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ORIGINAL PAPER

Extraction and Characterization of Nanocrystalline Cellulose from Doum (*Chamaerops humilis*) Leaves: A Potential Reinforcing Biomaterial

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Abstract The aim of this investigation was to extract nanocrystalline cellulose (NCC) from Moroccan Doum fibers (*Chamaerops humilis*) by chemical treatment to examine their potential for use as reinforcement fibers in bionanocomposite applications. The chemical composition, morphological and structural properties of the Doum fibers was determined at different stages of chemical treatment. Morphological (transmission electron microscopy and scanning electron microscopy), structural characterization (X-ray diffraction, Fourier transformed infrared), thermal characterization (thermogravimetric analysis). The suspension electrostatic stabilization (zeta potential) of NCCs was also carried out. The results of these characterization analysis found that average size of the NCC is 220 nm in length and 11 nm in diameter, with high crystallinity index (93 %), a thermal stability comparable to that of untreated Doum fibers (degradation temperature 340 °C), which is reasonably promising for the use of these nanofibers in reinforced-polymer manufacturing, and a good stability in water suspension that it allows their utilization such as reinforcement of the water-soluble polymers to prepare the bio-nanocomposite.

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Introduction

The use of renewable materials within bionanocomposite applications in packaging industry has gained interest in recent years, due to its sustainable, biodegradable and environment friendliness. Cellulose is the earth's most abundant biopolymer, extracted from renewable resources such as vegetal fibers and there chemical formula is $(C_6H_{10}O_5)_n$ [1]. Cellulose is a semi-crystalline material [2] and crystallinity of cellulose, like other properties, depends on the source of the material, but most native types of cellulose are 50–80 % crystalline. The structure of cellulose is composed of two main regions those are amorphous and crystalline [1]. As the name suggests, nanocrystalline cellulose corresponds to the crystalline regions of the cellulose, it was extracted from cellulose fibers by selectively dissolving the amorphous regions with acid hydrolysis [3] and the remaining crystalline regions can be liberated [4]. Previous works have shown that the dimensions of these nanofibers depend on the cellulose fiber source and on the acid hydrolysis conditions in terms of time, temperature and concentration of acid, for example, the size of NCCs extracted from cotton is between 10 and 50 nm and between 100 and 1000 nm in diameter and length respectively [5], while the size of NCC extracted from Sisal is between 3 and 5 nm and between 100 and 500 nm in diameter and length respectively [6].

Thus, the NCC as reinforcement in the polymeric matrix has several advantages such as their high specific area, great aspect ratio, low density, biodegradability,

renewability and low energy consumption in the extraction process [7, 8].

The Saw palm or Doum palm is a species of the family "*Chamaerops humilis*", it is a small plant whose leaves in a rosette, are palmate shaped fan, of origin of the regions bordering the western Mediterranean Sea: Italy, Spain, Libya, Algeria and Morocco. For a long time leaves Doum are traditionally used to replace armchair and mattresses and manufacture braided objects such as mats, baskets or ropes. In recent years, due to their good mechanical resistivity, Doum fibers are used to develop industrial applications of fiber in term of reinforcing the synthetic polymer matrix; for example, polypropylene [9], low density polyethylene [10] and epoxy resin [11].

Currently, to the best of our knowledge, there is no paper reporting on the nanocrystalline cellulose extract from Doum leaves (*C. humilis*). In this respect, the objective of this research is the extraction /characterization of the nanocrytalline cellulose from the Moroccan Doum plant as a substitute for synthetic fibers to make a good economic and ecologic composite materials. Once, the NCC was obtained. The chemical composition of the fibers after each chemical treatment was determinate according to ASTM Standard Methods. A thorough characterization of NCC was done using different characterization techniques such as atomic force microscopy (AFM), transmission electronic microscopy (TEM), scanning electronic microscopy (SEM), infrared spectroscopy (FTIR), X-ray diffraction (XRD) and thermogravimetric analysis (TGA).

Material and Experimental Details

Materials

Doum leaves gathered in the Rabat-Salé-Zemmour-Zeär region (Morocco) were used in this study. The chemical reagents and solvents used were sodium hydroxide (97 %), sodium chlorite (80 %), acetic acid (99.8 %), sulfuric acid (95–97 %), ethanol (99.8 %) and toluene (99.9 %) (purchased from Sigma–Aldrich).

Extraction of Nanocrystalline Cellulose

Washing with Hot Water

After grinding the raw materials until the grain size less than 0.5 mm, they were refluxed in water at 100 °C to remove hydrosoluble impurities, this mixture is let cool and then filtered and washed with distilled water. This treatment was performed three times.

Alkali Treatment

The alkali treatment was performed to purify the cellulose by removing lignin and hemicelluloses from Doum fiber. Following washing with water at reflux, the residue was treated with an alkali solution (4 wt% NaOH). The reaction mixture held in a round bottom flask for 3 h at reflux. The solid was then filtered and washed using excess distilled water. This treatment was carried out three times.

Bleaching Process

Following alkali treatment, the bleaching process was completed by adding the sodium chlorite solution (1.7 wt%) under acidic condition (pH 4–5) at reflux for 5 h under continuous agitation. The mixture is left to cool and then filtered by using additionally distilled water in surplus. This treatment was performed three times.

Acid Hydrolysis

The fiber results of the alkali and bleaching treatments was treated using sulfuric acid (64 wt%) for 1 h at 45 °C under continuous stirring. The hydrolyzed material was washed by centrifugation (Sorval WX Ultra Series Centrifuge) at 10,000 rpm and 10 °C for 20 min. The centrifugation step was repeated several times before the suspension was dialyzed against distilled water for several days until constant pH in the range of 6–7 was reached.

Following dialysis treatment, the resulting suspension was sonicated for 30 min before kept refrigerated for further used.

Chemical Composition

The chemical composition of the Doum fibres before and after the chemical treatment was determined according to the methods reported by the American Society for Testing and Materials (ASTM). The extractible, klason lignin, holocellulose and alpha-cellulose content were measured according to ASTM standard D1107-56, D1106-56, D1104-56 and D1103-60, respectively.

Characterization

Infrared Spectroscopy

The FTIR spectra were recorded on an *ABB Bomem FTLA 2000-102* FTIR instrument (ATR: SPECAC GOLDEN GATE) over the range of 4000–260 cm^{-1}.

Thermogravimetric Analysis

The thermogravimetric analysis (TGA) were performed on a Q500 (TA instrument) using a heating rate of 10 °C/min from room temperature to 700 °C under air. This yielded the onset temperature of decomposition, mass loss and maximum decomposition peak.

X-Ray Diffraction

The X-ray powder patterns were recorded using a diffractometer 'Bruker D8 Discover' with a CuKα radiation source ($\lambda = 1.54184$ nm) that operates an acceleration voltage of 45 kV and 100 mA current intensities.

Zeta Potential

Malvern Instruments Zetasizer (ZS) was used to measure the electrophoretic mobility and equivalent hydrodynamic size of NCC particles in different samples. Mobility values were converted to ζ-potentials using the Smoluchowski equation and the reported values are an average of 14 measurements.

Scanning Electron Microscopy

Scanning electron microscopy (FEI, Quanta 650-ESEM operating at 25 kV) was used to evaluate the Doum fiber morphology and to confirm the effect of the chemical treatments on the fiber morphology. For compared the diameter of the fibers before and after chemical treatment, a total of 50 fibers were measured by an image processing, ImageJ, using the SEM images.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) (TECNAI G220 S-TWIN) was used to determine the morphology and dimensions of the NCCs obtained from the Doum fibres. A drop of a dilute aqueous suspension (0.05 wt%) was deposited on the surface of a copper grid coated with a thin carbon film. The sample was dried before TEM analysis, which was carried out with an accelerating voltage of 100–120 kV. For seize diameter and length of NCC, a total of 50 fibers were measured by an image processing, ImageJ, using the TEM images and the results were reported as the mean value of the data from each measurement.

Atomic Force Microscopy

The topography and morphology of the NCCs were imaged using an AFM (Veeco Dimension ICON from Bruker). Prior to imaging, a droplet of the aqueous suspension was initially dried on a glass-slide and the scans were obtained in semi-contact mode in the air.

Results and Discussion

Chemical Composition

The Table 1 provide the chemical compositions of raw, alkali treated, and bleached Doum fibers.

As can seen in Table 1 the raw Doum fibers are composed of 43.2 % cellulose, 30.1 % hemicelluloses, 23.7 % lignin, and 12.5 % extractives, these results shows that vegetable fibers of Doum palm leaves are rich in cellulose. The results of this study corroborate with those reported in literature for Date Palm which are 35 % cellulose, 28 % hemicellulose and 27 % lignin [12]. The amount of hemicelluloses and lignin are lower in treated fibers compared to the raw fiber, whose NaOH was found to be efficient in removing the hemicelluloses from the fiber, as hemicelluloses content was decreased from 30.1 to 4.1 %, and most of the lignin content was removed by the bleaching treatment, in which it reacts with $NaClO_2$ to dissolve as a lignin chloride. Bleaching does not only remove lignin, but also some of the hemicelluloses. The final fiber obtained after the bleaching treatment was found to have the highest cellulose content (92.3 %).

Morphological Analysis

Vegetable fibers are essentially constituted by the microfibers cellulose, lignin and hemicelluloses; the color of these fibers is linked to the presence of chromophore groups of lignin [13] which indicates that the change of the fibers color after each chemical treatment reflects the change in the chemical composition of the fibers. The Fig. 1 represents the Doum fibers and the color change after each chemical treatment.

The SEM pictures of the raw Doum fibers and after bleaching treatment were taken to investigate the structure and morphology of these fibers. The raw Doum fibers were clearly composed of the individual fibers linked together as bundles by a cement material (Fig. 2a). The alkali and bleaching treatments allows to remove the hemicellulose and lignin present in the Doum fibers, leading to a defibrillation of raw fibers to the cellulose fibers (Fig. 2a). Therefore, the fiber diameter was reduced from 60 to 180 μm for the raw fibers to 60–180 μm for the bleached fibers. These results are comparable with the literature [2] which the diameter of the Agave fibers is in the range of 60–230, 9–110 and 7–12 μm to the raw state, after alkaline treatment and after bleaching treatment, respectively.

Table 1 Chemical composition of raw, alkali treated and bleached Doum fibers

	Raw fibers wt%	Alkali treated wt%	Bleached wt%
Extractible	12.5	–	–
Holocellulose	73.3	80.2	94.7
α-Cellulose	43.2	75.8	92.3
Lignin	23.7	20.1	4.1

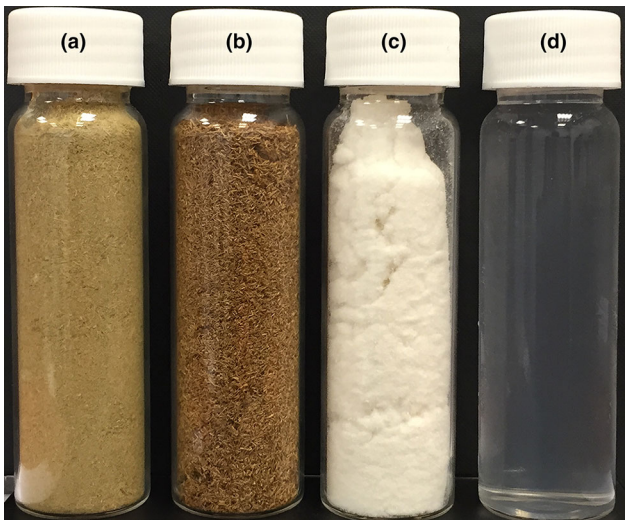

Fig. 1 Photographs of **a** raw, **b** alkali, **c** bleached and **d** acid hydrolyzed suspension Doum fibers

Acid hydrolysis of the purified cellulose fibers resulted in defibrillation of nanofibers. The AFM and TEM micrographs (Fig. 1c, d) show the separation of the nanofibers from the micro-sized fibers. The lengths and diameters of the NCCs were calculated by an image treatment, ImageJ, using the TEM images. The size of these nano-fibers is ranging from 6 to 17 nm in diameter and from 150 to 340 nm in length with an average 11 nm in diameter and 220 nm length (Fig. 3).

Infrared Spectroscopy

The FTIR spectroscopic analysis of the raw and chemically-treated Doum fibers is presented in Fig. 4, the comparison of these results allows us to know the effect of these treatments on the cellulose, hemicelluloses and lignin, which have been studied in the literature [2, 14].

The peak arranges 1640 cm^{-1} is mainly associated to the vibration binding of water molecules absorbed into cellulose fiber structure [2, 14, 15]. Alkali treatment consisted of removing the hydroxyl groups by reaction with sodium hydroxide, consequently it is observed the increasing of the concentration of OH^- groups, this mecanism is confirmed by ftir analysis by increasing of the peak intensity between 3300 and 3500 cm^{-1} attributed to the stretching vibration of OH group compared to this peak intensity of the raw fibers [16]. The peak at arrange to 1460 cm^{-1} reflected the C–C=C groups vibration of lignin, it reported that the intensity of this peak decreased after alkali treatment and vanished after bleached treatment which indicates the elimination of a most important portion of lignin [7]. The C–O–C glycosidic symmetric stretching and C–OH stretching vibration of cellulose backbone are at 1100 and at 1050 cm^{-1}, respectively. From the bleaching to the hydrolysis infrared curves, it is observed that there is a no difference between these peaks which indicates that the molecular structure of cellulose remains unchangeable after hydrolysis treatment [17].

From the FTIR analysis it has been concluded that there is a reduction of the amorphous compounds presents in the Doum fiber during the chemical treatments [16]. The peak observed in all of the spectra at 1050 cm^{-1} is due to the C–O–C pyranose ring skeletal vibration, the increase in the intensity of this band showed an increase in crystallinity of fibers after each chemical treatment [8].

X-Ray Diffraction

Figure 5 shows the X-ray diffraction patterns of the Doum fibers and those of treated at different stages of the chemical treatments. All these patterns showed the diffraction intensity at $2\theta = 22°$ and in the region $2\theta = 16°$. These two peaks of diffraction intensity indicated that all NCCs produced were of cellulose I type [3].

The crystallinity index (CrI) of fiber was determined by the method of Segal [18]:

$$CrI = \frac{I_{002} - I_{Am}}{I_{002}} \times 100$$

The intensity $\boldsymbol{I}_{002}$ of the diffraction of the plane 002 of maximum peak between $2\theta = 22°$ and $2\theta = 23°$, and $\boldsymbol{I}_{\mathrm{Am}}$ is the diffraction intensity at $2\theta = 18°$ which represents the amorphous portion while $\boldsymbol{I}_{002}$ represents both the crystalline and amorphous regions. The crystallinity index was determined for the different samples and the results are summarized in Table 2.

The crystallinity index for raw, alkali-treated, bleached, and acid-hydrolysed Doum fibres was found to be 63.3, 73.8, 87.6 and 93.2 %, respectively. These results demonstrate the increase in the degree of crystallinity

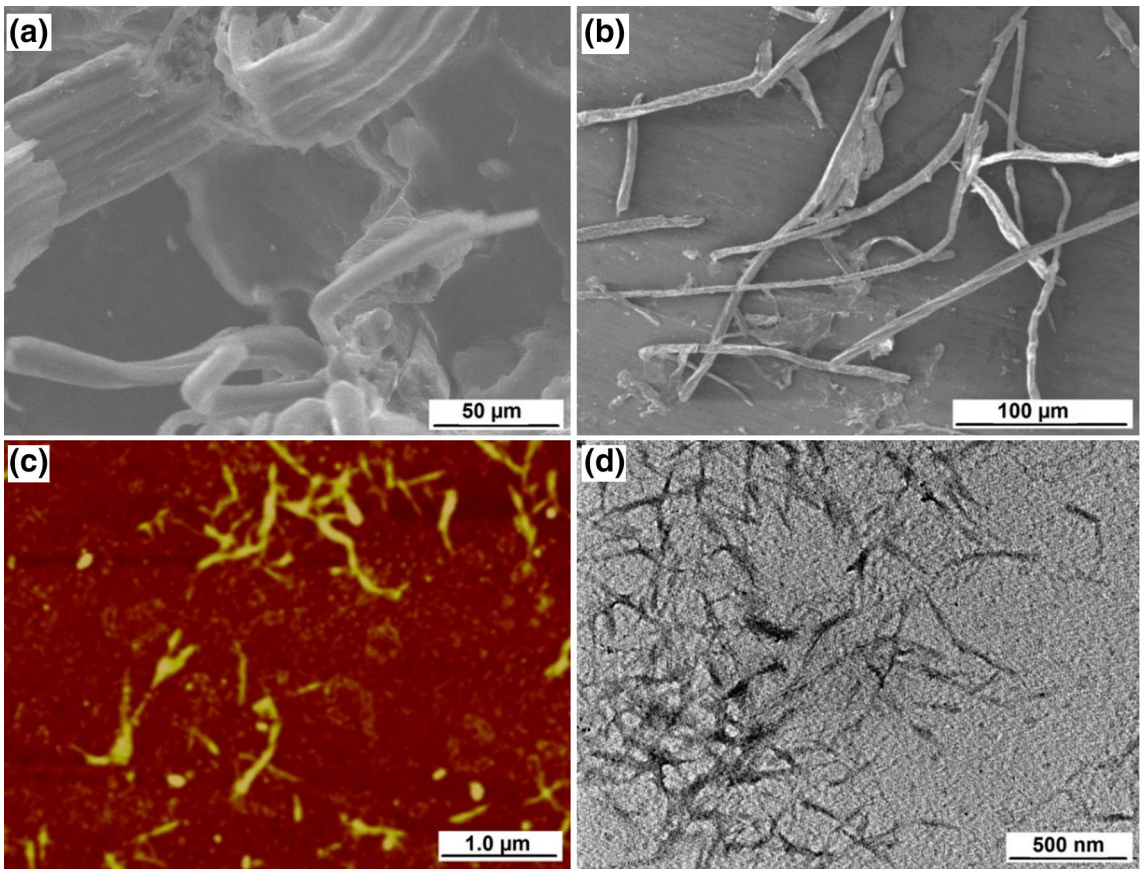

Fig. 2 **a** SEM micrographs of the untreated Doum fibers and **b** purified Doum fibers after bleaching treatments, **c** AFM and **d** TEM micrographs of the nanocrystalline cellulose

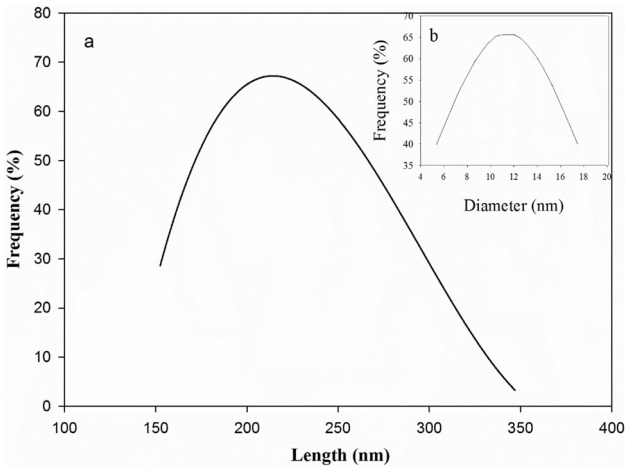

Fig. 3 Length (*a*) and diameter (*b*) distribution of the nanocrystalline cellulose

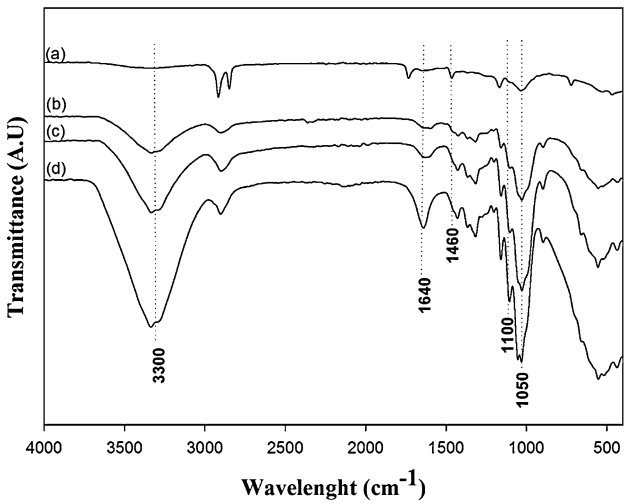

Fig. 4 FT-IR spectra of (*a*) raw, (*b*) alkali-treated, (*c*) bleached, and (*d*) acid hydrolyzed Doum fibers

after each chemical treatment by removing of the amorphous constituents of the fibers (lignin and hemicelluloses) [16]. Thus, the NCCs extracted from Doum fibers have a high degree of crystallinity compared to NCCs extracted from other fibers sources, with the same extraction conditions, for exemple the Agave fibers have a $CrI = 82\ \%$.

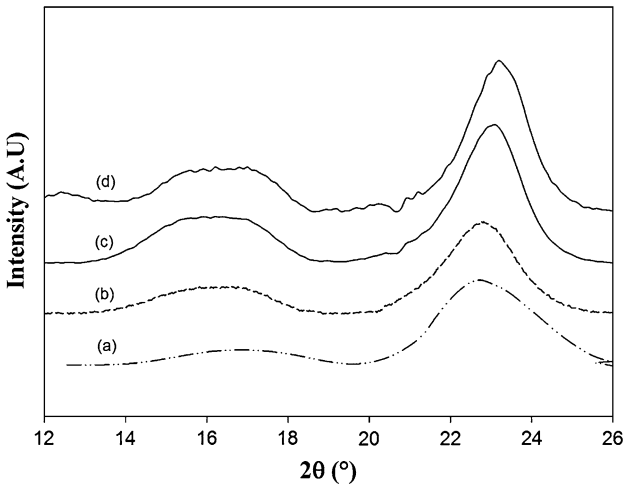

Fig. 5 X-ray diffraction curves for (*a*) raw, (*b*) alkali-treated fibers, (*c*) bleached, and (*d*) acid hydrolyzed Doum fibers

Table 2 Crystallinity index of Doum fibers at different stages of chemical treatment

Sample	CrI (%)
DM raw	63.3
DM alkali	73.8
DM-blanchit	87.6
DM-NCC	93.2

Thermogravimetric Analysis

Figure 6 shows the TGA and DTG results obtained from Doum fibers after each chemical treatment. An initial weight loss of approximately 6 % was observed for all samples upon heating to 100 °C [19]. These findings correspond to the vaporization and removal of moisture in the sample materials.

The TGA curves of the raw fibers showed three degradation steps related to hemicellulose, cellulose and lignin degradation, Because of the difference in their chemical structures these three essentially compounds of the natural fibers usually decompose at different temperatures which hemicellulose degraded at a temperature arrange of 280 °C, cellulose degraded at 324 °C while lignin degraded at temperature levels of 400 °C [20].

The degradation of the bleached fibers occurred from 285 to 360 °C with a maximum rate at 331 °C are attributed to cellulose degradation, the nanocrystalline cellulose also showed the same trend as the bleached fibers with the degradation occurring from 280 to 370 °C with a maximum rate at 331 °C. The increasing of the thermal stability of the fiber after bleaching treatment is a result of the elimination of the amorphous compounds and of the increase of their crystallinity [19]. It reported that the loss weight around of 400 °C for the all samples is consequence of char oxidation [20]. The residue weight at the temperature levels of 550 °C were 14, 2 and 0.5 wt% for raw, bleached and acid hydrolysed Doum fibers, respectively. The larger amount of residue at high temperatures found in the raw fibers as compared to that in the treated fibers was a result of the presence of ash as well as lignin [8, 20].

It can be concluded from these results that the nanocrystalline cellulose extracted from Doum fibers are characterized by a high thermal stability that allows their utilization in the reinforcing of the thermoplastic polymers.

Nanocrystalline Cellulose Suspension Stability

The zeta potential is a measure of the electrostatic intensity repulsion or attraction between particles that can be measured by pursuing the moving rate of charged particles across an electric field. The measurement of zeta potential

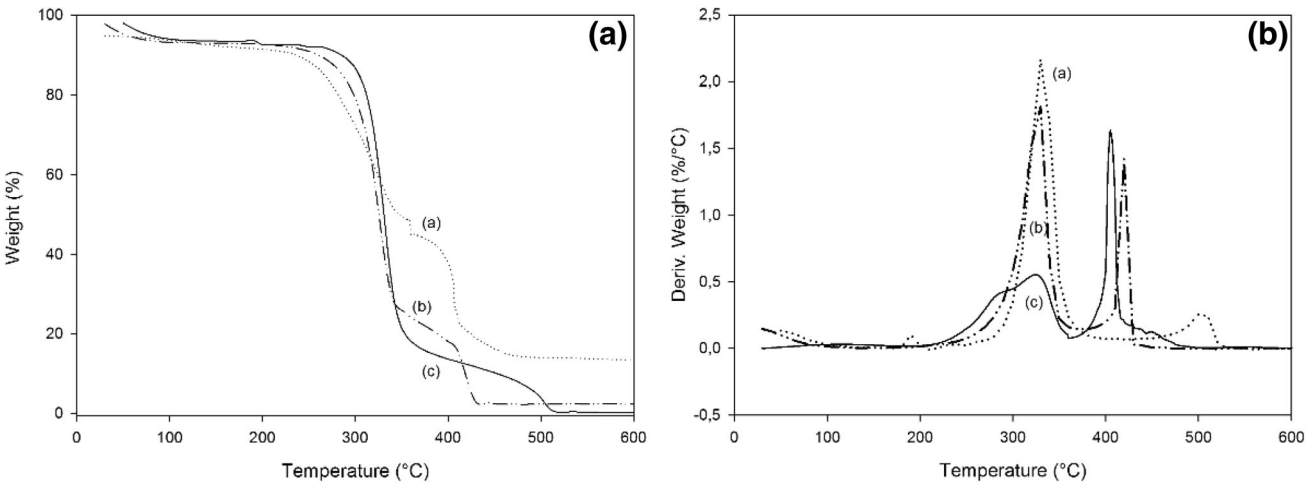

Fig. 6 **a** TGA and **b** DTG curves of (*a*) raw, (*b*) bleached, and (*c*) acid hydrolyzed Doum fibers

for a dilute aqueous suspension of NCCs (0.04 wt%) found that zeta potential is -31 mV, this suspension considered stable because, in general, when the absolute value of zeta potential is less than 15 mV it represents the presence of the agglomerations into the suspension, and when it's greater than 30 mV it indicates that there is sufficient mutual repulsion between particles which gives a stable suspension [21]. Thereby, the NCCs suspension extracted from the Doum fibers presents a sufficient electrostatic stabilization to ensure the stabilization of the suspension during storage for a long time [21] and allows their utilization such as reinforcement of the water-soluble polymers to preparing the nanocomposites or bionanocomposites.

Conclusion

In the present work, cellulose was extracted from Doum fibers by alkali treatment (4 % NaOH) and bleaching treatment (1.7 % $NaClO_2$). Nanocrystalline cellulose was isolated from this cellulose by acid hydrolysis (64 % H_2SO_4). The determination of the chemical composition demonstrated the effects of chemical treatments, based on XRD, FTIR, TGA, TEM, AFM and SEM analysis. The crystallinity index was increased from 63.3 to 87.6 %, the cellulose content was increased from 43.2 to 92.3 % and the diameter of the fibers was reduced averaging from 120 μm to 9 μm for the raw and bleaching fibers, respectively. The nanocrystalline cellulose extracted from Doum fibers is characterized by a size average 220 nm in length and 11 nm in diameter, high crystallinity (93 %), high thermal stability (degradation temperature 340°) and a good stability in water suspension.

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