

Near-Infrared Spectroscopy applied to rapid low cost characterization of *Cocos Nucifera* L. waste and its biotechnological potential for ethanol production

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TECHNOLOGY OF FOREST PRODUCTS

ABSTRACT

Background: This study evaluates the potential of coconut husk for bioethanol production after different pretreatments and assesses FT-NIRS as a qualitative screening tool. The biomass was subjected to dilute acid, autocatalytic and alkaline pretreatments to investigate changes in morphology, chemical composition, and CrI, as well as their effects on HY and TEY. Results from wet chemistry and XRD were compared with FT-NIRS spectral data to evaluate its applicability as a rapid screening tool.

Results: Significant differences ($p < 0.001$) among pretreatment groups were confirmed for cellulose, hemicellulose, lignin, and TEY, as well as for HY. The 0.2% H_3PO_4 pretreatment produced the largest effects on HY ($g = -5.24$, $p < 0.01$) and TEY ($g = -9.41$, $p < 0.01$), indicating a favorable balance between hemicellulose removal and cellulose preservation. PCA of FT-NIRS spectra revealed clear sample separation along PC2 according to increasing crystallinity index and hemicellulose reduction. Untreated bagasse (CrI = 21.46%, hemicellulose = 8.28%) appeared in the upper right corner, while 0.2% H_3PO_4 treated samples (CrI = 39.32%, hemicellulose = 2.45%) were located in the lower right corner.

Conclusions: Dilute phosphoric acid pretreatment enhances hemicellulose removal, glucose release, and ethanol yield by disrupting amorphous regions, reducing biomass recalcitrance. Despite limitations (small NIRS sample size, literature-based 85% fermentation efficiency, exploratory design), FT-NIRS effectively differentiates untreated from pretreated samples, supporting its use as a rapid, low-cost qualitative screening tool. Future studies require quantitative model development and fully randomized designs.

Keywords: Coconut husk valorization; lignocellulosic recalcitrance; enzymatic hydrolysis efficiency; biorefinery screening tool; FT-NIR spectroscopy.

HIGHLIGHTS

Sulfuric acid showed the strongest hemicellulose removal effects.
Phosphoric acid achieved the highest hydrolysis and ethanol yields.
Phosphoric acid greatly improved saccharification and ethanol potential.
PCA explained 96.1% variance and separated pretreatment groups.

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INTRODUCTION

Renewable energy sources are gaining increasing prominence in the Brazilian and global energy matrices due to their lower environmental impact and their potential to replace fossil fuels and their derivatives (Priya *et al.*, 2022). In this context, lignocellulosic biomass has attracted considerable attention as a renewable feedstock for the production of liquid and gaseous fuels, as well as other value-added products within biorefinery systems (Awasthi *et al.*, 2023).

Brazil has approximately 187,000 hectares dedicated to coconut cultivation, and the 2024 harvest resulted in the production of 2,105,345 tons of coconut fruits (IBGE, 2026), positioning the country among the world's leading producers. This scenario is closely associated with the expansion of the Brazilian coconut processing agroindustry, supported by Federal Law No. 14,975/2024, which established the National Policy for Incentivizing quality coconut production in Brazil (Brasil, 2024). The coconut processing agroindustry primarily produces coconut water, coconut milk, and coconut flour (D'Almeida and Albuquerque, 2025). However, these industrial activities generate large quantities of lignocellulosic residues, particularly coconut husk, which may represent up to 35% of the total fruit weight (Beveridge *et al.*, 2022).

The valorization of coconut husk for bioethanol production represents a promising strategy for the sustainable management of this agro-industrial residue. Bioethanol production from lignocellulosic biomass typically involves pretreatment, enzymatic hydrolysis, and fermentation processes (Awasthi *et al.*, 2023; Wu *et al.*, 2023). The efficiency of these processes is strongly influenced by the structural and compositional characteristics of the biomass, since variations in lignin content, cellulose crystallinity, and carbohydrate composition can significantly affect enzymatic digestibility and ethanol yield (Das *et al.*, 2023). This variability is particularly relevant for coconut husk biomass, whose composition may vary depending on species, cultivation conditions, and geographic origin. According to Esmeraldo *et al.* (2010), lignin, hemicellulose, and cellulose contents of coconut biomass have been reported to range from 17 to 64%, 0.15 to 25.9%, and 20 to 64%, respectively.

Accurate characterization of lignocellulosic biomass is therefore essential for optimizing pretreatment strategies and improving bioconversion efficiency. Currently, the structural and chemical composition of lignocellulosic biomass is mainly determined using conventional analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), element analyser, thermogravimetric analysis (TGA), scanning electron microscopy (SEM), x-ray diffraction (XRD) and nuclear magnetic resonance (NMR) (Abdulhameed *et al.*, 2025; Anuchi *et al.*, 2022; Lupoi *et al.*, 2014; Shapiro *et al.*, 2023). Although reliable, these approaches generally require extensive sample preparation, multiple analytical steps, and the use of toxic or aggressive reagents (Lupoi *et al.*, 2014). Moreover, they are labor-intensive and costly, which limits their applicability for rapid screening of large

numbers of biomass samples or for real-time monitoring in bioconversion processes (Bec and Huck, 2023; Lupoi *et al.*, 2014). Consequently, the development of rapid, non-destructive and cost-effective analytical methods capable of assessing biomass properties and process performance remains a key challenge for lignocellulosic biorefineries.

Among emerging analytical approaches, near-infrared spectroscopy (NIRS) has gained increasing attention for process monitoring, biomass analysis, and quality control. It enables the simultaneous detection of multiple chemical constituents without extensive sample preparation (Bec and Huck, 2023; Gudjónsdóttir *et al.*, 2024; Zhao *et al.*, 2025). Quantitative NIRS models achieve high accuracy through rigorous validation, including cross-validation and independent test set evaluation, which is essential for quantifying both precision and overall predictive reliability for routine applications. However, these models are sensitive to physical sample properties such as particle size, packing density, and temperature, all of which can introduce spectral variability and reduce prediction accuracy (Giussani *et al.*, 2025; Jeong *et al.*, 2024; Ramadan *et al.*, 2025). Furthermore, quantitative models depend heavily on accurate reference methods, which may propagate analytical errors into calibrations. Alternatively, qualitative NIRS avoids many of these issues by focusing on spectral pattern recognition. For lignocellulosic biomass such as coconut husk, this approach enables rapid assessment of pretreatment efficacy and batch consistency. Although qualitative NIRS does not provide absolute compositional values and remains sensitive to outliers, it offers a pragmatic alternative for industrial monitoring where rapid screening is prioritized over exact quantification (Tang *et al.*, 2025).

Most applications of NIRS in lignocellulosic biomass research have focused on well-established feedstocks, including sugarcane bagasse and woody biomass (Deknes *et al.*, 2025; Herdlevær *et al.*, 2022; Li *et al.*, 2021; Posom *et al.*, 2022). Although NIRS has been widely applied to various coconut-derived products, such as coconut water (Shen *et al.*, 2023), coconut milk (Suksangpanomrung, *et al.*, 2024), desiccated coconut (Pandiselvam *et al.*, 2022), and coconut endosperm thickness (Phuangsoambut *et al.*, 2023), studies specifically targeting coconut husk are still limited. Existing quantitative analyses are scarce (Rambo *et al.*, 2015), and to date, no published study has addressed the qualitative screening of this biomass. This knowledge gap is significant, given that coconut husk is an abundant and underutilized agricultural residue with considerable potential for applications in bioenergy and biocomposites. Addressing this gap requires the investigation of NIRS-based screening methodologies, intended not to replace high-precision quantitative models but rather to serve as a tool for evaluating physicochemical pretreatment-induced changes in coconut husk samples.

In this context, the main objective of this study is to investigate the use of coconut husk as a feedstock for bioethanol production and the influence of different pretreatments on its conversion efficiency, as well as to evaluate the potential of a NIRS-based screening methodology to evaluate morphological and compositional

changes in lignocellulosic biomass. To achieve this objective, four different pretreatment strategies were investigated to assess their effects on the conversion efficiency of coconut husk biomass, while NIRS was explored as a characterization tool capable of tracking structural and chemical modifications induced by these treatments. Therefore, we start from the following hypothesis: "Dilute phosphoric acid pretreatment significantly increases cellulose accessibility and improves biomass conversion efficiency for bioethanol production compared to other treatments, while FT-NIRS combined with PCA enables screening of pretreated lignocellulosic biomass for industrial qualitative assessment."

MATERIAL AND METHODS

Material

The coconut residue was collected at a point of sale of coconut water in Ponta Negra beach in the municipality of Natal (5°52'05.9"S, 35°10'55.3"W; Natal, RN, Brazil). The coconut residue was debarked; cut into small pieces (4 - 6cm), washed with tap water and dried for 72h at 60°C. Dried coconut coir was milled using a laboratory knife mill (Mill type willey, TE-680, Tecnal) and sieved using 20 mesh to standardize the material particle size. The coconut husk dust was then stored in plastic containers at room temperature until use.

METHODS

All experiments described in this section were conducted under intra-laboratory repeatability conditions. Each reported value represents the mean of independent replicates, and standard deviations (SD) are provided for all mean values presented in the results.

Pretreatments

Pretreatments were carried out according to four different conditions: three types of dilute acid pretreatment and one type of alkaline pretreatment. The ground coconut husk at 5% (w/v) load was mixed with 0.2% (w/v) H_3PO_4 solution and with distilled water for phosphoric acid pretreatment and autocatalytic pretreatment, respectively. Then, both suspensions were treated for 10 min at 186 °C. For sulfuric acid and alkaline pretreatments, the coconut husk dust at 10% (w/v) load was mixed with 2.0% (w/v) H_2SO_4 solution and 0.5% (w/v) NaOH solution, respectively. Then, both suspensions were treated for 30 min at 121 °C. Afterwards, the sediments were washed with tap water until constant pH. At the end, the collected solids were dried for 72h at 60°C and kept in plastic containers until use.

All pretreatments were applied to separate flasks containing biomass from a single harvested batch. The experimental unit was the individual flask. Each reported replicate is a technical replicate (triplicate measurements of the same pretreated sample). Independent biological replicates were not performed.

Chemical composition of lignocellulosic biomass

The chemical composition of untreated and pretreated coconut husk dust was analyzed, in quadruplicate, for cellulose, hemicellulose, Klason lignin and moisture contents. Determination of structural carbohydrates and lignin followed NREL (National Renewable Energy Laboratory) protocols (Sluiter *et al.*, 2012). The raw material was consecutively extracted with 95% ethanol (24h, 80 °C) and deionized water (24h, 110 °C). The extractive-free material was analyzed for chemical composition following the NREL protocols (Sluiter *et al.*, 2008). For the determination of moisture 3g of residue were placed in 3 beakers of 100 mL, previously weighed. The beaker was closed and placed in the oven at 100 °C for 24 h. The determination of polysaccharides was performed after concentrated acid hydrolysis in which 300 mg bagasse were hydrolyzed with 72% H_2SO_4 at 30 °C for 60 min followed by diluted acid hydrolysis using 3% H_2SO_4 at 121 °C for 60 min. Cellulose and hemicellulose content depend on carbohydrate and organic acids concentrations present in the hydrolysate. After hydrolysis, the reaction mixture was filtered through filter paper previously dried and weighed. The carbohydrate and organic acid concentrations were determined by high-performance liquid chromatography (HPLC-Shimadzu LC-10A series) equipped with a refractive index (RI) detector (10A). The HPLC analyses were performed at 65°C on the column SCR-101H Shimadzu and a 5 mmol.L⁻¹ sulfuric acid solution was used as mobile phase at 0.6 mL.min⁻¹ flow rate. The solid material retained on the filter paper was accounted as lignin.

Scanning electron microscopy (SEM)

Bagasse morphology was investigated by SEM before and after pretreatments to evaluate the variations in the lignocellulosic structure caused by pretreatments. Sample imaging was carried out using electron microscope, model tabletop microscope TM-3000 (Hitachi, Tokyo, Japan).

X-ray diffraction (XRD)

The bagasse crystallinity was evaluated by X-ray diffraction (XRD-6000, Shimadzu, Japan) using the Segal method. Copper K α radiation, 40 kV of voltage and 30 mA of electric current and a rate of 2.0 deg/min for a continuous scan from 10.0 to 35.0 were applied. The crystallinity index (CrI) for all samples were evaluated from the relationship between the intensity of the 002 peak (I_{002} , $2\theta = 22.5$) and the height of the minimum dip (I_{am} , $2\theta = 18.5$), the last one accounted to the amorphous structures in bagasse. The CrI was calculated according to Equation 1 (Senthamaraikannan *et al.*, 2026):

$$CrI(\%) = \left(\frac{I_{002} - I_{am}}{I_{002}} \right) \times 100 \quad (1)$$

It should be noted that the Segal method provides an apparent CrI, as the amorphous signal may include

contributions from amorphous cellulose as well as non-cellulosic components such as hemicellulose and lignin. Therefore, the CrI values obtained are considered relative indicators of cellulose structural organization and are most appropriate for comparative analysis among samples analyzed under the same experimental conditions.

Near-infrared spectroscopy (NIRS)

Five lignocellulosic fiber samples were analyzed in duplicate by FT-NIRS to identify the wavelength regions most correlated with changes in cellulose, hemicellulose, and lignin contents of young coconut husk biomass. Spectral analysis was performed using a Thermo-Nicolet Antaris II FT-NIR spectrophotometer equipped with a fiber-optic probe. The probe tip is beveled, in order to prevent diffuse reflection. The spectra acquisition was managed by the Software RESULT 3 of Thermo Scientific Co. and was performed at 8 cm⁻¹ resolution in the wavenumber range from 10000 cm⁻¹ to 4000 cm⁻¹. For each sample, in order to increase signal to noise ratio, 64 scans were averaged for each FT-NIR spectrum. The average spectra were used for subsequent mathematical treatment performed by Unscrambler Software version 10.3 (Camo software, Oslo, Norway). The second derivative spectrum was calculated after unit vector normalization with the Savitzky-Golay algorithm.

Principal component analysis (PCA)

PCA was applied to NIR spectral data of untreated and pretreated coconut husk samples using Jamovi software (version 2.6.24). Data suitability for factor analysis was confirmed by the Kaiser-Meyer-Olkin (KMO > 0.6) and Bartlett's test of sphericity ($p < 0.05$) (Shrestha, 2021). A scree plot was used to visualize the level of information retained by each component. A two-component score plot (PC1 vs. PC2) was generated, as PC2 was essential for visualizing differentiation among sample groups. No rotation was applied. All analyses considered a confidence level of 95%.

Enzymatic Hydrolysis and theoretical bioethanol yields

The enzymatic hydrolysis of untreated bagasse and pretreated coconut husk dust was carried out, in triplicate, at a substrate ratio of 5% (w/v) in 100 mM sodium citrate buffer (pH 4.8), under a 150 rpm constant stirring and at 50 °C during 48 h. The enzymatic load was 20 filter paper units (FPU) and 60 β-Glucanase units per gram of substrate added. To avoid microbial growth during the enzymatic hydrolysis, a 0.2% (w/v) sodium azide solution was added to the hydrolysis mixture. The amount of released glucose was measured by glucose oxidase/peroxidase reagent (BioSystems S.A., Barcelona, Spain) and total reducing sugars (TRS) content was measured by dinitrosalicylic acid reagent (Miller, 1959). Glucose and reducing sugars content were quantified in triplicate and the conversion value is expressed as an average with standard deviation. The hydrolysis yield (HY) was determined by Equation 2:

$$HY(\%) = \frac{\text{Released glucose(g/L)} \times \text{Reactional volume(L)}}{\text{Dry bagasse(g)} \times \text{cellulose content(g/g)} \times 1.11} \times 100 \quad (2)$$

where 1.11 is a correction factor that accounts for the conversion of cellulose into glucose (Dash *et al.*, 2025).

The theoretical ethanol yield (TEY) was estimated from the glucose concentration obtained in the enzymatic hydrolysate. For this calculation, glucose was considered the sole fermentable sugar contributing to ethanol production. The estimation was based on the stoichiometry of alcoholic fermentation ($C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$), which corresponds to a maximum theoretical yield of 0.51 g of ethanol per g of glucose (Alavijeh *et al.*, 2023). To account for process limitations typically observed during fermentation, an overall conversion efficiency of 85% from glucose to ethanol was assumed based on scientific literature reports (Beigbeder *et al.*, 2021; Zhao *et al.*, 2009). It was further considered that all glucose released during enzymatic hydrolysis was available for fermentation. Under these assumptions, TEY was calculated according to Equation 3:

$$TEY \left(\frac{\text{L of ethanol}}{\text{ton of bagasse}} \right) = \frac{\text{Dry bagasse(g)} \times \text{cellulose content} \left(\frac{\text{g}}{\text{g}} \right) \times HY \times 0.51 \times 0.85}{0.79 \left(\frac{\text{g}}{\text{mL}} \right)} \quad (3)$$

$$\times \frac{1}{1000} \frac{\text{L}}{\text{mL}} \times \frac{10^6}{1} \frac{\text{g}}{\text{ton of bagasse}}$$

where 0.51 is the conversion factor of ethanol from glucose (Alavijeh *et al.*, 2023); 0.85 is the process efficiency of ethanol from sugar (Zhao *et al.*, 2009) and 0.79 is the specific gravity of ethanol.

Data Processing and Statistical Analysis

To evaluate the effects of different pretreatment conditions on the chemical composition and enzymatic hydrolysis efficiency of coconut bagasse, data were subjected to statistical analysis using Jamovi software (version 2.6.24). Initially, the assumptions of normality of residuals and homogeneity of variances were assessed using the Shapiro-Wilk and Levene tests, respectively. In cases where a violation of normality was detected ($p < 0.05$), the skewness and kurtosis coefficients were further examined. In this study, skewness values ranged between -2 and +2, and an approximately normal distribution was confirmed through visual inspection of Q-Q plots. The Levene's test revealed heterogeneity of variances for most variables (cellulose, hemicellulose, lignin, and TEY), therefore a one-way analysis of variance (ANOVA) with Welch's correction was applied to these datasets to ensure robust comparisons among the control and experimental groups. For HY, which met the assumption of homogeneity, Fisher's ANOVA was employed. When significant differences were detected ($p < 0.05$), post-hoc comparisons were conducted using the Games-Howell test for variables with heterogeneous variances and the Tukey test for those with homogeneous variances. All analyses considered a confidence level of 95%.

Effect sizes for pairwise comparisons were estimated using Hedges' g to correct for bias due to small sample sizes ($n < 20$). Values were calculated from t -statistics obtained from post-hoc tests using Equations 4 to 6 (Hedges, 2025).

$$g = t \cdot \left(\sqrt{\frac{n_1 + n_2}{n_1 n_2}} \right) \cdot j \quad (4)$$

$$J = 1 - \left[\frac{3}{(df - 1)} \right] \quad (5)$$

$$df = n_1 + n_2 - 2 \quad (6)$$

Where n_1 and n_2 are the sample sizes of the two groups being compared, and J is the bias correction factor. Effect magnitudes were interpreted following the expanded guidelines proposed by Sawilowsky (2009): very small (0.01), small (0.2), medium (0.5), large (0.8), very large (1.2) and huge (> 2.0).

RESULTS

The chemical composition of untreated and pretreated coconut bagasse is presented in Table 1. The untreated residue presented the following chemical composition: $27.77\% \pm 0.59$ of cellulose, $8.26\% \pm 0.12$ of hemicellulose and 45.45 ± 0.94 of Klason lignin. In the untreated bagasse, hemicellulose and lignin constitute more than 50% of the total composition. In order to reduce lignin and hemicellulose contents, acid and alkaline pretreatments were performed.

Morphological changes in untreated and pretreated coconut bagasse were evaluated using SEM, XRD, and FT-NIRS. Figure 1 presents scanning electron micrographs of milled coconut bagasse, comparing untreated samples with those subjected to four different pretreatment conditions.

Table 1: Chemical composition of untreated and pretreated coconut (*Cocos nucifera* L.) bagasse. Values are expressed as mean $\pm$ standard deviation (n = number of replicates).

Bagasse Pretreatment	n	Chemical composition		
		Cellulose (%)	Hemicellulose (%)	Lignin (%)
Untreated	4	27.77 ± 0.59	8.26 ± 0.12	45.45 ± 0.94
2% (v/v) H_2SO_4	4	39.25 ± 1.01	2.81 ± 0.06	49.61 ± 2.15
0.2% (w/v) H_3PO_4	4	36.86 ± 4.19	2.45 ± 0.27	49.61 ± 2.15
0.5% (w/v) NaOH	4	31.53 ± 0.49	9.30 ± 0.30	39.93 ± 0.87
Autocatalytic	4	37.49 ± 2.97	2.41 ± 0.09	39.81 ± 2.71

The images reveal progressive surface disorganization and increasing exposure of cellulose fibers depending on pretreatment type and severity. Table 2 and Figure 2 show the crystallinity index (CrI) values, calculated using the XRD peak height method (Segal method), which is useful for comparing relative differences among samples.

Table 2: Crystallinity index (CrI) of untreated and pretreated coconut bagasse samples.

Bagasse	CrI(%)
Untreated	21.46
2% H_2SO_4	35.37
0.2% H_3PO_4	39.32
0.5% NaOH	31.23
Autocatalytic	41.52

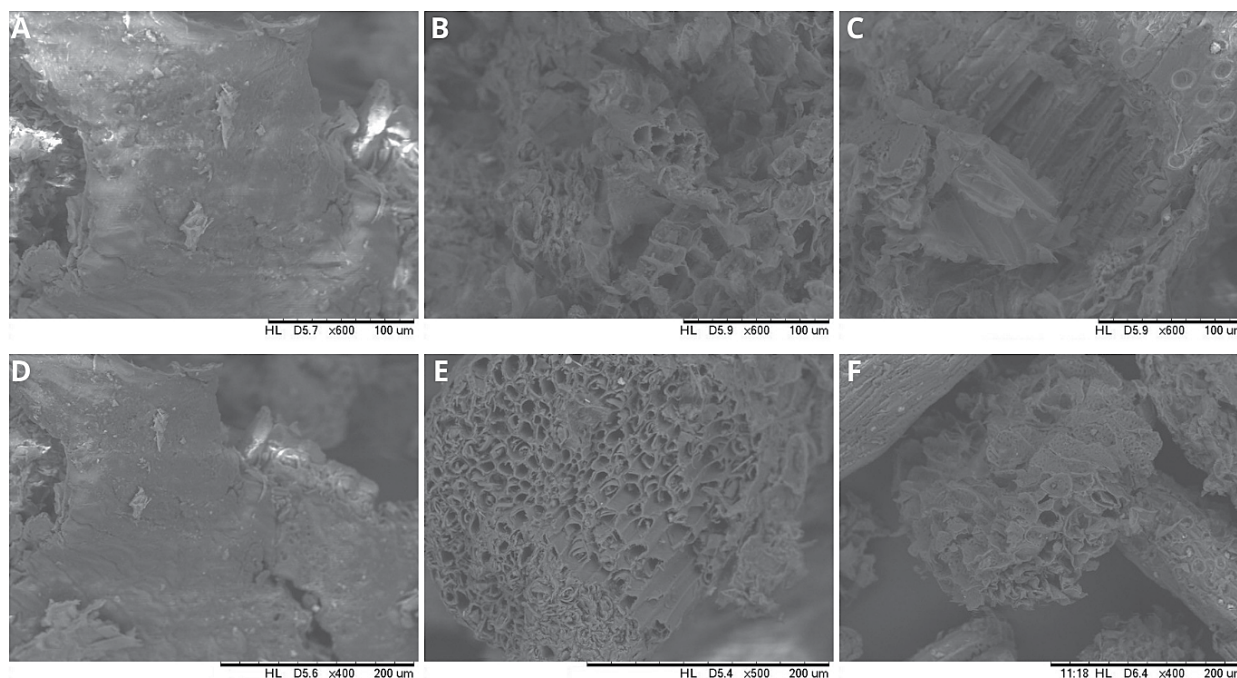


Figure 1: Scanning electron microscopy surface images of untreated and pretreated coconut bagasse samples: (A) untreated bagasse (600 $\times$ magnification; scale bar = 100 μ m); (B) 0.2% H_3PO_4 pretreatment (600 $\times$; scale bar = 100 μ m); (C) alkaline pretreatment (600 $\times$; scale bar = 100 μ m); (D) untreated bagasse (400 $\times$; scale bar = 200 μ m); (E) autocatalytic pretreatment (500 $\times$; scale bar = 200 μ m); (F) 2% H_2SO_4 pretreatment (400 $\times$; scale bar = 200 μ m).

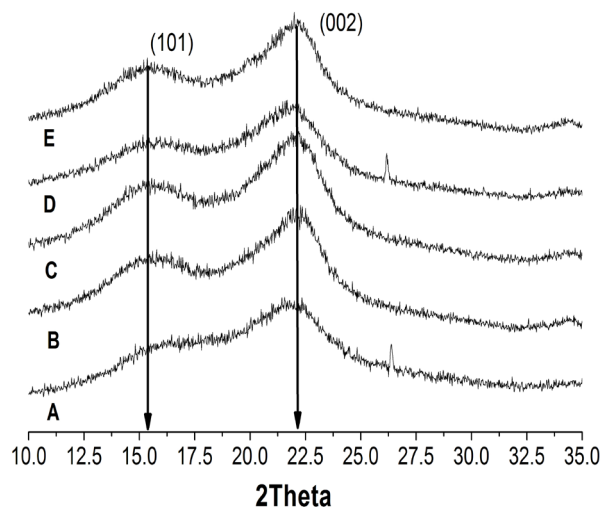


Figure 2: X-ray diffractograms of untreated and pretreated coconut bagasse. The main crystalline planes (101) and (002) of cellulose are indicated. Samples: (A) Untreated bagasse; (BA) Autocatalytic pretreatment; (C) 2% (w/v) H_2SO_4 ; (D) 0.5% (w/v) NaOH; and (E) 0.2% (w/v) H_3PO_4 .

The pre-processed NIR spectra in the 7200–5500 cm^{-1} region are shown in Figure 3. This spectral range captures information related to cellulose, hemicellulose, and lignin variations, along with features corresponding to amorphous, semi-crystalline, and crystalline structures in coconut bagasse.

Principal component analysis (PCA) was performed on the NIR spectral data to evaluate the qualitative discrimination among untreated and pretreated coconut husk samples. The Kaiser-Meyer-Olkin (KMO) measure was

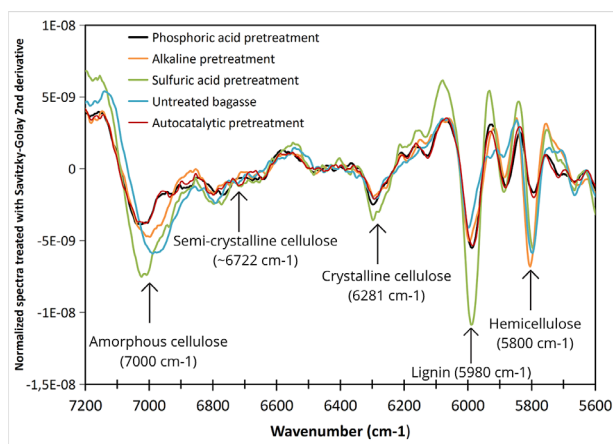


Figure 3: Normalized NIR spectra (Savitzky–Golay 2nd derivative) of untreated and pretreated coconut bagasse samples. Spectral regions associated with major lignocellulosic components are indicated: amorphous cellulose ($\sim 7000 \text{ cm}^{-1}$), semi-crystalline cellulose ($\sim 6722 \text{ cm}^{-1}$), crystalline cellulose (6281 cm^{-1}), lignin (5980 cm^{-1}), and hemicellulose (5800 cm^{-1}). Pretreatments evaluated: phosphoric acid (0.2% w/v H_3PO_4), alkaline (0.5% w/v NaOH), sulfuric acid (2% w/v H_2SO_4), and autocatalytic.

0.830, and Bartlett's test of sphericity was significant ($\chi^2 = 19107.6$, $\text{df} = 10$, $p < 0.001$), confirming the data suitability for factor analysis. Only the first principal component (PC1) exhibited an eigenvalue greater than 1 (4.807), accounting for 96.1% of the total spectral variance, while PC2 explained 2.5% (eigenvalue = 0.125). The scree plot (Figure 4A) revealed a sharp elbow after the first component. However, the score plot (Figure 4B) showed clear separation among sample groups along PC2, with untreated biomass (IN) positioned at the positive extreme of PC2, phosphoric acid pretreated biomass (PAP) and autocatalytic pretreated biomass (ACP) at the negative extreme, and alkaline pretreated biomass (AP) and sulfuric acid pretreated biomass (SAP) at intermediate positions.

After the characterization of the lignocellulosic material, the potential of coconut bagasse for bioethanol production was assessed. To evaluate this potential, the sugar conversion efficiency of untreated and pretreated samples was analyzed by quantifying TRS, partial reducing sugars (PRS), and glucose released after 48 hours of enzymatic hydrolysis (Table 3). Table 4 presents HY and TEY for each sample.

To verify the influence of changes in lignocellulosic biomass composition on HY and TEY, a statistical analysis was performed. The assumption of homogeneity of variances was assessed using Levene's test for all dependent variables. The results indicated a significant violation of homogeneity for most of the variables analyzed, including cellulose content ($F(4,15) = 4.10$; $p = 0.019$), hemicellulose content ($F(4,15) = 4.79$; $p = 0.011$), lignin content ($F(4,15) = 4.17$; $p = 0.018$), and TEY ($F(4,10) = 3.94$; $p = 0.036$). Conversely, HY met the assumption of homogeneity of variances ($F(4,10) = 3.26$; $p = 0.059$). Based on these findings, ANOVA with Welch's correction was performed for variables that violated homogeneity (cellulose, hemicellulose, lignin, and TEY), while Fisher's ANOVA was applied for HY.

Table 3: Sugar yields from untreated and pretreated coconut bagasse after 48 h of enzymatic hydrolysis. Values are reported as mean $\pm$ standard deviation (n = number of replicates).

Bagasse Pretreatment	n	Enzymatic hydrolysis sugar yields			
		TRS (g.L^{-1})	Glucose (g.L^{-1})	PRS (g.L^{-1})	Glucose (%)
Untreated	3	16.38 $\pm$ 0.69	4.21 $\pm$ 0.23	12.16 $\pm$ 0.47	25.72 0.55
2% H_2SO_4	3	5.12 $\pm$ 0.51	3.41 $\pm$ 0.02	1.72 $\pm$ 0.51	71.51 0.24
0.2% H_3PO_4	3	10.64 $\pm$ 0.91	8.73 $\pm$ 0.38	1.90 $\pm$ 0.69	78.71 1.39
0.5% NaOH	3	7.91 $\pm$ 0.78	4.69 $\pm$ 0.07	3.22 $\pm$ 0.79	55.27 0.73
Autocatalytic	3	8.76 $\pm$ 0.43	5.19 $\pm$ 0.20	3.57 $\pm$ 0.30	57.92 0.16

Table 4: Enzymatic hydrolysis yields of untreated and pretreated coconut bagasse. Values represent mean $\pm$ standard deviation (n = number of replicates).

Bagasse samples	n	Total Hydrolysis Yield (%)	Theoretical Ethanol yield (L/t of bagasse)
Untreated	3	27.33 1.82	41.64 2.77
2% H_2SO_4	3	15.65 0.13	33.70 0.29
0.2% H_3PO_4	3	40.41 2.16	86.34 4.62
0.5% NaOH	3	26.75 0.45	46.28 0.79
Autocatalytic	3	23.95 1.10	51.19 2.35

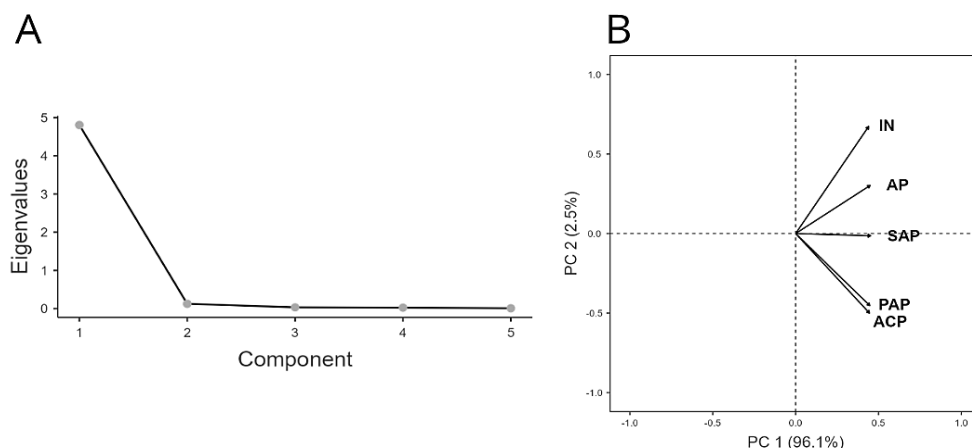


Figure 4: Principal component analysis (PCA) of FT-NIR spectral data from coconut husk samples subjected to different pretreatments. (A) Scree plot showing eigenvalues for the first five principal components (PCs); (B) Score plot of PC1 vs. PC2, where PC1 explains 96.1% of the total variance and PC2 explains 2.5%. Samples are coded by pretreatment conditions: IN (untreated), AP (alkaline pretreatment), SAP (sulfuric acid pretreatment), PAP (phosphoric acid pretreatment), and ACP (autocatalytic pretreatment). Spectral clustering indicates clear differentiation between untreated and pretreated samples, with PC1 capturing the major structural changes induced by pretreatments.

The results confirmed significant differences for cellulose content (Welch's $F(4,7.07) = 81.9$; $p < 0.001$), hemicellulose content (Welch's $F(4,6.87) = 1536.8$; $p < 0.001$), lignin content (Welch's $F(4,7.28) = 42.5$; $p < 0.001$), and TEY (Welch's $F(4,4.26) = 203.0$; $p < 0.001$). For HY, which met the assumption of homogeneity of variances, Fisher's ANOVA was applied and also indicated significant differences among groups (Fisher's $F(4,10) = 126.6$; $p < 0.001$). For multiple post-hoc comparisons, Games-Howell test (for data with heterogeneous variances) and Tukey test (for homogeneous data) were adopted. The results of post-hoc tests are presented in Table 5.

Hedges' g effect sizes for control vs. pretreatment comparisons are shown in Table 6. For cellulose, alkaline ($g = -6.06$, $p < 0.001$), autocatalytic ($g = -3.94$, $p < 0.05$), and 2% H_2SO_4 ($g = -12.13$, $p < 0.001$) pretreatments increased content with huge effects, while 0.2% H_3PO_4 pretreatment had no significant effect. All pretreatments reduced hemicellulose content with huge effect sizes (Table 6). For lignin, alkaline pretreatment reduced content ($g = 5.30$, $p < 0.001$) and 0.2% H_3PO_4 increased it ($g = -3.37$, $p < 0.05$).

Regarding HY, 0.2% H_3PO_4 increased saccharification ($g = -5.24$, $p < 0.01$), whereas 2% H_2SO_4 reduced it ($g = 7.25$, $p < 0.05$). For TEY, significant increases were observed only for 0.2% H_3PO_4 ($g = -9.41$, $p < 0.01$) and autocatalytic ($g = -2.97$, $p < 0.05$) pretreatments.

DISCUSSION

Acid pretreatments reduced hemicellulose content by approximately $69.00\% \pm 2.66\%$ and increased cellulose content by $36.37\% \pm 4.48\%$. This effect may be attributed to the disruption of covalent, hydrogen, and van der Waals interactions within amorphous cell wall components, which could facilitate hemicellulose hydrolysis while preserving the predominantly crystalline cellulose fraction (Worku *et al.*, 2024). Autocatalytic pretreatment also resulted in minor lignin solubilization. Conversely, the relative lignin content increased after treatment with 2% H_2SO_4 and 0.2% H_3PO_4 , a consequence of preferential hemicellulose removal. These observations are consistent with prior reports (Mathew *et al.*, 2011; Timung *et al.*, 2015).

Table 5: Mean differences and significance levels for post-hoc comparisons between untreated (control) and pretreated coconut bagasse samples. Significance levels are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Superscript letters denote the post-hoc test used: (a) Games-Howell, (b) Tukey. Negative values indicate an increase in the parameter relative to the control; positive values indicate a decrease.

Comparison	Cellulose (%) ^a	Hemicellulose (%) ^a	Lignin (%) ^a	HY (%) ^b	TEY (L/t) ^b
Control vs 0.5%NaOH	-3.76***	-1.04*	5.52***	0.58	-4.63
Control vs autocatalytic	-9.72*	5.85***	5.64	3.38	-9.55*
Control vs 0.2% H_3PO_4	-9.09	5.81***	-4.28*	-13.1**	-44.7**
Control vs 2% H_2SO_4	-11.49***	5.45***	-4.16	11.68*	7.94
0.5%NaOH vs autocatalytic	-5.96	6.89***	0.12	2.80	-4.91
0.5%NaOH vs 0.2% H_3PO_4	-5.33	6.85***	-9.80***	-13.70*	-40.1*
0.5%NaOH vs 2% H_2SO_4	-7.73***	6.49***	-9.68**	11.11***	12.58**
Autocatalytic vs 0.2% H_3PO_4	0.629	-0.04	-9.92*	-16.5**	-35.1**
Autocatalytic vs 2% H_2SO_4	-1.77	-0.40	-9.80**	8.30*	17.49*
0.2% H_3PO_4 vs 2% H_2SO_4	-2.40	-0.36	0.13	24.76**	52.64**

Table 6: Hedges' g effect sizes for comparisons between control and pretreatment groups. Negative values indicate an increase in the parameter relative to the control; positive values indicate a decrease. Asterisks indicate statistical significance from post-hoc tests: ***p < 0.001; **p < 0.01; *p < 0.05. Non-significant comparisons (p > 0.05) are shown without asterisks.

Pretreatment	Cellulose	Hemicellulose	Lignin	HY	TEY
Alkaline	-6.06***	-4.01*	5.30***	-0.31	0.34
Autocatalytic	-3.94*	32.10***	2.42	-1.97	-2.97*
0.2% H ₃ PO ₄	-2.64	24.31***	-3.37*	-5.24**	-9.41**
2% H ₂ SO ₄	-12.13***	48.54***	-2.18	7.25*	3.22

Alkaline pretreatment (0.5% NaOH) reduced lignin content from 45.45% to 39.93%. However, this degree of delignification remains insufficient for highly recalcitrant coconut bagasse. More severe alkaline conditions may be required to minimize non-productive enzyme adsorption onto lignin (Wu *et al.*, 2023; Dandash *et al.*, 2025).

Morphological analysis of young coconut bagasse (Figure 1A–1F) revealed significant fiber surface modifications following pretreatment. Among the conditions tested, phosphoric acid induced the most extensive surface disruption and internal fiber damage, surpassing the effects of sulfuric acid and autocatalytic pretreatment. Dilute sulfuric acid caused localized corrosion characterized by small surface cavities; however, the internal fibers remained largely intact. In contrast, phosphoric acid promoted widespread structural disassembly of the fiber matrix. This morphological deterioration correlated directly with improved hydrolysis yield (HY) and total ethanol yield (TEY). One possible explanation is that the structural disassembly induced by phosphoric acid may increase cellulose accessibility by exposing reactive sites and enlarging surface area and pore size, which could in turn facilitate solvent and enzyme diffusion (Arshad *et al.*, 2025). However, direct experimental evidence for these mechanisms was not obtained in the present study.

The crystallinity index (CrI) is another important parameter when evaluating lignocellulosic biomass following physicochemical pretreatments. The diffractograms of all samples (Figure 2) exhibited identical patterns, with the highest intensity observed in the 002 crystallographic plane, which represents the crystalline cellulose region (Senthamaraiyannan *et al.*, 2026). Untreated biomass displayed the lowest CrI (21.46%), attributable to its high content of amorphous hemicellulose and lignin. Since an increase in CrI reflects the removal of amorphous components such as hemicellulose and lignin (Sirijuncheon *et al.*, 2024), CrI values rose proportionally with hemicellulose removal. Autocatalytic pretreatment yielded the highest CrI, consistent with the simultaneous removal of hemicellulose and lignin. Alkaline pretreatment increased CrI to 31.23%, though to a lesser extent than acid pretreatments, reflecting its milder action. Collectively, the positive correlation between hemicellulose removal and CrI increase suggests that physicochemical pretreatments preferentially target amorphous components.

FT-NIR spectroscopy (Figure 3) enabled rapid qualitative discrimination among pretreatment conditions.

All samples displayed similar spectral profiles, but absorption band intensities varied according to component removal. Within the 7200–5500 cm⁻¹ region, the band at 5980 cm⁻¹ (assigned to lignin aromatic C–H first overtone) (Yeh *et al.*, 2004) and at 5800 cm⁻¹ (hemicellulose acetyl C–H first overtone) decreased after acid pretreatments, confirming removal of these constituents (Schwanninger *et al.*, 2011). Notably, bands associated with amorphous cellulose (7200–6950 cm⁻¹) were preferentially degraded following H₃PO₄ and autocatalytic pretreatments, consistent with increased CrI. Reductions in semi-crystalline and crystalline cellulose bands were not clearly observed, indicating that the evaluated pretreatments primarily affect amorphous structures. These findings agree with previous investigations (Herdlevær *et al.*, 2022; Nisgoski *et al.*, 2025; Tsuchikawa *et al.*, 2023).

PCA (Figure 4B) showed sample separation along PC2, which accounted for only 2.5% of the total spectral variance. Despite this low explained variance, untreated bagasse (CrI = 21.46%) was positioned in the upper right quadrant, whereas samples treated with H₃PO₄ (CrI = 39.32%) and autocatalytic (CrI = 35.37%) pretreatment were located in the lower right quadrant. A similar trend was observed for hemicellulose content. However, given the small proportion of variance captured by PC2, these clustering patterns should be interpreted with caution. The results suggest a possible association between PC2 scores and pretreatment-induced compositional changes, but no direct causal relationship can be inferred from the PCA alone. Overall, FT-NIRS coupled with PCA may serve as a useful qualitative screening tool for detecting pretreatment-induced changes in coconut husk biomass, provided that the limitations of low-variance components are recognized.

Nevertheless, FT-NIR spectroscopy remains qualitative in the absence of chemometric calibration against reference methods. Without such models, the technique cannot provide quantitative measurements. Therefore, to establish FT-NIRS as a definitive analytical tool capable of both rapid screening and accurate quantification, future studies must prioritize the development of extensive spectral libraries coupled with rigorous validation protocols.

Finally, to assess the potential of coconut biomass as a feedstock for bioethanol production, a statistical evaluation of its compositional and structural characteristics was conducted. A important finding is that extensive hemicellulose removal does not guarantee high enzymatic digestibility. Despite comparable hemicellulose reduction among acid pretreatments, only 0.2% H₃PO₄ significantly increased HY and TEY.

Effect size analysis (Table 6) using Hedges' g indicated that 2% H₂SO₄ pretreatment produced the largest effect on hemicellulose removal (g = 48.54, p < 0.001) but exerted a detrimental effect on HY (g = 7.25, p < 0.05). In contrast, 0.2% H₃PO₄ pretreatment showed a more moderate effect on hemicellulose removal (g = 24.31, p < 0.001) yet yielded the largest effects on HY (g = -5.24, p < 0.01) and TEY (g = -9.41, p < 0.01). However, several limitations of the effect size analysis must be acknowledged. The large Hedges' g values found in this study may be inflated due to the small

sample size, as Hedges' g is sensitive to variance estimates that become unreliable under low replication. Therefore, the absolute magnitudes should be interpreted with caution and the primary utility of the analysis lies in the consistent directional patterns: hemicellulose removal alone does not guarantee improved HY, whereas 0.2% H_3PO_4 pretreatment enhances conversion yields despite its more moderate effect on hemicellulose. Owing to the statistical instability inherent to small datasets, strong comparative rankings among pretreatments based solely on effect size magnitudes should be made with caution and future studies with larger sample sizes are required to obtain more stable and generalizable estimates.

Additionally, one possible explanation for the superior performance of phosphoric acid pretreatment is a dual mechanism: effective hemicellulose removal combined with amorphous region disruption and minimal cellulose degradation. This interpretation is supported indirectly by Silva *et al.* (2024), who reported that H_3PO_4 (1.5% w/v) caused only 8% cellulose loss compared to 38% for H_2SO_4 , resulting in 35% higher glucose release. Additionally, it is possible that sulfuric acid pretreatment may promote cellulose degradation or generate inhibitory compounds (e.g., furans, phenolic derivatives) that could reduce enzymatic and fermentation efficiency, although this was not directly measured in the present study.

Morphological analysis supports this interpretation: phosphoric acid induced more extensive fiber disassembly than sulfuric acid, which may improve enzyme accessibility. The autocatalytic pretreatment improved TEY only modestly ($g = -2.97$, $p < 0.05$), with a less pronounced effect than 0.2% H_3PO_4 . Alkaline pretreatment effectively reduced lignin ($g = 5.30$, $p < 0.001$) but did not improve HY or TEY, confirming that delignification alone, without adequate hemicellulose removal and amorphous cellulose disruption, is insufficient for this biomass. Furthermore, alkaline conditions could potentially degrade cellulose through peeling reactions (Mozdyniewicz *et al.*, 2013), which might reduce HY, though this remains speculative based on the current data. Further studies are therefore required to investigate whether alkaline pretreatment conditions can be optimized to enhance HY without promoting cellulose degradation.

Post-hoc comparisons (Table 5) confirmed that cellulose content increased significantly in all pretreatments except 0.2% H_3PO_4 , for which no significant difference was observed. Hemicellulose removal was significant for all acid and autocatalytic pretreatments, with no significant differences among them. For lignin, only alkaline pretreatment resulted in significant removal, whereas acid pretreatments exhibited minimal or non-significant delignification.

HY and TEY were significantly enhanced only by 0.2% H_3PO_4 ($p < 0.01$ for both) and by autocatalytic pretreatment ($p < 0.05$ for TEY). These results collectively indicate that dilute phosphoric acid is the most promising pretreatment for young coconut husk valorization. Although TEY provides an essential benchmark for assessing practical yields and identifying process inefficiencies, it does not incorporate the complex physicochemical and biological constraints inherent to lignocellulosic conversion. Specifically,

inhibitory compounds generated during pretreatment (furans, phenolic compounds, and organic acids) can substantially impair microbial metabolism and fermentation performance, resulting in actual yields below theoretical predictions. Therefore, while TEY remains indispensable for initial feasibility assessments, comprehensive process evaluation must integrate experimental data on sugar release, fermentation efficiency, and inhibitor profiles to enable realistic production estimates.

CONCLUSIONS

Dilute phosphoric acid (0.2% H_3PO_4) pretreatment significantly improved HY and TEY from young coconut husk, outperforming sulfuric acid, autocatalytic, and alkaline pretreatments. This superior performance is attributed to effective hemicellulose removal combined with amorphous region disruption and minimal cellulose degradation, as confirmed by compositional, morphological (SEM), and crystallinity (XRD) analyses.

FT-NIR spectroscopy coupled with PCA effectively distinguished untreated from pretreated samples based on spectral patterns, supporting its use as a qualitative screening tool for pretreatment-induced changes in lignocellulosic biomass. In order to confirm and extend these findings, future studies should focus on quantitative model development, experimental validation of fermentation efficiencies, and the use of fully randomized designs with independent biological replicates.

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DATA AVAILABILITY

The datasets supporting the conclusions are included in the article.

AUTHORSHIP CONTRIBUTION:

Project Idea: RJAN,
Database: RJAN
Processing: RJAN
Analysis: RJAN, VV, JAO, GRM
Writing: RJAN, VV, JAO, GRM, ESS
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REFERENCES

- ABDULHAMEED, A.S.; ABDULLAH, S.; AL-MASUD A.A.; *et al.* Development and characterization of a chitosan/acid-modified lignocellulosic biomass (Eggplant residue, *Solanum melongena* L.) biocomposite for brilliant green dye removal. *Cellulose*, v. 32, n. 14, p. 8567–8590, 2025. <https://doi.org/10.1007/s10570-025-06712-7>
- ALAVIJEH, R. S.; SHAHVANDI, A.; OKORO, O. V.; *et al.* Biorefining of corn stover for efficient production of bioethanol, biodiesel, biomethane, and value-added byproducts. *Energy Conversion and Management*, v. 283, p. 116877, 2023. <https://doi.org/10.1016/j.enconman.2023.116877>

- ANUCHI, S. O.; CAMPBELL, K. L. S.; HALLETT, J. P. Effective pretreatment of lignin-rich coconut wastes using a low-cost ionic liquid. *Scientific Reports*, v. 12, n. 1, p. 6108, 2022. <https://doi.org/10.1038/s41598-022-09629-4>
- ARSHAD, N.; PANAKKAL, E. J.; KALIVARATHAN, P. B.; *et al.* Emerging technologies in pretreatment and hydrolysis for high-solid-loading bioethanol production from lignocellulosic biomass. *Fermentation*, v. 11, n. 11, p. 613, 2025. <https://doi.org/10.3390/fermentation11110613>
- AWASTHI, M. K.; SAR, T.; GOWD, S. C.; *et al.* A comprehensive review on thermochemical, and biochemical conversion methods of lignocellulosic biomass into valuable end product. *Fuel*, v. 342, p. 127790, 2023. <https://doi.org/10.1016/j.fuel.2023.127790>
- BEC, K.; HUCK, C. Analytical advantages of near-infrared (NIR) spectroscopy. Wiley Analytical Science, 2023. Available at: <https://analyticalscience.wiley.com/content/article-do/analytical-advantages-near-infrared-nir-spectroscopy>. Accessed in: May 30th 2026.
- BEIGBEDER, J. B.; MEDEIROS, D. J. M.; LAVOIE, J. M. Optimization of yeast, sugar and nutrient concentrations for high ethanol production rate using industrial sugar beet molasses and response surface methodology. *Fermentation*, v. 7, n. 2, p. 86, 2021. <https://doi.org/10.3390/fermentation7020086>
- BRASIL. Law n° 14,975, of 18 September 2024. Establishes the National Policy for the Promotion of Quality Coconut Cultivation. *Diário Oficial da União*: section 1, Brasília, DF, 19 September 2024. Available at: www.planalto.gov.br/ccivil_03/_ato2023-2026/2024/lei/114975.htm. Accessed in: January 27th 2026.
- D'ALMEIDA, A. P.; DE ALBUQUERQUE, T. L. Coconut husk valorization: innovations in bioproducts and environmental sustainability. *Biomass Conversion and Biorefinery*, v. 15, n. 9, p. 13015–13035, 2025. <https://doi.org/10.1007/s13399-024-06080-5>
- DANDASH, A. A.; ABU-JDAYIL, B.; TANNOUS, J. H. Lignin extraction from various biomass sources: a comprehensive review of characteristics, applications, and future prospects. *The Chemical Record*, v. 25, n. 8, p. 1–32, 2025. <https://doi.org/10.1002/tcr.202500045>
- DAS, N.; JENA, P. K.; PADHI, D.; *et al.* A comprehensive review of characterization, pretreatment and its applications on different lignocellulosic biomass for bioethanol production. *Biomass Conversion and Biorefinery*, v. 13, n. 2, p. 1503–1527, 2023. <https://doi.org/10.1007/s13399-021-01294-3>
- DASH, D.; MUND, N. K.; NAYAK, S.; *et al.* Efficient release of fermentable glucose from the Agave leaf biomass using phosphoric acid as the pretreating agent for bioethanol production. *Biomass Conversion and Biorefinery*, v. 15, n. 11, p. 17479–17491, 2025. <https://doi.org/10.1007/s13399-025-06704-4>
- DEKNES, L. B.; ABRANTES, K. K. B.; FALCIONI, R.; *et al.* Efficiency of near-infrared spectroscopy in quantifying lignin in black liquor-impregnated reforestation wood. *Polymers*, v. 17, n. 19, p. 2614, 2025. <https://doi.org/10.3390/polym17192614>
- ESMERALDO, M. A.; BARRETO, A. C. H.; FREITAS, J. E. B.; *et al.* Dwarf-green coconut fibers: A versatile natural renewable raw bioresource. Treatment, morphology, and physicochemical properties. *BioResources*, v. 5, n. 4, p. 2478–2501, 2010. <https://doi.org/10.15376/biores.5.4.2478-2501>
- GIUSSANI, B.; EL GHOUCH, S. O.; MONTI, M.; *et al.* Understanding variability and calibration challenges in NIR miniaturized sensors: the impact of particle size and analytical session in almond powder analysis. *Measurement*, v. 256, p. 118481, 2025. <https://doi.org/10.1016/j.measurement.2025.118481>
- GUDJÓNSDÓTTIR, M.; HILMARSDÓTTIR, G. S.; ÖGMUNDARSON, Ó.; *et al.* Near-infrared spectroscopy and chemometrics for effective online quality monitoring and process control during pelagic fishmeal and oil processing. *Foods*, v. 13, n. 8, p. 1186, 2024. <https://doi.org/10.3390/foods13081186>
- HEDGES, L. V. Effect sizes for experimental research. *British Journal of Mathematical and Statistical Psychology*, v. 79, n. 1, p. 31–45, 2025. <https://doi.org/10.1111/bmps.12389>
- HERDLEVÆR, K. M.; LØHRE, C.; NODLAND, E.; *et al.* Comparison of calibration models for rapid prediction of lignin content in lignocellulosic biomass based on infrared and near-infrared spectroscopy. *Results in Chemistry*, v. 4, p. 100496, 2022. <https://doi.org/10.1016/j.rechem.2022.100496>
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. Produção de coco-da-baia, Available at: www.ibge.gov.br/explica/producao-agropecuaria/coco-da-baia/br. Accessed in: January 28th 2026.
- JEONG, H.; YANG, S. J.; PEERAPATTANA, J.; *et al.* Comparative evaluation of accuracy in near-infrared and Raman spectroscopic determinations of component concentration in packed solid mixtures under various packing densities. *Analytica Chimica Acta*, v. 1331, p. 343310, 2024. <https://doi.org/10.1016/j.aca.2024.343310>
- LUPOLI, J. S.; SINGH, S.; SIMMONS, B. A.; *et al.* Assessment of lignocellulosic biomass using analytical spectroscopy: an evolution to high-throughput techniques. *BioEnergy Research*, v. 7, n. 1, p. 1–23, 2014. <https://doi.org/10.1007/s12155-013-9352-1>
- MATHEW, A. K.; CHANEY, K.; CROOK, M.; *et al.* Dilute acid pre-treatment of oilseed rape straw for bioethanol production. *Renewable Energy*, v. 36, n. 9, p. 2424–2432, 2011. <https://doi.org/10.1016/j.renene.2011.01.030>
- MILLER, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, v. 31, n. 3, p. 426–428, 1959. <https://doi.org/10.1021/ac60147a030>
- MOZDYNIOWICZ, D. J.; NIEMINEN, K.; SIXTA, H. Alkaline steeping of dissolving pulp. Part I: cellulose degradation kinetics. *Cellulose*, v. 20, n. 3, p. 1437–1451, 2013. <https://doi.org/10.1007/s10570-013-9926-2>
- NISGOSKI, S.; VIEIRA, H. C.; DOS SANTOS, J. X.; *et al.* Discrimination of caatinga species based on bark using near infrared spectroscopy. *CERNE*, v. 31, e-103490, 2025. <https://doi.org/10.1590/0104776020251013490>
- PANDISELVAM, R.; MAHANTI, N. K.; MANIKANTAN, M. R.; *et al.* Rapid detection of adulteration in desiccated coconut powder: vis-NIR spectroscopy and chemometric approach. *Food Control*, v. 133, p. 108588, 2022. <https://doi.org/10.1016/j.foodcont.2021.108588>
- PHUANGSOMBUT, A.; PHUANGSOMBUT, K.; MEETIM, J.; *et al.* Application of miniaturized near-infrared spectrometer for indirectly determining flesh thickness of intact polished coconut. *Postharvest Biology and Technology*, v. 198, p. 112224, 2023. <https://doi.org/10.1016/j.postharvbio.2022.112224>
- POSOM, J.; PHUPHAPHUD, A.; SAENGPRACHATANARUG, K.; *et al.* Real-time measuring energy characteristics of cane bagasse using NIR spectroscopy. *Sensing and Bio-Sensing Research*, v. 38, p. 100519, 2022. <https://doi.org/10.1016/j.sbsr.2022.100519>
- PRIYA; P. S. D.; VERMA, Y.; MUHAL, R. A.; *et al.* Biofuels: An alternative to conventional fuel and energy source. *Materials Today: Proceedings*, v. 48, p. 1178–1184, 2022. <https://doi.org/10.1016/j.matpr.2021.08.227>
- RAMADAN, A.; ABATZOGLOU, N.; GOSSELIN, R. Addressing temperature variations of miniaturized NIR spectrometers: Advancing quantitative models for pharmaceutical analysis. *Journal of Pharmaceutical and Biomedical Analysis*, v. 264, p. 116959, 2025. <https://doi.org/10.1016/j.jpba.2025.116959>
- RAMBO, M. K. D.; ALVES, A. R.; GARCIA, W. T.; *et al.* Multivariate analysis of coconut residues by near infrared spectroscopy. *Talanta*, v. 138, p. 263–272, 2015. <https://doi.org/10.1016/j.talanta.2015.03.014>
- SAWILOWSKY, S. S. New effect size rules of thumb. *Journal of Modern Applied Statistical Methods*, v. 8, n. 2, p. 597–599, 2009. <https://doi.org/10.22237/jmasm/1257035100>
- SCHWANNINGER, M.; STEFKE, B.; HINTERSTOISSER, B. Qualitative assessment of acetylated wood with infrared spectroscopic Methods. *Journal of Near Infrared Spectroscopy*, v. 19, n. 5, p. 349–357, 2011. <https://doi.org/10.1255/jnirs.942>
- SENTHAMARAIKANNAN, P.; SUNESH, N. P.; DIVAKARAN, D.; *et al.* Characterization of biomass based novel microcrystalline cellulose from Eucalyptus teriticornis leaf. *Scientific Reports*, v. 16, n. 1, p. 3480, 2026. <https://doi.org/10.1038/s41598-025-18347-6>
- SHAPIRO, A. J.; O'DEA, R. M.; EPPS III, T.H. thermogravimetric analysis as a high-throughput lignocellulosic biomass characterization method. *Sustainable Chemistry & Engineering*, v. 11, n. 49, p. 17216–17223, 2023. <https://doi.org/10.1021/acscschemeng.3c03769>
- SHEN, X.; WANG, T.; WEI, J.; *et al.* Potential of near-infrared spectroscopy (NIRs) for efficient classification based on postharvest storage time, cultivar and maturity in coconut water. *Foods*, v. 12, n. 12, p. 2415, 2023. <https://doi.org/10.3390/foods12122415>
- SHRESTHA, N. Factor analysis as a tool for survey analysis. *American Journal of Applied Mathematics and Statistics*, v. 9, n. 1, p. 4–11, 2021. <https://doi.org/10.12691/ajams-9-1-2>
- SILVA, N. V. F.; MENEZES, F. F.; GONÇALVES, A. R.; *et al.* Modulating the properties and structure of lignins produced by alkaline delignification of sugarcane bagasse pretreated with two different mineral acids at pilot-scale. *International Journal of Biological Macromolecules*, v. 263, p. 130111, 2024. <https://doi.org/10.1016/j.ijbiomac.2024.130111>

SIRIJUNCHEUN, S.; SEITHTANABUTARA, V.; WANTALA, K.; *et al.* Impact of alkali impregnation on physicochemical properties of macro-scale sugarcane bagasse. *Biomass Conversion and Biorefinery*, v. 14, n. 13, p. 14611–14626, 2024. <https://doi.org/10.1007/s13399-023-03762-4>

SLUITER, A.; HAMES, B.; RUIZ, R.; *et al.* Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory, 2012. Available at: <https://www.nrl.gov/docs/gen/fy13/42618.pdf>. Accessed in: May 30th 2026.

SLUITER, A.; RUIZ, R.; SCARLATA, C.; *et al.* Determination of extractives in biomass. National Renewable Energy Laboratory, 2008. Available at: https://www.researchgate.net/publication/309045937_Determination_of_Extractives_in_Biomass_Laboratory_Analytical_Procedure_LAP. Accessed in: May 30th 2026.

SUKSANGPANOMRUNG, P.; RITTHIRUANGDEJ, P.; HIRIOTAPPA, A.; *et al.* Rapid, non-destructive prediction of coconut composition for sustainable UHT milk production via near-infrared spectroscopy. *Journal of Food Composition and Analysis*, v. 128, p. 106009, 2024. <https://doi.org/10.1016/j.jfca.2024.106009>

TANG, C.; LI, M.; JIANG, B.; *et al.* High-throughput profiling of sweet potato vine biomass for cellulosic ethanol production using near-infrared spectroscopy and chemometrics. *Microchemical Journal*, v. 213, p. 113679, 2025. <https://doi.org/10.1016/j.microc.2025.113679>

TIMUNG, R.; MOHAN, M.; CHILUKOTI, B.; *et al.* Optimization of dilute acid and hot water pretreatment of different lignocellulosic biomass: A comparative study. *Biomass and Bioenergy*, v. 81, p. 9–18, 2015. <https://doi.org/10.1016/j.biombioe.2015.05.006>

TSUCHIKAWA, S.; INAGAKI, T.; MA, T. Application of near-infrared spectroscopy to forest and wood products. *Current Forestry Reports*, v. 9, n. 6, p. 401–412, 2023. <https://doi.org/10.1007/s40725-023-00203-3>

WORKU, L. A.; BACHHETI, R. K.; BACHHETI, A.; *et al.* Understanding the biochemical changes at molecular level during biomass pretreatment: a comprehensive analysis. *Cellulose*, v. 31, n. 12, p. 7281–7312, 2024. <https://doi.org/10.1007/s10570-024-06081-7>

WU, W.; LI, P.; HUANG, L.; *et al.* The role of lignin structure on cellulase adsorption and enzymatic hydrolysis. *Biomass*, v. 3, n. 1, p. 96–107, 2023. <https://doi.org/10.3390/biomass3010007>

YEH, T.-F.; CHANG, H.; KADLA, J. F. Rapid prediction of solid wood lignin content using transmittance near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, v. 52, n. 6, p. 1435–1439, 2004. <https://doi.org/10.1021/jf034874r>

ZHAO, Y. L.; DOLAT, A.; STEINBERGER, Y.; *et al.* Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. *Field Crops Research*, v. 111, n. 1–2, p. 55–64, 2009. <https://doi.org/10.1016/j.fcr.2008.10.006>

ZHAO, Y.; ZHU, Y.; REN, Y.; *et al.* Predictive modeling of lignocellulosic content in crop straws using nir spectroscopy. *Plants*, v. 14, n. 10, p. 1430, 2025. <https://doi.org/10.3390/plants14101430>