

Isolation and Characterization of Nanocellulose Obtained from Industrial Crop Waste Resources by Using Mild Acid Hydrolysis

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ABSTRACT Cellulose, microcrystalline cellulose and nanocellulose were prepared from three agricultural waste resources: pineapple leaf (PALF), banana rachis (BR), and sugarcane bagasse (SCB). Each waste resource was first converted into microcrystalline cellulose which was subsequently converted into cellulose nanoparticles by using mild (30% w/v) and strong (60% w/v) sulfuric acid concentrations for extraction. Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA) were used to characterize each waste resource and extracted cellulosic materials. Furthermore, nanocelluloses were studied by zeta potential, size analysis, and transmission electron microscopy (TEM). Cellulose nanowhiskers were successfully obtained and isolated with a 33% average yield by applying a mild acid treatment. Substrates BR and SCB proved to be more promising agricultural waste resources in terms of their crystalline cellulosic content and properties.

KEYWORDS Cellulose, pineapple leaf, sugarcane bagasse, banana rachis, whiskers

1 INTRODUCTION

According to the Food and Agriculture Organization (FAO), in the year 2012 nearly 2 million metric tons (MT) of banana, 2.5 million MT of pineapple, and 4 million MT of sugarcane were produced in Costa Rica. However, high crop production creates high amounts of agricultural waste, such as rachis in banana, leaves in pineapple, and bagasse in sugarcane. These agricultural waste resources have relatively high cellulose contents: 32–44% in sugarcane bagasse, 60–65% in banana rachis, and 80–83% in pineapple leaf fibers [1, 2]. Therefore, these agricultural waste resources might show great potential as a source of cellulose, its derivatives, and nanocellulose. Research on nanocellulose (NC) in the form of cellulose nanofibers (CNF), cellulose nanocrystals (CNC) and cellulose nanowhiskers (CNW) from different natural resources has been the subject of several reviews and book chapters published in recent years [3–7]. Several research papers have been published in the last few years on isolation and characterization of nanocellulose from agricultural wastes such as sugarcane bagasse [8–11],

banana pseudostem and rachis [12–14], and pineapple fibers [15–17]. Isolation of the nanocellulose from these lignocellulosic materials requires precise control of process parameters such as the extent of chemical treatment, temperature, hydrolysis time, and liquid/solid ratios. Chemical treatment using sulfuric acid solutions has been the preferred method to extract and isolate nanocellulose, although a peroxide/organosolv procedure has also been reported [13]. Hydrolysis treatment by using concentrated sulfuric acid 60–65% (w/v) at 45 °C, and for 30–45 minutes has been widely used. These conditions have been applied to agricultural residues, inspired by extensive studies on NC isolated from wood cellulose [18, 19]. For example, based on the premise that using a sulfuric acid concentration of 64% (w/v) may result only in limited reductions in the size of banana rachis microfibrils, high acid concentrations have been preferred [12]. On the other hand, it has been determined that using a hydrolysis time greater than 60 minutes negatively affects the extraction of CNW from sugarcane bagasse in the presence of sulfuric acid 60% (w/v), which caused degradation of cellulose [20]. Similar observations have been recently reported for CNW isolated from pineapple leaves using similar acid concentrations [21].

The main purpose of this work was to isolate and characterize NC from sugarcane bagasse, banana

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rachis, and pineapple leaf fibers by applying ultrasonic-assisted mild acid hydrolysis to microcrystalline cellulose (MCC) extracted from the above-mentioned agricultural waste resources. A full comprehensive characterization of each lignocellulosic substrate, their isolated cellulose, MCC and NC was carried out by means of X-ray diffraction (XRD), transmission electron microscopy (TEM), dynamic light scattering (DLS), and thermogravimetric analysis (TGA).

2 MATERIALS AND METHODS

2.1 Materials

Lignocellulosic substrates, including banana rachis (BR), sugarcane bagasse (SCB), and pineapple leaves (PALF), were kindly supplied by the Del Monte Company (Limón, Costa Rica), Inprotsa Farm (Alajuela, Costa Rica), and Cutris Sugarcane Mill (Alajuela, Costa Rica), respectively. Lignocellulosic substrates were freshly collected at each plantation. Reagent grade chemicals, such as sodium hydroxide, chlorhydric acid, sodium chlorite and sulfuric acid, were obtained from Fisher Scientific (PA, USA).

2.2 Isolation of Nanocellulose

Each lignocellulosic substrate was subjected to an alkali and bleaching treatment in order to remove pectin, hemicellulose, and lignin. Each fresh substrate was dried for four weeks inside a solar dryer, milled, and sieved down to 0.85 mm (20 mesh), then treated for 4 hours with sodium hydroxide 2% (w/v) at 80 °C, and then for another 4 hours with sodium chlorite 2.5% (w/v) at room temperature. The obtained intermediate material, hereafter referred to as CELL, was dried and weighed. The CELL intermediates were designated according to the original source as CELL-BR, CELL-SCB, and CELL-PALF. The MCC samples from each CELL intermediate were obtained by refluxing each CELL with hydrochloric acid 2 N at 90 °C and for 15 minutes, and then washed with distilled water to neutralize. Each MCC was dried in an oven at 60–80 °C, weighed, and labeled accordingly (MCC-BR, MCC-SCB, and MCC-PALF). Finally, NC samples were obtained by placing 12 g of each MCC sample (60 mesh) in a reflux in the presence of 300 ml of sulfuric acid 30% (w/v) for 5 hours at 50 °C. Another nanocellulose sample was also obtained by treating a MCC sample with sulfuric acid 60% (w/v) for 30 minutes at 50 °C. At the end of the hydrolysis reaction, each suspension was washed with distilled water and centrifuged four times at 8500 rpm for 15 minutes at 10 °C. Then, dialysis was carried out for four days until the suspension was neutralized, and ultrasonication was

carried out with the help of Misonix 3000 equipment for 10 minutes. Each NC was dried and weighed. Nanocellulose sample obtained with 30% (w/v) sulfuric acid treatment was designated as NC30-BR, NC30-SCB, and NC30-PALF, while nanocellulose obtained with 60% (w/v) sulfuric acid treatment was designated as NC60-BR, NC60-SCB, and NC60-PALF.

2.3. Characterization

Chemical properties of BR, SCB, and PALF were determined based on corresponding ASTM methods. Substrates and their cellulosic derivatives were analyzed using wide-angle X-ray scattering (WAXS) in a Bruker DS Advance diffractometer with $\text{CuK}\alpha$, 2θ scanning between 5 and 40° at 1° per minute. Crystallinity indices (CI) from WAXS datum were estimated using a height ratio method. Thermogravimetric analysis (TGA) was performed in a TA Instruments Q500 TGA, from 50 to 800 °C at 20 °C/min under inert atmosphere. Fourier transform infrared spectroscopy (FTIR) was used to elucidate chemical structure with a Thermo Scientific Nicolet iS10 equipped with attenuated total reflectance (ATR) attachment. Apparent size distribution and zeta potential for each NC was determined with a Malvern Instruments Zetasizer Nano ZS90. Micrographs of NC were taken with a Hitachi HT7700 transmission electron microscope (TEM) operated at 100 kV.

3 RESULTS AND DISCUSSION

3.1 Chemical and Structural Characterization

Cellulosic materials were characterized in terms of their chemical composition, crystallinity, and thermal behavior. Chemical composition of the lignocellulosic substrates is shown in Table 1. Substrate BR has an initial advantage as production of nanocellulose is concerned due to the lowest lignin and the highest cellulose content in comparison to PALF and SCB.

Table 1 Chemical composition of the substrates (wt% \pm 0.01).

Chemical property	BR	PALF	SCB
Humidity	6.40	8.90	8.62
Ashes	2.65	4.64	5.21
Cold water extractives	22.41	35.01	9.03
Hot water extractives	28.25	38.50	12.49
α -Cellulose	49.12	45.32	47.35
Lignin	20.23	25.63	24.41
Hemicellulose	18.57	17.43	17.86
Holocellulose	67.69	62.75	65.21

However, all substrates show similar holocellulose contents, as the holocellulose is made of α -cellulose and hemicellulose.

Structural characterization of each substrate, CELL, MCC and NC was performed by means of FTIR (Figure 1) and XRD (Figure 2). In all cases, a relative reduction and broadening of the peak around 3400 cm^{-1} can be

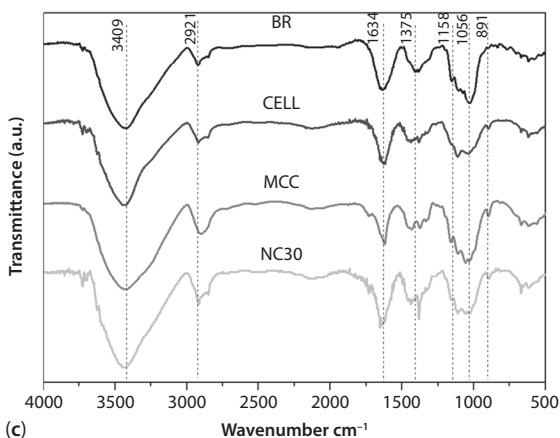
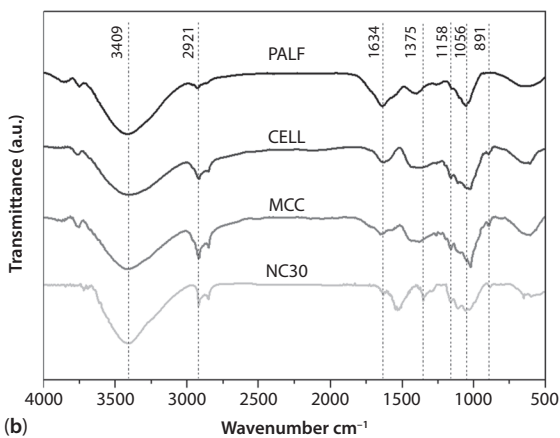
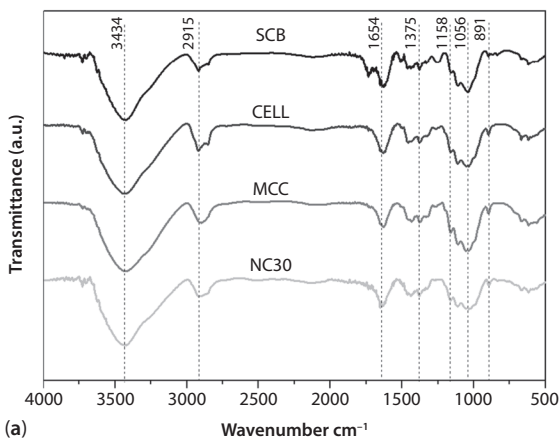


Figure 1 FTIR of the substrates and cellulosic materials: (a) SCB, (b) PALF, and (c) BR.

observed in the FTIR spectra (Figure 1) as lignin removal and cellulose hydrolysis proceeded. In addition, the signal originating from the conjugated carbonyl group in lignin at $1660\text{--}1665\text{ cm}^{-1}$ also gradually weakened as the chemical treatment progressed [22]. Basically, all materials experienced chemical changes during the extraction and hydrolysis processes up to the extraction of each

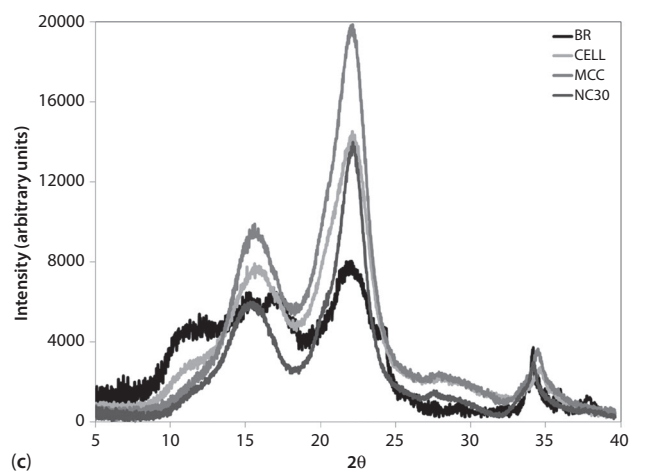
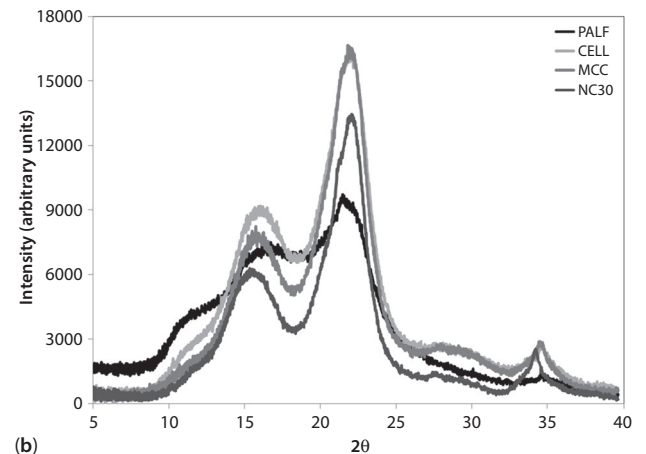
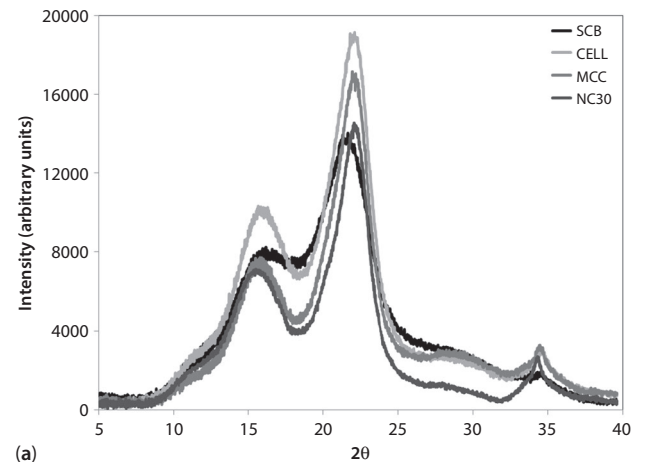


Figure 2 XRD of the substrates and cellulosic materials: (a) SCB, (b) PALF, and (c) BR.

Table 2 Crystalline index and yield values.

Material	CI (%)	Yield (wt%)
Pineapple		
PALF	35	
CELL	59	86
MCC	70	60
NC30	72	32
Banana		
BR	46	
CELL	66	85
MCC	71	59
NC30	81	32
Sugarcane		
SCB	47	
CELL	63	86
MCC	73	62
NC30	73	35

nanocellulose. It is worth mentioning that the BR spectra shows a band around 1300 cm^{-1} , which has been ascribed to cellulose type II [23].

The XRD data of all samples are presented in Figure 2. It can be observed that BR appears to be the less uniform while SCB is more uniform in terms of the crystalline structure and order. All samples show three crystallographic planes characteristics of cellulose I [4, 24, 25]: $2\theta = 16^\circ$ related to (110) plane, $2\theta = 22^\circ$ related to the (200) plane, and $2\theta = 35^\circ$ related to (004) plane. Besides, BR shows an X-ray reflection around $2\theta = 12^\circ$ characteristic of cellulose type II, which was also noticed in the FTIR spectra. The simultaneous presence of cellulose I and cellulose II is possible and has been reported in pineapple residues [23]. Furthermore, BR shows less ordered crystalline structure than the other two substrates; PALF shows a shoulder at $2\theta = 12^\circ$ while SCB presents a more pure crystalline type I structure. On the other hand, CELL-BR shows a reduction of such reflection at 12° becoming a shoulder. In general, as the chemical treatment and extraction progress, the XRD patterns become sharper and the diffusive peaks shown in the lignocellulosic starting materials disappear. This is more drastically viewed in the case of the BR substrate, then PALF and then SCB. This behavior is represented through the crystallinity index (CI) trends presented in Table 2. Both SCB and BR substrates exhibit greater CI values in comparison to PALF substrate. A similar trend is observed when the CELL and MCC samples obtained from SCB, BR, and PALF substrates are analyzed. Further chemical treatment to obtain NC did not result in an increase of

the crystallinity index for NC30-SCB and NC30-PALF compared with their MCC counterparts. However, NC30-BR showed an increase in the extent of crystallinity as compared to MCC-BR. On the other hand, all extents of yield were similar to each other.

Crystallinity index values for steam-exploded and bleached banana and PALF fibers have been reported to be 83.8 and 89.3%, respectively [15]. More recently, nanocellulose fibers from sugarcane bagasse have been reported to have a 75% crystalline index when using a combination of chemical, mechanical, and enzymatic extraction treatments [26].

3.2 Particle Size and Morphology

Average particle size and zeta potential were determined for nanocellulose extracted from all three substrates using sulfuric acid 30 and 60% (w/v). Clear differences in particle size were observed for the three types of NC. The NC30-PALF showed the smallest average particle size (250 nm), followed by NC30-SCB (459 nm), and then NC30-BR showing the highest particle size (730 nm). At higher acid concentrations all particle sizes were reduced; 160 nm, 296 nm, and 334 nm for NC60-PALF, NC60-SCB, and NC60-BR, respectively. In contrast, their average zeta potential values increased [12] from -27 mV for NC30 to -43 mV for NC60. During the acid hydrolysis with H_2SO_4 , cellulose-OH groups react and the nanocellulose is partially sulfonated, forming anionic sulfate ester groups (-OSO₃-). As the concentration of the acid is increased, (-OSO₃-) groups added to the material are increased in the same way, raising the zeta potential value. Aspect ratio was reduced in the case of the NC60 particles. These results reflect the differences between sources of each of the lignocellulosic substrates. Subjecting the substrates to the same experimental parameters resulted in different particle sizes and shapes.

The TEM micrographs of NC extracted from each MCC by using sulfuric acid 30% (w/v) and by using sulfuric acid 60% (w/v) are depicted in Figures 3 and 4, respectively. These results are in accordance with what was previously observed in this work with dynamic light scattering (DLS) analysis; smaller particles with lower aspect ratios were obtained at higher acid concentrations. NC30 materials exhibited whisker-like shape, although some degree of aggregation was observed, possibly due to incomplete hydrolysis. NC60 samples showed irregular shapes due to extensive acid hydrolysis. A concentration of sulfuric acid of around 64% (w/v) has been extensively reported in the literature based on the premise that using lower acid concentration would prevent the formation of nanocellulose and their yield would be lower. However, in this work it has been demonstrated that it is possible

to use milder acid concentrations in order to obtain cellulosic whiskers with reasonable quantities. Recent studies reported lower sulfuric acid concentrations in order to extract nanocellulose from a variety of vegetable sources; a 50% acid concentration value was used to extract nanocellulose from sugarcane bagasse [27],

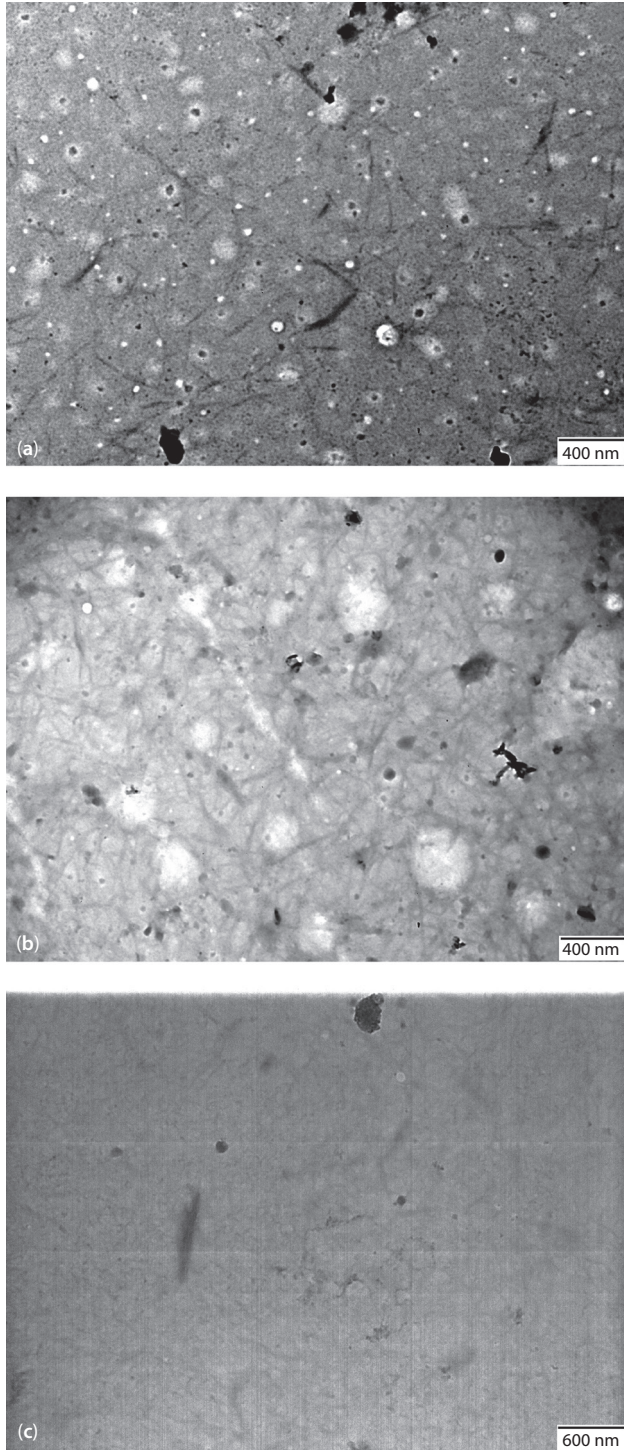


Figure 3 TEM micrographs of NC30 from (a) SCB, (b) PALF, and (c) BR.

while a combination of ball milling with a 47% sulfuric acid concentration was employed to extract nanocellulose from commercial cellulose [28].

3.3 Thermal Properties

Thermal stability of each lignocellulosic and cellulosic material was assessed by means of their TGA (Figure 5)

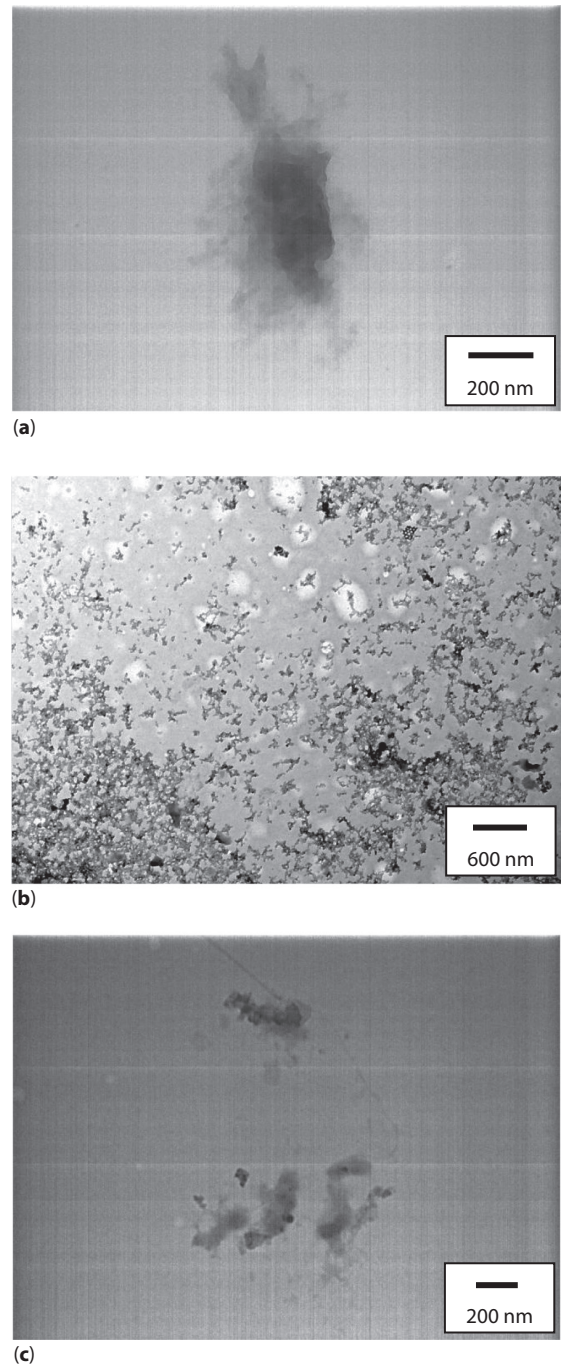


Figure 4 TEM micrographs of NC60 from (a) SCB, (b) PALF, and (c) BR.

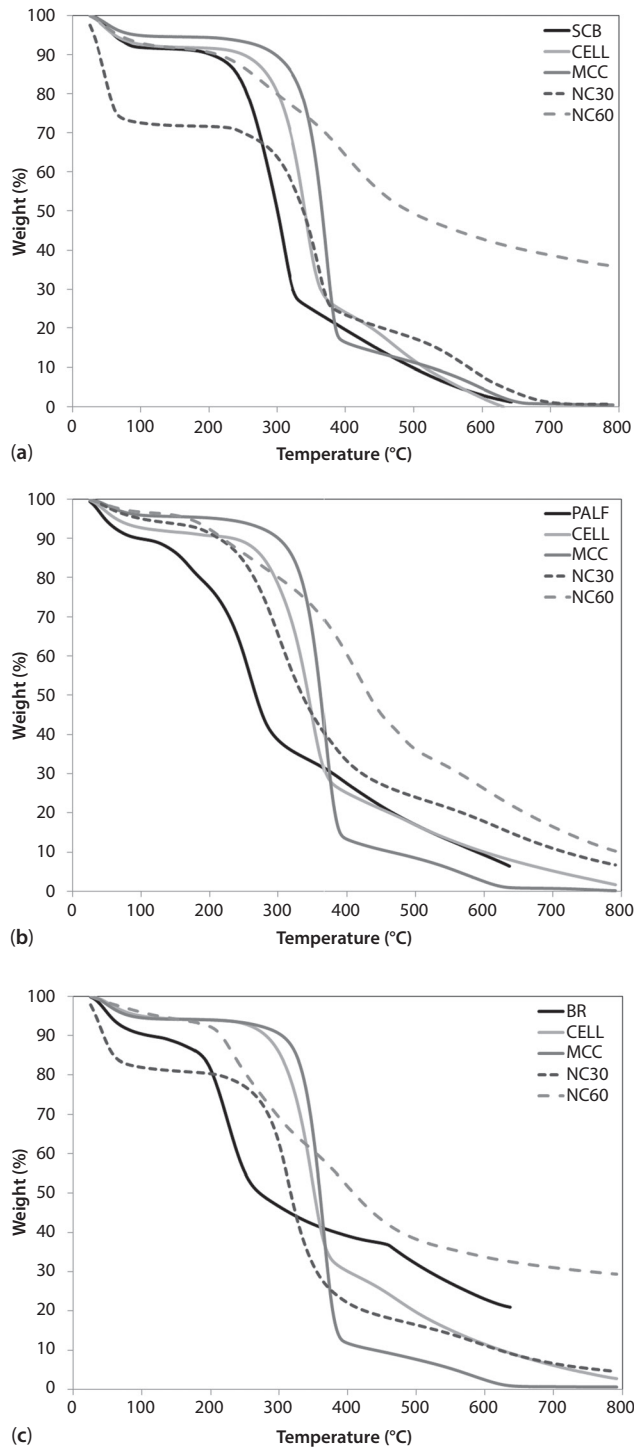


Figure 5 TGA of the substrates and cellulosic materials: (a) SCB, (b) PALF, and (c) BR.

and DTGA (Figure 6) traces. As seen in those figures, removal of the lignin fraction and refining of the crystalline fraction of cellulose yield improved thermal stability, reaching its maximum in MCC samples. A similar behavior has been reported in sugarcane bagasse by Mandal and Chakrabarty [10] and by Teixeira *et al.* [20].

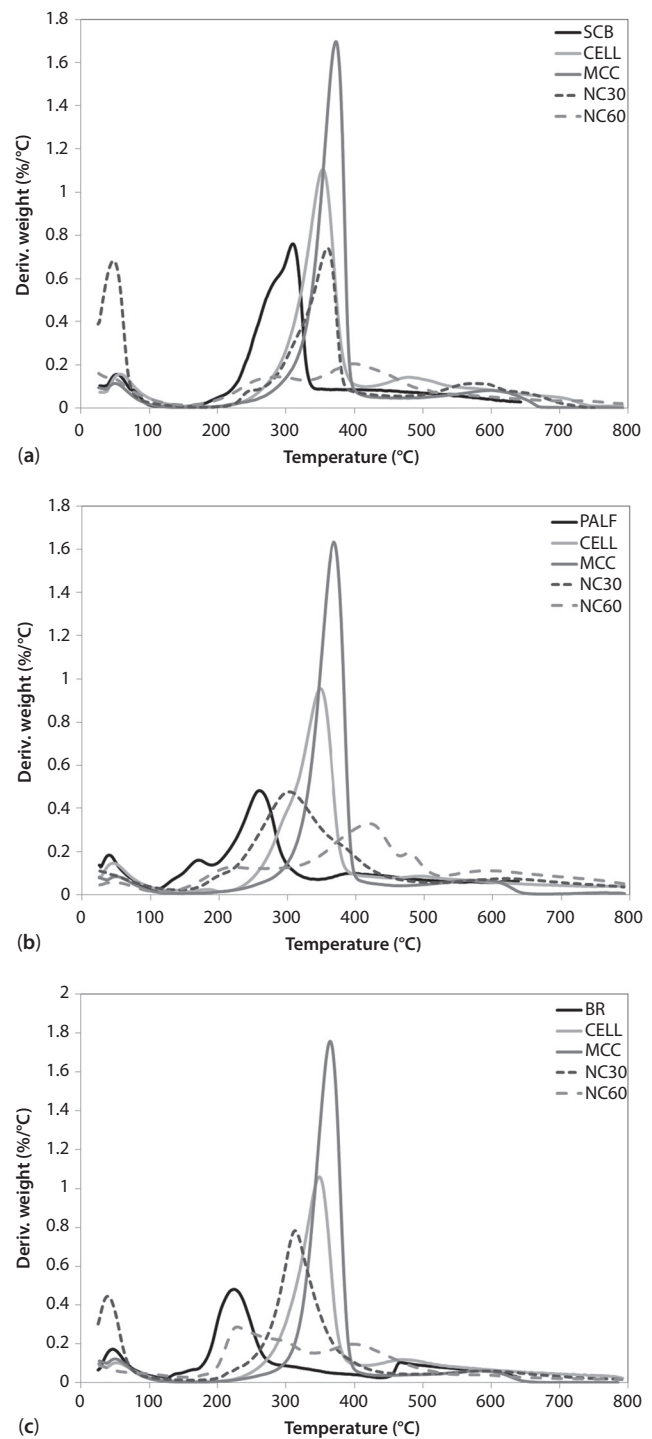


Figure 6 DTGA of the substrates and cellulosic materials: (a) SCB, (b) PALF, and (c) BR.

All materials exhibited water (moisture or chemically bonded) loss around 100 °C. PALF substrate proved to be a more heterogeneous material than the other two substrates, which is in agreement with its chemical composition described in Table 1. The PALF had higher amounts of cold and hot water extractives than BR and

SCB had. On the other hand, SCB exhibited a well-defined TGA trace with less inflection points, which corresponds to a less complex lignocellulosic material as compared to PALF and BR (Figure 5). Thermal stability is reduced in the case of each NC material due to the acid hydrolysis degradation of the cellulose and the incorporation of sulfate groups into the amorphous regions of the polymer [10]. Reduction of thermal stability is even more drastic when using higher acid concentrations, resulting in lower degradation onset temperatures and broadening of the degradation range. Although smaller particles were obtained at higher acid concentrations, their thermal stability and aspect ratio were lower.

4 CONCLUSIONS

It was feasible to extract nanocellulose from three different types of agricultural crop waste resources by using mild acid conditions. Using low acid concentration yielded cellulose nanowhiskers, while using high acid concentrations produced cellulose nanoparticles. The SCB substrate proved to be a more promising raw material compared to PALF and BR in terms of thermal stability, crystalline index, crystal uniformity, and substrate complexity.

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