



Integrated Production of Fermentable Sugars and Phenolic Compounds from Sisal Residue

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Abstract

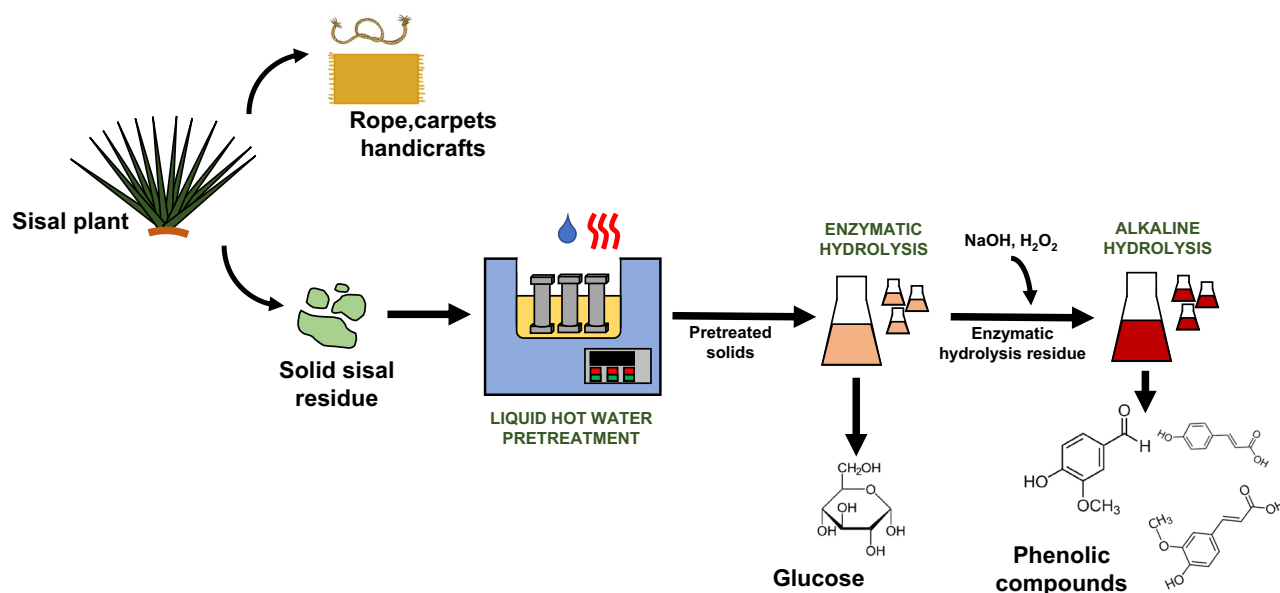
Abstract In the present study, sisal (*Agave sisalana*) defibration residue was used as a raw material to obtain fermentable sugars and phenolic compounds through integrated processes. Hydrothermal pretreatments under different temperature conditions (160, 180, and 200 °C) and incubation times (30, 60, and 90 min) were performed to promote cellulose enrichment and, consequently, enzymatic digestibility. The enzymatic hydrolysis residue was then used as a precursor for phenolic compounds via alkaline hydrolysis, evaluating the effects of sodium hydroxide concentrations (2%, 4%, and 6%, w/v) and hydrogen peroxide concentrations (0%, 1%, and 2%, v/v). In general, increasing pretreatment severity led to greater losses of extractives, ash, and hemicellulose at the expense of increased cellulose content and crystallinity index. The best pretreatment condition was 180 °C for 60 min, which increased cellulose content from 16.43% to 38.91% and glucose release from 3.0 to 17.2 g/L (conversion = 79.7%) at 5% solids. Under high solid loadings, enzymatic hydrolysis of pretreated sisal residue was also successful, yielding glucose levels above 90 g/L (at 30% solids). The production of phenolic compounds (mainly vanillin) was achieved by increasing sodium hydroxide and eliminating hydrogen peroxide, reaching a maximum concentration of 2,115.6 mg/L using 4% sodium hydroxide and 20% solids. Therefore, this study contributes to understanding the potential of sisal as a precursor to value-added products and may contribute to the development of technologies using plants from arid zones.

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Graphical Abstract



Keywords Valorization · Hydrothermal · High solid loading · Alkaline hydrolysis

Introduction

The deterioration of the Earth's climate due to human activity makes it urgent to use natural resources efficiently [1]. Arid and semi-arid regions are those that particularly lack attention from public policies, since their socio-economic scenario is fragile. Even so, several of these regions (such as the Caatinga and savannas) present biodiversity, with plants of high productivity throughout the year and high income potential, which can be directed from energy generation to the production of cosmetics [2]. The ability of these plants to adapt to harsh conditions should be seen as an asset for a sustainable path, but more important is the proper management of all products throughout the production chain [3].

Agave sisalana, also called sisal, is one of the main crops in arid regions because it is responsible for the production of natural fibers used for making ropes, handicrafts, and textile products [4]. Sisal is a plant native to Mexico, but currently Brazil, Tanzania, and Kenya are the main players, contributing to the global production of approximately 400 kton per year [5]. Sisal is one of the agave species with the greatest adaptability to water scarcity and is characterized by a short stem and thick and elongated leaves from which the natural fiber is recovered [6]. However, the mechanical defibration stage generates a high volume of waste, which is commonly disposed of inappropriately.

According to Vieira et al. [7], the mass of waste (considering juice, mucilage, and short fibers) corresponds to over 95% of the mass of sisal leaves. Although progress has been made in reusing sisal fiber residue as fertilizer, repellent, or ground cover [4, 8], its accumulation in the soil leads to severe acidification, pollutes the surface, and contributes to methane emissions via anaerobic digestion [7]. For this reason, scientific researchers have focused on implementing technologies to manage the volume of waste generated in sisal processing, potentially transforming a problem into job creation and a source of income for communities near cultivation areas.

Sisal solid residues contain cellulose and hemicellulose, which can be converted into biofuels (ethanol and n-butanol) through biotechnological routes, which are generally more environmentally friendly [9]. Pretreatments are the first step in the process, enriching cellulose in the biomass and facilitating enzyme accessibility to polysaccharides. During hydrothermal pretreatment, water reaches the subcritical region, promoting water autoionization and breaking acetyl and glucuronic groups in hemicellulose, acidifying the bulk liquid and contributing to the removal of amorphous components (mainly hemicellulose) and the reorganization of biomass components [10]. It is noteworthy that hydrothermal pretreatments require less water for washing than chemical pretreatments, which would also be advantageous considering the context of sisal production [11]. Some hydrothermal pretreatment

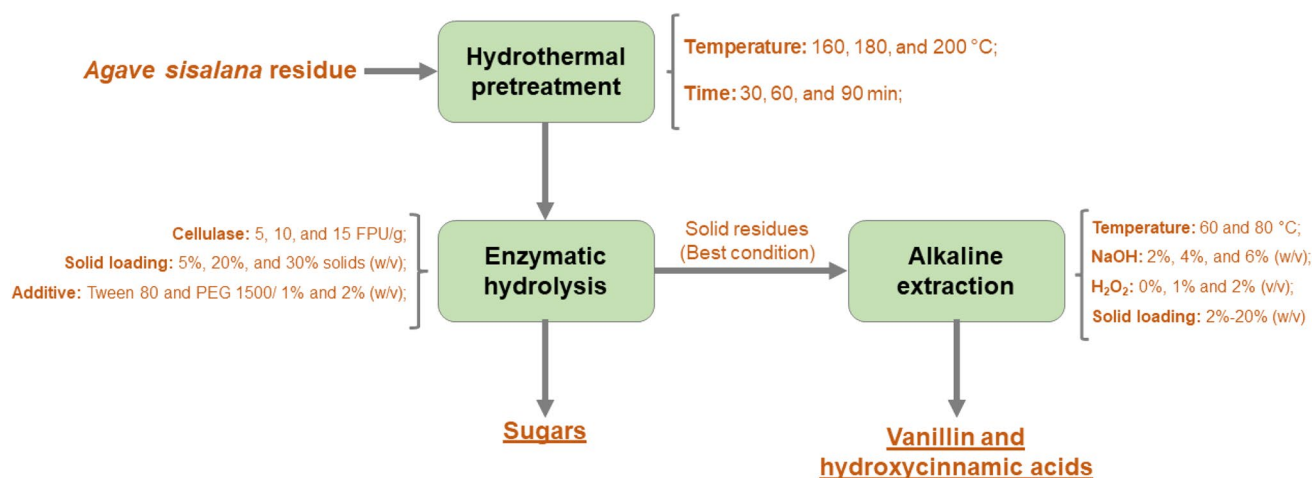


Fig. 1 Workflow performed in the fractionation of sisal residue

technologies have already shown success with agave-derived biomass [10, 12–15], but there are few reports involving sisal residues. Therefore, there is no clear understanding of how hydrothermal process variables, such as time and temperature, affect the yield, chemical composition, and digestibility of pretreated sisal residue. Nor has any progress been made regarding the reuse of lignin-enriched sisal solid residue after enzymatic hydrolysis.

Bound phenolic compounds comprise molecules linked to polysaccharides and integrated into the native lignin structure, being released under mild conditions with the aid of alkaline agents (sodium hydroxide, potassium hydroxide, sodium carbonate, etc.) [16, 17]. Although cellulases and hemicellulases can break down polysaccharides, lignin is preserved and can be the source of a number of high-value phenolic compounds. For example, hydroxycinnamic acids are precursors of potent antioxidants for cosmetics and are released in high concentrations during the alkaline hydrolysis of cereal residues [18]. According to L'udmila et al. [19], p-coumaric acid is among the main products derived from lignin, and can reach a market price of up to US\$ 60 per kilogram. In Tarabanko et al. [20, 21], the authors reported that the addition of oxidizing agents during alkaline hydrolysis can improve vanillin production, an ingredient widely used in food and cosmetic formulations. However, the performance of alkaline hydrolysis using sisal residues as raw material is unknown, whether in the presence or absence of an oxidizing agent. The identification of the phenolic compounds released would be interesting from an academic perspective and could represent an opportunity for technological innovation, especially when integrated with the use of polysaccharides. To the authors' knowledge, no cascade valorization scheme has been carried out in the literature involving sisal waste focusing on the release of

sugars and recovery of phenolic compounds via alkaline hydrolysis.

Therefore, to contribute to the exploration of the plant's potential, this study investigated the fractionation of sisal residue through an integrated process comprising hydrothermal pretreatment, enzymatic hydrolysis, and alkaline hydrolysis to produce glucose and phenolic compounds. During the hydrothermal pretreatment, the temperature and incubation time conditions were varied to improve cellulose enrichment and enzymatic digestibility. Once the best enzymatic hydrolysis conditions were determined, the solid residue was digested with sodium hydroxide and hydrogen peroxide, enabling the quantification and identification of phenolic components in the hydrolysate.

Materials and Methods

Chemicals and Biomass

Sodium hydroxide, sodium azide, sulfuric acid, acetic acid, citric acid, sodium citrate, hydrogen peroxide, glucose, xylose, arabinose, monobasic sodium phosphate, dibasic sodium phosphate, sodium carbonate, polyethylene glycol 1500 (PEG 1500), and Tween 80 were purchased from Synth (São Paulo, Brazil). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3,5-dinitrosalicylic acid (DNS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, acetonitrile, and the glucose oxidase/peroxidase kit (GODPOD) were purchased from Sigma-Aldrich (MO, USA). HPLC standards (including formic acid, levulinic acid, furfural, hydroxymethylfurfural (HMF), gallic acid, catechin, vanillic acid, syringic acid,

vanillin, p-coumaric acid, ferulic acid, quercetin, and eugenol) were also acquired from Sigma-Aldrich (MO, USA).

Solid sisal residue was collected in the city of Pedra Grande, Rio Grande do Norte, Brazil (5°07'56.3"S 35°54'31.7"W). The sisal residue was initially dried at 45 °C for 5 days to reduce moisture content and simplify biomass processing. The dry residue was then ground in a knife mill to a 48-mesh (0.8 mm) particle size and stored in plastic bags.

Fractionation of Sisal Residue

The sisal residue fractionation steps are summarized in Fig. 1. Hydrothermal pretreatments were performed under different temperatures and incubation times. Under the best conditions, enzymatic hydrolysis tests were performed, and the residue from this step was used as a precursor for phenolic compounds via alkaline hydrolysis and fast pyrolysis.

Hydrothermal Pretreatment

Hydrothermal pretreatment of sisal residue was performed using the liquid hot water method. The residue (4 g) was mixed with 20 mL of water in 304 stainless steel cylinders with screw caps and placed in a preheated oil bath at 160, 180, and 200 °C under different times (30–90 min). The operating conditions were selected based on the studies of Lü et al. [22] and Singh et al. [23]. After the incubation time, the cylinder was opened using a vise, and the pretreated material was collected and washed with distilled water using a vacuum filtration system. The pretreated material retained on the filter paper was placed in an air circulation oven at 60 °C for 24h, and the solid yield was recorded. The best hydrothermal pretreatment condition was defined based on the balance between chemical composition (Sect. "Physico-chemical characterization") and sugar release during enzymatic hydrolysis (Sect. 2.2.2). The severity factor for each pretreatment ($\log_{10}R_0$) was calculated from Eq. 1:

$$\log_{10}R_0 = \log_{10} \left(t \times e^{\left(\frac{T-T_{ref}}{14.75} \right)} \right) \quad (1)$$

where t is the incubation time, T is the hydrothermal pretreatment temperature, and T_{ref} is the reference temperature (equal to 100 °C).

Enzymatic Hydrolysis

Enzymatic hydrolysis tests were performed to evaluate the release of fermentable sugars after hydrothermal

pretreatments. First, using 25 mL Erlenmeyer flasks, pretreated sisal samples (0.25 g) were mixed with 50 mM sodium citrate buffer (pH 5.0), 0.05% (w/v) sodium azide, and 15 FPU/g cellulase to reach a final volume of 5 mL. The Erlenmeyer flasks were placed in a rotary incubator at 50 °C and 120 rpm for 48h. After incubation in boiling water (~100 °C), the supernatants were separated from the solid residues by centrifugation and then stored for quantification of released sugars. Glucose concentrations were determined using the GODPOD kit. The solid residue was washed thoroughly with water and then dried in an air circulation oven at 60 °C for 24h. Tests were also performed with untreated sisal residue as a control. After defining the best pretreatment condition, enzymatic hydrolysis tests were conducted by varying the presence of additives (PEG 1500 and Tween 80), solid loading (20% and 30%), and the feeding approach. The values of cellulose conversion were estimated from Eq. 2.

$$\text{Cellulosic conversion}(\%) = \frac{\text{released glucose (g)}}{\text{cellulose(g)} \times 1.11} \times 100 \quad (2)$$

Production of Phenolic Compounds from Enzymatic Hydrolysis Residue

Phenolic compounds were produced from the enzymatic hydrolysis residue using alkaline hydrolysis and fast pyrolysis. Alkaline hydrolysis was performed in capped test tubes containing enzymatic hydrolysis residue (0.04–0.4 g) and 2 mL of liquid phase containing different concentrations of sodium hydroxide (2%–6%, w/v) and hydrogen peroxide (0%–2%, v/v). The test tubes were left in a thermostatic bath (60 and 80 °C) for 2h. Then, the supernatants were collected by centrifugation at 1,500×g and diluted for future analysis of total phenolic compounds and for high-performance liquid chromatography (HPLC) (Sect. "HPLC"). The yield of total phenolic compounds was calculated from the concentration of phenolic compounds released (C_{phen}), the volume of alkaline hydrolysis (V), and the mass of residue from enzymatic hydrolysis (m), according to Eq. 3.

$$\text{Total phenolic compounds}(\%) = \frac{(C_{phen} \times V)}{m} \times 100 \quad (3)$$

Analytical Methods

Physicochemical Characterization

The chemical composition analyses of sisal residues consisted of determining the contents of cellulose,

Table 1 Chemical characterization of untreated and pretreated hydrothermal sisal residues

Sample/Severity	Yield (%)	Cellulose (%)	Hemicellulose (%)	Hemicel- lulose loss (%)	Klason lignin (%)	Extractives (%)	Ashes (%)
Untreated	—	16.43 ± 1.28	8.74 ± 0.37	—	9.68 ± 0.47	40.33 ± 1.24	10.12 ± 0.14
160 °C, 30 min (3.24)	55.1	25.24 ± 2.65	7.85 ± 1.73	50.5	22.86 ± 2.26	15.63 ± 1.04	4.22 ± 0.21
160 °C, 60 min (3.54)	52.3	30.13 ± 1.23	8.89 ± 0.48	46.8	19.62 ± 1.06	15.04 ± 1.86	5.01 ± 0.18
160 °C, 90 min (3.72)	45.6	35.70 ± 2.28	9.82 ± 1.29	48.8	20.31 ± 1.11	10.68 ± 2.46	3.91 ± 0.28
180 °C, 30 min (3.83)	43.3	37.96 ± 0.73	11.02 ± 1.89	45.4	18.73 ± 0.64	15.13 ± 0.76	5.43 ± 0.23
180 °C, 60 min (4.13)	44.1	35.70 ± 2.51	10.34 ± 1.01	47.8	20.92 ± 1.56	12.37 ± 1.31	3.08 ± 0.39
180 °C, 90 min (4.31)	40.8	38.79 ± 1.34	9.53 ± 0.85	55.5	22.33 ± 1.39	11.32 ± 0.98	2.98 ± 0.17
200 °C, 30 min (4.42)	44.5	34.84 ± 1.59	9.75 ± 1.01	50.4	23.81 ± 2.00	13.73 ± 0.77	3.01 ± 0.25
200 °C, 60 min (4.72)	40.2	38.91 ± 1.03	10.39 ± 0.28	52.3	25.71 ± 1.51	12.81 ± 0.83	2.41 ± 0.04
200 °C, 90 min (4.90)	38.3	34.75 ± 1.01	9.17 ± 0.22	59.8	29.51 ± 0.14	16.85 ± 1.43	2.69 ± 0.22

hemicellulose, Klason lignin, extractives, and ashes according NREL protocols [24–26]. The values of hemicellulose loss were calculated using Eq. 4.

Using an XRD-6000 X-ray diffractometer (Shimadzu, Japan) and a range of 10–60°, diffractogram profiles and the crystallinity indices were obtained to assess the degree of crystallinity of sisal residue after hydrothermal pretreatment. The crystallinity index (CrI) was calculated in Eq. 5 from the intensity values of the plane peak 002 (I_{002} ; 20.9°) and of the valley between planes 002 and 001 (I_{am} ; ~15.7°).

$$\text{Hemicellulose loss}(\%) = \frac{\text{Solid yield} \times \text{Hemicellulose content in pretreated biomass}}{\text{Hemicellulose content in untreated biomass} \times 100} \quad (4)$$

$$\text{CrI}(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \quad (5)$$

Quantification of Total Phenolic Compounds and DPPH Assay

Total phenolic compounds were determined by the Folin–Ciocalteu method based on the protocol of Fujita et al. [27]. First, samples from the alkaline hydrolysis of sisal residue were diluted with distilled water, and then 125 µL aliquots were mixed with 625 µL of 0.2 N Folin solution in test tubes. After 5 min, 500 µL of 75 g/L sodium carbonate was added, and the tubes were left to stand in a light-free environment at room temperature for 60 min. Absorbance measurements were performed on a K37-VIS spectrophotometer (KASVI, Brazil) at 760 nm using gallic acid as a standard.

The DPPH analysis was initiated by adding 0.4 mL of 0.06 mM DPPH (dissolved in ethanol) to cuvettes containing 0.8 mL of the alkaline hydrolysate sample, as described

by Brand-Williams et al. [28]. After agitation and 25 min of rest, measurements were taken on a spectrophotometer at 517 nm, and the results were expressed in terms of mmol Trolox equivalents per mL of sample.

HPLC

HPLC analyses were performed on an LC-10 platform (Shimadzu) to determine some substances. For quantification of sugars and organic acids, samples were injected into a

Shim-pack SCR 101-H column (7.9 × 300 mm) and eluted using 5 mM sulfuric acid at 0.6 mL/min and 50 °C. In this analysis, a refractive index detector was used, and sugar and organic acid concentrations were determined from previously prepared calibration curves. The quantification of furans and phenolic compounds was performed using a Shim-pack C18 column (4.5 × 150 mm) and SPD detector (at 280 nm). Elution was performed using a mixture of 1% (v/v) acetic acid and acetonitrile, as described by Maduzzi et al. [18]. As with the methodology for sugars, the identification and quantification of phenolic compounds were carried out by verifying the residence time and using calibration curves for each compound (gallic acid, catechin, syringic acid, vanillic acid, vanillin, p-coumaric acid, ferulic acid, quercetin, and eugenol).

Statistical Analyses

The results of chemical characterization, enzymatic hydrolysis, and phenolic compound production were expressed as the arithmetic mean and standard deviation. Comparison of

Fig. 2 XRD profile of untreated and hydrothermal pretreated sisal residues

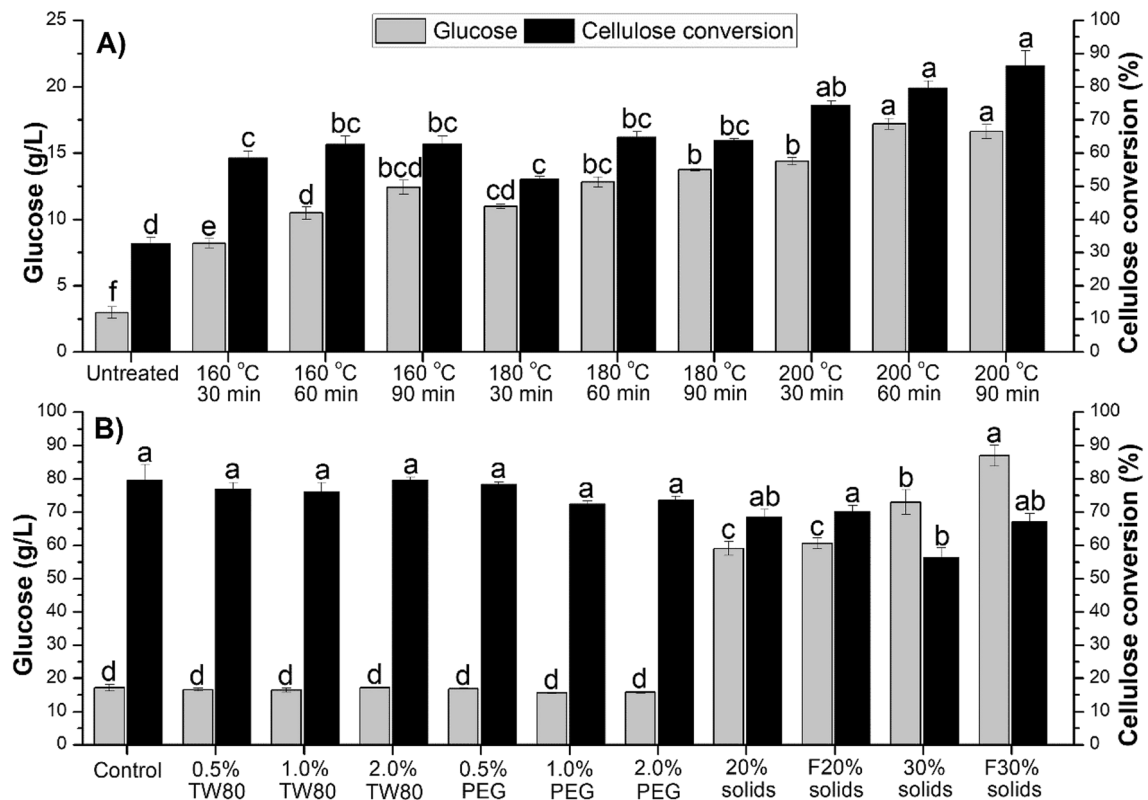
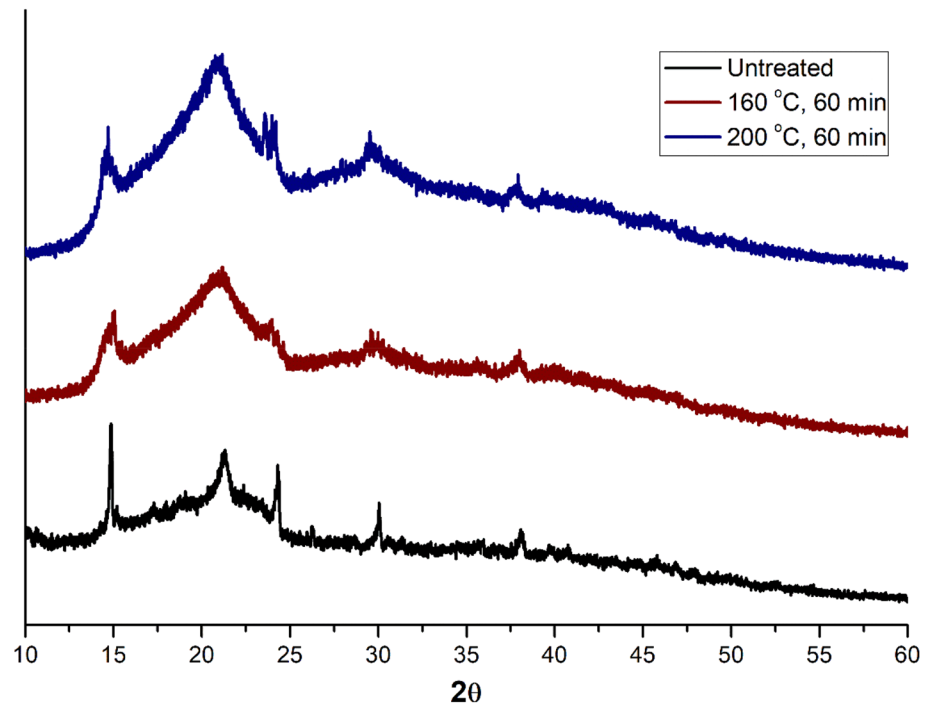


Fig. 3 Effects of hydrothermal pretreatments **A** and enzymatic hydrolysis operating conditions on glucose release and cellulose conversion of sisal residue. All experiments were conducted in a

rotary incubator at 50 °C and 120 rpm. For a given response, equal letters indicate no statistical difference using a 5% significance level ($p < 0.05$)

process results was performed using Tukey's post hoc test using Statistica 7.0 software (StatSoft, USA) at a significance level of 5% ($p < 0.05$).

Results and Discussions

Characterization of sisal Residue After Hydrothermal Pretreatments

The chemical characterization results of untreated and pretreated sisal samples are shown in Table 1. Extractive values changed significantly after hydrothermal pretreatment. The untreated sisal residue contained 40.33% extractives, a limiting factor for achieving high sugar content in enzymatic hydrolysis. It is noteworthy that sisal residue comes from the plant leaf and therefore has a low content of structural components, such as lignocellulose, and accumulates phytochemicals to support photosynthesis and protect against environmental stresses [29]. However, hydrothermal pretreatments were effective in removing extractives, resulting in pretreated residues with less than 17% extractives. In addition to lipophilic substances, sisal residues are rich in carboxylic acids, such as malic acid and citric acid, due to Crassulacean Acid Metabolism (CAM), and can be solvated in the liquid medium or even hydrolyzed and oxidized [30, 31]. In this study, the loss of extractives was also accompanied by a reduction in solid yield, which reached a minimum value of 38.3% at 200 °C and 90 min. The maximum solid yield of 55.1% was obtained in the hydrothermal pretreatment at 160 °C for 30 min, the lowest-severity condition. As expected, sisal residue is a material rich in ash (10.12% in the untreated material), since it derives from the plant's leaves. In other studies with sisal residue, the ash content was even higher, exceeding 14% [32]. After hydrothermal pretreatment, the ash content was less than 3%, particularly at 200 °C. As with extractives, mineral materials are released and solubilized in liquid media during hydrothermal pretreatment, thereby improving the chemical composition. Although hemicellulose values in the biomass remained virtually unchanged, hemicellulose was digested during the hydrothermal pretreatment. The residue pretreated at 180 °C for 30 min contained 11.02% hemicellulose, but hemicellulose loss amounted to 45.4%. After pretreatment at 200 °C for 90 min, hemicellulose loss reached 59.8%. This perception that the pretreatment was not efficient for hemicellulose is due to the low initial polysaccharide content in the residue and to the redistribution of components resulting from the abrupt removal of extractives.

In turn, the Klason lignin and cellulose contents increased in the pretreated sisal residue samples. Except for the pretreatments at 160 °C for 60 min and 180 °C for 30 min,

Klason lignin values exceeded 20%, representing a more than twofold increase compared to the untreated material (only 9.68%). Although undesirable, this occurrence was expected given the pretreatment operating method. Hydrothermal pretreatment can partially disrupt the lignin structure (especially under more severe conditions). Still, the macromolecule is insoluble in water and can repolymerize after cooling, becoming more recalcitrant [33]. At least, hydrothermal pretreatment redistributes lignin and consequently modifies biomass morphology, often associated with improved enzymatic hydrolysis performance [34]. Regarding cellulose, the polysaccharide content increased significantly with pretreatment, from 16.43% to values above 25%, with a positive effect of temperature. For example, using a 60-min retention time, the cellulose values were 30.13%, 35.70%, and 38.91% at 160, 180, and 200 °C, respectively. Unlike hemicellulose, cellulose has a predominantly crystalline structure and is therefore resistant to hydrothermal pretreatment conditions, which explains its retention and the increase in cellulose in pretreated biomasses [34, 35]. XRD analyses confirmed this trend of cellulose enrichment, as shown in Fig. 2. The sample pretreated at 160 °C for 60 min showed a slight increase in CrI from 37.9% to 38.3%. However, an even higher CrI value was recorded in the sample pretreated at 200 °C for 60 min (51.0%). Another indicator of the loss of amorphous elements due to hydrothermal pretreatment is the reduction in peaks associated with inorganic oxides [32].

Enzymatic Digestibility of Untreated and Pretreated Sisal Residues

Hydrothermal pretreatments significantly improved enzymatic hydrolysis performance (Fig. 3A). The untreated sisal residue showed only 2.98 g/L glucose and 32.7% cellulosic conversion, while the lower-severity hydrothermal pretreatment (160 °C, 30 min, $\log R_0 = 3.24$) showed 8.20 g/L glucose and almost twice the conversion (58.6%). Overall, glucose release increased with the severity of the hydrothermal pretreatment, reaching a maximum of 17.20 g/L at 200 °C and 60 min. The cellulosic conversion behavior was similar, reaching a maximum of 86.3% at 200 °C and 90 min. Although there are no reports of hydrothermal pretreatment, the cellulosic conversion values of the present study (58.6–86.3%) are comparable to those from the literature on chemical pretreatments. Kashuk et al. [36] investigated the release of sugars from sisal residue pretreated by the Kraft process and observed conversion values of 89–98%. In a study on acid-alkaline pretreatment of sisal residue, Dash et al. [37] reported cellulosic conversion values above 80%. Recently, our research team investigated the enzymatic digestibility of sisal residue modified by a sequence of acid and alkaline pretreatments, achieving maximum cellulosic

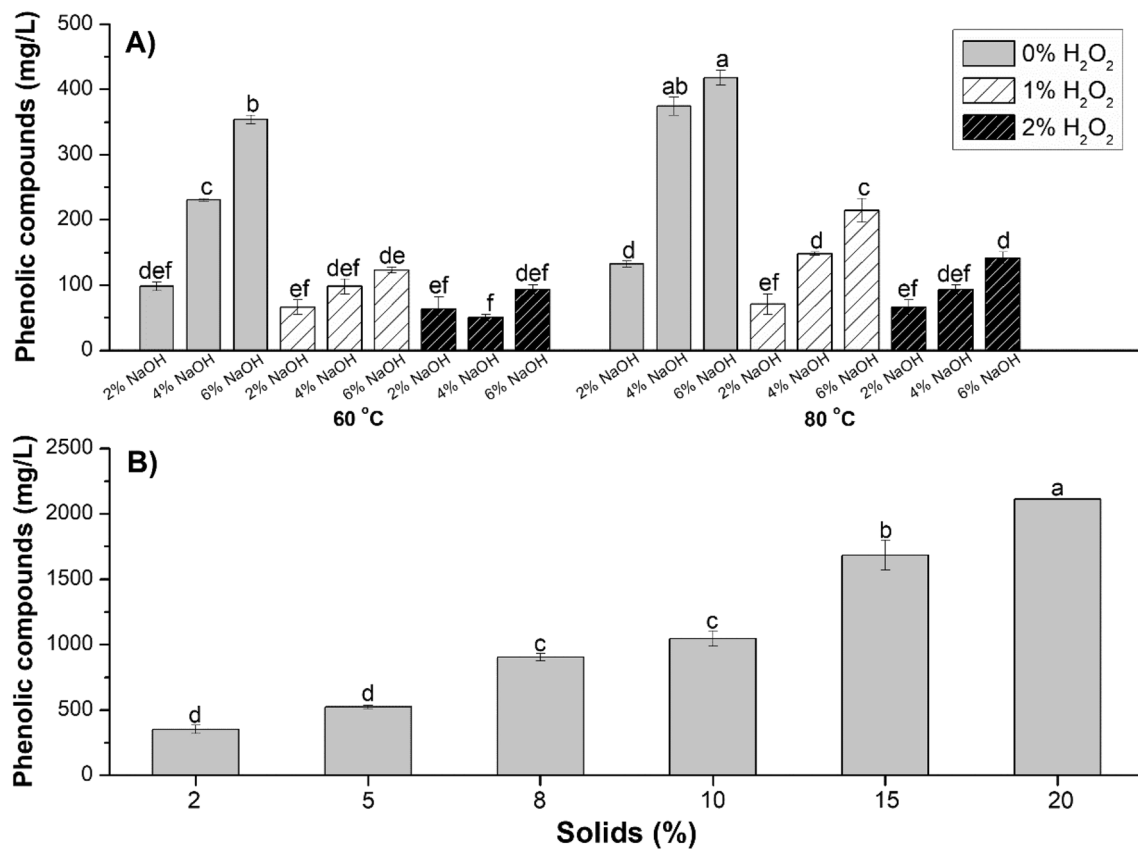


Fig. 4 Release of phenolic compounds from enzymatic hydrolysis residue under different alkaline hydrolysis conditions at 2% solids **A**. Release of phenolic compounds from enzymatic hydrolysis residue

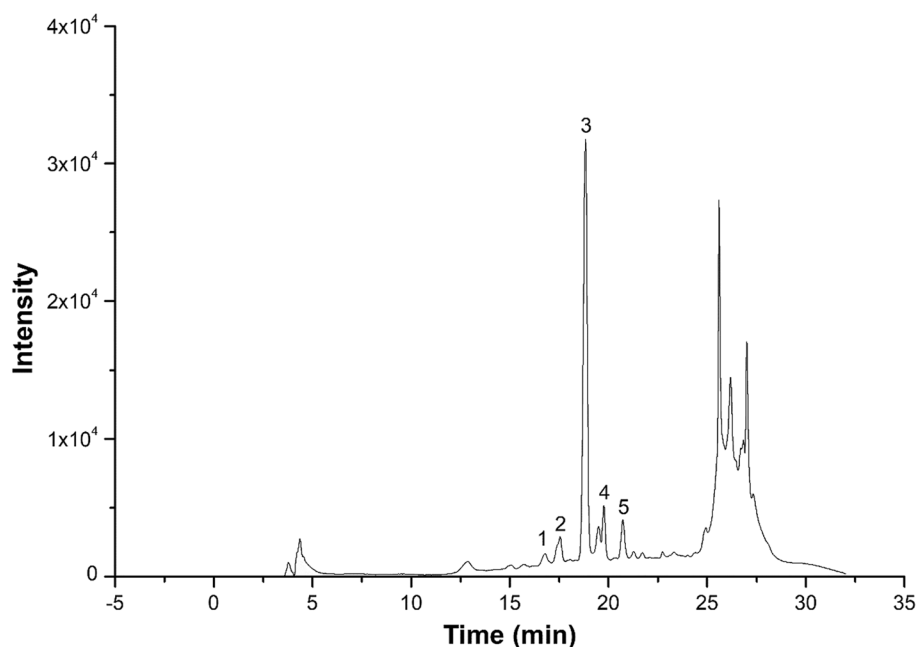
under different solid loadings using 6% sodium hydroxide without hydrogen peroxide and temperature of 80 °C **B**. Equal letters indicate no statistical differences using a 5% significance level ($p < 0.05$)

conversion of 91.2% [32]. These results highlight the role of hydrothermal pretreatment in boosting the enzymatic digestibility of sisal residue. Given the goal of maximizing glucose release and the decline in cellulose content at 200 °C and 90 min, the condition of 200 °C and 60 min was selected for the next step. Another justification is the reduction in energy consumption during sisal residue processing and the higher solid yield. The liquid fraction of the pretreatment was not studied, but it is also a source of sugars. If the slurry were used for bioethanol production, adopting less severe conditions might result in lower concentrations of furfural and HMF, which are known fermentation inhibitors [23].

Figure 3B shows the enzymatic hydrolysis results in tests with additives and increased solid loading. Tween 80 and PEG 1500 were chosen for this step because they can reduce cellulase loss due to non-productive adsorption, thereby increasing the availability of cellulases for attack on cellulose. Other authors report that surfactants can act to mitigate cellulase loss at the gas–liquid interface and improve thermal stability under heat stress [13, 18, 38].

However, the results obtained did not meet expectations, regardless of surfactant dosage, with release around 17 g/L and conversion at 79% using 5% solids. The team hypothesizes that the benefits were not manifested because the pretreated sisal residue still has a relatively low lignin content. Based on del Río et al. [39], the syringyl/guaiacyl ratio (S/G ratio) of sisal residue is considered high (~3.3), making this biomass more susceptible to biological conversion into sugars, and surfactant addition is unnecessary. This would also entail additional costs for enzymatic hydrolysis. In tests with high solid loadings, slight difficulty in homogenization was observed due to the water retention capacity of the pretreated residue, but this did not compromise the performance of enzymatic hydrolysis. In the conventional approach, glucose release was 59.12 g/L for 20% solids and 73.00 g/L for 30% solids, corresponding to cellulosic conversions of 68.4% and 56.3%, respectively. The fed-batch approach reduced the problem of lack of free water, and the metrics improved, making it possible to obtain 60.58 (conversion equal to 70.1%) and 86.97 g/L (conversion

Fig. 5 HPLC profile of the alkaline hydrolysate of the enzymatic hydrolysis residue obtained using 4% (m/v) NaOH, 0% H₂O₂, 20% solids, 80 °C, and 1h incubation



equal to 67.1%) using a total solid loadings of 20% and 30%, respectively. Using agave bagasse as a feedstock, Caspeta et al. [40] also presented good enzymatic hydrolysis results under high solid loadings (~80 g/L glucose). Therefore, it is clear that sisal residue is a potential biomass for second-generation biofuel schemes, being particularly strategic because it is cultivated in arid and semi-arid regions.

Production of Phenolic Compounds from Enzymatic Hydrolysis Residue

The fed-batch approach with 30% solids yielded the best sugar release results, serving as a carbon source for fermentation processes or as a precursor for platform molecules. Furthermore, the post-hydrolysis residue can be converted into a value-added product, such as bioactive phenolic compounds [16, 41]. Due to the digestion of polysaccharides by enzymes, the post-enzymatic hydrolysis residue is rich in lignin, which can be broken down into phenolic compounds by exposure to an alkaline environment. In Fig. 4A, phenolic compound production ranged from 63.9 to 417.9 mg/L at 2% solids, where increasing the sodium hydroxide dosage and temperature improved process performance. In the absence of hydrogen peroxide and at a temperature of 60 °C, increasing the dosage from 2 to 4% sodium hydroxide increased the release of total phenolics by 2.3-fold. The total phenolic content increased gradually when 1% hydrogen peroxide and 80 °C were used, from 70.8 mg/L at 2% sodium hydroxide to 214.7 mg/L at 6% sodium hydroxide. In the present case, the phenolic compounds are covalently linked to the cell wall by ester bonds and to lignin by ether bonds [42]. Both covalent bonds are sensitive to the concentration of the alkali agent

as reported by Ares-Péon et al. [43]. In turn, the increase in temperature implies a greater supply of energy necessary for the rupture of phenolic-polysaccharide bonds and the depolymerization of lignin, which explains the higher results obtained at 80 °C. In alkaline environments, hydrogen peroxide releases reactive oxygen species, which break C–C covalent bonds and can aid in lignin depolymerization [20]. However, due to the conditions applied in the present study, the process may have been biased towards the rupture of aromatic rings in carboxylic acids [44], resulting in lower quantification in the Folin method. In terms of yield, the maximum value is 2.08% (w/w) at 80 °C with 6% sodium hydroxide and no oxidant; this condition was selected for evaluating the impact of the solid loading.

As expected, increasing solid loading leads in greater availability of phenolic compounds, and therefore, better results were observed at 20% solids (2,114.6 mg/L) (Fig. 4B). This phenolic compound yield is 1.06% (w/w), a decrease compared to tests with 2% solids, but still an attractive value given the mild nature of alkaline hydrolysis. Faustino et al. [45] reported phenolic yields of up to 1.09% (w/w) using sulfite pretreatment liquor as a feedstock. In a previous study by the group, phenolic compound release reached 5% (w/w) from corncob enzymatic hydrolysis residue [41]. Increasing solid loadings here not only improves productivity but can also aid in subsequent downstream processing steps. After HPLC identification, the major phenolic compound in the alkaline hydrolysate using 20% solids was vanillin (1,279.3 mg/L). Vanillin is a compound derived from the G unit of lignin and is widely used in the food and pharmaceutical industries due to its pleasant fragrance and flavor. Catechin (62.0 mg/L), vanillic acid (76.9 mg/L),

p-coumaric acid (125.5 mg/L), and ferulic acid (114.3 mg/L) were also present in the alkaline hydrolysate (see Fig. 5).

In terms of antioxidant activity, the 50-fold diluted alkaline hydrolysate showed DPPH radical inhibition of 45.2%, which is equivalent to 9.55 μmol Trolox per mL. Since vanillin does exhibit a low DPPH radical scavenging response [46], the manifestation of antioxidant activity was possibly promoted by vanillic acid and hydroxycinnamic acids [47, 48]. Previous studies have investigated the potential of agave and its derivatives for producing bioactive compounds, primarily because it is a material rich in extractives. Indeed, the literature reports good biological activity of saponins from *Agave sisalana* [49, 50], but this is the first time the potential of phenolic compounds bound to the lignocellulosic matrix has been evaluated. The results obtained here are even more interesting, given that the source was a residue from enzymatic hydrolysis, aligning with the idea of a circular economy and zero waste.

Conclusions

This study demonstrated successful fractionation of sisal residue into two value-added products. Hydrothermal pretreatments favored the removal of extractives, ash, and hemicellulose, while the cellulose and Klason lignin contents increased significantly as a function of process severity. The sisal residue pretreated at 200 °C for 60 min exhibited high enzymatic digestibility, achieving attractive sugar release values for the production of 2G ethanol (~90 g/L glucose) under high solid loadings (30% solids in fed-batch). The solid residue from enzymatic hydrolysis was subjected to alkaline hydrolysis and proved to be an excellent source of phenolic compounds, particularly vanillin. In summary, the results presented here provide valuable insights into the processing of agave sisalana biomass and may pave the way for new technologies for the valorization of plants from semiarid and arid regions.

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Data Availability Data and materials are available on request.

Declarations

Conflict of interest The authors declare there are no conflict of interest.

Ethical Approval This article does not include any studies involving humans or animals conducted by the authors.

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