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RESEARCH ARTICLE

Sequential extraction of carbohydrates and lignin from agricultural waste and their structural characterization

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ABSTRACT

This work reports on the extraction and characterization of carbohydrates such as cellulose, cellulose nanofibres, hemicellulose, and lignin from agricultural waste, i.e. maize stalks and sugarcane bagasse. The chemical compounds were extracted by hot water extraction (HWE) followed by alkaline extraction with 10 wt.% of sodium hydroxide (NaOH). Cellulose nanofibres (CNF) were isolated by mechanical grinding the cellulose fraction using a supermass colloider. The characteristics of the different fractionated components were investigated using nuclear magnetic resonance (NMR) spectroscopy, elemental analysis, thermogravimetric analysis (TGA), Fourier transform infrared (FTIR) spectroscopy and X-Ray diffraction (XRD). The removal of the hemicellulose, lignin and the extractives were confirmed quantitatively by TAPPI standard methods. It was observed that the cellulose content and its crystallinity increased after treating maize stalks and sugarcane bagasse with hot water extraction and alkali treatment. The thermal stability of cellulose also improved after treatments. Transmission Electron Microscopy (TEM) results confirmed that web-shaped CNF were successfully extracted from the cellulose obtained from sugarcane bagasse and maize stalks. The dimensions of the CNF ranged from 5 to 30 nm in width and a few microns in length. In future studies, the fractionated hemicellulose, lignin and (micro and nano) cellulose will be used as reinforcements in the development of biocomposite materials.

1. Introduction

The extraction of high value chemicals from agricultural residues has attracted tremendous interest because agricultural residues are abundantly available and is a non-food renewable resource. The utilization of agricultural residues for the value-added applications has shown potential

in addressing socio-economic problems and climate changes (Chen *et al.*, 2015). Agricultural residues are mainly composed of cellulose, hemicellulose, and lignin as main components. Other components include waxes, pectins and oils which are present in small quantities (P. Zhang *et al.*, 2015). The complex cross-linked structure of agricultural residues is attributed to bonds

between the components which prevent their separation, depolymerisation and conversion into valuable products (Chen *et al.*, 2015; P. Zhang *et al.*, 2015).

The chemical components of agricultural residues such as cellulose, hemicellulose and lignin are usually extracted from flax, kenaf, coconut, sisal, etc (Sheltami *et al.*, 2012). From these, despite their abundance, sugarcane bagasse and maize stalks have received minimum attention for the extraction of these components(Motaung & Anandjiwala, 2015). Maize stalks and sugarcane bagasse are by-products of maize and sugarcane processing and are abundantly available. Both maize and sugar are among the largest produced agricultural products in South Africa and are planted in various provinces, such as Mpumalanga, KwaZulu Natal, Eastern Cape and Free State. The agricultural sector produces maize and sugar for economic generation and minimum attention is paid to the beneficiation of the maize stalks residues and sugarcane bagasse. However, after harvesting, both maize stalks remain on the field as a ′trash blanket′. Currently, these agricultural residues do not a have direct application; hence, they are underutilized for low value products such as animal feeding, energy generation, fertilizer and/or disposed of in dumping site or incinerated. This is due to the lack of knowledge and understanding about the value addition of these materials (Motaung & Mtibe, 2015; Mtibe, Linganiso, *et al.*, 2015). Like any other agricultural residues, maize stalks and sugarcane bagasse contain high-value resources such as cellulose, hemicellulose and lignin, which can be used in various applications such as reinforcements in composites materials. For instance, Thakur *et al.* (Thakur *et al.*, 2013) developed green composites from novel urea-formaldehyde (UF) and pine fibres. The findings revealed that incorporating fibres into UF improved mechanical properties due to good interfacial adhesion between polymer matrix and fibres. However, it was also reported that the properties increased with increasing fibre loading.

Extracting cellulose, hemicellulose and lignin from agricultural residues is a highly energy-intensive process, as the bondsetween these components need to be broken (Mohtar *et al.*, 2017). Various pre-treatments have been documented in the literature for the extraction of these components from agricultural residues (Kumar *et al.*, 2016, 2020; Rana *et al.*, 2019; Singha & Thakur, 2009). Some of the pre-treatments include organosolv, chemical, physical, physico-chemical, enzyme hydrolysis and ionic liquids (Barana *et al.*, 2016; Mohtar *et al.*, 2017; Raj & Shah, 2017; D. Yang *et al.*, 2013; P. Zhang *et al.*, 2015). For instance, Kumar *et al.* (Kumar *et al.*, 2012) extracted high pure cellulose from sugarcane bagasse using chemical treatments and subsequently reinforced functionalized with dimethyl sulfate (DMS). Fourier transform-infra red (FTIR) and X-ray diffraction (XRD) studies confirmed that high-quality cellulose was extracted from sugarcane baggase. Even though these pre-treatments have shown good results in the extraction of individual components of agricultural residues, it is still difficult to utilize a one-step process in an attempt to extract different components of agricultural residues (Li *et al.*, 2016). In recent years, researchers have been working on simultaneous extraction of fractionates using combined pre-treatment methods (Mohtar *et al.*, 2017; P. Zhang *et al.*, 2015).

Various authors have reported multi-step pre-treatment methodto isolate hemicellulose, lignin and cellulose (Barana *et al.*, 2016; Mohtar *et al.*, 2017; D. Yang *et al.*, 2013; P. Zhang *et al.*, 2015). The pre-treatment of agricultural residues using ionic liquids and alkali treatment has been a research hotspot (Mohtar *et al.*, 2017; D. Yang *et al.*, 2013). Other authors have reported the extraction of various agricultural residue components using acid and alkali treatments (Barana *et al.*, 2016). The use of ionic liquids was preferred due to their advantages, such as being a green technology, recyclable, eco-friendly and non-flammable. The significant drawbacks of ionic liquids are their high cost, and the research is still in the infancy stage. However, alkali pre-treatment is commonly employed to efficiently solubilize hemicellulose (Kargarzadeh *et al.*, 2012; Mokhena & Luyt, 2014, 2017; Mtibe, Mokhothu, John, *et al.*, 2018). It is worth noting that besides solubilising hemicellulose, it also solubilizes lignin and other extractives (Barana *et al.*, 2016; Flauzino Neto *et al.*, 2013; Silvério *et al.*, 2013).

As mentioned, multi-step treatments of agricultural residues to isolate individual components are commonly performed by ionic liquid treatment

and alkali treatment (Mohtar *et al.*, 2017; D. Yang *et al.*, 2013). However, as a continuation to previous studies reported by Mohtar *et al.* (Mohtar *et al.*, 2017) and Yang *et al.* (D. Yang *et al.*, 2013), the present study investigates the simultaneous extraction of hemicellulose, lignin, cellulose and cellulose nanofibres from sugarcane bagasse and maize stalks using hot water and alkali treatment following by mechanical grinding. In this case, hot water was used as a cheaper and environmentally viable way to recover hemicellulose, as compared to the conventional methods, such as ionic liquid and harsh chemicals (e.g., alkali treatment using NaOH or KOH and diluted acids). Ionic liquids are expensive, limiting their application for industrial scale-up, whereas harsh chemicals generate huge amounts of toxins since they are performed in replicates with numerous washing steps. The extracted constituents were characterised using a range of techniques such as Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), nuclear magnetic resonance (NMR) spectroscopy, elemental analysis and X-Ray diffraction (XRD). In addition, the chemical compositions of the agricultural residues prior and post-treatment were analysed using TAPPI standard methods. Moreover, nano-scale cellulose was further characterized by transmission electron microscopy (TEM).

2. Methods and materials

2.1. Materials

The post-harvested maize stalks (MS) were supplied by a farm in Cofimvaba in Eastern Cape, South Africa. Sugarcane bagasse (SB) was provided by Illovo in Durban, South Africa. Sodium hydroxide (NaOH) pellets, ethanol, hydrochloric acid (HCl) and sulphuric acid (H_2SO_4) of 99.9 %, 99.9%, 37%, and 98% purity, respectively were obtained from Minema Chemicals, South Africa. Sodium chlorite ($NaClO_2$), and dimethyl sulfoxide (DMSO) of 80% was purchased from Sigma Aldrich, South Africa.

2.2. Chemical compositions

The lignin and cellulose contents of the maize stalks and sugarcane bagasse were analysed according to the TAPPI standard methods T222 om-88 and T19 m-54, respectively. The lignin content of the samples was analysed by reaction with sulphuric acid (72%). On the other hand, the cellulose content of the samples was analysed by the reaction with the mixture of 6.0 mL acetyl acetone, 2 mL 1.4 dioxan, and 1.5 mL HCl (37%). The hemicellulose content was determined according to the method reported by Abdel-Halim (Abdel-Halim, 2014).

2.2.1 Fractionation of maize stalks and sugarcane bagasse

Maize stalks and sugarcane bagasse were washed with tap water and air dried overnight. They were then subjected to hot water treatment at a temperature of 170 ºC in a 7 litre electrically heated rotating digester for 30 minutes. This resulting solution was filtered, and the hemicellulose filtrate was collected and stored in the cold room at 4 ºC prior to hemicellulose extraction.

The solid cake obtained after the filtration process was subjected to further treatment with 10 wt.% NaOH at 170 ºC in the same digester for 30 minutes. At the end of the treatment, the resulting black liquor was collected and stored in the cold room at 4 ºC until required.

2.2.2. Extraction of hemicellulose

The extracted liquid after hot water treatment was treated with a mixture of ethanol and water (3:1) for 24 h to precipitate hemicellulose as reported by Zhang *et al.* (P. Zhang *et al.*, 2015) and Yang *et al.* (D. Yang *et al.*, 2013). After 24 h, the white precipitate of hemicellulose was separated and further purified by washing with ethanol and acetone. After that, the residue was dried in a vacuum oven at 40 ºC for 24 h.

2.2.3. Extraction of lignin

Lignin was isolated according to the method reported by Yang *et al.* (D. Yang *et al.*, 2013). The lignin dissolved in black liquor was precipitated with 6M HCl followed by washing with acidified water at a pH of 2.0 in a Büchner funnel and dried in a vacuum oven at 40 °C for 24 h.

2.2.4. Extraction of cellulose and nanofibres

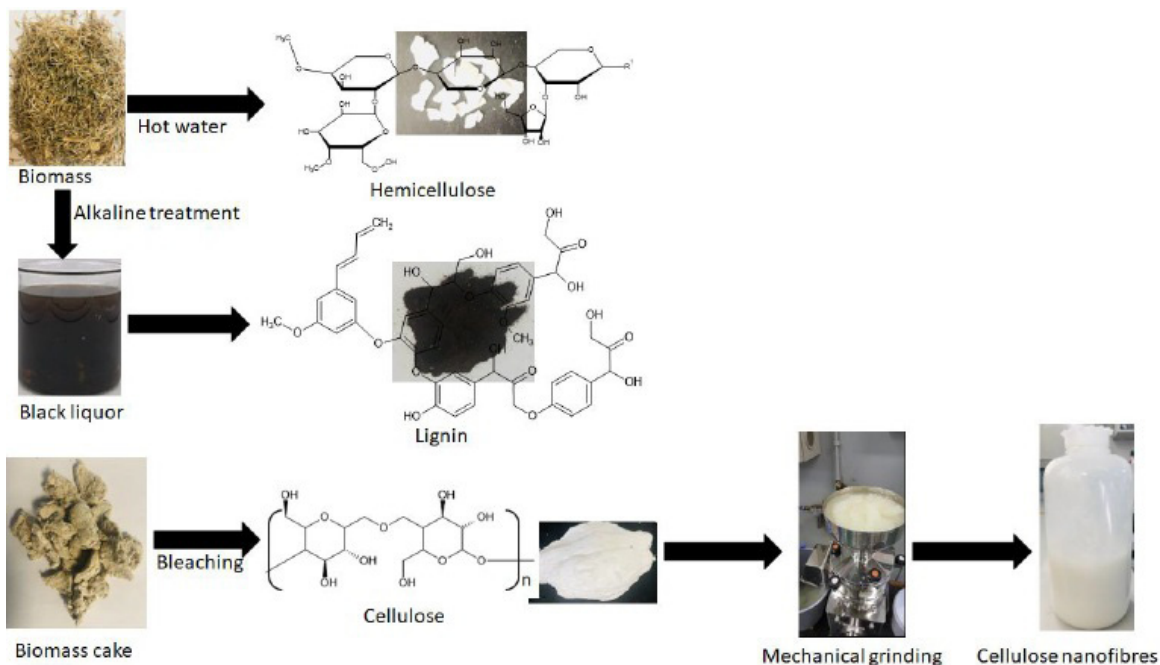Cellulose was extracted from solid cake obtained after alkaline treatment. The solid cake was treated

Figure 1. A schematic diagram for the extraction of cellulose, hemicellulose, lignin and nanocellulose.

with 1.5 wt.% $NaClO_2$ at 80 °C for 1 h. After the treatment, the material was washed with water to remove the excess chemicals and achieve a neutral pH. In the case of cellulose nanofibers extraction, 2% of the cellulose pulp was agitated in a blender for 15 minutes. The dispersed material was then ground using an MKCA6-3 supermass colloider (Masuko Sangyo Co, Ltd., Japan)] at a speed of 1500 rpm for 20 min.

A schematic of the extraction processes applied to these agricultural residue components is shown in Fig. 1.

2.3. Characterization

2.3.1. Elemental analysis

Elemental analysis was performed to determine untreated materials' total carbon, hydrogen, nitrogen, oxygen and sulphur content and extracted individual components. The analysis was carried out using an Elemental Vario Analyser (Elementar, Germany)

2.3.2. Nuclear Magnetic resonance (NMR)

The solution state ^{1}H was obtained using a Bruker Ultrashield 400 Plus spectrometer using 50 mg

of sample dissolved in 1.0 mL of C2D6OS. Tetramethylsilane (TMS) was used as a standard to calibrate the shifts of the peaks.

2.3.3. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the untreated sugarcane bagasse, untreated maize stalks, cellulose, hemicellulose and lignin were obtained using an ATR 4000 spectrophotometer (Perkin-Elmer, USA). All samples were scanned over a range of 4000–600 cm^{-1} with an average of 4 scans with a resolution of 4 cm^{-1}.

2.3.4. X-Ray diffraction (XRD)

The XRD profiles of the agricultural residues were measured using BRUKER AXS X-ray Diffractometer D8 Advance (Bruker, Germany) equipped with PSD (position sensitive detector), Vantec-1 detector and Cu-Kα radiation (λKα$_1$ = 1.5406 Å). Scattering radiation was detected in a 2θ = 5–90º rate of 1s per step.

2.3.5. Thermal gravimetric analysis (TGA)

Thermogravimetric analysis was carried out on a thermogravimetric analyser (PerkinElmer,

Buckinghamshire, UK) at a heating rate of 10 °C/min using nitrogen as a purge gas. The TGA programme was conditioned to increase the temperature linearly from room temperature to 600 °C under nitrogen flow. The temperature of the sample was monitored and the sample's weight loss was expressed in terms of percentage weight loss.

2.3.6. Transmission Electron Microscopy (TEM)

The dimensions of the cellulose nanofibres were determined using transmission electron microscopy (TEM). Cellulose nanofibres were diluted with water to 0.01% (w/v). The suspension was deposited on a carbon grid, stained with 2 wt% uranyl acetate and allowed to dry at ambient temperature. The grids were observed with a Philips CM 200 microscope operated at an accelerating voltage of 80 kV.

3. Results and discussions

3.1. Chemical compositions

3.1.1. Determination of cellulose, lignin, hemicellulose, and extractives

It was observed that the chemical compositions of both the maize stalks and sugarcane bagasse were similar. The cellulose, hemicellulose and lignin contents were on average 40%, 21% and 23%, respectively. After treatments, the cellulose content of both maize stalks and sugarcane bagasse were 71.4 % and 60.3 %, respectively. This indicate that the treatments were effective in removing both lignin and hemicellulose. On the contrary, hemicellulose and lignin contents were drastically decreased after the treatments. The cellulosic materials such as sugarcane bagasse and maize stalks consist of semi-crystalline

cellulose, amorphous hemicellulose, and lignin. However, amorphous materials are easily dissolved in chemicals such as NaOH whereas, crystalline materials are difficult to dissolve in most chemicals (Abraham *et al.*, 2011, 2013; Deepa *et al.*, 2011). It was noticed that when untreated sugarcane bagasse and maize stalks were subjected to hot water extraction and alkali treatment, hemicellulose was partially hydrolysed, and lignin depolymerised into phenolics and sugars. The solubility of hemicellulose and lignin was attributed to the cleavage of α-ether bonds between lignin and hemicellulose during the treatments (Mtibe, Linganiso, *et al.*, 2015). It was also clearly observed from Table 1 that these treatments did not wholly remove hemicellulose and lignin. Similar findings were reported by Abraham *et al.* (Abraham *et al.*, 2011, 2013). Their studies reported that alkali treatment removed a certain amount of non-cellulosic constituents such as lignin, hemicellulose, and extractives, leading to swelling of the crystalline domains and alkalisation of the peripheral hydroxyl groups and therefore resulting in exposure of short length crystallites. The authors also reported that alkali treatment assists in surface modification.

3.1.2. Ultimate analysis

Table 2 shows the results of the ultimate analysis of untreated maize stalks, untreated sugarcane bagasse, cellulose, lignin, and hemicellulose extracted from maize stalks and sugarcane bagasse. The analysis of organic materials such as biomass relies on the chemical formula and their constituents. As mentioned earlier, agricultural residues consist of cellulose, hemicellulose, and lignin as major constituents. Like any other organic material, the materials studied are composed of carbon, hydrogen, nitrogen, and oxygen. All the materials studied showed the highest value of carbon and oxygen. Lignin extracted from both maize stalks and sugarcane

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)
Maize stalks (MS)	41.38 ± 1.56	20.46 ± 1.91	22.97 ± 1.18	9.05 ± 0.92
Sugarcane bagasse (SB)	40.13 ± 0.81	22.31 ± 0.34	23.3 ± 0.57	7.03 ± 0.47
MS after treatments	71.4 ± 2.0	5.87 ± 0.69	1.2 ± 0.4	-
SB after treatments	60.3 ± 1.9	6.7 ± 1.51	2.44 ± 0.5	-

Table 1. Chemical composition of untreated and treated maize stalks and sugarcane bagasse.

Sample	C (%)	H (%)	N (%)	O (%)
Maize stalks untreated	44.84	7.35	1.55	46.26
Maize-hemicellulose	38.52	6.76	0.63	54.09
Maize-lignin	55.61	6.09	2.06	36.24
Maize-cellulose	40.74	7.69	0.17	51.4
SB untreated	44.13	6.95	0.24	48.68
SB-hemicellulose	37.71	6.52	0.34	55.43
SB-lignin	54.56	6.36	0.56	38.52
SB-cellulose	40.42	6.66	0.21	52.71

Table 2. Elemental analysis of untreated maize stalks and SB, cellulose, hemicellulose, and lignin extracted from maize stalks and sugarcane bagasse

bagasse showed the highest carbon content (55%) and lowest oxygen content (36-39%) compared to all other materials studied, which could be due to the complex structure of the lignin. This indicates that lignin from agricultural waste can be used as a source of carbon for producing carbon-based fillers. These results were similar to those reported by Zhao *et al.* (Zhao *et al.*, 2011). On the other hand, the opposite was observed in the case of the hemicellulose extracted from both maize stalks and sugarcane bagasse. Hemicellulose presents a carbon content of 38% and oxygen content of 54%. In all materials studied, the values of hydrogen ranged between 6 and 8%. Moreover, in all the materials (except untreated and lignin extracted from maize stalks), nitrogen values were less than 1%. The trace amount of nitrogen can probably be associated with the dry matter used as nourishment.

3.2. NMR analysis

^{1}H NMR spectra of lignin extracted from sugarcane bagasse and maize stalks are shown in Fig. 2 (a) and (b). The signal at 1.5 ppm represents the proton exhibiting aliphatic and aromatic acetates (Uma Maheswari *et al.*, 2020). The peaks in the range of 1.5 to 2.4 ppm denote protons for aliphatic acetates and aromatics. The 3.4 to 4.0 ppm signals are attributed to methoxy groups (Uma Maheswari *et al.*, 2020). The signals in the range from 4 to 5 ppm were assigned to Hγ of β-O-4, and H$_\beta$ (Uma Maheswari *et al.*, 2020). The signal in the range of 5.1 to 6 represented Hα in benzyl aryl ethers and Hα of α-O-4 (X-F Sun, H Wang, G Zhang, P Fowler, 2010). Lastly, the signals in the range of 6 to 8 represented

aromatic proton in the syringyl units, aromatic proton in the guaiacyl units and aromatic proton in p-hydroxyphenyl units (Uma Maheswari *et al.*, 2020). Other studies reported similar result (Lei *et al.*, 2013; Oliveira *et al.*, 2006; X. F. Sun *et al.*, 2011), where lignin was extracted from various sources.

^{1}H NMR of hemicellulose extracted from maize stalks and sugarcane bagasse by hot water extraction (HWE), which was obtained by precipitation in a mixture of ethanol and water, are shown in Fig. 3a) and b), respectively. The signals for ^{1}H NMR of hemicellulose were assigned based on the previously reported literature data (Jin *et al.*, 2009; F. Peng *et al.*, 2009). As seen in Fig. 3(a) and (b), the signal corresponding to the methyl group, was observed at 1.8 ppm. A sharp peak at 2.5 ppm is assigned to the acetyl group attached to hemicellulose. The methyl protons of 4-O-methyl-α-D-glucuronic acid produced a signal at 3.4 ppm (H. Peng *et al.*, 2012). The signals in the region from 3.0 to 4.5 ppm observed in both spectra were assigned to the protons attached to C5, C2, C5 and C4 of the anhydroxylose units of arabinose and xylose (Jin *et al.*, 2009). A weak signal corresponding to unsubstituted internal xylose was clearly observed at 4.4 ppm in both Fig. 3(a) and (b). Double weak signals in the region between 4.8 to 5.4 ppm correspond to anomeric protons of terminal arabinofuranose linked to *C*-3 and *C*-2 of xylans (F. Peng *et al.*, 2009). Similar results were reported for hemicellulose extracted from various sources (F. Peng *et al.*, 2012; H. Peng *et al.*, 2012; J. X. Sun *et al.*, 2004; X. F. Sun *et al.*, 2005, 2011; Yu *et al.*, 2010; X. M. Zhang *et al.*, 2011).
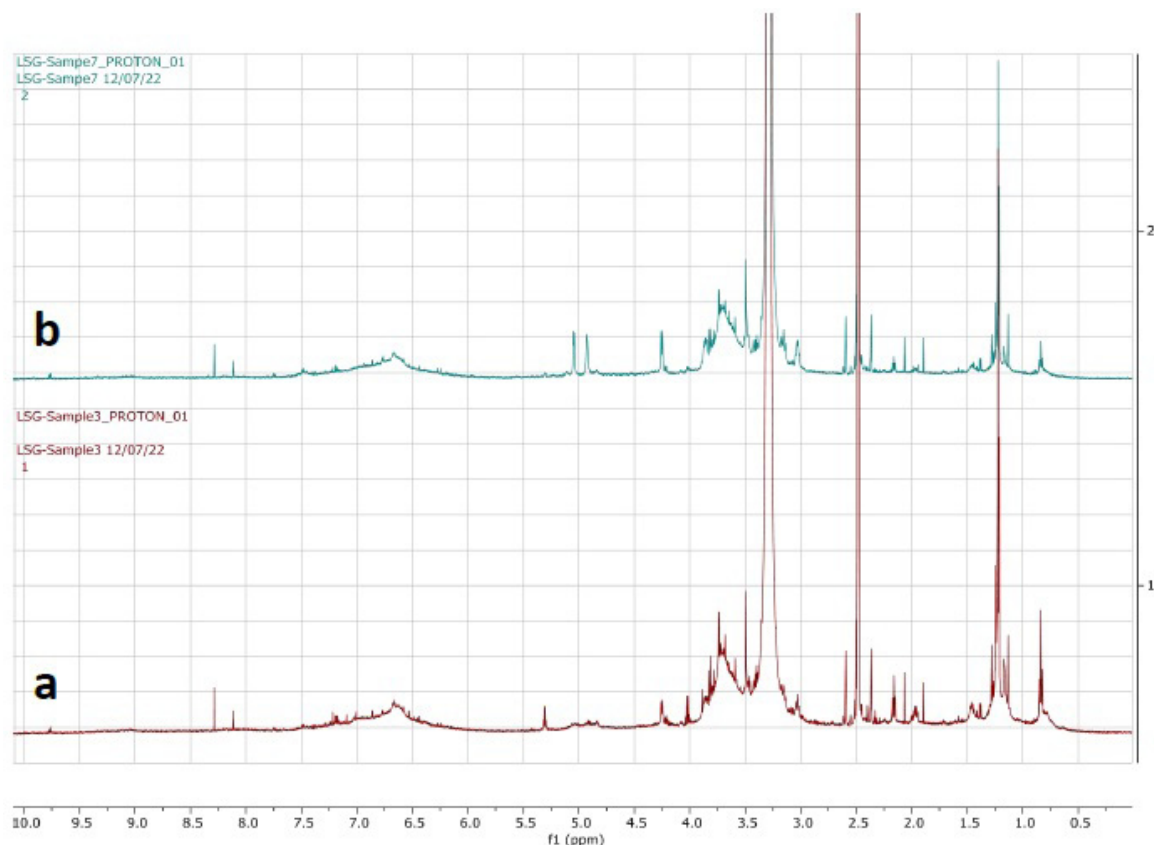
Figure 2. ^{1}H NMR spectra (in DMSO) of the lignin extracted from **(a)** maize stalks and **(b)** sugarcane baggase.

3.3. FTIR analysis

Fig. 4a and 4b show the FTIR spectra of untreated maize stalks and sugarcane bagasse, and the extracted hemicellulose, lignin and cellulose from the maize stalks and sugar cane bagasse. As mentioned previously, agricultural residues are composed of cellulose, lignin, and hemicellulose; therefore, they are composed of alkanes, esters, aromatics, ketones, and alcohols. The broad absorption bands in the region 3318-3311 cm^{-1} were observed in all spectra, and correspond to the OH stretching of lignin, cellulose, and hemicellulose. The strong absorption bands observed at wavenumbers 2899-2892 cm^{-1} correspond to CH stretching vibrations of lignin, cellulose, and hemicellulose. The C=O stretching vibration which represents carboxylic acid groups of ferulic and p-coumaric acids of lignin and hemicelluloses was clearly observed at wavenumbers 1722-1729 cm^{-1} in the spectra of untreated materials, hemicellulose, and lignin.

These observations were similar to those reported by various researchers. The absorption bands at wavenumbers 1643-1646 cm^{-1} were observed in all the spectra and represent water absorption which is attributed to the bending modes of water molecules in the cellulosic fibres. The absorption bands in the region 1021-1036 cm^{-1} in all spectra correspond to C-O-C stretching vibrations. The bands at 899 cm^{-1} corresponded to CH rock vibrations of cellulosic fibres and were observed in all ranges.

It was clearly observed that there were some differences in the spectra of lignin hemicellulose and cellulose. For instance, in the case of lignin, the absorption bands at 1590 cm^{-1}, 1500 cm^{-1} and 1460 cm^{-1} correspond to aromatic lignin structures were observed in both Fig. 4a and 4b. These observations were in agreement with those reported by Moghaddam*et al.* (Moghaddam *et al.*, 2014), Isaac *et al.* (Isaac *et al.*, 2018), Zhao *et al.* (Zhao *et al.*, 2011), Saha *et al.* (Saha *et al.*, 2017),
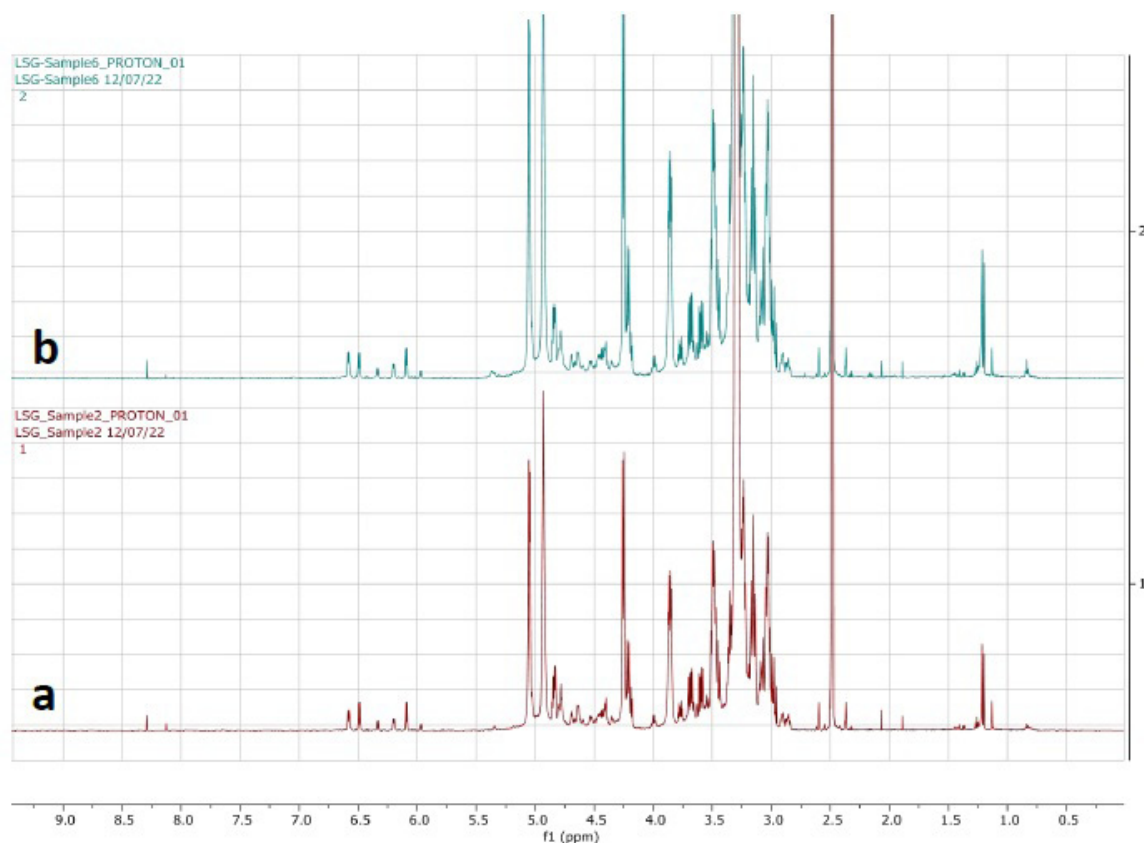
Figure 3. ^{1}H NMR spectra (in DMSO) of the hemicellulose extracted from **(a)** maize and **(b)** sugarcane baggase.
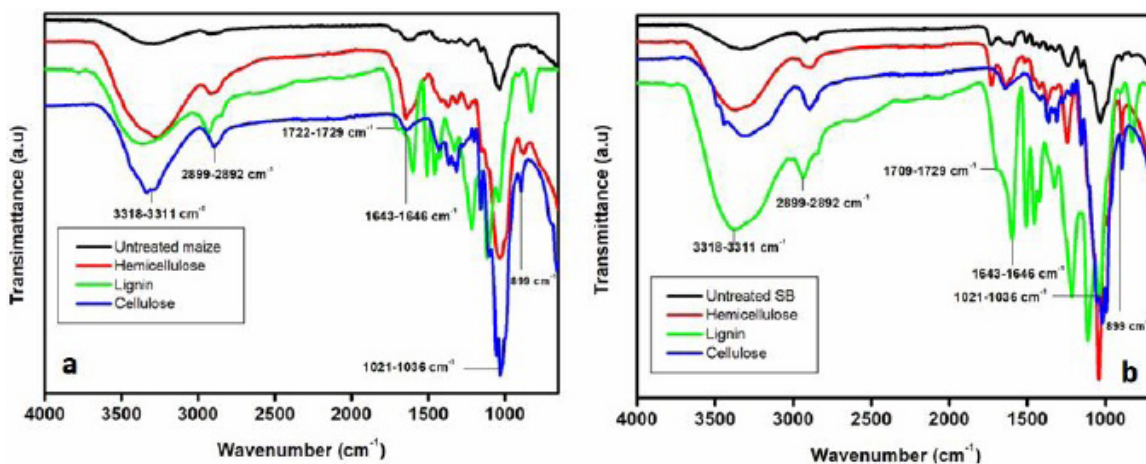

Figure 4. FTIR spectrum of **(a)** untreated maize stalks and **(b)** sugarcane bagasse, together with the hemicellulose, cellulose and lignin extracted from each material.

Maheswari *et al.* (Uma Maheswari *et al.*, 2020), Yang *et al.* (S. Yang *et al.*, 2016). The absorption band at 1410 cm^{-1} is assigned to C-H deformation in lignin (Moghaddam *et al.*, 2014). Other bands at 1320 cm^{-1} and 1260 cm^{-1} represent C-O stretching of syringyl ring breathing and C=O stretching of

guaiacyl ring, respectively (Moghaddam *et al.*, 2014). The absorption band at 1218 cm^{-1} is assigned to ring breathing with C-O stretching of the syringyl and guaiacyl structures (Moghaddam *et al.*, 2014). The bands at 1160 cm^{-1} and 1120 cm^{-1} correspond to ester bonds associated with

p-hydroxypheny propane units and ether-O for syringyl structures, respectively (Moghaddam *et al.*, 2014; Saha *et al.*, 2017). The absorption band at 990 cm^{-1} represent –CH=CH– group out of plane deformation (Saha *et al.*, 2017). Lastly, the band at 830 cm^{-1} correspond to aromatic C–H out-of-plane deformation (Moghaddam *et al.*, 2014).

In the case of hemicellulose, the absorption bands at 1475 cm^{-1}, 1410 cm^{-1}, 1386 cm^{-1} and 1300 cm^{-1} are assigned to CH and C-O or OH bending vibrations in hemicelluloses (F. Peng *et al.*, 2009). The bands between 1200 cm^{-1} and 100 cm^{-1} represent stretching vibrations of side groups (C-OH) and the glycosidic bond vibration (C-O-C). These results were similar to those reported by Peng *et al.* (F. Peng *et al.*, 2009) and Jin *et al.* (Jin *et al.*, 2009). The band at 900 cm^{-1} is assigned to the β-glycosidic linkages between the sugar units.

The functional groups present in cellulose were similar to those of untreated materials. The difference between the two spectra was the intensity of the peaks. In the case of cellulose, the peaks were more visible. However, it was noticed that carbonyl group was not visible in the case of cellulose. This could be due to the complete removal of lignin and hemicellulose from the material. Similar results were reported by numerous researchers (Flauzino Neto *et al.*, 2013; Motaung & Mtibe, 2015; Mtibe, Mandlevu, *et al.*, 2015), indicating that the treatment of biomass with sodium hydroxide removes lignin and hemicellulose.

3.4. X-ray diffraction analysis

The XRD profiles of the untreated maize stalks and untreated sugarcane bagasse and cellulose extracted from both maize stalks and sugarcane baggase are shown in Fig. 5a and 5b.

Fig. 5a and b show XRD data of untreated maize stalks, sugarcane bagasse, and cellulose extracted from those sources. All the materials studied displayed similar patterns with significant peaks at around 16, 22.4 and 34.9º. These peaks are attributed to miller indices of (110), (200) and (004). These results are similar to those reported by French and Ling *et al.* Peaks at 16 and 22.5º are more visible compared to the peak at 34.9º. French and Ling *et al.* reported that the peak at 34.9º corresponds to the composites of several reflections. The reflection peak at 2θ =22.4º became more intense in the case of cellulose. This could be due to the increase in crystallinity of cellulose. Other studies also reported these observation (Motaung & Mtibe, 2015; Mtibe, Linganiso, *et al.*, 2015). The increase in crystallinity was presumably due to the removal of non-cellulosic amorphous lignin and hemicellulose induced by HWE and alkali treatment performed during the purification process. It is worth noting that the peaks of cellulose became more evident in comparison to untreated samples. This suggests that the presence of non-cellulosic amorphous materials such as lignin and hemicellulose negatively affect the crystallinity of the cellulose. A similar trend was seen in the study reported by Mtibe and co-workers (Mtibe, Linganiso, *et al.*, 2015) and Motaung
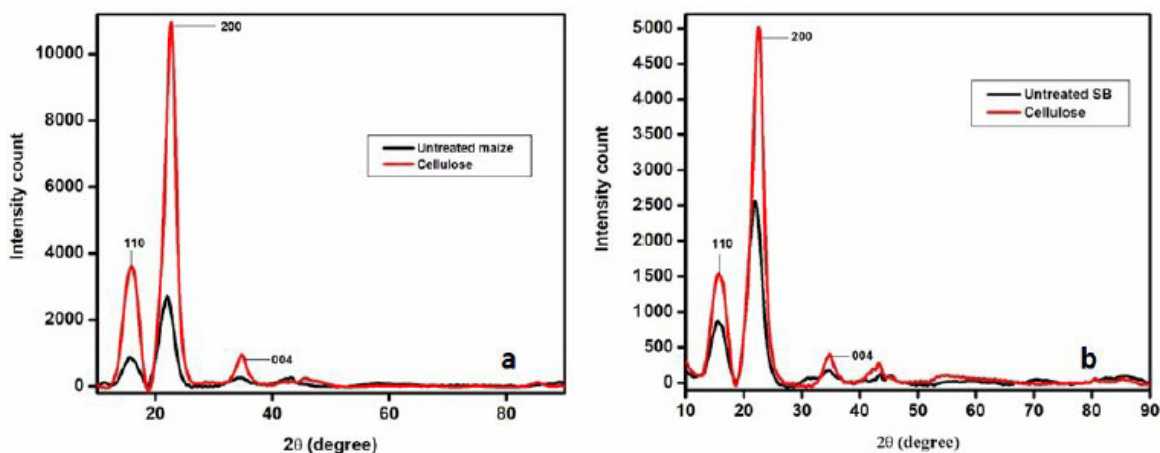

Figure 5. XRD spectrum of **(a)** untreated maize stalks and **(b)** sugarcane bagasse, together with cellulose extracted from each material.

and Anandjiwala (Motaung & Anandjiwala, 2015). Mtibe *et al.* (Mtibe, Linganiso, *et al.*, 2015) extracted cellulose from maize stalks using sodium hydroxide, sodium chlorite and potassium hydroxide, whereas Motaung and Anandjiwala (Motaung & Anandjiwala, 2015) treated sugarcane bagasse with sodium hydroxide and sulfuric acid. Interestingly, it was noticed that cellulose obtained from maize stalks showed higher intensity in comparison to that obtained from sugarcane bagasse. This could be due to the nature of the material, such as chemical compositions.

3.5. Thermogravimetric analysis

The TGA of untreated maize stalks, sugarcane bagasse, and lignin, cellulose and hemicellulose are shown in Fig. 6a and 6b. Due to the chemical structures of cellulose, hemicellulose and lignin, their decomposition occurred at different temperatures. In all cases, a small mass loss in 50-120 ºC was observed, which was attributed to evaporation of moisture and low molecular weight materials. All the materials studied had a moisture content of ≤ 10% except for the hemicellulose extracted from sugarcane bagasse. Similar results were reported by García *et al.* (García *et al.*, 2012). The thermograms of the untreated materials showed a shoulder before the main decomposition peaks at the temperatures 318 ºC and 203 ºC for sugarcane bagasse and maize stalks, respectively. These shoulders represent the presence of acetyl groups of hemicellulose. The shoulders were not visible

in cellulose due to the removal of amorphous lignin and hemicellulose induced by HWE and alkali treatment as confirmed by XRD, FTIR and chemical analysis. Similar observations were reported by Mtibe *et al.* (Mtibe, Linganiso, *et al.*, 2015). The α-cellulose in untreated maize stalks and sugarcane bagasse decomposed at 335 °C and 380 °C, respectively. It was noticed that decomposition of lignin commenced before the main decomposition peak and persisted until 600 ºC. The decomposition temperature of cellulose obtained from both the maize stalks and sugarcane bagasse shifted to higher temperatures due to the removal of thermally less stable non-cellulosic components. At about 430 °C, cellulose decomposed completely, and a char residue of less than 12% remained at 600 °C. Moreover, the decomposition temperature of isolated lignin and hemicellulose shifted to lower temperatures with high char residues after decomposition. This indicates that both lignin and hemicellulose are thermally less stable when compared to cellulose. The char residue in the case of lignin was due to the aromatic nature of the lignin complex (Abraham *et al.*, 2013). Hemicellulose and lignin showed higher char contents in comparison to the untreated materials and the cellulose obtained from both maize stalks and sugarcane bagasse, as seen in Fig. 6. During the heating of all materials in the temperature range between 150 °C to 600 °C, a huge mass loss was observed which was attributed to the release of volatiles such as carbon dioxide and methane from the hemicellulose, lignin and cellulose (García *et al.*, 2013).
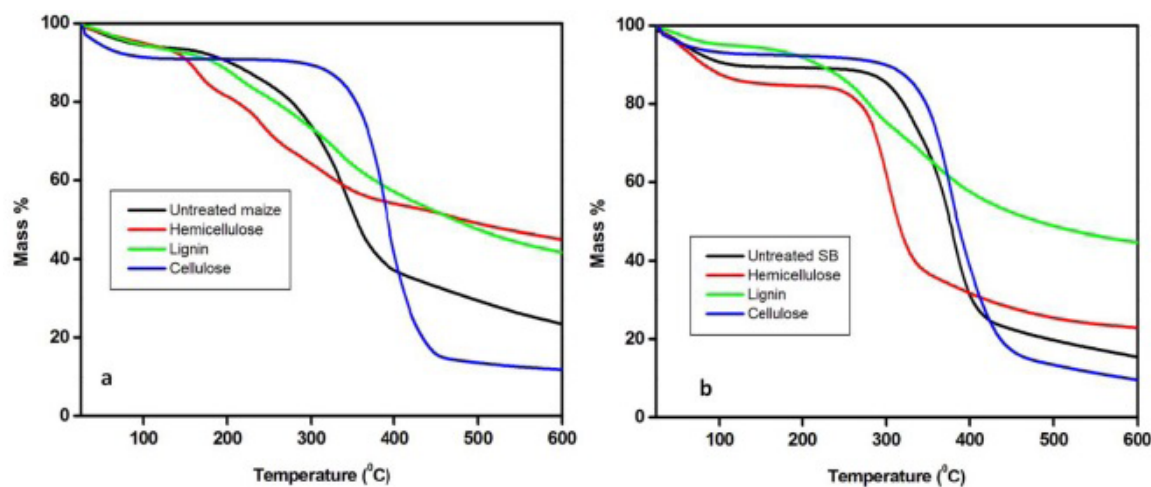

Figure 6. TGA spectrum of **(a)** untreated maize stalks and **(b)** sugarcane bagasse, together with the hemicellulose, cellulose and lignin extracted from each material.

3.6. Nanocellulose

Fig. 7 shows TEM images of cellulose nanofibres obtained from cellulose that was extracted from sugarcane bagasse and maize stalks. The mechanical treatment resulted in the defibrillation of nanofibres from the cell wall (Kumar *et al.*, 2016, 2021; Mtibe *et al.*, 2019; Mtibe, Linganiso, *et al.*, 2015; Mtibe, Mokhothu, & Linganiso, 2018). The isolation of individual nanofibres from bundles of cellulose fibres obtained from sugarcane bagasse and maize stalks, as well as nanofibres aggregates, are seen in Fig.7a and 7b. The agglomeration of nanofibres could be due the drying process during sample preparation for TEM analysis (Jonoobi *et al.*, 2011; Mokhena *et al.*, 2018; Mokhena & Luyt, 2014). TEM results observed that the diameters of the nanofibres extracted from both maize stalks and sugarcane bagasse ranged from 5 to 30 nm, with the lengths reaching a few micrometres. The dimensions of cellulose nanofibres depend on different factors such as source, pre-treatment, and mechanical grinding parameters. In our case, there was no significant difference between the dimensions of maize and bagasse. This could probably be related to the same phyco-chemical treatments used in this study.

4. Conclusions

Lignin, hemicellulose and cellulose were successfully extracted by hot water treatment,

10 wt.% alkali treatment and 1.5 wt.% NaClO$_2$. Cellulose nanofibres were successfully extracted by mechanical grinding using a supermass colloider. TAPPI standards quantitatively confirmed the chemical compositions of cellulose, hemicellulose, lignin and extractives. The extraction of different components of the agricultural residues was confirmed by FTIR and NMR spectroscopy for functional groups and molecular structure determination, respectively. The crystallinity and themal stabilities of the extracted materials were determined by XRD and TGA, respectively. As anticipated, cellulose showed higher crystallinity and thermal stabilities in comparison to hemicellulose and lignin counterparts. TEM observation confirmed the successful extraction of cellulose nanofibres produced from cellulose obtained from maize stalks and sugarcane bagasse with the diameters ranging from 5 to 30 nm and lengths in microns. Therefore, sugarcane bagasse and maize stalks represent an altenative and promising biomass resource for the extraction of hemicellulose, cellulose, lignin and cellulose nanofibres. Further research is required to explore these agricultural waste materials for various applications, such as reinforcing elements in developing green biocomposites.

Acknowledgements

Figure 7.   TEM images of cellulose nanofibres extracted from **(a)** maize stalks and **(b)** sugarcane bagasse.

Compliance with ethical standards

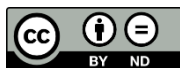**Conflict of interest:** The authors declare no conflict of interest

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