Industrial Crops and Products 62 (2014) 280–285

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Isolation of cellulose nanofibrils from mandarin (*Citrus unshiu*) peel waste

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ARTICLE INFO

Article history:
Received 28 February 2014
Received in revised form 14 July 2014
Accepted 7 August 2014

Keywords:
Cellulose
Nanofibril
Mandarin peel waste
Citrus unshiu
Hydrothermal treatment

ABSTRACT

Cellulose obtained from mandarin (*Citrus unshiu*) peel waste was purified by the removal of oil, coloring substances, and pectin. Two pectin-removing methods, namely multistep and hydrothermal treatments, were investigated to compare their purification efficiencies. The multistep treatment consisted of three steps: removal of metal in pectin, depolymerization, and dissolution of pectin. In contrast, the hydrothermal treatment comprises a single step process involving a solution of 0.18 wt% hydrochloric acid. Fourier-transform infrared spectroscopy and neutral sugar content analysis of the purified cellulose from the mandarin peel waste indicated that the hydrothermal treatment was more effective for the purification of cellulose, as compared to the multistep treatment. The crystal width of the purified cellulose was determined from X-ray diffraction analysis, and it revealed a smaller crystal width (2.5 nm) than that of wood cellulose (3.9 nm). After the pectin removal, the purified cellulose from the mandarin peel waste was fibrillated by sonication to obtain cellulose nanofibrils, yielding cellulose fibers with widths of 2–3 nm, as observed by atomic force microscopy. The observed fiber width corresponded to the crystal width, indicating that the cellulose nanofibrils were completely individualized by sonication.

1. Introduction

The production of citrus juice on an industrial level leads to a considerable quantity of solid and liquid residues (globally around 8–20 million tons per year), which is considered waste or is used as a supplement in agricultural practices. During citrus juice production, only about half of the fresh citrus weight is transformed into juice (Pascual and Carmona, 1980), generating a great amount of residue (peel, pulp, seeds, leaves, and whole citrus fruits that do not meet quality requirements), which has a moisture content of approximately 78 wt% (Garcia-Castello et al., 2006; Megías et al., 1993). In general, citrus juice residues have no economic value, even though they are rich in soluble sugars, cellulose, hemicellulose, pectin, and essential oils that could form the basis of several industrial processes (Rezzadori et al., 2012). This huge amount of waste is, in most cases, burned or disposed of (Lapuerta et al., 2008).

This study focused on the utilization of cellulose in citrus juice residue. Cellulose is the main constituent of the cell wall in all plants, and is a linear polymer of poly-β(1 → 4)-D-glucose units with a syndiotactic configuration (Dufresne et al., 1997). Cellulose chains are organized into crystalline microfibrils surrounded by a non-cellulosic matrix in the plant cell wall (Frey-Wyssling, 1954). The cellulose nanofibrils are extremely thin fibers; for example, the thickness of the nanofibrils contained in wood cell walls is 3–4 nm. Their characteristic structure allows for their use in novel applications such as reinforcement nanomaterials for plastics (Yano et al., 2005), drug delivery systems (Roman et al., 2009), biosensors (Zhang et al., 2010), and packaging (de Azeredo, 2009). Cellulose obtained from agricultural citrus juice waste is expected to be a rich source of nanofibrils.

Cellulose nanofibrils and their aggregated nanofibers have been isolated from various kinds of fruits such as strawberries, pears, rambutans (Niimura et al., 2010), apples (Ifuku et al., 2011), and bananas (Zuluaga et al., 2007). Niimura et al. (2010) examined the isolation of 1–2-nm thick cellulose nanofibrils from fruit tissue, the thicknesses of which were smaller than wood-derived nanofibrils, a major source of nanofibrils. This characteristic fruit-based nanofibril shape is anticipated to expand the applications of cellulosic materials.

Since the cellulose nanofibrils in fruit tissues are embedded with pectin (Marín et al., 2007; Choi et al., 2013), which is mainly composed of galacturonic acid, its isolation requires the imple-

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http://dx.doi.org/10.1016/j.indcrop.2014.08.007
0926-6690/

mentation of pectin removal processes (Ifuku et al., 2011; Habibi et al., 2009). To remove pectin from citrus juice residue, a multistep chemical treatment consisting of three processes, including inorganic substance removal, pectin depolymerization, and its dissolution, has been reported as being effective for this purpose (May, 1990). This multistep treatment has been previously applied toward the analysis of chemical constituents present in citrus juice residue. However, it was not as useful for the purification of cellulose, since the treatment requires a significant amount of chemical reagents and long reaction times. On the other hand, hydrothermal treatment (Martínez et al., 2010) has emerged as an alternative to purify cellulose by a simple procedure. Most non-cellulosic materials such as pectin and hemicellulose were removed from fruits by these treatments, but residues from pectin and hemicellulose were detected. Regardless of the pectin and hemicellulose residue, cellulose nanofibrils were obtained from fruits by fibrillation treatment. To isolate cellulose nanofibrils from fruits, pectin and hemicellulose do not need to be completely removed. The development of a simple technique, capable of removing most pectin and hemicellulose, is important.

The purpose of this study was to design an efficient method for the isolation of cellulose nanofibrils from mandarin (*Citrus unshiu*) peel waste. After the extraction of oil and coloring substances, pectin was removed by two methods, namely multistep and hydrothermal treatments. The efficiencies of the pectin removal methods were compared using Fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). The fibrillation behavior of the purified cellulose was observed by atomic force microscopy (AFM).

2. Experimental

2.1. Materials

The mandarin (*C. unshiu*) peel waste from juice production was kindly provided by Hiroshima Cooperative stock company, Japan. The materials were stored in a freezer at −60 °C. Before extraction, they were pulverized into particles using a cutter mill (3M-7-40, Masuko Sangyou Co., Ltd., Japan). Powdered bleached wood pulp (W-400, Nippon Paper Chemicals Co., Ltd., Japan) was used as wood cellulose.

2.2. Purification of cellulose

Fig. 1 shows the flow chart of the cellulose purification process. Oil and coloring substances found in the mandarin peel waste were extracted according to the previous report (Taboada et al., 2010). The extraction was conducted with a solution of water/ethanol (15:85, v/v) at 85 °C for 20 min. The concentration of the solid was 10 wt%. The extracted mandarin peel waste was collected by centrifugation (8000 rpm, 10 700 × *g*, 5 min, 25 °C), and then extracted again with the water/ethanol solution. The extraction was repeated until the supernatant liquid became transparent. Finally, the extracted mandarin peel waste was washed with distilled water. The washed mandarin peel waste was collected by centrifugation (8000 rpm, 10 700 × *g*, 5 min, 25 °C). This washing treatment was repeated five times.

After the extraction of oil and coloring substances, pectin was removed by two methods, namely multistep (Taboada et al., 2010) and hydrothermal treatments. The multistep treatment consisted of three steps including the removal of metal in pectin, depolymerization, and dissolution of pectin. First, the metal in pectin was removed with a 0.98 wt% solution of ammonium oxalate at 20 °C for 1 h. Second, the water-insoluble pectin was depolymerized with 0.18 wt% hydrochloric acid at 80 °C for 1 h.

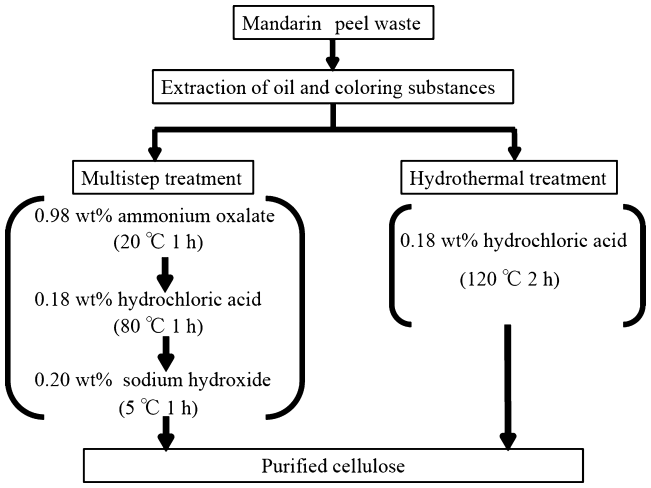

Fig. 1. Flow chart of cellulose purification process.

Finally, the depolymerized pectin was removed with 0.20 wt% sodium hydroxide at 5 °C for 1 h. In each step, the supernatant was removed after centrifugation (8000 rpm, 10 700 × *g*, 5 min, 25 °C). Each step was repeated three times. After the multistep treatment, the samples were washed five times with distilled water by centrifugation.

In the hydrothermal treatment, the extracted mandarin peel waste was added to a 0.18 wt% hydrochloric acid at a solid concentration of 1.0 wt%. The treatment was conducted at 120 °C for 2 h at 0.12 MPa using an autoclave (MC-3032S, ALP Co., Ltd., Japan). After treatment, the samples were washed with distilled water five times by centrifugation.

2.3. Fibrillation of purified mandarin peel waste

The purified cellulose from the mandarin peel waste was fibrillated by sonication (US-150T, NISSEI Co., Ltd., Japan) of a 0.0001 wt% water suspension for 15 s. The non-fibrillated fraction was removed by centrifugation at 18 000 rpm. The wood cellulose was fibrillated by the wet-disk milling (MKCA6-2, Masuko Sangyou Co., Ltd., Japan) of a 3 wt% water suspension. The disk milling was repeated 15 times and performed at 1800 rpm.

2.4. FT-IR spectroscopy

Freeze-dried samples were analyzed using FT-IR spectroscopy (Spectrum GX, Perkin Elmer Inc., USA) in ATR mode. All spectra were obtained by accumulation of 128 scans in the range of 500–4000 cm^{-1}. The resolution was 4 cm^{-1}.

2.5. Neutral sugar content

The neutral sugar content in the samples was analyzed after acid hydrolysis (Talebnia et al., 2008). A dried sample (50 mg) was hydrolyzed with 600 μL sulfuric acid (72 wt%) at 50 °C for 90 min. After dilution with 16.8 mL distilled water, the sample was hydrothermally treated at 120 °C for 1 h at 0.12 MPa using the autoclave. After acid hydrolysis, the sample was neutralized with calcium carbonate. The neutral sugar content in the hydrolyzed samples was measured using high-performance liquid chromatography (HPLC) with refractive index (RI) detection (RI-2031 plus, JASCO Co., Ltd., Japan). The measurement was conducted at 80 °C using an HPLC column (HPX-87P, Bio-Rad Laboratories Inc., USA). Ultra-pure water was used as the eluent. The flow rate was 0.6 mL/min.