Near-infrared spectroscopic analysis for rapid evaluation of major chemical components in sugarcane bagasse

S. Kasemsumran[1,*], S. Jungtheerapanich[1], K. Ngowsuwan[1], W. Thanapase[1] and S. Miyata[2]

[1] Laboratory of Non-destructive Quality Evaluation of Commodities, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok 10900, Thailand

[2] Japan International Research Center for Agricultural Sciences, Tsukuba 305-8686, Japan

* Corresponding author: aapspk@ku.ac.th

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ABSTRACT

In this study, near-infrared (NIR) spectroscopy was employed to determine cellulose in terms of glucan, hemicellulose in terms of xylan, and lignin contents in sugarcane bagasse as biomass for energy purpose utilization. We investigated by using three sample groups which were consisting of (A) twenty simulated samples prepared by mixing cellulose, hemicellulose, and lignin standards in many ratios to expand the range of contents for these analyses, (B) forty-seven sugarcane bagasse samples of wild species of *Saccharum spontaneum* and *Erianthus,* and their hybrids obtained from Khon Kaen Field Crops Research Center, and (C) seventy sugarcane bagasse samples collected from various sugar factories in Thailand. All samples were measured in the NIR region of 1,100–2,500 nm using reflectance mode. Partial least square (PLS) regression models for the quantitative determination of glucan, xylan, and lignin contents in sugarcane bagasse samples were calculated from data of NIR spectra and of analyzed contents detected by reference methods. The best PLS calibration models for cellulose (correlation coefficient (R) = 0.94, standard error of prediction (SEP) = 4.31%), xylan (R = 0.88, SEP = 1.50%), and lignin (R = 0.94, SEP = 2.08%) in sugarcane bagasse samples obtained from the model using actual bagasse samples, in which they developed from multiplicative scattering correction, second derivative, and second derivative pretreated NIR spectra, respectively. This study shows that the matrix of actual sugarcane bagasse in the NIR calibration model was necessary for getting the accurate results than those obtained by using a more comprehensive concentration range of analyzes obtained from the NIR calibration model, including the simulated samples. Essentially, NIR spectroscopy can be used to predict the necessary chemical constituents of sugarcane bagasse prior to converting biomass to substitute energy with fast detecting and reducing the use of chemicals.

Keywords: Near-infrared spectroscopy, biomass, glucan, xylan, lignin, sugarcane bagasse

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INTRODUCTION

Thailand is one of the important agricultural countries in the world. They produce many kinds of agricultural products for human and animal consumption. By-products from agriculture and agro-industry are agricultural and industrial wastes, such as bagasse and rice husk, which can be converted into energy (Soccol *et al*., 2010). These materials are called “biomass”. Due to petroleum oil is considered the main fuel source of the world, it is reducing and has a higher price every year. Finding alternative energy from oil is, therefore, essential. The sugarcane is a member of the Gramineae (Poaceae) family, tribe Andropogenae and the genus Saccharum, which is the most substantial harvest for sugar production

and energy production. In Thailand, the industry of sugarcane production grew to 127 million metric tons per year and became the world's second-largest sugar exporter (Ratanasumarn and Chitprasert, 2020). Biomass from sugarcane production can be divided into top trashier and bagasse. However, the sugarcane mill produces a lot of bagasse as residue, which is easy to collect and deliver to a renewable energy factory. Therefore, bagasse has a high potential to utilize as a renewable source of energy. There are many advantages to generating bioenergy using bagasse when compared to fossil fuels, including lower greenhouse gas emissions, energy cost savings, and waste management.

Sugarcane bagasse is a solid fibrous part of sugarcane from the process of juice extraction from sugarcane, which is the main fibrous by-product, and its yield is about 30% of the weight of sugarcane (Asagekar and Joshi, 2014; Arni, 2018; Xu *et al*., 2020). Sugarcane bagasse has many advantages such as low cost and being rich in carbohydrates, which consists of high cellulose (40–45%), hemicellulose (25–30%), and lignin (18–25%) (as the lignocellulosic biomass), but low in protein (2–3%) and small amounts of extractive and mineral salts (Soccol *et al*., 2010; Rocha *et al*., 2015; Santo *et al*., 2018). Sugarcane bagasse is used to produce power as a fuel to generate electricity (Khaenson, 2018), to make paper, to produce ethanol (Betancur and Pereira Jr., 2010; Dias *et al*., 2012), to produce animal feed (Okano *et al*., 2006), to fertilize plants, and others (Mussatto *et al*., 2006; Arni, 2018). Sugarcane bagasse is the lignocellulosic biomass. It is mainly composed of three macromolecular biopolymers known as cellulose and hemicellulose (carbohydrate polymers), and lignin (aromatic polymer).

As the bioenergy sources, the conversion of lignocellulosic biomass to ethanol, including four major steps of i) pretreatment, ii) depolymerization (saccharification) of cellulose and hemicelluloses to soluble monomer sugars by a process known as hydrolysis, iii) conversion of these monomeric sugars to valuable products such as ethanol in a fermentation process and iv) separation and purification of the products. In the chemical pretreatment method using acid, hemicelluloses will be targeted, whereas in alkali-catalyzed pretreatment, mainly lignin is removed (Dashtban *et al*., 2009). Ethanol production can be integrated with a combined heat and power plant using the removed lignin to reduce the production cost. The bioconversion of lignocellulosic residues (sugarcane bagasse) to ethanol is more complicated than the bioconversion of starch-based residues and thus requires assessment of their composition essential for bioenergy conversion processes. For example, the i) pretreatment and iii) fermentation process for bioethanol production requires this chemical compositional information, reactor design and process adjustment for the fast pyrolysis of biomass need this information well. Each step in the bioconversion process has to be optimized to improve the yield (Dashtban *et al*., 2009; Liu *et al*., 2010). Chemical compositional changes in sugarcane bagasse are subjected to different resources and their impacts on the conversion process to bioenergy. Therefore, it is necessary to know the chemical composition of sugarcane bagasse in order to lead to the selection of suitable energy production processes.

The conventional methods used to determine the chemical composition of bagasse are combined with several methods such as the chemical extraction and CHONS analyzer, in which these methods are time-consuming, expensive, labor-intensive, and not practically feasible for the analysis of large populations of samples. On the other hand, near-infrared (NIR) spectroscopy is a powerful technique owing to rapid analysis, non-destructive, and minimal sample preparation. For example, NIR has been used for predicting soluble sugars in sugarcane, the fiber content of sugarcane stalk, stalk soluble sugar, and bagasse hydrolyzed sugar (Wu *et al*., 2015; Phuphaphud *et al*., 2019). Matt *et al*. (1996) employed the NIR technique by creating various predictive equations including ethanol extractives, ash, lignin, uronic acids, arabinose, xylose, mannose, galactose, glucose, C, H, N, and O from wood biomass feedstocks. Nicole *et al*. (2008) applied the NIR method to classify biomass samples, including red oak, yellow poplar tree, walnut family tree, switchgrass, corn cob, and bagasse. Wolfrum and Sluiter (2009) studied to improve the quality of the NIR model for the

prediction of chemical composition in dilute-acid pretreated corn stoves. Recently, the rubberwood properties testing by mean of the visible and NIR spectroscopic prediction for the hemicellulose, cellulose, N, C, H, and moisture contents in waste rubberwood as biomass for energy utilization were reported (Phumichai *et al*., 2020). The sugarcane bagasse is taken into account in our present study to implement the NIR technique for the most available biomass sources.

Therefore, this research aimed to employ the NIR spectroscopic method to determine cellulose (glucan), hemicelluloses (xylan), and lignin contents in sugarcane bagasse. The predictive performance between 1) a global NIR calibration model developed from different sugarcane bagasse samples of using the artificial, wild, and hybrid species and those obtained from sugarcane mills, and 2) a local NIR calibration model developed from sugarcane bagasse samples of the wild and hybrid species and those obtained from sugarcane mills, were investigated and compared. This comparison process could be further associated with the guideline to collect calibration samples by sugarcane germplasm to develop the best NIR calibration model for accurate quantitative analysis of those major chemical components in sugarcane bagasse.

MATERIALS AND METHODS

Sample Preparation

Three groups of sugarcane bagasse samples were employed in this study. One was the simulated samples as a group of A (n=20) prepared by mixing of cellulose and xylan from birch wood (Sigma-Aldrich, Steinheim, Germany) and lignin powder from beechwood (Wood Chemistry Lab., Tokyo University) in twenty ratios by using a D-optimal mixture design (Esbensen, 2010) to expand the range of contents for cellulose (35–65%), xylan (15–30%) and lignin (15–35%), respectively. The second was the samples as a group of B (n=47) obtained from different parts (leaf, sheath, stem, and whole tree) of wild species *Saccharum spontaneum* and *Erianthus* species and their hybrids provided by Khon Kaen Field Crops Research Center (KKFCRC) and others. The last one was the samples collected from various sugar factories in Thailand (group of C; n=70). The sugarcane bagasse samples were dried, ground, and sieved. Before NIR measurement, the samples were again ground with a Cyclotec 1093 (1–mm) (FOSS, HillerØd, Denmark) to obtain the regular size. The moisture content of all samples was controlled at ≤12%. Figure 1 shows ground samples of three groups preparing for NIR measurement.

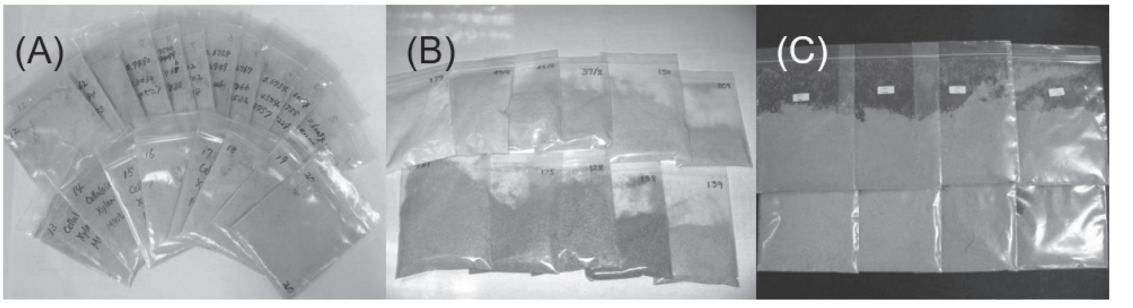

Figure 1 Sugarcane bagasse samples: (A) group A prepared by mixing standard samples, (B) group B obtained from wild species of *Saccharum spontaneum* and *Erianthus* and their hybrids, and (C) group C collected from sugar factories

NIR Spectral Acquisition

Each sample (1.50 ± 0.02 g) was packed in a closed cup for a powder sample (diameter of 2.85 cm). They were kept at 25°C before the NIR measurement. The NIR reflected spectra were recorded by InfraAlyzer 500 (BRAN+LUEBBE, Norderstedt, Germany) spectrometer in the region of 1,100–2,500 nm, at 2 nm resolution. The instrument

was daily calibrated by using two reference standards before the measurement begins. A medium-absorbance reference (dark halo material) was used to display the noise inherent in the instrument from all causes not attributable to the sample itself, the operator, or drift. A permissible noise level (PNL) was computed from the average of ten scans and was displayed along with the actual level. A calibration reference (white polystyrene material) was used to confirm wavelength accuracy. Typically, the sharp peak at 1,680 nm is used together with the built-in software of this NIR instrument to make any required adjustment. Each sample was divided into 3 sections for three scans. It took about 10 min to finish scanning each sample. The averaged NIR spectral data of each sample was used for data analysis. Figure 2 shows the procedures for collecting NIR spectra.

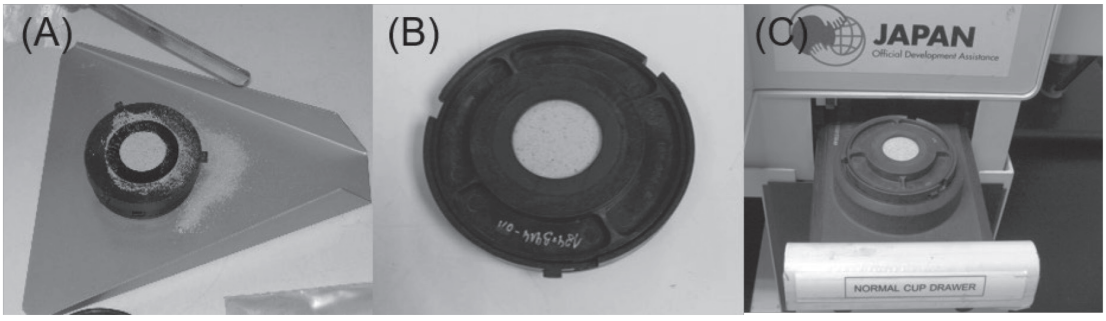

Figure 2 Sample preparations for NIR measurement: (A) filling sample, (B) packing sample in a closed cup, and (C) placing a sample cup in the drawer for NIR measurement

Reference Analysis

Cellulose and hemicellulose contents in terms of glucan and xylan were determined by using a method of the National Renewable Energy Laboratory (Sluiter *et al*., 2008). The quantitative analysis method for lignin content was the acetyl bromide method (Iiyama and Wallis, 1989). The distribution contents of glucan, xylan, and lignin in three groups of samples are shown in Table 1.

Table 1 Distribution of the analyte contents in the sugarcane bagasse sample for three groups

Sample group	Content	Minimum (%)	Maximum (%)	Mean (%)	SD (%)
A (n=20)	Glucan	14.82	35.16	25.46	6.91
	Xylan	14.84	30.00	22.87	5.44
	Lignin	35.14	64.84	51.68	8.23
B (n=47)	Glucan	11.82	42.81	26.16	6.82
	Xylan	7.74	26.15	19.13	3.69
	Lignin	7.08	23.15	15.07	4.25
C (n=70)	Glucan	34.90	47.53	41.92	3.26
	Xylan	17.20	26.95	23.13	1.86
	Lignin	17.20	28.30	24.04	1.89

Note: A = mixing standard samples, B = wild species and their hybrids, C = sugarcane bagasse from sugar factories, SD = standard deviation

Data Analysis

Data pretreatment is an essential step to build most types of calibration models in NIR analysis. With a well-designed pretreatment method, the performance of the model can be greatly improved. The overlap spectral data is generally obtained in the NIR region. It is possible to separate the overlapping spectra to provide a more significant correlation between the light absorption and structural molecules by using the second derivative (2D) pretreatment. In our case, the scan results were scattered due to the pathlength of the controlled particle size ground sample vary a little from sample to sample. To reduce the scattering, multiplicative scatter correction (MSC) was implemented on NIR data. The NIR spectra were subjected to an individual pretreatment of 2D (seven-point Savitsky-Golay filter) and MSC before developing the calibration models and then made the comparisons. Calibration models were calculated using the partial least square (PLS) regression method and validated by a separate test set of samples using Unscrambler (Ver. 9.8: CAMO AS, Trondheim, Norway). Two calculation types were carried out, type I consisted of 100 samples from groups A, B, and C for a calibration set and 37 samples from group C for a prediction set, in which they were randomly selected. Type II contained 80 samples from groups B and C for a calibration set and employed the same samples as type I for the prediction set.

The calibration models of type I and type II were subjected because this study describes a NIR method using a comprehensive calibration model to predict those three analyte contents in bagasse samples. As the fact that sugarcane bagasse is the fibre that remains after the sugars have been extracted. It is a by-product of the sugarcane industry with approximately 32–34% cellulose (glucan), 19–24% hemicellulose (xylan), 25–32% lignin, 6–12% extractives, and 2–6% ash (Haghdan *et al*., 2016). Due to the lignin content generally presents in the low level. Therefore, sample group A was prepared to increase the lignin content by mixing the standard of glucan (14.82–35.16%), xylan (14.84–30.00%), and lignin (32.14–64.84%). Moreover, sample group B was included to get the low contents of glucan (11.82%), xylan (7.74%), and lignin (7.08%). By having sample groups A and B into C, the calibration samples used for a comprehensive NIR model development can go beyond the natural composition ranges of bagasse (Table 1). Table 2 shows the distribution of glucan, xylan, and lignin contents in the samples for the calibration set and prediction set. Note that the maximum and minimum contents of analysts were kept in the calibration set.

Table 2 Distribution of the analyte contents in the sugarcane bagasse samples for model development

Calculation	Sample group	Content	Minimum (%)	Maximum (%)	Mean (%)	SD (%)
Type I	Calibration ABC (n=100)	Glucan	11.82	47.53	31.22	9.57
		Xylan	7.74	30.00	21.19	4.15
		Lignin	7.08	64.84	25.35	14.61
	Prediction C (n=37)	Glucan	35.00	47.08	41.94	3.10
		Xylan	19.30	26.20	23.16	1.66
		Lignin	18.40	27.13	24.06	1.72
Type II	Calibration BC (n=80)	Glucan	11.82	47.53	32.65	9.63
		Xylan	7.74	26.95	20.77	3.68
		Lignin	7.08	28.30	18.76	5.65
	Prediction C (n=37)	Glucan	35.00	47.08	41.94	3.10
		Xylan	19.30	26.20	23.16	1.66
		Lignin	18.40	27.13	24.06	1.72

Note: A = mixing standard samples, B = wild species and their hybrids, C = sugarcane bagasse from sugar factories, SD = standard deviation

RESULTS AND DISCUSSION

Figure 3 shows the 137 original NIR spectra of all samples in the region of 1,100–2,500 nm. The spectrum pattern for each group was mainly similar. Therefore, the second derivative spectrum of averaged sugarcane bagasse samples for sample groups A (solid line), B (dot line), and C (dash line) was done and compared in Figure 4. It can be seen that the feature of averaged second derivative spectrum for each sample group was mainly like to each other. The peak intensity of the major bands was varying from the differences in chemical compositions, mainly due to cellulose, hemicellulose, and lignin. At the same time, some peaks of sample group A shifted to the longer wavelengths in the wavelength ranges of 1,700 and 2,000–2,400 nm. This shifting may concern the different plant sources of cellulose and xylan standards from birch wood, and lignin standards from beech wood, blending for sample group A. In contrast, sample groups B and C used the actual bagasse from sugarcane. Naturally, the composition of this lignocellulose is variable and depends on the plant source (Dashtban *et al*., 2009). In Figure 4, the O–H polymeric overtone band and O–H/C–O polymeric combination band clearly showed at 1,430 nm (all groups) and 2,075 nm (group B and C), 2,100 nm (group A), respectively. These bands were associated with cellulose, hemicellulose, starches, and sugars as C–H and O–H–related bands. The highest intensity was obtained from the second derivative spectrum of group B. It was noted that sample group B was obtained from the Khon Kaen Field Crops Research Center. They prepared the bagasse samples by using the machines at the laboratory level. Therefore, their bagasse samples may consist of the remaining starches and sugars higher than other sample groups got from the factory. The O–H stretching bands of water showed at 1,920 nm. It illustrated that sample groups B and C contained the same range of moisture content that was higher than the moisture content found in sample group A (≤ 7%). The assignment for lignin peaks at 2,270 and around 2,326–2,336 nm corresponding to C–H stretching and C=O combination, and C–C/C–H in aromatic structure in lignin molecule, respectively (Workman Jr. and Weyer, 2008).

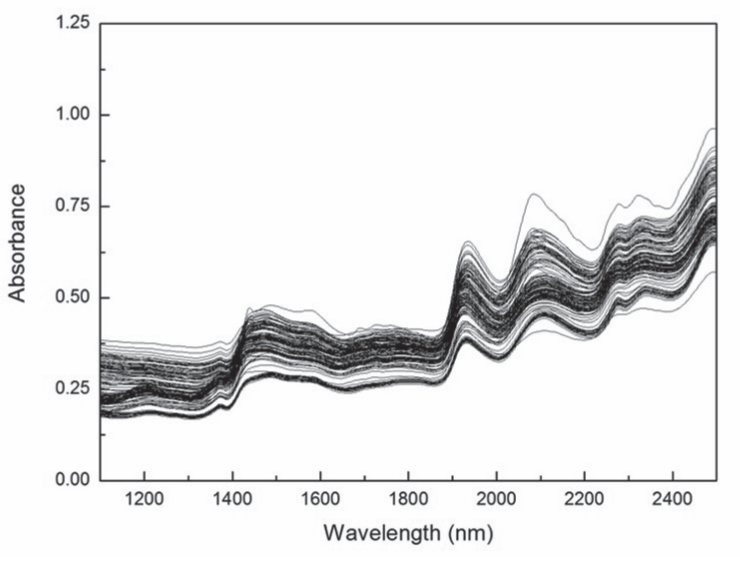

Figure 3 The original NIR spectra of all samples in the region of 1,100–2,500 nm

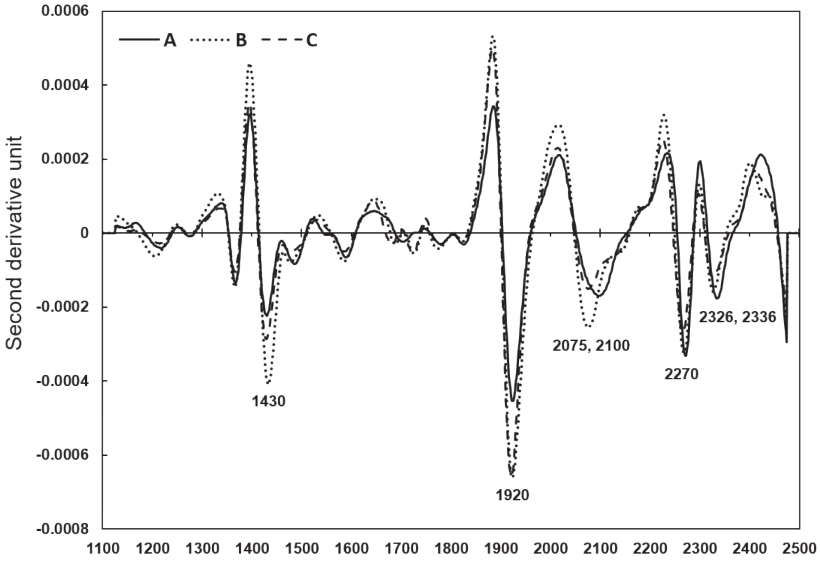

Figure 4 The second derivative spectra of averaged sugarcane bagasse sample groups A, B, and C in the region of 1,100–2,500 nm

The statistical results of PLS calibration models for the determination of glucan, xylan, and lignin content in sugarcane bagasse obtained by different pretreated NIR spectral data for calculation types I and II were compared in Tables 3 and 4, respectively. The highest predictive performance of the PLS calibration model was selected by considering a model that yielded the lowest values of the standard error of prediction (SEP) and the bias of prediction. In calculation type I, the best PLS calibration models for glucan, xylan, and lignin in sugarcane bagasse samples were developed from MSC pretreated spectra using factor numbers 7, 10, and 6, respectively. As for calculation type II, the best PLS calibration models for glucan, xylan, and lignin in sugarcane bagasse samples were developed from MSC pretreated spectra (factor number 6), second derivative pretreated spectra (factor number 8), and second derivative pretreated spectra (factor number 3), respectively. These models yielded the lowest SEP (type I: 4.53% for glucan, 1.62% for xylan, and 2.71% for lignin; type II: 4.31% for glucan, 1.50% for xylan, and 2.08% for lignin) and bias value with high correlation coefficient (R). The difference between calculation types I and II was that type I contained a set of simulated samples prepared by mixing standard powder of glucan, xylan, and lignin. They were not actual sugarcane bagasse. The ranges of analyte content were expanded by including a simulated sample set, which illustrated the developed model with a higher R-value than those obtained from models in calculation type II. However, the SEP values obtained from the best models in type II were lower than those obtained from the best models in type I. It was maybe that the matrix of the simulated sample was different from the actual sugarcane bagasse sample, which may raise the prediction error. Therefore, the best PLS calibration models for predicting glucan, xylan, and lignin content in bagasse were obtained from type II with the lowest SEP value, in which all samples in type II were only actual sugarcane bagasse.

Table 3 Partial least square calibration results of calculation type I for predicting glucan, xylan, and lignin contents in sugarcane bagasse samples

Content	Pretreatment	F	Calibration			Prediction	
			R	SEC (%)	Bias (%)	SEP (%)	Bias (%)
Glucan	None	4	0.90	4.20	-1.53×10^{-7}	4.59	–1.11
	MSC*	7	0.93	3.41	-1.11×10^{-6}	4.53	–0.10
	Second derivative	4	0.90	4.06	-6.29×10^{-7}	4.61	–0.83
Xylan	None	13	0.90	1.83	-1.92×10^{-5}	1.98	0.49
	MSC*	10	0.88	1.97	-4.31×10^{-6}	1.62	0.23
	Second derivative	7	0.88	1.94	1.34×10^{-7}	1.64	0.42
Lignin	None	5	0.98	3.16	2.07×10^{-6}	3.78	–1.54
	MSC*	6	0.99	2.42	5.00×10^{-6}	2.71	–0.38
	Second derivative	4	0.98	2.81	6.39×10^{-7}	2.90	–0.68

Note: F = factor number, R = correlation coefficient, SEC = standard error of calibration, SEP = standard error of prediction, MSC = multiplicative scattering correction, * selected partial least square calibration model

Table 4 Partial least square calibration results of calculation type II for predicting glucan, xylan, and lignin contents in sugarcane bagasse samples

Content	Pretreatment	F	Calibration			Prediction	
			R	SEC (%)	Bias (%)	SEP (%)	Bias (%)
Glucan	None	8	0.95	3.04	5.38×10^{-6}	4.39	–0.40
	MSC*	6	0.94	3.22	1.13×10^{-9}	4.31	–0.51
	Second derivative	4	0.92	3.82	2.02×10^{-6}	4.46	–1.16
Xylan	None	11	0.88	1.75	4.34×10^{-6}	1.84	0.02
	MSC	9	0.86	1.87	-6.76×10^{-6}	1.85	–0.10
	Second derivative*	8	0.88	1.71	1.55×10^{-7}	1.50	0.02
Lignin	None	4	0.94	1.97	-1.15×10^{-7}	2.33	–0.74
	MSC	3	0.94	1.93	-4.41×10^{-7}	2.43	–0.81
	Second derivative*	3	0.94	1.94	-1.43×10^{-7}	2.08	–0.38

Note: F = factor number, R = correlation coefficient, SEC = standard error of calibration, SEP = standard error of prediction, MSC = multiplicative scattering correction, * selected partial least square calibration model

The scatter plots of the best calibration model for the determination of glucan, xylan, and lignin in bagasse built from calculation type II were given in Figure 5. They showed the predictive performances acceptable for most applications for predicting glucan and lignin content ($0.91 \leq R \leq 0.95$) and approximate prediction of xylan ($0.81 \leq R \leq 0.90$) (Williams *et al*., 2019). We can compare our results to the previously reported by Rodríguez-Zúñiga *et al*. (2014), in which they

developed NIR models for the prediction of cellulose, hemicellulose, and lignin in sugarcane bagasse by using only 68 calibration samples. They reported the root mean squared error of prediction (RMSEP) to predict cellulose, hemicellulose, and lignin with 4.1%, 3.8%, and 3.5%, respectively. By that of our obtained the best results in type II as RMSEP value for prediction of cellulose, we got a very little higher RMSEP of 4.31%. However, we obtained the higher predictive performance for the model prediction of hemicellulose and lignin with the RMSEP values of 1.50% and 2.08%, respectively.

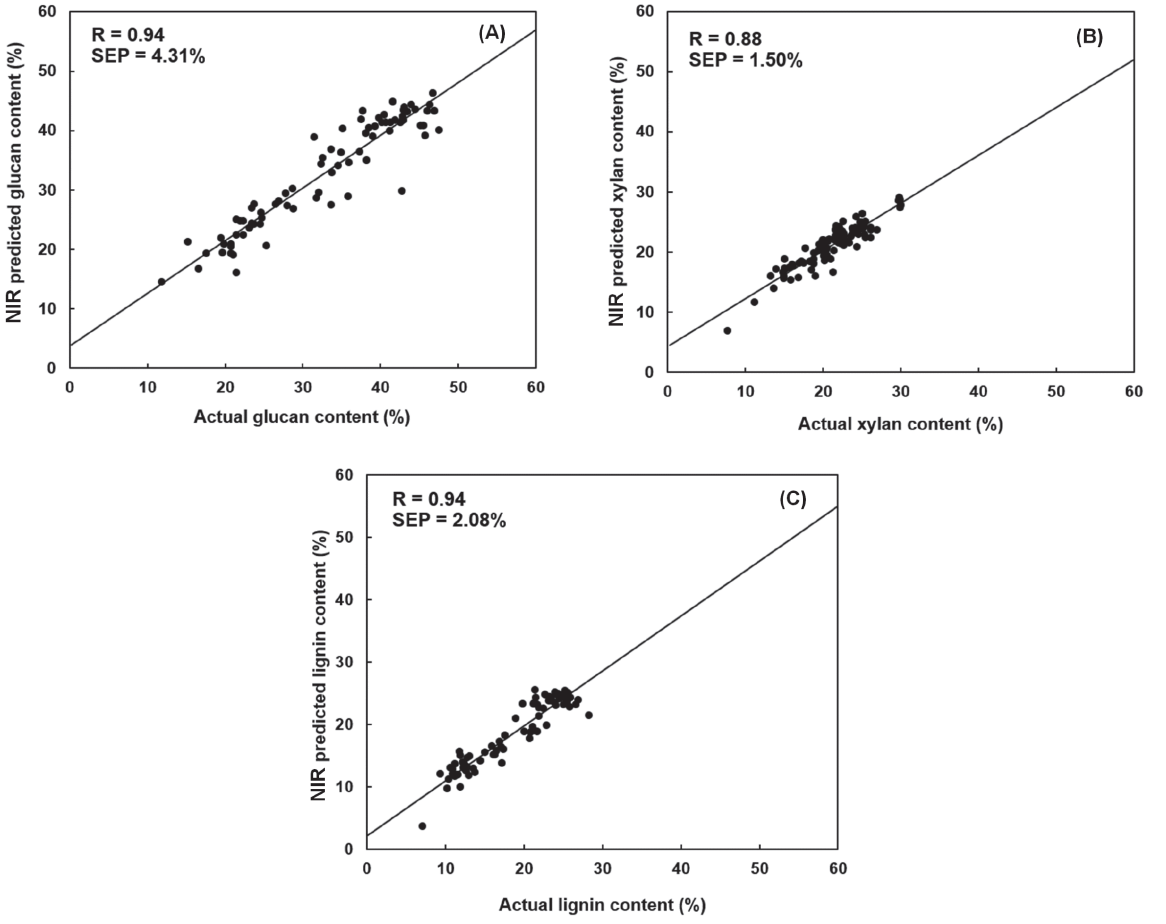

Figure 5 Scatter plots for (A) actual and NIR predicted analyte contents in bagasse samples for the best calibration models using MSC pretreatment for glucan prediction and second derivative pretreatment for (B) xylan and (C) lignin predictions (calculation type II). R = correlation coefficient, SEP = standard error of prediction

CONCLUSIONS

In this study, we have demonstrated the possibility of employing the NIR spectroscopic method for the quantitative determination of glucan, xylan, and lignin in sugarcane bagasse. These major chemical components in the bagasse sample could be obtained in minutes using the NIR analysis before their conversion to bioenergy. The PLS calibration models developed from pretreated NIR spectra (MSC for glucan and second derivative method for xylan and lignin) of actual sugarcane bagasse in the whole NIR region provided the best predictive performance with high correlation and lowest SEP values of 4.31%, 1.50%, and 2.08% for glucan, xylan and lignin predictions, respectively. These developed calibration models are recommended for quantitative analysis of the unknown bagasse samples with the range of those calibration set for glucan of 11.82–47.53%, xylan of 7.74–26.95%, and lignin of 7.08–28.30%. From the results, it can be concluded that the matrix of actual sugarcane bagasse in the NIR information is important for the development of an accurate model for prediction than those obtained by the result of using the NIR information of artificial bagasse sample with a broader concentration range of analyzes.

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