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High quality fluorescent cellulose nanofibers from endemic rice husk: Isolation and characterization

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ABSTRACT

Cellulose nanofibers (CNFs) with high crystallinity and purity were isolated from two endemic rice husk varieties using a hydrothermal approach followed by acid-alkali treatments and mechanical disruption. The CNFs isolated had a mean diameter of ~35 nm. The TGA and DTG profiles showed good thermostability of the CNFs. The CNFs also showed a prominent photoluminescence peak at 404 nm with high quantum yield (~58%). This is the first report on the native fluorescence property of nanocellulose in absence of any conjugated fluorescence molecule/dye. The CNFs further demonstrated appreciable hemocompatibility in the hemolysis test, exhibiting its potential for biomedical applications.

1. Introduction

Biopolymeric sugar crystals originating from amylose, cellulose, and lignin have shown tremendous potential as "green composites" owing to their sustainability, renewability, biocompatibility, and biodegradability (Gericke, Trygg, & Fardim, 2013; Jayaramudu et al., 2013). Cellulose is easily considered as an inexhaustible biomass resource due to its ample presence in the form of plant and bacterial celluloses. Cellulose is basically a homo-polymer of β-D-glucopyranose units, which exhibit a unique structural hierarchy, derived from their biological origin. They are composed of nanofiber assemblies with a diameter that ranges from 2–20 nm, and length ranging up to a few micrometers. At the molecular level the repeating β-D-glucopyranose molecules are covalently linked through acetal functions between the equatorial —OH group of C4 and the C1 carbon atom (β-1,4-glucan) (Abdul Khalil, Bhat, & Ireana Yusra, 2012).

So far, materials from different biological sources like cotton, ramie, sisal, flax, wheat straw, potato tubers, sugar beet pulp, soybean stock, banana rachis etc. have been studied for production of a variety of cellulosic nanoparticles (Siró & Plackett, 2010). The hierarchical structure of the plant celluloses, in such cases, have been broken down using different extraction methods to yield cellulosic nano-materials having interesting properties (Lavoine, Desloges, Dufresne, & Bras, 2012; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011). These materials are commonly referred to as nanocelluloses, which are characterized by rod-like or fibrous morphologies with different geometries, depending on the biological source it is derived from (Bras, Viet, Bruzzese, & Dufresne, 2011; Chornet & Overend, 1989). Steam explosion or hydrothermal treatment is an effective synthesis technique that can be modulated and combined with chemical treatments to convert biomass into by-products of desired dimensions and functionalities (Kokta, 1991). The hydrothermal treatment of lignocelluloses for different time intervals leads to the breakdown of the composite biopolymer structures, resulting in defibrillation of the cellulosic fibers (Cristobal et al., 2008; Leung, Lam, Chong, Hrapovic, & Luong, 2013). Cellulose has also been isolated using different chemical treatments such as acid hydrolysis, TEMPO mediated oxidation, alkaline pre-treatments, enzymatic hydrolysis etc., which results in the hydrolytic cleavage of the glycosidic bonds in the amorphous regions of the cellulose, releasing individual crystallites. These derived nanofibers have high aspect ratio, Young's modulus, tensile strength and a very low coefficient of thermal expansion [(Leung et al., 2013, Tanpichai et al., 2012). They have been found to have wide degree of applicability as nanofillers for composite reinforcement, soft-tissue replacement, artificial bones, dental prostheses, food packaging, building materials, targeted delivery

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vehicles for therapeutics, measurement of residual dipolar couplings, reinforcing additives for plastic and polymeric materials etc. (Moon et al., 2011, Leung et al., 2013).

In this work, cellulose nanofibers (CNF) have been isolated from rice-husk of endemic autumn (*Ahu*) and summer (*Boro*) rice varieties, respectively, by hydrothermal treatment in a teflon-lined reactor followed by multistep acid-alkali treatments. The CNFs derived, have been characterized for microstructural properties like morphology, organization as well as thermal stability. We also report the discovery of a novel optical fluorescence property in these isolated CNFs. Such fluorescence property has previously been reported only with composite cellulosic nanosystems, conjugated/grafted with known fluorescent molecules (Díez et al., 2011). The effect of the preparative chemical treatments for the isolation of CNFs on its natural biocompatibility was also studied.

2. Materials and methods

2.1. Isolation of nanocellulose from rice husk

The CNFs were isolated based on previously published techniques with certain modifications (Daifullah, Awwad, & El-Reefy, 2004). The rice husk raw material was extensively washed with distilled water to remove dust and impurities, dried and milled to very fine particles. The samples were then pre-soaked in 2% NaOH for 14 h (fibre to liquor ratio 1:11) and subsequently autoclaved in a Teflon lined-autoclave at 20 lb. pressure and 210 ± 5 °C, for a period of 8 h. The procedure was repeated for two subsequent cycles following which the fibers were washed in MilliQ water until it was rid of alkali. The steam exploded fibers were bleached using a mixture of sodium hydroxide-acetic acid and 8% sodium hypochlorite solution. After the bleaching, the fibers were thoroughly washed in MilliQ water and dried. The obtained nanofibers were suspended in 10% HCl solution and sonicated for 2 h at 40 °C. The resultant fibers were washed several times to neutralize the pH and dried. The as-prepared CNFs are hereby termed as CNFa for the autumn (*Ahu*) variety and CNFb for the summer (*Boro)* variety.

2.2. Chemical estimation

The chemical composition of the rice husk and the CNF were determined according to the methods reported by the Technical Association of Pulp and Paper Industry (TAPPI). The cellulose and hemicellulose contents were assessed according to TAPPI standard T203 OS-74 while the lignin content was measured according to TAPPI standard T222 OS-83 (Zobel et al., 1966).

2.3. Characterization of the CNFs

The as-prepared CNFs were studied under a JEOL JEM-2100 transmission electron microscope (TEM). The CNFs were also subjected to Energy-dispersive X-ray spectroscopy (EDX) analysis using the Oxford Instrument model 7572. For the aforementioned analysis, a droplet of the dilute suspension of CNFs was deposited on a carbon-coated copper microgrid (400 mesh) and allowed to dry under vacuum, prior to examination. Atomic Force Microscopy (AFM) imaging was used to characterize the topographical features of CNFs obtained after the thermochemical and mechanical treatments. The AFM was done in tapping mode using a Nanonics NSOM and SPM System (MultiView 2000) and the drive frequency of the cantilever was about 200–300 kHz with a scan rate of 0.5–3 Hz (~2 Hz). The X-ray diffraction (XRD) study was carried on a RIGAKU miniflex instrument and Ni filtered Cu Kα radiation (λ = 1.5406 Å) was employed for the study. The Fourier transform infrared (FTIR) spectroscopy was conducted on a Perkin Elmer Spectrum 100 instrument. For the analysis, the CNFs were ground into a fine powder along with KBr and subsequently pressed into a pellet using a Qwik Handi-Press Kit. The thermo gravimetric analysis (TGA) studies of the samples were carried out using a Perkin Elmer STA 6000 instrument. The spectra were recorded under ambient nitrogen atmosphere at a heating rate of 20 °C/min. For the comparative analysis of the spectroscopic and thermogravimetric properties of the CNFs, carboxymethyl cellulose (HiMedia) was used as commercial cellulose, in the aforementioned experimental studies.

2.4. Hemolysis test

The hemolysis test was carried out according to Ryadnov, Degtyareva, Kashparov, and Mitin (2002) where mammalian blood sample from goat was collected in a vial containing heparin as anticoagulant. The blood sample was centrifuged at 2000 rpm and the supernatant was discarded. The precipitate containing the erythrocytes was washed twice with PBS (pH 7.4) for 10 min. Two milliliters each of the erythrocyte suspension were distributed in three vials and the volume made up to 50 ml. Two of the vials were incubated with 10 mg of the as-prepared CNF (a and b), for 1 h at 37 °C. Post-incubation, the vials were centrifuged at 2000 rpm for 10 min and the absorbance of the supernatant was recorded at 415 nm on a UV–visible spectrophotometer. The absorbance of PBS was recorded as a negative control. The hemolysis percentage was calculated using the following equation: $(A_{sample} - A_{media}/A_{100} - A_{media}) \times 100$. Where, A_{100} is the absorbance of erythrocytes suspension with 100% hemolysis which was obtained by suspending erythrocytes in PBS containing 0.2% Triton X-100.

3. Results and discussion

CNFs were extracted from two endemic rice (*Oryza. sativa L. ssp. indica*) varieties: CNFa from autumn (*Ahu*) and CNFb from summer (*Boro*) varieties, by a hydrothermal process, followed by bleaching and acid treatments. The CNFs obtained after the acid treatments and drying were translucent-white in appearance. The chemical composition of the rice husk varieties and the derived CNFs were chemically analyzed and are represented in the Table S1. The native cellulose content of the autumn and summer, raw rice husk varieties estimated at 37.1% and 32.8% respectively, were enhanced to 94.2% and 89.6% in the derived CNFa and CNFb samples (Table 1). The lignin content of the rice husks were substantially lowered following the thermochemical treatment of the raw fibers, resulting in the breakdown of the lignocellulosic structure and improved defibrillation (Park et al., 2004).

Fig. 1a and b shows the TEM images of the CNFa and CNFb. The high-pressure and temperature treatments followed by chemical treatments resulted in the defibrillation of the cellulose nanofibers, evident in the TEM images revealing the separation of these nanofibers. The average diameter of the CNFs, calculated from the electron micrographs were found to be in the range of 30–40 nm (Fig. 1c and d). The EDX profile tabulated (Fig. S1 a, b) shows the absence of elemental impurities in the isolated CNFs. The

Table 1
Compositional analysis of the Raw rice husk fibers from *autumn (ahu)* variety (Raw a) and *summer (boro)* variety (Raw b) and the derived cellulose nanofibers (CNFa, CNFb).

Sample	Cellulose (%)	Hemicellulose (%)	Total lignin content (%)
Raw a	37.1	29.4	24.14
Raw b	32.8	31.5	29.50
CNF a	94.2	1.7	1.9
CNF b	89.6	2.1	2.5