Carbohydrate Polymers 93 (2013) 144–153

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Property evaluations of dry-cast reconstituted bacterial cellulose/tamarind xyloglucan biocomposites

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ARTICLE INFO

Article history:
Received 30 October 2011
Received in revised form 30 March 2012
Accepted 24 April 2012
Available online 4 May 2012

Keywords:
Bacterial cellulose
Xyloglucan
Polysaccharide interactions
Biocomposite
Surface properties
Hydrophilicity

ABSTRACT

We describe the mechanical defibrillation of bacterial cellulose (BC) followed by the dry-cast generation of reconstituted BC films (RBC). Xyloglucan (XGT), extracted from tamarind seeds, was incorporated into the defibrillated cellulose at various compositions, and new films were created using the same process. Microscopy and contact angle analyses of films revealed an increase in the microfibre adhesion, a reduced polydispersity in the diameters of the microfibrils and increased hydrophobic behaviour as a function of %XGT. X-ray diffraction analysis revealed changes to the crystallographic planes of the RBC and the biocomposite films with preferential orientation along the (1 1 0) plane. Compared with BC, RBC/XGT biocomposite with 10% XGT exhibited improvement in its thermal properties and in Young's modulus. These results indicated a reorganisation of the microfibres with mechanical treatment, which when combined with hydrocolloids, can create cellulose-based materials that could be applied as scaffolding for tissue engineering and drug release.

1. Introduction

The versatility of biopolymers allows for new applications in the food, pharmaceutical and biotechnology industries. The structures and functionalities of polysaccharides, proteins and lipids allow their utilisation in biomimetic and nanotechnology systems, including biosensors, transistors or mechanical modifiers. Cellulose is one of the most studied biopolymers, and several sources of this biopolymer have been evaluated. The backbone chains of cellulose, consisting of long linear chains of 1,4-β-D-glucopyranose, are organised by hydrogen bonds that form a hard network structure. This linearity yields multiple elementary fibrils that aggregate into larger bundles, which can contain crystalline and amorphous regions. The degrees of crystallinity and the crystal dimensions are dependent on the origin of the cellulose or on the modification (chemical or physical) to which it was submitted (Guo & Catchmark, 2012; Sugiyama, Vuong, & Chanzy, 1991; Tischer, Sierakowski, Westfahl, & Tischer, 2010; Wong, Kasapis, & Tan, 2009; Woodcock & Sarko, 1980). Specifically, cellulose from bacterial sources exhibits higher crystallinity and has distinct advantages over cellulose from other sources. These advantages include high purity (pretreatment is not required for the extraction of lignin or hemicelluloses) and high surface area when compared with plant cellulose (Guo & Catchmark, 2012). Therefore, the great interest in bacterial cellulose (BC) is justified by its many potential applications. These applications include medical devices (Nge, Nogi, Yano, & Sugiyama, 2010) and wound dressings to prevent infection caused by bacterial or fungal agents that can occur as skin heals (Czaja, Young, Kawecki, & Brown, 2007; Jonas & Farah, 1998).

Many of the functional properties of cellulose depend on its capacity to interact with diverse molecules or macromolecules of varying polarity. The adsorption and adhesion phenomena depend on the organisation of glucan chains located at the surface of the cellulose microfibrils (Mazeau, 2011). The association of hydrophilic structures, including hydrocolloids such as xyloglucan, with cellulose can modify the mechanical and chemical properties of the cellulose (Zhou, Rutland, Teeri, & Brumer, 2007). The structural description of the cellulose microfibril surfaces or that of cellulose associated with other molecules can be performed using several techniques. Recent studies include atomic force microscopy (AFM), scanning electron microscopy (SEM), contact angle (CA) measurements and small- and wide-angle X-ray diffraction (SAXS and WAXS) (Castro et al., 2011; Elazzouzi-Hafraoui et al., 2008;

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http://dx.doi.org/10.1016/j.carbpol.2012.04.062

Huang, Chen, Lin, Hsu, & Chen, 2010; Klechkovskaya et al., 2003; Woehl et al., 2010). The results have provided information regarding the morphology on various scales, the hydrophilicity-related properties, the microfibril dimensions and the relative crystallinity of these cellulose samples.

Recent advances in computer modelling and surface force analysis have improved our understanding of the cellulose chains and the cellulose–xyloglucan interactions at the molecular level (Mazeau, 2011; Zhang, Brumers, Agren, & Tu, 2011). *In vitro*, these complex structures are important to many applications particularly in the biotech industries. Some studies have demonstrated that cellulose-based films, after processing, show selective permeability, allowing the passage of water vapour while preventing the passage of microorganisms (Chang & Zhang, 2011; Klemm et al., 2009).

A commercial xyloglucan product isolated from tamarind seeds (*Tamarindus indica*) has important applications, especially in pharmaceutical formulations to obtain gels (Salazar-Montoya, Ramos-Ramírez, & Delgado-Reyes, 2002; Yamanaka et al., 2000), and as a drug vehicle for controlled-release systems (Burgalassi, Chetoni, Panichi, Boldrini, & Saettone, 2000; Coviello, Matricardi, Marianecci, & Alhaique, 2007; Jó, Petri, Beltramini, Lucyszyn, & Sierakowski, 2010; Kawasaki et al., 1999; Miyazaki, Kawasaki, Endo, & Attwood, 2001). This biopolymer is formed by a 1,4-β-D-glucopyranosyl backbone partially substituted with 1,6-α-D-xylopyranosyl side chains, some of which are further substituted with 1,2-α-D-galactopyranosyl residues (Freitas et al., 2005; Hayashi, 1989; Jó, Petri, Valenga, Lucyszyn, & Sierakowski, 2009; Muller et al., 2011; Stupp et al., 2008; York, Harvey, Guillen, Albersheim, & Darvill, 1993).

In addition, because xyloglucan (XG) has storage or structural functions in plants, its association with cellulose has been widely studied in relation to biosynthesis and the growth process of higher plant organisms (Zhou et al., 2007). These studies have enabled new discoveries and applications based on the cellulose–xyloglucan interaction. For example, the development of XG as a molecular anchor to tether chemical functionality to cellulose opened new possibilities for industrial plant fibre modification in which it is used as a dispersing agent or to build nanostructured surfaces (Muller et al., 2011; Zhou et al., 2007). In all cases, the cellulose–xyloglucan interaction is slightly dependent on its structural details as observed by Lopez et al. (2010). The authors observed that the adsorption is dependent on M_w and on the side chains.

The development of biocomposites from cellulose and other additives has been widely explored. However, the methodologies typically used include a pretreatment of the cellulose, which involves a rigorous process of solubilisation and reconstruction of the cellulose membrane. Thus, the purpose of this research is to use bacterial cellulose (BC) in the development of films by first mechanically defibrillating and reconstructing the cellulose *via* a dry-cast process to produce RBC. In addition, the inclusion of tamarind XG as a substitute for defibrillated BC pulp was explored at varying percentages to produce biocomposite films with distinct properties. The structural, morphological, thermal and mechanical stabilities were evaluated using several techniques. The results obtained can promote new methods for the production of cellulose-based materials for use in, among other applications, tissue engineering support and drug release.

2. Materials and methods

2.1. Polysaccharide sources

Prior to the dry-cast process, the xyloglucan was obtained from *T. indica* seeds by aqueous extraction from tamarind kernel powder (TKP, Balasanka Mills, India) with an approximately 55–65% recovery of the polysaccharide (Menon et al., 2010). The chemical composition of the TKP used in the present work has been previously reported (Jó et al., 2009, 2010).

The TKP was dispersed in distilled water at 30 °C and stirred overnight, then sonicated for 30 min using a solid 25-mm probe (VCX 750, Sonics & Materials, Inc., Newton, CT, USA). The insoluble fraction was separated by centrifugation at 6311 × *g*, and the soluble fraction was precipitated with the addition of commercial ethanol (98%, Dipalcool Distribuidora de Álcool Ltda, Almirante Tamandaré, PR, Brazil), washed with acetone and dried in an oven at 50 °C. Then, the purified xyloglucan (XGT) was characterised by gel permeation chromatography (GPC), in a Viscotek 270 Dual Detector GPC, with PWxl columns (2500, 4000 and 6000 g/mol), at 30 °C using 0.1 mol/L $NaNO_3$ with 0.02% (w/v) NaN_3 as an eluent and a *dn*/*dc* increment of 0.144.The never-dried bacterial cellulose (BC) cultured from *Acetobacter xylinum* was donated by Membracel Produtos Tecnológicos Ltda®, located at Almirante Tamandaré, PR, Brazil. Because the BC was received in the presence of hypochlorite and acetic acid, neutralisation was performed, and the membranes were dialysed against distilled water. The conductivity and pH were monitored until constant values (~4.8 μS/cm and ~6.8) were obtained. Finally, the material was washed several times with ultrapure water. For comparative purposes, dried commercial BC from the same company was used as a control in all experiments.

2.2. Achievement of dry-cast cellulose biocomposites

The never-dried BC (99 wt% of water) (Klemm et al., 2009) was submitted to mechanical treatment to yield a pulp using a blade mixer over 30 min. To this, 2 L ultrapure water was added for each 20 g of wet BC.

Various volumes of an aqueous XGT solution were incorporated into the pulp to yield final compositions of 10, 20 or 30 wt%.

The dispersions, deposited on polypropylene petri dishes, were oven dried for 48 h at 37 °C to yield RBC or biocomposite films.

2.3. Biocomposite characterisations

2.3.1. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) analysis

The SEM images were obtained using a JEOL JSM-6360LV microscope (JEOL Ltd., Tokyo, Japan) at 10 kV and at a magnification of 10,000×. All samples were covered by a thin layer of gold (<10 nm) to improve the conductivity of the surface.

The AFM analyses were performed on an Agilent microscope (Agilent Technologies, Santa Clara, CA, USA) using Pico Image software (Agilent Technologies, Santa Clara, CA, USA). The tapping mode images were obtained with Vistaprobes® (Nanoscience Instruments, Inc., Phoenix, AZ, USA) silicon tips (nominal spring of 48 N/m and resonance frequency of ~180 kHz), and the scanning was over 2.0 μm × 2.0 μm and 5.0 μm × 5.0 μm. The data treatment and presentation were accomplished with the help of Gwyddion Software (Czech Metrology Institute).

2.3.2. Contact angle (CA) analysis

The CA analysis was obtained in a DataPhysics GmbH tensiometer (Filderstadt, Germany), model OCA 15plus. A study of the hydrophilicity on the surface of the biocomposites was performed using the sessile drop method with ultrapure water as the solvent. The measurements were conducted at 25 °C using a 500-μL Hamilton syringe (Bonaduz, Switzerland) and a needle with an internal diameter of 1.37 mm, an external diameter of 1.65 mm and a length of 38.1 mm.

The surface free energy was obtained using Young's equation with the contact angle results. The calculation was performed with