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Soybean straw nanocellulose produced by enzymatic or acid treatment as a reinforcing filler in soy protein isolate films

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ABSTRACT

This work is a comparative study of the application of mercerized soybean straw (MSS) and nanocellulose produced by acid (CNCs) or enzymatic hydrolysis (CNFs) as reinforcing fillers in soy protein isolate (SPI) films. CNCs presented average dimensions of about 10 nm-thick and 300 nm-long with a crystallinity index of 57%, whereas CNFs have similar diameters, though with greater lengths (> 1 μm), lower crystallinity index (50%) and greater thermal stability. Incorporation of 5% of CNCs and CNFs (g/100 g of SPI) improved the SPI film tensile strength by 38 and 48% respectively, and decreased the SPI film elongation at break when compared to control films. The SPI-CNC films showed the lowest values for solubility, probably due to their higher crystallinity (63%). On the other hand, the water vapor permeability was solely reduced with CNF addition, which can be attributed to their higher aspect ratio (length/diameter) and a better incorporation into the protein matrix.

1. Introduction

Owing to recent technological innovations, biodegradable polymers have gradually replaced synthetic polymers, helping to overcome serious environmental issues. Although the complete substitution of synthetic polymers by their biodegradable counterparts is virtually impossible, several attempts to produce biodegradable films with acceptable processability and end-use properties (mechanical and barrier features), have been done (Azeredo & Waldron, 2016; Jimenez, Fabra, Talens, & Chiralt, 2012; Shi & Dumont, 2014; Wihodo & Moraru, 2013; Zink, Wyrobnik, Prinz, & Schmid, 2016).

Soy proteins are among the various sources of recently investigated renewable materials (Brandenburg, Weller, & Testin, 1993; Koshy, Mary, Thomas, & Pothan, 2015). However, soy proteins processed in the form of films are characterized by having low water vapor barrier properties and moderate mechanical resistance when compared to synthetic polymers films (Krochta, 2002; Kumar, Sandeep, Alavi, Truong, & Gorga, 2010). Several methods for improving the properties of soy protein-based films, such as thermal, chemical, enzymatic, irradiation treatments and incorporation of reinforcing fillers, have been evaluated with different efficacies (Wihodo & Moraru, 2013). Particularly, the use of reinforcing fillers has been proved to be a satisfactory approach, which consists in the addition of organic or inorganic nanometric sized particles prepared from silver (Zhao, Yao, Fei, Shao, & Chen, 2013), montmorillonitte clays (Kumar et al., 2010), carbon (Li et al., 2016), or nanofibers extract from cellulosic materials (Samir, Alloin, & Dufresne, 2005; Zhang et al., 2016).

Cellulose nanofibers, or nanocellulose, are assumed as suitable reinforcing fillers. These fibers can originate after underwent chemical or enzymatic hydrolysis, followed by mechanical treatment of different lignocellulosic materials. The used extraction mode influences the dimensions, the composition, and the properties of the resulting nanocellulose, which comprises two groups: (i) cellulose nanocrystals (CNCs) or cellulose whiskers, originated from acid hydrolysis, and (ii) cellulose nanofibrils (CNFs) or micro/nanofibrillated cellulose, resulted from enzymatic hydrolysis and/or mechanical processes (Nechyporchuk, Belgacem, & Bras, 2016).

Concentrated sulfuric acid is commonly employed in acid hydrolysis reaction, which hydrolyzes the amorphous regions of the cellulose structure. CNCs typically have a rigid structure associated with a high degree of crystallinity (64–74%), bearing negatively charged sulfate groups on their surface. In general, CNCs have diameters of 4–15 nm

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and between 500 nm and 2 μm long, greatly dependent on the applied extraction procedure (Alemdar & Sain, 2008; Flauzino Neto, Silvério, Dantas, & Pasquini, 2013; Samir, Alloin, Sanchez, El Kissi, & Dufresne, 2004).

The enzymatic and mechanical methods (e.g., high-pressure homogenizer), on the other hand, afford strongly entangled bundle of fibrils with diameter in the nanoscale (5–110 nm) and length of few micrometers (de Campos et al., 2013; Kaushik, Singh, & Verma, 2010; Martelli-Tosi, Torricillas, Martins, Assis, & Tapia-Blácido, 2016; Paakko et al., 2007). For CNF preparation, xylanases and cellulases are the most used enzymes which are also usually applied in the second generation biorefineries. Xylanases are important because they can degrade the hemicelluloses remained in mercerized soybean straw (MSS), breaking the linear polysaccharide β-1,4-xylan into xylose (Saha, 2003). Cellulases acts in synergism with xylanases, cleaving the cellulose molecules into shorter polysaccharides or sugars via hydrolysis of β-1,4-D-glycosidic linkages (Coughlan, 1985). Therefore, by using appropriate defibrillation mechanical treatments, CNFs and microfibers may be resulted as a co-product of second generation processes.

Soybean straw is a rich source of cellulose fibers and an abundant agricultural residue. The world generates over 240 million tons annually of soybean straw most disposed as waste in landfills or incinerated (FAOSTAT, 2017). The raw soy straw (around 50% of the soybean mass) has an average composition of 35% cellulose, 21% insoluble lignin, 17% hemicelluloses, 11% ash, and 1% acid soluble lignin, with small fractions of protein, pectin, and glucuronic acid substitutes (Cabrera et al., 2015; Wan, Zhou, & Li, 2011). In our previous study, we reported that microfibers as fillers, prepared from chemically pretreated soybean straws, were important in enhancing the mechanical properties of soy protein isolate (SPI) films (Martelli-Tosi et al., 2017). It was shown that films containing soybean straw treated with NaOH 17.5% and H_2O_2 presented lower solubility and have no effect on SPI films water vapor permeability. The hypothesis of the present study is that nanocellulose obtained from more environment-friendly processes, as enzymatic hydrolysis, can be properly used as reinforcement nanofiller, improving SPI films properties, as similar as CNCs. Hence, the main aims of this work were: (1) to extract and characterize nanocellulose, by chemical (CNCs) or enzymatic (CNFs) hydrolysis from MSS (pre-treated with NaOH 17.5% and H_2O_2), and (2) to verify how incorporation of these fibers affects the mechanical and thermal properties of SPI cast films.

2. Materials and methods

2.1. Materials

Soybean straw was provided by Embrapa Soja (Londrina, Brazil). Hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), ethanol (PA) and acetone were all purchased from Dinâmica Química Contemporânea LTDA (Brazil). The enzymatic cocktail Optimash™VR was obtained from DuPont Inc. (Newark, USA).

2.2. Production of soybean straw nanofibrils (CNFs) and nanocrystals (CNCs)

Prior to nanocellulose extraction, the soybean straw was submitted to chemical treatments following the procedure presented by Martelli-Tosi et al. (2017), in which 100 g of soybean straw was suspended in a solution of 17.5% NaOH (w/v) at room temperature (20–30 °C) for 15 h. After this period, the material was filtered and washed with distilled water until neutral pH was achieved. The resulting fibers were bleached with a mixture of 4% H_2O_2 (w/v), 2% NaOH (w/v), and 0.3% $MgSO_4.7H_2O$ (as a stabilizer) at 90 °C for 3 h. The suspension was cooled to room temperature, and the fibers filtered, washed with distilled water until neutral pH was reached, and rinsed with ethanol and acetone. The final product was dried at 50 °C in an assisted air circulation oven (Marconi, Brazil). This sample was identified as mercerized soybean straw (MSS).

CNFs were produced by hydrolysis of MSS with the enzymatic cocktail Optimash™ VR (93 μL/g of MSS), equivalent to approximately 134 U of endoglucanase and 446 U of xylanase per gram of MSS), followed by mechanical homogenization at 14,000 rpm in an Ultraturrax disperser (T18 IKA, China) for 5 min, plus 3 min of probe-type sonication (Branson 450, Switzerland). Method which was described in detail in a previous publication (Martelli-Tosi et al., 2016). The reducing groups, remained from enzymatic treatments in the CNF suspension, were quantified according to the dinitrosalicylic acid (DNS) method (Miller, 1959).

CNCs were obtained by MSS acid hydrolysis according to the procedure reported by Flauzino Neto et al. (2013), with some modifications. A mixture of 30 mL of H_2SO_4 64% (w/v) per gram of MSS was heated to 70 °C and kept under stirring for 40 min. The suspension was diluted tenfold in cold water to cease the hydrolysis reaction, and centrifuged at 7000 rpm for 10 min to remove the acid in excess. The precipitate was dialyzed with tap water until a neutral pH was achieved (5–7 days). The resulting material was subject to the same mechanical treatments as applied for the CNF suspensions.

The CNFs and the CNC colloidal suspensions were stored in a refrigerator at 4 °C with the addition of some drops of chloroform to prevent any bacterial growth before the film were produced.

2.3. Morphologies, zeta potential values and yields of soybean straw CNFs and CNCs

The CNF and the CNC morphologies were observed under a JEOL transmission electron microscope (JEM 100CXII, Tokyo, Japan) operating at 80 kV. After sonication in an ultrasonic bath for 2 min, a drop of diluted suspension was deposited on a carbon-coated grid and dried at room temperature for 24 h. The grid was stained by 2 min immersion in an aqueous solution of 1.5% uranyl acetate and dried at room temperature. The fiber dimensions were determined directly from the TEM images using ImageJ analysis software (NIH, Bethesda, USA). Around 30 measurements were acquired for each sample.

The zeta potential values of the suspensions of the CNF and the CNC suspensions were determined in a Zetasizer Nano ZS instrument (Malvern Instruments, UK) coupled to a ZEN 1020 dip cell at 25 °C. The measurements were repeated three times for each sample, and three samples were analyzed for each suspension (CNFs or CNCs).

The CNF and CNC yields were determined from the mass of the nanocellulose suspension obtained after drying the CNFs or the CNCs were dried to constant mass in an oven at 50 °C. The nanocellulose yields (g of CNCs or CNFs/100 g of MSS) were calculated according to the relation (Martelli-Tosi et al., 2016):

$$\text{Yield of CNFs or CNCs (\%)} = (\text{dried mass/MSS}) \times 100 \quad (1)$$

where MSS is the amount of mercerized soybean straw that was initially used to extract the CNFs or the CNCs.

2.4. Preparation of nanocomposite films

The composite films were prepared from three different formulations: (i) a control formulation, processed from an aqueous SPI suspension at 5% w/w, to give the SPI 5%; (ii) an SPI suspension (at 5%) added with the CNC suspension (at 0.25% w/v, from acid hydrolysis), to give the nanocomposite SPI 5%_CNCs, and (iii) an SPI suspension added with the CNF suspension (at 0.25% w/v, from enzymatic hydrolysis), to give the nanocomposite SPI 5%_CNFs. Therefore, the final concentration of nanocellulose in the nanocomposites was 5 g of CNCs or CNFs/100 g of SPI.

All of the mixtures were homogenized in an Ultraturrax