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Cellulose nanofibers produced from banana peel by enzymatic treatment: Study of process conditions

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ABSTRACT

Cellulose nanofibers (CNFs) were isolated from banana peel bran via alkaline treatment followed by enzymatic treatment with xylanase. The influence of process conditions such as pH, temperature, and concentrations of the enzyme and substrate on the properties of the CNFs was evaluated with a 2^{4-1} fractional factorial design with three central points. Enzyme at 70 U/g of bran, substrate at 15%, pH 6.0, and temperature between 35 and 55 °C favored enzymatic hydrolysis. Transmission electron microscopy (TEM) images confirmed that treatment with xylanase effectively isolated cellulose fibers at the nanometer scale. Fourier transform infrared spectroscopy (FTIR) showed that a fraction of amorphous compounds was removed. X-ray diffraction revealed that the CNFs presented high crystallinity index (66.2%). The CNFs had a diameter of 3.7 nm, their aspect ratio was in the range of long nanofibers, and their suspension was stable (−29.1 mV). These features make the CNFs potentially applicable as reinforcing agents in composites. The results evidenced that enzymatic hydrolysis with xylanase successfully afforded CNFs from banana peel, a residue that constitutes a potential source of biodegradable materials of commercial interest.

1. Introduction

Cellulose, the main component of the cell walls of plant fibers, has been extensively explored because it resembles synthetic polymers with the advantage that it originates from natural, renewable, and biodegradable resources. Cellulose is an ideal material to produce nanoparticles for use as reinforcing agent in composite materials. It presents good mechanical strength and stiffness, interesting thermal and electrical properties, and high degree of crystallinity (Bhattacharya et al., 2008; Cherian et al., 2008; Deepa et al., 2011; Siqueira et al., 2010a). Recently, interest in obtaining nanometric cellulose fibers from natural sources has increased. Bananas are a popular fruit that grows in tropical and subtropical regions (Pelissari et al., 2014). Cultivation and industrialization of banana fruit generates a considerable amount of waste with high lignocellulosic content. One example of such waste is banana peel, a byproduct of banana processing during food production (Elanthikkal et al., 2010). Banana peel is a source of cellulose. Banana peel processing not only adds value to this byproduct, but it also helps to reduce the environmental impact of this waste (Rosa et al., 2010). Molina (2013) has suggested the integral use of banana: its peel could be used to produce nanofibers that could be introduced as reinforcing agents in films produced from the banana pulp.

A series of processes are necessary to isolate cellulose nanofibers (CNFs). There are many ways to extract CNFs, all of which lead to different types of fibrillar material with characteristics that will depend on the raw material (cellulose), pretreatment, and disintegration process (Chen et al., 2011). In general, plant materials are lignocellulosic, which makes them resistant to bioconversion and requires pretreatment to increase their digestibility and render cellulose more accessible for hydrolysis. Chemical treatment can remove the amorphous fractions (hemicellulose and lignin) present in the structure of a plant fiber. Alkali treatment causes the structure to swell, modifying the physical features of the fiber wall and consequently increasing the surface area that is exposed to hydrolysis in the cellulose fibers (Andrade-Mahecha et al., 2015; Castro and Pereira Jr, 2010).

Different techniques afford cellulose nanoparticles from plant sources. CNFs are commonly prepared by chemical treatment, but new techniques to isolate CNFs are currently being developed. Enzymatic hydrolysis can help to isolate cellulose fibers from plant cell walls. Because enzymatic hydrolysis dismisses the

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need for solvents and chemicals, the mild conditions of this process make it economically attractive and environmentally friendly (Meyabadi and Dadashian, 2012; Siqueira et al., 2010a; Yu et al., 2008). Xylanases are usually employed in enzymatic hydrolysis. These enzymes initially promote catalytic hydrolysis of the hemicellulose fractions present in the plant fiber. Then, they attack the glycosidic bonds β-1,4 located between the glucose units comprising cellulose, which culminates in hydrolytic cleavage. Hydrolysis usually produces CNFs in colloidal suspensions (Hubbe et al., 2008; Pääkko et al., 2007).

To develop a new technique to isolate CNFs with sustainable characteristics, Tibolla et al. (2014) studied the production of CNFs from banana peel bran by enzymatic hydrolysis at fixed conditions of pH (5.5), temperature (45 °C), and concentrations of the substrate (25%, w/v) and enzyme (50 U/g of bran). The authors compared their results with results obtained by acid hydrolysis. Enzymatic hydrolysis proved to be a very promising technique to prepare CNFs: the resulting nanofibers were longer, and they had smaller diameter and greater aspect ratio. In addition, the CNFs presented higher negative surface charge, which is important to prevent the nanofibers from agglomerating.

Previous studies have shown that CNFs obtained by enzymatic hydrolysis have potential application as reinforcing agents in composites. However, the efficiency of enzymatic hydrolysis depends on factors such as the hydrolysis time (h), the concentrations of substrate (%) and enzyme (U/g of bran), pH, and temperature. These factors often interact with one another, so it is important to optimize the hydrolysis process to improve its yield (Meyabadi and Dadashian, 2012). Here, experiments were performed with a fractional factorial design 2^{4-1} with three central points.

This study aimed to analyze how process conditions (pH, temperature, and concentrations of enzyme and substrate) employed during the enzymatic treatment of unripe banana peels of the variety "Terra" (*Musa paradisiaca*) influenced the properties of CNFs produced via hydrolysis by xylanase.

2. Materials and methods

2.1. Materials

The banana peel bran was prepared from unripe banana peels (mature green) of the variety "Terra" (*Musa paradisiaca*), according to the methodology described by Pelissari et al. (2012). The fruit was obtained from the southeastern region of Brazil; the crop was harvested in March 2013, but it was not subjected to any post-harvest treatment. All the chemicals used in this work were reagent grade. Xylanase enzyme, kindly provided by Novozymes (Araucária – PR, Brazil), was used to produce CNFs by enzymatic hydrolysis.

2.2. Pretreatment of the banana peel bran

Plant materials contain a large amount of amorphous compounds, so it was necessary to delignify the bran. An alkaline treatment was conducted according to the method described by Tibolla et al. (2014). This process was performed in 5% w/v KOH alkaline solution at a bran/solution ratio of 1:20. Vigorous stirring and room temperature were employed for 14 h. Then, the substrate was subjected to successive washings with deionized water and centrifuged after each washing (10,000 rpm, 5 °C, 15 min). This process removed hemicellulose and lignin, to improve the next step of enzymatic hydrolysis. Fig. 1a depicts the sequence of steps used to obtain the unripe banana peel bran. Fig. 1b shows the bran delignification process (alkaline treatment) and the resulting residue applied during enzymatic hydrolysis.

2.3. Production of cellulose nanofibers (CNFs)

Producing cellulose nanofibers is complex and depends on numerous factors, so a statistical study that considered different process conditions was conducted herein. On the basis of the paper by Rodrigues and Iemma (2014), the experiments were performed by employing a 2^{4-1} fractional factorial design with three central points as represented by the experiment matrix shown in Table 1. The independent variables were temperature (T), pH, and concentrations of the enzyme [E] and substrate [S]; the analyzed response variables were length, diameter, aspect ratio, zeta potential, yield, and crystallinity. The ranges of the variables had been defined in preliminary tests. Analysis of the effects of the fractional factorial design on all the evaluated responses allowed to establish conditions for the validation tests that would provide CNFs with maximum length, minimum diameter, and higher aspect ratio, zeta potential, yield, and crystallinity.

Enzymatic hydrolysis was conducted according to the method adapted from Tibolla et al. (2014). Erlenmeyer flasks containing the substrate (i.e., banana peel bran at concentrations of 15, 25, or 35%) and 0.1 M acetate buffer (pH of 4.0, 5.0, or 6.0) were placed in the thermostatic shaker (temperature of 35, 45, or 55 °C) for 10 min, for the medium to adapt. Then, the enzyme xylanase (concentration of 30, 50, or 70 U/g of bran) was added to the mixture and left at the desired temperature for 24 h, under agitation (150 rpm). The suspensions were placed in a thermostatic bath at 80 °C for 30 min, to denature the enzyme. Next, the residual pulp was washed with deionized water, and the solid was separated by centrifugation (10,000 rpm, 5 °C, 15 min) and suspended in deionized water. At the end of these procedures, a colloidal suspension of CNFs was obtained and stored at 4 °C in a sealed container.

The effects of the independent variables were evaluated at 10% significance (Rodrigues and Costa, 2014) by using the software Protimiza Experimental Design Statistical. In the case of fractional factorial design and biological processes, which are complex procedures, it is better to accept $p < 0.1$ than leave out an important factor. In this case, a validation test was required.

2.4. Characterization

For the XRD and FTIR analyses, an amount of each suspension (suspension from alkaline treatment (ATS) and suspension of CNFs) was dried in a freeze-dryer (Equipamentos Terroni, model LS 3000, São Paulo, Brazil). The freeze-dried samples were stored at 4 °C in sealed containers.

2.4.1. Physicochemical and size particle analysis of the bran

The chemical composition of the bran was determined in terms of the ash, total extractives (polysaccharides including hemicelluloses), lignin, and cellulose contents. The ash content was obtained by using AOAC (AOAC, 2005). The total extractives content was obtained by digestion with water and alcohol according to NREL/TP 520-42619 (Sluiter et al., 2008b). The lignin content was determined by digestion with sulfuric acid (72% w/w) in combination with high pressure at 121 °C according to NREL/TP 520-42618 (Sluiter et al., 2008a). The cellulose content was obtained by digestion with acetic acid (80% w/w) and nitric acid (70% w/w) (Sun et al., 2004). A laser diffraction analyzer (Laser Scattering Spectrometer Mastersizer S, model MAM 5005–Malvern Instruments Ltd., Surrey, England) was used to determine the particle size of the banana peel bran; ethanol was used as solvent. An ultrasound device was coupled to the equipment to increase the dispersion of the sample. Measurements were performed at 25 °C, in triplicate.

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