NIRS determination of carbohydrates from hydrothermal-treated rice straw

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ABSTRACT: Increasingly stringent environmental standards and the need for sustainable development are forcing pulp and paper mills to study methods to maximize the use of process wastes. Several studies have shown that "hydrothermal process" wastes might serve as raw material to obtain monosaccharides and oligosaccharides. High-performance liquid chromatography (HPLC) traditionally has been used to study these carbohydrates. However, HPLC has a series of disadvantages that could be avoided by use of other methods. This study evaluated the potential use of near infrared spectroscopy (NIRS) as a substitute analysis method for HPLC, using rice straw as raw material. The results showed that NIRS analysis can be used as a prediction method for monosaccharides and oligosaccharides. This method has the additional advantage of being a fast, nondestructive technique that does not require pretreatment of samples, and therefore, decreases the costs and the environmental impact of the analyses process.

Application: NIRS technology can be used as an alternative to HPLC to predict the amount of carbohydrates in hydrothermal-treated rice straw.

Because paper pulp is obtained preferentially from the cellulose fraction of plant biomass, a number of other, potentially useful lignocellulosic components are lost in the process [1,2]. The need for process economy, sustainability in the exploitation of raw materials, and reduction of waste has prompted the integration of energy valorization lines for byproducts into the production of cellulose pulp to exploit the major residual fractions of the raw material, which consist mainly of hemicelluloses and lignin.

Various methods for the fractional valorization of lignocellulosic materials from forest plants (particularly conifer and leafy wood) are available. A number of methods for exploiting lignocellulose from forest and agricultural plants also are available. In fact, these lignocellulosic materials can be valorized in a global or integral manner in a single step with treatments such as combustion [3], gasification, pyrolysis [4], or liquefaction [5] for energy production. These treatments afford total valorization by selective fractionation of the main biomass components (i.e., fractional valorization) in much the same manner as delignification, polysaccharide hydrolysis, and mixed methods for the individual recovery of these components [6,7].

The greatest shortcoming of fractional exploitation methods is that they are unable to isolate cellulose, hemicelluloses, and lignin without altering their chemical structures [8]. Prominent among available choices, particularly in terms of future applicability, is depolymerization of hemicelluloses by autohydrolysis, also known as the "hydrothermal process," which requires no acids; instead, the hydrolytic agent is produced by the reaction medium itself [9].

The paper industry is facing major challenges to achieving the goal of sustainable development, including the efficient recovery of byproducts and waste and the use of alternative raw materials [10,11]. Fractional exploitation and paper quality analysis involves using chemical reagents [12] that join the typical waste of the paper industry. Many tests also use destructive techniques and large numbers of samples [13], and analyses can take some time to perform; as a result, large amounts of product that do not meet specifications may be released before an abnormality in the process or end-product is detected.

The oil industry has introduced the use of infrared (IR) analyzers to correlate photometric measurements made at variable wavelengths with technical properties and specifications for some fractions. For example, some commercially available analyzers for gasolines can determine the octane number (motor [MON] and research [RON]), composition, and proportion of oxygen-containing additives, vapor pressure, and distillation curve, among other properties, in near-real time [14].

The paper industry currently is assessing near IR spectroscopy (NIRS) as a nondestructive technique for determining pulp and paper properties [15]. So far, this technique has been used to predict quality indicators such as kappa number [16], yield, and cellulose content [17,18].

Some studies have shown that functional groups, such as carbonyl and carboxyl, and molecular weight are important in this context as they are involved in the characterization of glucose and closely related to paper properties [19,20].

The purpose of this work was to assess NIRS analysis as a means to study carbohydrates from hydrothermal fractionated rice straw in comparison with using high-performance liquid chromatography (HPLC) as the analysis technique.

EXPERIMENTAL

Conditioning of the raw material

The rice straw used in this work was *Oryza sativa* var. *Senia* from the "Arroz de Valencia" designation of origin. The straw was allowed to dry in the sun to a constant moisture. Straw samples were collected to determine their moisture content, and the remainder was turned over daily to facilitate uniform drying. Once dry, the straw was ground in a hammer mill and sieved to obtain (a) the 4-6 cm size fraction, free of impurities such as stones, sand, and dust; (b) the coarse fraction (larger than 6 cm); and (c) the fine fraction (smaller than 4 cm). The 4-6 cm fraction was used for hydrothermal fractional treatment. This fraction was chosen to carry out the trials because it was more suitable for scale up of the process.

Characterization of the raw material

The raw material was characterized chemically in accordance with the applicable TAPPI Standard Methods for the different components: lignin (TAPPI T-222, "Acid-insoluble lignin in wood and pulp"), α-cellulose (TAPPI T-203 0S-61, "Alpha-cellulose in pulp"), ethanol-benzene extractables (TAPPI T-204, "Solvent extractives of wood and pulp"), and ash (TAPPI T-211, "Solvent extractives of wood and pulp"). By exception, holocellulose was determined with the method developed by Wise [21]. The chemical composition of the rice straw we used was 60.7% holocellulose; 41.2% α-cellulose; 21.9% Klason lignin; 2.2% soluble lignin; 0.56% ethanol-benzene extractables, and 9.2% ash.

Hydrothermal treatment

The amounts of water and rice straw needed to obtain a liquid:solid ratio of 6, 7, or 8 mL/g was placed in a 15 L batch reactor that was heated by an outer jacket containing electrical wires.

The reactor contents were stirred by rotating the reaction vessel via a motor connected through a rotary axle to a control unit, which included the required instruments for measurement and control of pressure and temperature. Once the mixture was heated at the selected temperature, the reactor was depressurized, and the liquid and solid fractions were separated for subsequent treatment or analysis.

Experimental design

The experimental design we used consisted of a series of points (tests) around a central composition point (central test) that were used to estimate the quadratic terms of a polynomial model [22]. A total of 15 tests were required for the three independent variables studied (i.e., temperature [T], time [t], and liquid:solid ratio [R]).

The values of the independent variables were normalized by using the following equation to facilitate direct comparison of coefficients and determine the effects of the independent variables on each dependent variable:

$$X_n = 2 \cdot \frac{X - \overline{X}}{X_{\max} - X_{\min}}$$

where X_n is the normalized value of temperature (T), time (t), or liquid:solid ratio (R); X is the absolute experimental value of the variable concerned; $\overline{X}$ is the mean of the extreme values of X; and X_{max} and $X_{\min}$ are its maximum and minimum values, respectively.

Characterization of the fractions of the hydrothermal treatment

The carbohydrates studied in the samples tested were monosaccharides, disaccharides, and low molecular weight oligosaccharides. The chemical composition of lignocellulosic material was determined by means of HPLC techniques.

Aliquots were taken from the liquid fraction of a 15 L batch reactor, filtered through a 0.45 µm membrane, and injected directly into HPLC device to determine monosaccharides (glucose, xylose, and arabinose), furfural, hydroxymethyl furfural 5 (HMF), acetic acid, and formic acid. A second aliquot of the liquid phase (25 mL) was subjected to quantitative posthydrolysis with 4% sulfuric acid for 60 min at 121°C before analysis by HPLC. The increase in the concentrations of monosaccharides and acetic acid caused by this posthydrolysis was a measure of the concentration of oligomers and acetyl groups attached to the oligosaccharides.

Chromatographic determination was carried out using an Agilent 1100 HPLC chromatograph equipped with a column of ion exchange resin Aminex HPX-87H under the following conditions: 0.05 mol/L mobile phase flow rate; 0.01 M sulfuric acid; 0.6 mL/min flow rate; 40°C column and detector temperature; and 5 µL injection volume. All samples analyzed by HPLC were filtered through cellulose acetate membranes of 45 µm pore size.

NIR spectroscopy analysis

All samples were scanned twice with a Foss NIRSystems 6500 monochromator (Foss NIRSystems, Silver Spring, MD, USA) using WinISI II 1.5 software (Infrasoft International, Port Matilda, PA, USA). The visible NIRS (VNIRS) and NIRS spectra (400-2500 nm) were collected at 2 nm intervals. Samples were placed in a rectangular cell with a 4.5 cm² × 5.5 cm² quartz window.

Spectral data analysis

Spectral data analysis and calibration equations were performed with WinISI II 1.5 software. Spectral data of the calibrations set were subjected to different spectral pretreatments combined with different mathematical pretreatments. Standard normal variate and detrending scatter correction were used as spectral pretreatments. As mathematical pretreatments, first and second derivatives were used to reduce baseline variation and enhance spectral features.

We used modified partial least square regression (MPLSR) [23] to relate spectra with the measured values of glucose, xylose, etc. Multivariate regression was carried out using the partial least squares (PLS) algorithm. The residues obtained

Sample	X_T (°C)	X_t (min)	X_R (mL/g)	Oligomers (%)	Glucose (%)	Xylose (%)	Arabinose (%)
1	170	10	7	0.80	0.97	2.30	0.56
2	190	20	8	0.34	1.37	2.96	0.73
3	150	20	8	0.98	0.84	2.02	0.43
4	190	20	6	0.28	1.37	2.79	0.70
5	150	20	6	0.69	0.82	1.96	0.39
6	190	0	8	0.52	1.32	2.63	0.66
7	150	0	8	0.67	0.65	1.81	0.33
8	190	0	6	0.59	1.27	2.58	0.64
9	150	0	6	1.07	0.61	1.71	0.28
10	170	20	7	0.73	1.04	2.33	0.60
11	170	0	7	0.85	0.92	2.27	0.53
12	170	10	8	0.77	0.99	2.30	0.57
13	170	10	6	0.82	0.95	2.30	0.54
14	190	10	7	0.52	1.90	3.82	0.95
15	150	10	7	1.04	1.03	2.66	0.51

I. Oligomers and monomers content in the liquid phase with respect to the original raw material. X_T, X_t, X_R = values of temperature, time, and liquid: solid ratio, respectively.

after the calculation of each regression term in the modified partial least squares (MPLS) regression were standardized, dividing by the standard deviation before the calculation of the next regression term [24]. According to Shenk and Westerhaus 1991, the number of terms was fixed to obtain a minimun error value before overfitting. Validation in all cases was based on full cross-validation.

The best pretreatment method was chosen based on the method that produced models with higher R^2 values and smaller standard error of cross-validation (SECV). The standard error of the reference methods (SEL) was calculated as the uncertainty estimate of the reference method. For this, two subsamples of each sample were analyzed. The formula used to calculate this error was:

$$SEL = \sqrt{\left(\left(\sum y_1 - y_2\right)/2N\right)}$$

where y_1 and y_2 are the values obtained for a sample and its repetition, and N is the number of data pairs used to calculate the SEL.

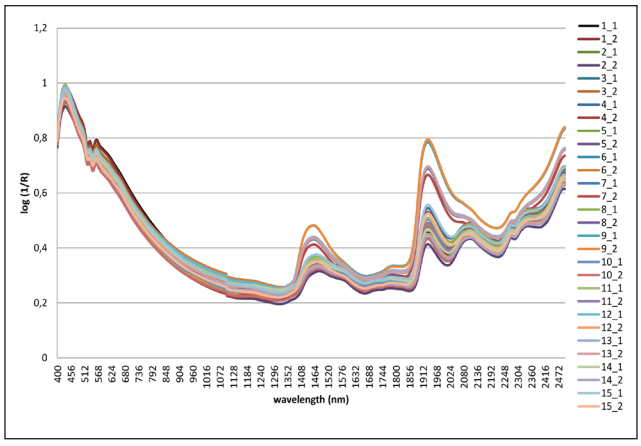
RESULTS AND DISCUSSION

The operational variables used in the 15 tests were temperature: 150°C–190°C; treatment time (as the time elapsed after the operating temperature was reached), 0-20 min; and liquid:solid ratio of 6-8. **Table I** shows the respective values and the results of the tests as the averages of three determinations per dependent variable related to the liquid and solid fraction obtained in each test. The composition of the hemicellulose in rice straw (percent by weight based on dry material) was 13% xylose, 1.6% mannose, 0.4% galactose, 4.0% arabinose, <0.2% acetyl groups, and <0.2% uronic acids.

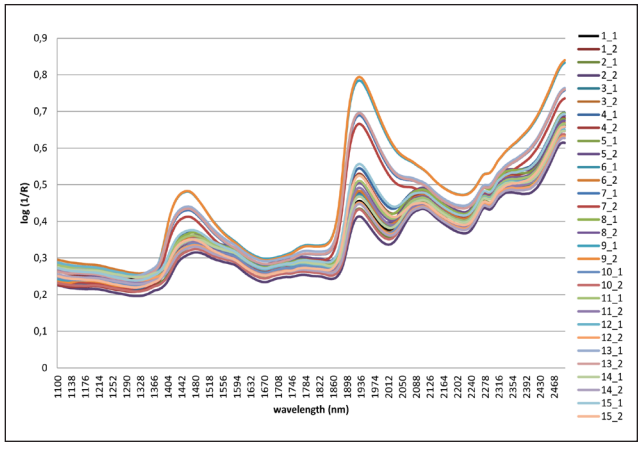
Hydrothermal treatment causes the dissolution of hemicellulosic polysaccharides present in the raw material. This process is enhanced by means of acidification that occurs through the release of acetyl groups of hemicelluloses. Polysaccharides were released from their constituent monomers because they are not completely stable in the cooking medium. Furfural and hydroxymethylfurfural could be formed during the process as a result of dehydration of pentoses and hexoses, respectively. These degradation substances were detected only in low concentrations (<0.15%) during the most extreme hydrothermal fractionation.

Operating to 190°C, for 10 min, and a liquid:solid ratio of 7 resulted in high values of glucose (1.9%), xylose (3.82%), and arabinose (0.95%) (Table I), allowing for a savings in capital when not operating with the maximum time and/or liquid:solid ratio. To determine the relevance of the simultaneous analysis of visible NIRS (VNIRS), with the possibility to perform forecasting based only with NIRS, a series of trials were carried out using both analysis methods. The VNIRS trials were carried out in a spectral region of 400-2500 nm and the NIRS samples were carried out in a spectral region of 1100-2500 nm.

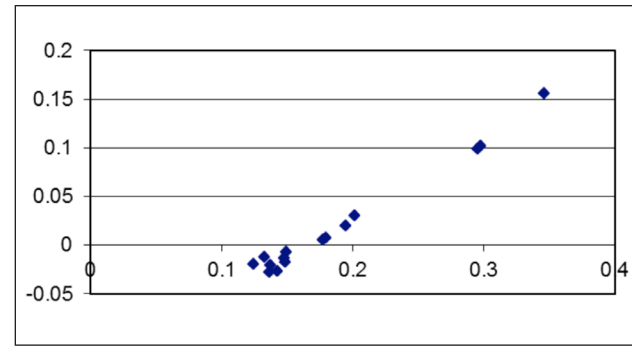
Figures 1 and **2** show the NIR and VNIR spectra of the 15 rice straw samples that were subjected to hydrothermal treatment; the measurements were carried out in duplicate. The figures show an inverse relationship between the values that reaches log (1/R) and the monosaccharide concentration. This relationship is valid because the amount of monosaccharides released and detected by HPLC increase when the hydrolysis is performed at more severe conditions, and therefore, the carbohydrates detected by VNIRS and NIRS de-



1. Near infrared (NIR) and visible NIR (VNIR) spectra of 30 rice straw samples.



2. NIR spectra of 30 rice straw samples.



3. Correlation of the partial least squares (PLS) terms with spectral data.

crease.

All spectral data were summarized in a few variables (PLS terms of multivariate regression) with greater correlation with reference values (**Fig. 3**). In Fig. 3, PLS terms 1 and 2 are represented, and each point corresponds to the spectra of each sample.

The error obtained in the calculation of the amount of oligosaccharides by HPLC was 0.08 (**Table II**). The errors for the NIRS equations by the first derivative in the trials carried out with VNIRS and NIRS were 0.10 and 0.14, respectively. The errors by the second derivative in the trials carried out with VNIRS and NIRS were 0.12 and 0.14, respectively. Taking into account the similarity of the SEL and SECV values, the results showed that both methods, VNIRS and NIRS, can be used to replace HPLC.

For glucose, the errors obtained by the first derivative in

		SECV	R^2	SEL
Oligomers (g/L)	First derivative_VNIRS	0.10	0.89	0.08
	Second derivarite_VNIRS	0.12	0.86	
	First derivative_NIRS	0.14	0.82	
	Second dericative_NIRS	0.14	0.80	
Glucose (g/L)	First derivative_VNIRS	0.10	0.82	0.09
	Second derivarite_VNIRS	0.09	0.85	
	First derivative_NIRS	0.12	0.77	
	Second dericative_NIRS	0.11	0.80	
Xylose (g/L)	First derivative_VNIRS	0.32	0.85	0.11
	Second derivarite_VNIRS	0.37	0.80	
	First derivative_NIRS	0.42	0.74	
	Second dericative_NIRS	0.43	0.73	
Arabinose (g/L)	First derivative_VNIRS	0.18	0.96	0.14
	Second derivarite_VNIRS	0.19	0.95	
	First derivative_NIRS	0.16	0.97	
	Second dericative_NIRS	0.20	0.94	

II. Comparison between the errors obtained by NIR spectroscopy (NIRS) and VNIR spectroscopy (VNIRS), and the standard error of high performance liquid chromatography (HPLC), the reference method. (SECV = standard error of cross-validation; R^2 = liquid: solid ratio^2; SEL = standard error of reference methods.)

the trials carried out with VNIRS and NIRS were 0.10 and 0.12, respectively. The errors for the second derivative in the trials carried out with VNIRS and NIRS were 0.09 and 0.11, respectively. The reference error (SEL) used to compare the performance of both mathematical methods was 0.09. These results show that the glucose content of different hydrothermal treatments can be predicted by means of direct measurement of rice.

The error obtained in the calculation of the amount of xylose by HPLC was 0.11. The errors obtained by means of VNIRS analysis system were 0.32 and 0.37, with the first and second derivative, respectively, and the errors obtained by means of NIRS analysis system were 0.42 and 0.43 with the same derivatives, respectively. This NIRS error, although three times higher than that obtained with the reference method, is inside the accuracy and precision levels required in NIRS trials. This difference might have occurred because the HPLC results did not include xylose dehydrated to furfural [25].

The error obtained in the calculation of the amount of arabinose by HPLC was of 0.14. The errors obtained by means of VNIRS analysis system were 0.18 and 0.19 with the first and second derivative, respectively, and the errors obtained by means of NIRS analysis system were 0.16 and 0.2 with the same derivatives, respectively. The prediction of the amount of arabinose obtained with both of the methods of analysis studied was good enough to use both of them as alternatives to the methods of analysis by HPLC.

Nearly all of the R^2 values obtained showed regression coefficients higher than 0.8, and support the use of the NIRS prediction method for the determination of certain sugars present in the raw matter studied.

CONCLUSIONS

The results of the 30 samples analyses show than the errors obtained in the measures carried out by HPLC (SEL), VNIRS, and NIRS (SECV) were similar when the oligosaccharides analyzed were glucose and arabinose. However, when the oligosaccharide analyzed was xylose, the errors obtained by HPLC (SEL) were lower than those detected by VNIRS and NIRS (SECV). The results obtained using VNIRS and NIRS spectral scanning do not show a significant variation between the systems used.

Although the number of samples used in the feasibility study is smaller, the use of NIRS technology can be viewed as a viable alternative to the HPLC technology to predict the amount of oligosaccharides present in rice straw subjected to hydrothermal treatment. The NIRS technology also has the additional advantage of being a fast, nondestructive technique that does not need sample pretreatment, therefore decreasing the cost and the environmental impact by removing the sample pretreatment reagents. **TJ**

ABOUT THE AUTHORS

This topic was my Ph.D. topic, and I continue working in this area of research. Previously, I studied rice straw pulp obtained by various methods, and now I have studied the relationship between the predictions of carbohydrates from hydrothermal treatment using HPLC and NIRS.

The most difficult aspect of this research was to set chemometric correlation from multivariate NIR analysis. We addressed this aspect with the use of specific software.

To me, the most interesting result of this study was that NIRS analysis can be used as a substitute analysis method for HPLC to predict monosaccharides and oligosaccharides. In paper mills, the use of NIRS as a substitute for HPLC has the additional advantage of being a fast, nondestructive technique that does not need any sample pretreatment. Therefore, it decreases the costs and the environmental impact of the analyses process.

The next step will be the study of the differentiation by NIRS of pulps obtained by different pulping processes and the relationship between spectral data analysis and pulp properties.

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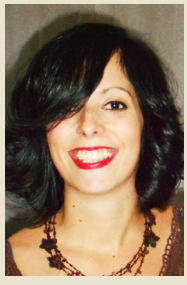
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