ABSTRACT: In this work, a combination of mechanical and chemical pretreatments using urea on corn residues (leaves and stems) was evaluated to obtain total reducing sugars (TRSs). The residues were characterized via high-performance liquid chromatography (HPLC) to quantify biomass composition. During the mechanical pretreatment, the particle size of the biomass was reduced to 0.5, 1, and 2 mm. The chemical pretreatment was performed with urea solution at different concentrations (2, 5, and 10% w/v) and a fixed biomass-to-solvent ratio of 1:25 (g/mL) as well as stirring at 150 rpm for 20 h. The effect of temperature on the pretreatment results was evaluated by varying such operating variables in 30 and 50 °C. After both pretreatments, hydrolysis was carried out in an autoclave using sulfuric acid at 1% v/v at 121 °C for 1 h. The content of TRS was quantified using 3,5-dinitrosalicylic acid (DNS) method and biomass after pretreatment was characterized via Fourier transform infrared (FT-IR). For both leaves and stems, the HPLC technique reported the presence of 47.4 g of cellulose, 40.04 g of hemicellulose, and 26.38 g of lignin. It was found that the highest amount of TRS (36.50 g/L) was obtained with a urea concentration of 2% at 50 °C using a particle size of 0.5 mm. The production of TRS was significantly higher for pretreated biomass than that for raw corn residues, confirming the importance of both mechanical and chemical pretreatments to reach better delignification results.

1. INTRODUCTION
In recent years, the search for biofuels as an alternative energy source has motivated the use of agricultural products due to their high sugar content; however, this type of feedstock put at risk the food industry, representing the main drawback in their application. In this context, several works have addressed the use of agricultural wastes to face competitiveness between food and energy industries. The large availability and low cost are the main advantages of using these residues to produce alternative energy sources. In the Department of Bolivar (Colombia), approximately 300–400 tons of corn is produced throughout the year, generating a huge amount of waste that represents 54% of the total corn crops (0.18 ton of leaves and stems/ton of corn crops). The corn residues are normally discharged into the environment causing environmental problems such as accumulation, spread of pests, and indiscriminate burning of garbage.

The use of corn waste for biofuel production via fermentation of total reducing sugars is limited by its complex internal structure that provides resistance to the rupture of linked biopolymers (cellulose, hemicellulose, and lignin). Lignin is responsible for supporting the corn biomass structure and offers a barrier to the degradation of cellulose and hemicellulose into TRS due to its high mechanical resistance. In this sense, mechanical and chemical pretreatments must be performed to break down the structure and allow the extraction of reducing sugars from corn leaves and stems. The use of urea solution during chemical pretreatments has aroused scientific interest owing to the rapid delignification of biomass as well as the high production yield of TRS. Along with urea, different chemical agents have been used to degrade lignin and hemicellulose from lignocellulosic residues such as organic solvents, alkali, and diluted acids. The main advantages and disadvantages of using urea for this purpose compared with others are summarized in Table 1.

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2. EXPERIMENTAL SECTION

2.1. Raw Material and Characterization. The corn residues (leaves and stems) were collected from a farmer in Maria La Baja, a municipality located in North-Colombia. Separately, these lignocellulosic materials were washed with distilled water to remove any impurity that may affect further treatments. A sample of each corn residue was collected for chemical compositional analysis after sun drying until constant weight and size reduction were achieved. For compositional measurement, high-performance liquid chromatography (HPLC) was selected according to the NREL/TP-510-42618 procedure, which provides the content of hemicellulose, cellulose, and lignin that are present in the samples. The novelty of this work lies in the experimental optimization of operating conditions that affect TRS production when using biomasses with high lignin content such as corn leaves and stems.

2.2. Pretreatments. The dried corn residues (leaves and stems) were combined, and the resulting mixture refers to the corn biomass and urea. The temperature and stirring rate were adjusted to 9–11 °C at 150 rpm, respectively. Once the reaction time ended, the liquid phase was collected by filtering and the pH adjusted to 9–11 by adding the NaOH solution. The production of TRS was quantified with 3,5-dinitrosalicylic acid (DNS), a colorimetric-based method that reduced the 3,5-dinitrosalicylic acid (yellow) by the action of glucose or another reducing sugar on 3-amino-nitrosalilic acid (brick red). The UV–vis spectroscopy is used to measure the absorbance of this substance at 540 nm before calibrating with a stock solution of glucose (0.4 g/L).

2.3. Acid Hydrolysis. The solid phase collected after filtering in the pretreatment section was washed thoroughly to reach a high yield of total reducing sugars, which was focused on determining optimal conditions for a combined mechanical and chemical pretreatment with urea of corn wastes by evaluating the influence of process variables such as urea concentration, temperature, and particle size. The advantage of this work lies in the experimental optimization of operating conditions that affect TRS production when using biomasses with high lignin content such as corn leaves and stems.

3. RESULTS AND DISCUSSION

3.1. Biomass Characterization. The raw corn biomass composed of leaves and stems was characterized by the HPLC technique to determine the contents of cellulose, hemicellulose, lignin, and ashes. Table 2 summarizes the composition of this lignocellulosic material, in which cellulose mostly contributed to stem composition with 28.3%, followed by hemicellulose with 20.9%. For leaves, similar results were observed for both cellulose and hemicellulose. Compared with other works, it was observed that the contents of these compounds are similar to those found in the literature.14 First, 10 g of treated biomass was placed in an Erlenmeyer flask and urea solution was added until the desired biomass-to-solvent ratio of 1:25 g/mL was achieved. Then, the flask was placed in a shaker (Labcondo Corp.) for the degradation of lignin during 20 h of continuous mixing of biomass and urea. The temperature and stirring rate were fixed at 50 °C and 150 rpm, respectively. Once the reaction time ended, the liquid phase was collected by filtering and the pH adjusted to 9–11 by adding the NaOH solution. The production of TRS was quantified with 3,5-dinitrosalicylic acid (DNS), a colorimetric-based method that reduced the 3,5-dinitrosalicylic acid (yellow) by the action of glucose or another reducing sugar on 3-amino-nitrosalilic acid (brick red). The UV–vis spectroscopy is used to measure the absorbance of this substance at 540 nm before calibrating with a stock solution of glucose (0.4 g/L).

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characteristics of soil, use of fertilizers, and cultivation techniques.

3.2. Mechanical and Chemical Pretreatments. To evaluate the effects of both mechanical and chemical pretreatments on the TRS production from corn biomass, it was important to determine the amount of TRS that can be obtained without any pretreatment. In this context, acid hydrolysis was conducted in control samples, i.e., biomass subjected to mechanical pretreatment only (0.5, 1, and 2 mm) and raw corn wastes (without physical transformation). The total reducing sugars produced after hydrolysis are summarized in Table 3 for all control samples. The TRS amount was higher for mechanical pretreated biomass than that for raw material, confirming improvements in converting cellulose and hemicellulose via acid hydrolysis. For different particle size reductions, it was observed that 2 mm sized particles achieved the highest TRS production (21.4 ± 0.9 g/L), followed by 1 mm (20.6 ± 0.7 g/L) and 0.5 mm (20.1 ± 0.8 g/L). The particle size of lignocellulosic biomass has proved to alter sugar yields based on experimental results reported in the literature. Similar results were obtained by authors in previous work using empty fruit bunches and kernel shell biomass with mechanical and chemical pretreatments.17 The production of TRS was the highest when the particle size of biomass was reduced to 0.5 mm, enhancing the conversion of lignocellulosic material into reducing sugars. Zhang et al.20 conducted a literature review related to the relationship between biomass particle size and hydrolysis sugar yield, indicating that most contributions present negative effects of small-sized particles while other works achieved positive or neutral effects.

The results of total reducing sugars quantification for corn biomass subjected to chemical pretreatment with urea at a temperature of 50 °C are shown in Table 4. The biomass sample pretreated with 2% w/v of urea solution was identified as S2%, while the samples S5 and S10% refer to urea solutions of 5 and 10% w/v, respectively. The total reducing sugars were calculated as the sum of reducing sugars quantified after pretreatment and acid hydrolysis. After the delignification of corn leaves and stems using chemical pretreatment, it was observed that reducing sugar achieved the highest value (6.9 g/L) at the particle size of 1 mm and urea concentration of 10%. The measurement of reducing sugars after acid hydrolysis indicated that a lower particle size (0.5 mm) and urea concentration (2%) are optimum in obtaining the highest TRS.

The improvements derived from biomass pretreatment are observed when comparing raw biomass and sample S2% because the amount of total reducing sugars present in the pretreated samples is greater than that obtained in the samples without chemical pretreatment. This increase in the amount of TRS is due to the degradation of the polymeric structure of the lignocellulosic material by breaking down the cellulose–hemicellulose–lignin links using urea solution. Such an agent avoids the association of cellulose molecules once the bond has disintegrated, thus facilitating delignification.21 The decrease in TRS quantification when using a high concentration of urea is directly related to the chemical degradation of xylose and glucose that occurs in the delignification and the generation of inhibitory side compounds during the partial hydrolysis of hemicellulose such as derivates of furan (furfural and hydroxymethyl-furfural), whose degradation produces aliphatic acids of low molecular weight (another type of inhibitors).22,23

The effect of temperature on the total reducing sugars production was assessed by varying temperature to 30 °C, and the results are listed in Table 5. The samples S10% were not included in this analysis because of the poor TRS production achieved at higher temperatures. A higher quantification of TRS was observed at a temperature of 50 °C compared with that at 30 °C, suggesting that higher temperature contributes to achieving better hydrolysis performance. At a temperature of 30 °C, the optimum conditions of urea concentration and particle size to achieve TRS in 30.8 g/L were 2% w/v and 2 mm, respectively. However, no significant difference was observed in TRS (30 g/L) for 2% w/v and 0.5 mm. Yoo et al.24 suggest that this phenomenon may occur because the thermal effect contributes to breaking down the internal structures of the lignocellulosic material, enabling the delignification. Besides, higher sugar production with less concentrated urea solutions owing to less thermal stress is required to drive urea molecules and initiate degradation. Similar trend was reported by Pessoa et al.25 for temperature effects on the acid hydrolysis of sugarcane bagasse. They evaluated three different temperatures (140, 150, and 160 °C).

### Table 3. Effect of Size Reduction on the Production of TRS from Corn Biomass

| particle size (mm) | TRS (g/L) |
|-------------------|-----------|
| 0.5               | 20.1 ± 0.8|
| 1                 | 20.6 ± 0.7|
| 2                 | 21.4 ± 0.9|
| Raw               | 18.3 ± 0.4|

### Table 4. Total Reducing Sugar Production from Pretreated and Hydrolyzed Biomass at 50 °C

| particle size (mm) | reducing sugars after pretreatment (g/L) | reducing sugars after hydrolysis (g/L) | total reducing sugars (g/L) |
|-------------------|-----------------------------------------|--------------------------------------|-----------------------------|
|                   | S2%          | S5%          | S10%         | S2%          | S5%          | S10%          | S2%          | S5%          | S10%          |
| 0.5               | 3.4          | 4.2          | 6.4          | 33.1         | 26.5         | 20.7          | 36.5         | 30.8         | 27.2          |
| 1                 | 3.4          | 4.1          | 6.9          | 30.8         | 23.6         | 22.2          | 34.2         | 27.79        | 29.2          |
| 2                 | 3.2          | 3.1          | 5           | 29.4         | 25.4         | 21.3          | 32.6         | 28.6         | 26.4          |
| raw               | 2.7          | 3.2          | 5.1          | 29.4         | 25.3         | 15.2          | 32.2         | 28.5         | 20.3          |
| standard deviation| 0.3          | 0.6          | 0.9          | 1.7          | 1.2          | 3.2           | 1.9          | 1.3          | 3.8           |

### Table 5. Total Reducing Sugar Production from Pretreated and Hydrolyzed Biomass at 30 °C

| particle size (mm) | reducing sugars after pretreatment (g/L) | reducing sugars after hydrolysis (g/L) | total reducing sugars (g/L) |
|-------------------|-----------------------------------------|--------------------------------------|-----------------------------|
| 0.5               | 2.9          | 2.4          | 27          | 30          | 27.5          |
| 1                 | 3.3          | 2.8          | 26.2         | 23.5         | 29.5         | 26.4          |
| 2                 | 2.8          | 2.2          | 27.9         | 26.2         | 30.8         | 28.5          |
| raw               | 2.5          | 2.1          | 26.9         | 19.5         | 29.4         | 21.7          |
| standard deviation| 0.3          | 0.3          | 0.7          | 2.9          | 0.6          | 3.0           |
and found that the highest TRS recovery occurred at 150 °C, while lower temperatures cause incomplete hydrolysis of the material and higher temperature degrades the sugars. The standard deviation calculated for all experiments suggested a higher dispersion of results for a temperature of 50 °C than 30 °C. Satarn et al.26 evaluated the influence of acid concentration on the reducing sugar production from corn stover and found a sharp increase between 0 and 1% v/v of sulfuric acid. They obtained 18.5 g/L of TRS at the acid concentration of 1% v/v, value that is similar to the 19.5 g/L (see Table 5) reported in this work for corn biomass without physical pretreatment.

Based on the TRS quantification with varying temperature, particle size, and urea concentration, the identified optimum conditions for corn biomass treatment are particle size of 0.5 mm, urea solution of 2%w/v, and temperature of 50 °C. These results were compared with those found in the literature as shown in Table 6. Wang et al.8 assessed the effect of solid loading (30–70%), temperature (60–80 °C), and the ratio of soybean flour to corn stover (1:40–1:10) on the enzymatic saccharification of corn stover via high-solid pretreatment and reported the optimum treatment conditions of 80 °C, 97% urea concentration, and biomass-to-urea ratio of 1:1 (g/mL). Ojeda et al.7 evaluated the pretreatment of corn stover fractions such as stems and leaves to increase the TRS production. They obtained maximum values of TRS for stems exhibited the presence of 47.4 g of cellulose, 40.04 g of hemicellulose, and 26.38 g of lignin in the characterization via the DNS method. The corn biomass comprising leaves and stems (corn stover) and a lower concentration of urea was required to achieve the maximum values of TRS.

### Table 6. Comparison of Operating Conditions for the Biomass Pretreatment in the Present Study with Those in Previous Works

| Conditions | Wang et al. | Ojeda et al. | Julio-Altamiranda et al. | This Work |
|------------|-------------|--------------|--------------------------|-----------|
| Raw material | corn stover | corn leaves and stems | empty fruit bunches and kernel shells | corn leaves and stems |
| Urea concentration (% w/v) | 97 | 5 | 4–6 | 2 |
| Temperature (°C) | 80 | 80 | 50 | 80 |
| Biomass/urea solution ratio (g/mL) | 1:1 | 1:25 | 1:25 | 1:25 |

This work attempted to fill the knowledge gap related to the optimum conditions for corn waste delignification under chemical and mechanical pretreatments, which have proved to enhance the reducing sugar production. To identify these conditions, different urea solutions (2, 5, 10% w/v), temperatures (30, 50 °C), and particle sizes (0.5, 1, and 2 mm) were evaluated, and the total reducing sugars were measured using the DNS method. The corn biomass comprising leaves and stems exhibited the presence of 47.4 g of cellulose, 40.04 g of hemicellulose, and 26.38 g of lignin in the characterization via HPLC. Such content of lignin was treated via chemical pretreatment using a urea solution to break down the cross-links for a further hydrolysis reaction. The highest amount of TRS (36.50 g/L) was obtained with a urea concentration of 2% at 50 °C using a particle size of 0.5 mm, and they were selected as optimum conditions. Under these conditions, the measurement of reducing sugars after mechanical and chemical

![Figure 1. FT-IR spectra of the S2% sample (2% w/v urea solution, 0.5 mm particle size, and 50 °C).](https://dx.doi.org/10.1021/acsomega.0c00645)

![Figure 2. FT-IR spectra of the S10% sample (10% w/v urea solution, 2 mm particle size, and 50 °C).](https://dx.doi.org/10.1021/acsomega.0c00645)

CH₃ and C–O–C stretching vibrations, which confirm the presence of cellulose. The functional groups attributed to hemicellulose were identified at 1163, 1248, 1420, and 1508 cm⁻¹, corresponding to the C=O stretching vibrations and the C–O stretching of carbonyl groups and xylene, respectively. The lignin content was confirmed by the presence of the peak at 1456 cm⁻¹ attributed to the C–H deformation of lignin, CH₂ symmetric angular deformation, and CH₃ deformation. The sample S10% showed similar absorbance peaks assigned to cellulose and hemicellulose; however, more peaks characteristic of lignin (1458, 1516, 1604, and 2916 cm⁻¹ of C–H deformation, aromatic skeletal vibrations C=C and O–H stretching) confirm the low performance of the delignification process.7 Besides, peak at 3410 cm⁻¹ confirms the presence of inhibitory compounds.

### 4. CONCLUSIONS

This work attempted to fill the knowledge gap related to the optimum conditions for corn waste delignification under chemical and mechanical pretreatments, which have proved to enhance the reducing sugar production. To identify these conditions, different urea solutions (2, 5, 10% w/v), temperatures (30, 50 °C), and particle sizes (0.5, 1, and 2 mm) were evaluated, and the total reducing sugars were measured using the DNS method. The corn biomass comprising leaves and stems exhibited the presence of 47.4 g of cellulose, 40.04 g of hemicellulose, and 26.38 g of lignin in the characterization via HPLC. Such content of lignin was treated via chemical pretreatment using a urea solution to break down the cross-links for a further hydrolysis reaction. The highest amount of TRS (36.50 g/L) was obtained with a urea concentration of 2% at 50 °C using a particle size of 0.5 mm, and they were selected as optimum conditions. Under these conditions, the measurement of reducing sugars after mechanical and chemical
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