSM were 0.71 and 0.80 respectively. Nawi et al. (2013) further improved the measurement of Brix value from internode samples using the Vis/SWNIRS based on the SSM. The prediction accuracy obtained from this study was good with R^2 and root mean square error prediction (RMSEP) values of 0.91 and 1.45 Brix respectively. However, the accuracy of this study could be further improved if the FRS was used instead of the Vis/SWNIRS. Lu et al. (2000) reported that a greater spectral region beyond 1,100 nm could give improved prediction results in predicting sugar content. Lammertyn et al. (1998) and Moons et al. (1997) found that longer wavelengths in the region between 700 and 2,500 nm may be needed in predicting the sugar content of apple fruits. Thus, it was hypothesized that the FRS could improve the prediction accuracy of Brix values from internode samples by both CSSM and SSM.

Therefore, the aim of this study was to investigate the capability of the FRS to determine sugarcane quality from sugarcane internode samples. To our knowledge, no studies have been reported on using NIR spectroscopy methods in the full wavelength range from 400 to 2,500 nm to predict sugar content from sugarcane internode samples. The specific objectives of this study were (1) to investigate the feasibility of using the FRS to predict the Brix values from internode samples based on the CSSM and SSM; (2) to compare the prediction accuracy between the CSSM and SSM under the same experimental setups.

Materials and methods

Sugarcane stalk samples

A total of 125 internodes were extracted from 11 sugarcane stalk samples. The stalk samples were supplied by the Bureau of Sugar Experimental Station (BSES), Bundaberg, Queensland in June 2012. The stalk samples were collected from three commercial varieties, namely, Q155, Q208 and Q190. They were grown under commercial conditions with the fertilisation based on soil test and the six easy steps of nutrition guidelines (Schroeder et al. 2009).

After removal of all leaf materials, the stalk samples were topped and perpendicularly cut at the node portion into individual internodes using pruning shears. Each internode was cut into four sections (S1, S2, S3 and S4) of approximately the same length, representing the node and the internode areas (*Plate 1* and *Figure 1*). Each cut section was subjected to two different scanning methods, namely, cross sectional scanning method (CSSM) and skin scanning method (SSM). The CSSM was applied by scanning the cross sectional surface of each section while the SSM was applied by scanning the skin surface of the section (Nawi et al. 2012b).

Plate 1. Photo of prepared internodes

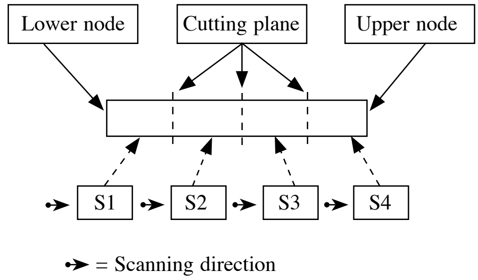

Figure 1. Schematic diagram of the four scanned internodes

Spectral reflectance measurements

The NIR reflectance of the internode samples were measured using a full-range spectroradiometer (FRS) manufactured by FieldSpec®, Analytical Spectral Devices (ASD), Boulder, USA. The spectral range for FRS was between 350 and 2,500 nm with a resolution of 1.4 nm in the 350 – 1,000 nm range and 2 nm in the 1,000 – 2,500 nm range. The measurements were carried out using the 25° field-of-view (FOV) optics of the spectroradiometer. The probe was located 50 mm above the sample at 45° angle. The distance between the probe and the samples were maintained by fixing the probe to a tripod and the samples were positioned in a fixed sample holder. The FRS was set to record the average reading of 20 scans for each spectrum.

Even though the aim of this study was to predict sugar content using spectroscopic methods in the field, the spectral measurement for this preliminary investigation was conducted in a dark room. The dark room measurements were undertaken to make sure that the influences of environmental factors (sunlight intensity, time of measurement, weather conditions and temperature) on the spectral measurement were minimised (Wu et al. 2008).

In this study, two halogen lamps (Lowell Pro-Lam 14.5 V tungsten bulb, Ushio Lighting, Inc., Japan) were used to provide illumination to the dark room. The lamps were placed about 800 mm away from the samples at 45° angle. Relative reflectance spectra were calculated by dividing sample radiance with reference radiance from a spectralon white reference panel for each wavelength (Abdel-Rahman et al. 2010). All spectral data were transformed into ASCII format and processed using the RS3 software for Windows (Analytical Spectral Devices, Boulder, USA) designed with a graphical user interface.

Brix measurement

After the spectral acquisition, each section was squeezed using a clamp to extract the juice. The extracted juice samples from the same internode were then mixed to provide an average Brix value for the internode. The Brix was measured using a handheld °Brix refractometer (Model: RHB-32ATC, from Huake Instrument Co., Ltd, Baoan, Shenzhen, China; the °Brix range was 0 – 32% with automatic temperature compensation). The spectral and Brix measurement were carried out on the same day. Even though the standard sugarcane quality is determined as CCS (derived from Brix, Pol and fibre), only the Brix value was used in this study. Brix was chosen because it is the easiest, least expensive quality parameter to be measured with little preparation (Staunton et al. 2011).

Statistical data analysis and model development

Prior to the multivariate analysis, the reflectance spectra (R) were converted to absorbance units using the log (1/R) transformation. In order to avoid low signal-to-noise ratio, the first 50 nm data points were removed from the original spectral data, resulting in only the wavelength region between 400 and 2,500 nm being analysed in this study. The spectral data were then pre-processed for optimal performance. Several pre-processing methods were tested including multiplicative scatter correction (MSC), first and second derivatives, and standard normal variate (SNV) (Nawi et al. 2013). After some trials and computations, MSC was found to be the best pre-processing method for this study. The MSC method is mainly used for removing the effects of light scattering on spectra (Lu 2001).

Once the data pre-processing had been completed, principal component analysis (PCA) was performed to transform the spectral data to a new coordinate system to maximize the spectral features and reduce the data dimensionality (Lu et al. 2000). Partial least square (PLS) method (Purcell et al. 2005) was then performed to build the calibration models for both CSSM and SSM. The maximum number of latent variables (LVs) for the PLS models used in this study were set at ten. The calibration models were developed using 75% of the data and the remaining 25% were used for prediction (Nawi et al. 2012a). Samples for the prediction models were selected by taking one of every four samples from the entire sample set, taking care to ensure that each set included samples that covered the entire range of reference Brix values. This external validation method (Fernández-Novales et al. 2009) was adopted in order to ensure that the samples used in the prediction model were not used in the calibration model.

The pre-processing method and model developments were performed using with the statistical software program for multivariate calibration 'The Unscrambler' (version 9.2, Camo Process AS, Oslo, Norway). In this study, two statistical parameters, root mean square error (RMSE) and coefficient of determination (R^2) were used to measure the performance of both calibration and prediction models.

Results and discussion

Statistical characteristics of Brix and spectral overview

Table 1 summarises the statistical characteristics of the Brix values for both calibration and prediction data sets. Both data sets showed similar means, ranges and standard deviations, indicating that the selection of the data for each set was appropriate. A relatively wide range of Brix values shown in the table indicated the variation of Brix along the height of the stalk samples with the highest Brix value belonging to the first internode and the lowest Brix value belonging to the last internode (Nawi et al. 2013).

The absorbance spectra of three internode samples having high (22.2), middle (18.2) and low (11) Brix values as measured by the FRS using the CSSM are shown in *Figure 2*. These spectra were pre-processed using MSC method. The curves of these three absorbance spectra were similar between each other with several obvious absorption peaks around 680, 980, 1,200, 1,460 and 1,920 nm. The absorption peak at 680 nm was due to chlorophyll content (Abbott et al. 1997). The absorption peaks around 980, 1,200, 1,460 and 1,920 nm were related to water content in the stalks (Lu et al. 2000).

Prediction of sugarcane Brix using CSSM

Table 2 shows the performance of both calibration and prediction models in predicting Brix values using the CSSM from the internode samples. To explore the effects of different wavelength regions on calibration and prediction models, the wavelength range of the FRS (400 to 2,500 nm) was divided into three regions;

Table 1. Statistical characteristics of Brix in internode samples

Data set	Sample no.	Max	Min	Mean	SD
Calibration	95	22.2	12.2	18.4	2.39
Prediction	30	21.9	11.0	18.2	2.60

SD = Standard deviation

Table 2. PLS model performances for different wavelength ranges for CSSM

Wavelength (nm)	Calibration		Prediction	
	R2	RMSEC	R2	RMSEP
400 – 1,000	0.94	0.86	0.91	1.10
400 – 1,800	0.92	0.96	0.92	1.03
400 – 2,500	0.89	1.12	0.49	2.51

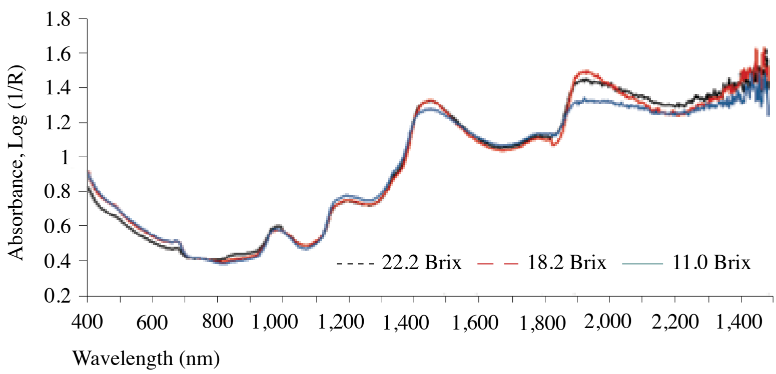

Figure 2. Typical spectral absorption curves for sugarcane as measured by CSSM at different Brix values

400 – 1,000 nm, 400 – 1,800 nm and 400 – 2,500 nm. The table shows that the correlations of the NIR spectral data with the Brix values ranged from 0.49 – 0.92, depending on the wavelength regions. The RMSEPs ranged between 1.03 and 2.51 Brix. The wavelength regions between 400 and 2,500 nm had lower R^2 values due to the presence of spectral noise beyond 1,800 nm (*Figure 2*). By removing these noisy regions from the analysis, the R^2 increased to 0.92. Park et al (2003) claimed that the signal to noise ratio was very low beyond 1,800 nm, which is typical for devices equipped with fibre optics. For the visible and shortwave NIR regions (400 – 1,000 nm), the R^2 and RMSEP were 0.91 and 1.10 Brix respectively (*Table 2*).

The results of the calibration and prediction models for the CSSM in the range of 400 to 1,800 nm are presented in the scatter plots in *Figures 3a* and *3b*. In the figures, the ordinate and abscissa represent the predicted and measured values of the Brix. The R^2 for Brix prediction obtained in this study was better (0.92) than the previous published studies. For example, Nawi et al. (2012a) used the FRS and Vis/SWNIRS to predict Brix from whole stalk samples based on the CSSM in the field. The R^2 values of Brix prediction obtained using the FRS and Vis/SWNIRS were 0.76 and 0.68 respectively.

In general, the prediction accuracies obtained in their study were not high, possibly due to the variation between sub-samples, reflecting the heterogeneous nature

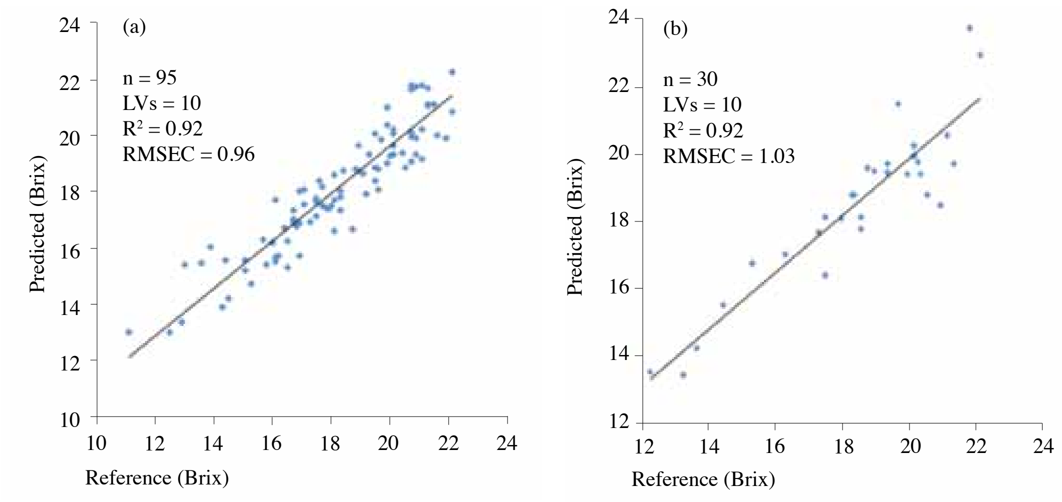

Figure 3. Scatter plots for CSSM: (a) calibration model (b) prediction model

of the raw sugarcane stalk. Moreover, since the experiment was conducted in the field and relied on ambient lighting, variation in weather conditions may have also influenced the accuracy of results. In contrast, this study had better prediction accuracy since it was carried out in the dark room with minimal environmental influences. In terms of equipment performance, the study found that the FRS had better prediction accuracy than the Vis/SWNIRS.

In another study, Nawi et al. (2012b) applied the CSSM on internode samples and reported an acceptable correlation (R^2 = 0.71) between spectral data collected by the Vis/SWNIRS and Brix values. However, this preliminary study only used 25 internode samples. The limited number of internode samples reduced the robustness of the model which may also have limited the model accuracy. Overall, using the FRS with a larger sample number (n = 125) had successfully improved the performance of the spectroscopic methods in predicting Brix values based on the CSSM.

Prediction of sugarcane Brix using SSM

The performance of both calibration and prediction models in predicting Brix values by the SSM at different wavelength regions are shown in *Table 3*. The table shows

Table 3. PLS model performances for different wavelength ranges for SSM

Wavelength mm	Calibration		Prediction	
	R^2	RMSEC	R^2	RMSEP
400 – 1,000	0.84	1.30	0.79	1.58
400 – 1,800	0.84	1.31	0.82	1.50
400 – 2,500	0.86	1.21	0.78	1.63

that the correlations of the calibration models varied from 0.84 to 0.86 while the RMSECs varied from 1.21 to 1.31 Brix. The prediction results for R^2 were slightly lower than the calibration models, ranging between 0.78 and 0.82 and RMSEPs were slightly higher than RMSECs ranging between 1.50 and 1.63 Brix. Similar to the CSSM, the wavelength region from 400 to 1,800 nm had the highest prediction accuracy with R^2 and RMSEPs values of 0.82 and 1.50 Brix respectively. In contrast, the wavelength region from 400 – 2,500 nm had the lowest R^2 value of 0.78 with RMSEP of 1.63 Brix.

Similar to the CSSM model, the results of the calibration and prediction models for the wavelength region from 400 – 1,800 nm as measured by the SSM are presented in the scatter plots in *Figures 4a* and *4b*. Overall, this study reported better results (R2 = 0.82) than the previous work reported by Nawi et al. (2012b) who used a similar method.

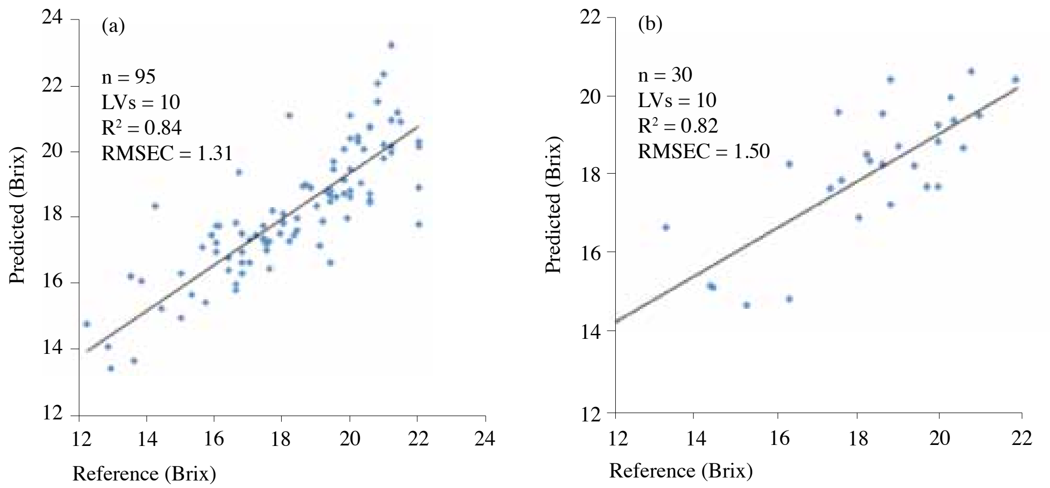

Figure 4. Scatter plots for SSM: (a) calibration model (b) prediction model

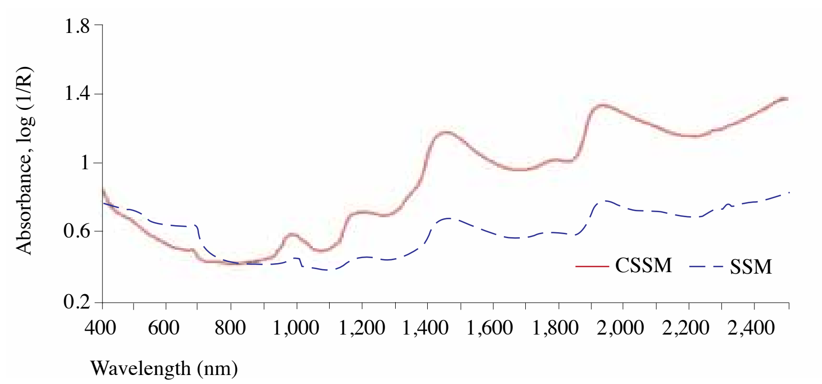

Figure 5. Comparison of typical absorbance spectrums between SSM and CSSM

Nawi et al. (2012b) reported good prediction accuracy (R^2 = 0.80) for predicting Brix values from spectral data collected by the Vis/SWNIRS using the SSM. However, in another study, Nawi et al. (2013) predicted the Brix values from internode samples using the Vis/SWNIRS based on SSM and obtained higher accuracy (R^2 = 0.91) than this study. The higher accuracy reported in their study was probably due to the inclusion of a larger sample number (n = 291) than this study (n = 125). Furthermore, their study was conducted using a purpose-built measurement box, which was designed to reduce the environmental variations during the spectral measurement.

Comparison between SSM and CSSM

A comparison of the average absorbance spectra of the internode samples as measured by the SSM and CSSM is shown in *Figure 5*. Even though the trend of the curves for both scanning methods was similar, it can be seen that the curve representing the CSSM was higher than the SSM curve. The difference indicated that the skin surface had a lower absorption level than internode flesh because of the presence of the skin. The low absorption by the skin was due to the fact that the whole skin surface was covered by a thin protective layer of waxy material which was moisture resistant (Huang et al. 2005). Furthermore, the diffuse reflectance of the skin surface was related to the surface geometry (size and shape) of individual internodes, while the cross sectional surface did not have this problem since measurements were taken from a flat cross sectional surface. This finding was consistent with a study conducted by Lu et al. (2000) on peeled and unpeeled apples.

The different absorption levels may have influenced the prediction accuracy of both scanning methods. The higher absorption level for the CSSM caused this scanning method to have higher prediction accuracy than the SSM. However, both scanning methods could be applied in the field for different measurement activities. For example, the CSSM has the potential to be used to screen the quality of sugarcane crops in the field for breeding and clonal evaluation programmes. For on-the-go quality measurement, the SSM has the potential to be applied to scan the moving billets on the elevator of a sugarcane harvester. However, to realize the potential application of the SSM on a harvester, more research is needed to develop a reliable measurement and sampling system which is suitable to be mounted on a sugarcane harvester.

Conclusion

This study demonstrated that the FRS coupled with PLS models could be applied to predict Brix values from internode samples using either the CSSM or SSM. It has been shown that the CSSM gives better prediction results than that of SSM. The prediction of Brix values from the spectral data using the CSSM was good, with R^2 and RMSEP values at 0.92 and 1.03 respectively. For the SSM, the prediction accuracy was also good with R^2 and RMSEP values at 0.82 and 1.50 respectively. This study suggested that the spectroscopy method has the potential to be used for measuring sugarcane quality from stalk samples in the fields. The study also identified the need to develop a reliable quality measurement system on a sugarcane harvester. However, further studies are needed to examine the influence of different varieties, crop ages, planting regions and year of harvests on the model performances.

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Abstrak
Potensi spektroskopi pantulan inframerah (NIR) dekat untuk meramal kandungan gula tebu daripada sampel ruas telah dikaji. Spektra NIR diukur menggunakan spektroradiometer berskala penuh (FRS) dalam sempadan gelombang antara 350 nm dan 2,500 nm berdasarkan kaedah pengimbasan keratan rentas (CSSM) dan pengimbasan kulit (SSM). Model statistik telah dibangunkan dengan menggunakan kaedah *partial least square* (PLS) untuk menterjemah data spektra dan membina model penentu ukur untuk kandungan gula (Brix) tebu. Kedua-dua CSSM and SSM mempunyai ketepatan jangkaan yang bagus dalam meramal kadar Brix, dengan nilai korelasi penentuan (R^2) masing-masing sebanyak 0.92 dan 0.82 dan nilai *root mean square error of prediction* (RMSEP) masing-masing sebanyak 1.03 dan 1.50 Brix. Keputusan ini menunjukkan bahawa FRS boleh digunakan untuk meramal kandungan gula daripada ruas tebu menggunakan CSSM atau SSM. Bagaimanapun, CSSM didapati memberikan ketepatan ramalan yang lebih baik berbanding dengan SSM. Keputusan ini menunjukkan kaedah spektroskopi mempunyai potensi untuk digunakan bagi penentuan kandungan gula tebu secara cepat daripada sampel batang di ladang.

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