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Effect of cellulose nanocrystals from sugarcane bagasse on whey protein isolate-based films

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ABSTRACT

Whey protein isolate (WPI) has been utilized as edible film or food packaging material. However, WPI films are hydrophilic due to highly polar amino acids which provide a moderate barrier to water vapor and low mechanical properties. To overcome these drawbacks, cellulose nanocrystals (CNCs) extracted from sugarcane bagasse were incorporated with whey protein. FTIR and TGA were used to confirm the changes in chemical structures and to observe the thermal properties, respectively. The CNCs had sizes of 200–300 nm and diameters of 20–40 nm using TEM and AFM technique, respectively. Different amounts of CNCs (0–8 wt% based on WPI) were added into whey protein solution and formed films. The lightness and transparency of the films tended to decrease with increasing WPI content. The water activity (a_w) and water solubility of those films increased, whereas their water contact angle values decreased, implying that the film became more hydrophilic when the cellulose nanocrystal was added. The addition of CNCs increased the tensile strength and Young's modulus and reduced the water vapor permeability of WPI-based CNC films. However, the CNCs did not change the oxygen permeability of the film. Therefore, the obtained WPI films provided good mechanical performance and may be promising as an alternative product for film packaging.

1. Introduction

Whey protein isolate (WPI) is a valuable by-product of the cheese production and it consists of protein content > 90% (*w*/w) (Mulvihill & Ennis, 2003). It has been studied for film formation and coating application. Several authors have investigated the properties of whey protein isolate films as transparency, flexibility, odorless, excellent barrier to oxygen transmission and providing moderate mechanical properties (Ramos et al., 2013; Sothornvit, Hong, An, & Rhim, 2010). Additionally, these films have demonstrated mechanical and barrier properties better than competitive protein-based (such as corn, zein, wheat, gluten and soy protein isolate) or polysaccharide-based (such as starch, cellulose, carrageenan and pectin) films (Ramos, Fernandes, Silva, Pintado, & Malcata, 2012). However, the limitation of whey protein isolate films to reach the expansive commercial applications was due to low mechanical and high water vapor permeability properties because of their hydrophilic nature (Khwaldia, Pérez, Banon, Desobry, & Hardy, 2004; Kim & Ustunol, 2001). Therefore, incorporation of cellulose nanocrystals (CNCs) in a biopolymer to improve these properties may be useful because CNCs have strong hydrogen bonding and a high surface area (Qazanfarzadeh & Kadivar, 2016).

Thailand is the second largest sugar exporter in the world (Thailand: Sugar Annual | USDA Foreign Agricultural Service, 2017) and > 20 million tons of sugarcane bagasse (SCB) is created annually. Generally, SCB is used as boiler fuel to produce steam which in turn is used in the sugar production industry. Moreover, SCB is also utilized in ethanol and pulp production. SCB consists of approximately 40–50% cellulose (Hajiha & Sain, 2015). Therefore, SCB is an interesting source for CNC extraction. Acid hydrolysis is the most well-known process for CNCs extraction. It breaks and removes the disordered and amorphous regions of cellulosic fibers leaving well-defined crystals in the form of CNCs (Deepa et al., 2011; Habibi, Lucia, & Rojas, 2010).

CNCs have been shown to be of benefit in food packaging by improving the mechanical and water barrier properties of alginate film (Sirviö, Kolehmainen, Liimatainen, Niinimäki, & Hormi, 2014).

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Moreover, adding CNC and titanium dioxide nanoparticles increased the tensile strength, Young's modulus and water sensitivity of nanocomposite wheat gluten-based film (El-Wakil, Hassan, Abou-Zeid, & Dufresne, 2015). Qazanfarzadeh and Kadivar (2016) reported improved physical, mechanical and barrier properties of WPI film incorporated with oat husk nanocellulose. Therefore, to the best of our knowledge, this is the first report to extract CNCs from SCB for application in WPI films. Therefore, this study aimed to synthesize and characterize CNCs from SCB produced using acid hydrolysis and to investigate the effect of cellulose nanocrystals from sugar bagasse on the properties of nanocomposite whey protein film to determine its further utilization as a food packaging material.

2. Materials and methods

2.1. Materials

The sugarcane bagasse (SCB) used in this study was obtained from Kaset Thai International Sugar Corporation Public Company Limited, Nakhonsawan, Thailand. Whey protein isolate (WPI) was purchased from Mighty International Co., Ltd., Thailand. Glycerol was provided from Sac Scienec-Eng Ltd., Part, Thailand. Sodium chlorite ($NaClO_2$) was purchased from Ajax Finechem Pty., Ltd., New Zealand. Glacial acetic acid and sulfuric acid were bought from QRec Chemical Co., Ltd., New Zealand. Dialysis tubing with a 12–14 kDa molecular weight cut-off was used.

2.2. Extraction of cellulose from SCB

Cellulose extraction followed the methodology of Saelee, Yingkamhaeng, Nimchua, and Sukyai (2016). Sugarcane bagasse was treated with steam explosion at 195 °C for 15 min. Then, it was bleached around 7 times with 1.4% (*w/v*) sodium chlorite until the color of samples became white (Mandal & Chakrabarty, 2011). The untreated, steam-exploded and bleached SCB were chemically analyzed according to standard TAPPI methods (TAPPI, 2003) being alpha cellulose (T203 om-98), lignin (T222 om-98) and holocellulose (the combination of alpha-cellulose and hemicellulose) analysis using the acid chlorite method (Browning, 1967). The hemicellulose content was calculated using holocellulose minus the alpha cellulose content.

2.3. Preparation of cellulose nanocrystals

About 2 g of cellulose was dispersed in 40 mL of 60% (*v*/v) sulfuric acid (solid-liquid ratio 1:20) (Kumar, Negi, Choudhary, & Bhardwaj, 2014) at 45 °C for 75 min with an agitation speed of 700 rpm using a turbine. To stop the reaction, 400 mL of cold water was added. Acid removal for cellulose suspension was carried out using a centrifuge (TOMY MX-305, Japan) at 15,000 rpm and 4 °C for 15 min. Dialysis was applied against distilled water to remove acid residue until pH 7 was obtained. The sample was then dispersed in a sonicator bath (Elmasonic Model S100H, Germany) for 2 h at 30 °C and was kept at 4 °C for further use.

2.4. Characterization of cellulose nanocrystals

2.4.1. Transmission electron microscopy (TEM)

Transmission electron microscopy is an analytical method to show the general morphology of CNCs. The solution of CNCs was dropped into a carbon-coated copper grid, coated with 1.5% uranyl acetate for 5 min and analyzed using a transmission electron microscope (Hitachi model HT7700; Japan) with an electric potential for analysis of 80 kV.

2.4.2. Atomic force microscopy (AFM)

An atomic force microscope (Asylum Research model MFP-3D AFM (Bio), USA) was used to characterize the morphology and the dimensional image of the CNCs. The solution of CNCs was diluted and dropped on the surface of a microscope slide. Then, it was allowed to dry at room temperature (RT) and analyzed. The dimension of the sample was measured using Gwyddion software.

2.4.3. Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR)

The ATR-FTIR spectra of the CNCs were recorded using a spectrophotometer (Thermo Scientific Nicolet IR200, USA). The sample was heated at 50 °C until dried and cut or ground into small pieces. The samples were analyzed at wavenumbers in the range 400–4000 cm^{-1}.

2.4.4. X-ray diffraction (XRD)

The crystallinity of the sample after acid hydrolysis was obtained using an X-ray diffractometer (Bruker model D8 Advance, USA) with Cu Kα radiation. The condition was set at a speed of 5°/min and the sample was scanned at θ in the range 5–40°. The crystallinity index (Crl) calculation was determined using Eq. 1 (Segal, Creely, Martin, & Conrad, 1959). I_{002} is both crystalline and amorphous and I_{am} is an amorphous material.

$$CrI\ (\%) = \frac{(I_{002} - I_{am})}{I_{002}} x100 \quad (1)$$

2.4.5. Thermogravimetric analysis (TGA)

The thermal properties of the fibre in each step were measured using a thermogravimetric analyzer (Mettler Toledo, TGA/SDTA 851e, Switzerland). Samples of 2–5 mg were weighed and heated from 25 to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere.

2.5. Preparation of cellulose nanocrystal combined with WPI for film application

Whey protein isolate was prepared (5% by weight). The plasticizer used in this study was glycerol, which was 50% solid. The CNCs varied from 0 to 8% by wt. and then was added into the whey solution to form film. To control the solution under neutral conditions, 2.0 M of NaOH was used to adjust the pH to 7. The solution was properly mixed with an overhead stirrer (IKA RW 20 digital, Malaysia) in a water bath at 90 °C for 30 min and cooled down at RT for 15 min. The solution was degassed in a sonicator bath for 10 min and 30 g was poured into a Teflon mold (10 × 10 × 1.5 cm^3), after which it was stored in a controlled incubator at 50 °C (Binder model FED115, Germany) for 15 h to form the film. The film was removed from the mold and controlled in the standard condition, (50% relative humidity, 25 °C and 48 h) before testing the film's characteristics. The WPI films at 0, 2, 5 and 8% CNC were named as WPI, WPI-2, WPI-5 and WPI-8, respectively.

2.6. Characterization of whey protein film incorporated with cellulose nanocrystals

2.6.1. Water activity (a_w)

Film was analyzed for its water activity by keeping it in a desiccator that contained saturated magnesium nitrate ($Mg(NO_3)_2$) at 25 °C for at least 7 days. Then, 2 g of sample was measured using a water activity meter (Testo 650 Water Activity System, Testo Inc., USA).

2.6.2. Solubility

First, the film was cut into rectangular (1 × 1 cm) pieces and dried in an oven at 70 °C for 24 h. The sample was placed into the tube filled with distilled water (10 mL) at RT for 24 h. Filter paper number 1 was prepared by drying at 150 °C for 1 h. and weighed after it had dried. Then, the film was poured onto the filter paper and dried at 105 °C for 3 h. Later, it was kept in a desiccator containing silica gel and weighed after drying. The % solubility was calculated using Eq. 2: