Carbohydrate Polymers 87 (2012) 2031–2037

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

High performance edible nanocomposite films containing bacterial cellulose nanocrystals

Johnsy George [a,*], Siddaramaiah [b]

[a] Defence Food Research Laboratory, Siddarthanagar, Mysore 570 011, India
[b] Sri Jayachamarajendra College of Engineering, Mysore 570 006, India

ARTICLE INFO

Article history:
Received 29 August 2011
Received in revised form 3 October 2011
Accepted 6 October 2011
Available online 14 October 2011

Keywords:
Edible nanocomposites
Cellulose nanocrystals
Gelatin

ABSTRACT

Bacterial cellulose obtained from *Gluconacetobacter xylinus* in the form of long fibers were acid hydrolyzed under controlled conditions to obtain cellulose nanocrystals. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) confirmed the formation of rod like cellulose nanocrystals having an average diameter and length of 20 ± 5 nm and 290 ± 130 nm respectively. These nanocrystals were used to prepare gelatin nanocomposite films and characterized for elucidating its performance. The formation of percolated networks of cellulose nanocrystals within gelatin matrix resulted in improving the mechanical properties of nanocomposites. The moisture sorption and water vapor permeability (WVP) studies revealed that the addition of cellulose nanocrystals reduced the moisture affinity of gelatin, which is very favorable for edible packaging applications. Results of this study demonstrated the use of bacterial cellulose nanocrystals (BCNCs) in the fabrication of edible, biodegradable and high-performance nanocomposite films for food packaging applications at relatively low cost.

1. Introduction

Various biodegradable polymers have been explored for the development of edible films as an effort to reduce packaging waste created by non-degradable petroleum based food packaging materials (Tharanathan, 2003). The edible films not only provide physical protection to foods but also act as mass transfer barriers for moisture, oxygen, carbon dioxide, lipids, flavors and aromas between food products and the atmosphere (Marcuzzo, Sensidoni, Debeaufort, & Voilley, 2010). Among various biopolymers, protein based films gained more interest in the development of edible coatings due to their nutritive values and better properties (Gennadios, 2004). Gelatin is one such protein based biopolymer, which is widely studied for packaging applications due to their film forming ability and environmental appeal (Sobral, Menegalli, Hubinger, & Roques, 2001). However, the use of gelatin in most of the food packaging applications is limited because of their poor mechanical properties and water sensitivity. A recent technique pursued to overcome some of the limitations of biopolymers is the use of nanomaterials, which can reinforce biopolymers by the formation of nanocomposites (Dufresne, 2006). Owing to the reinforcement provided by the nanometer-sized particles dispersed in the biopolymer matrix, these nanocomposites exhibit markedly improved mechanical, thermal, optical, and physicochemical properties as compared to pure biopolymer (Petersson & Oksman, 2006). Among such nanomaterials, cellulose nanocrystals mainly obtained from plant sources are widely used for improving the properties of biopolymers (Eichhorn et al., 2010). These cellulose nanocrystals are highly crystalline and exhibit excellent properties like high tensile strength and modulus (Uddin, Araki, & Gotoh, 2011). However, for edible applications bacterial cellulose is more preferred over plant cellulose as it is edible, available in relatively pure form and having better properties than that of plant cellulose (Lin & Lin, 2004). In this context, bacterial cellulose that can be converted in to nanocrystals is gaining great importance in the field of edible nanocomposites.

Bacterial cellulose produced in substantial quantities by *Gluconacetobacter xylinus* is well known for its superior properties compared to plant cellulose (Iguchi, Yamanaka, & Budhino, 2000). This cellulose is widely used in several applications like scaffold for tissue engineering (Svensson et al., 2005), acoustic diaphragms (Nishi et al., 1990), ion exchange membranes (Choi et al., 2004), electronic devices (Nogi & Yano, 2008), etc. Apart from these applications, bacterial cellulose is widely used in food applications also, where thick sheets of cellulose were cooked in sugar syrup and added with desserts, fruit cocktails and jellies (Okiyama, Motoki, & Yamanaka, 1992; Vandamme, De Baets, Vanbaelen, Joris, & De Wulf, 1998). Bacterial cellulose, with its distinctly soft texture and high fiber content is a suitable thickening/stabilizing agent in several processed foods. Bacterial cellulose, having high water

* Corresponding author. Tel.: +91 821 2473150; fax: +91 821 2473468.
E-mail address: g.johnsy@gmail.com (J. George).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.10.019

holding capability is used for making composite gels with dolphin fish surimi, for the production of high fiber, low fat and healthy fish based food products (Lin, Chen, & Chen, 2011). The gel like properties of bacterial cellulose combined with its complete indigestibility in the human intestinal tract had made this an attractive food base and dietary fiber (Stephens, Westland, & Neogi, 1990). Recently bacterial cellulose composites containing plant polysaccharides such as pectin were reported to be used as a model system for the fermentation of complex cell wall dietary fibers (Mikkelsen, Gidley, & Williams, 2011). The main advantage of bacterial cellulose is that it can be easily processed into microfibrils, nanofibrils and nanocrystals by employing a top-to-down approach and further used as a reinforcing component in edible nanocomposite films.

The most commonly employed method for the processing of cellulose nanocrystals is by employing acid hydrolysis (Araki, Wada, Kuga, & Okano, 1998; Hirai, Inui, Horii, & Tsuji, 2009). Each cellulose microfibril can be considered as a string of cellulose crystals, linked along the microfibril by disordered or paracrystalline regions (Azizi Samir, Alloin, & Dufresne, 2005). The acid treatment leads to the removal of the para-crystalline domains that are regularly distributed along the microfibers, and leads to the formation of rod-like cellulose nanocrystals (Habibi, Lucia, & Rojas, 2010). These nanocrystals can be physically incorporated into various polymer matrices to form polymer nanocomposites. Cellulose nanocrystals have attracted a great deal of interest in the nanocomposites field due to their appealing intrinsic properties such as nanoscale dimensions, high surface area, unique morphology, low density renewability, biodegradability and high mechanical strength. The conversion of cellulose fibers into nanocrystals results in the formation of whiskers with large aspect ratio, mainly due to their nanoscale dimensions. This superior aspect ratio plays a critical role in their reinforcement potential when used for the fabrication of polymer nanocomposite materials. Most studies reported in the past about edible composite films; use either microcrystalline cellulose or cellulose fibers of plant origin, as a reinforcing component in biopolymers to enhance the mechanical properties (Dogan & McHugh, 2007; Muller, Laurindo, & Yamashita, 2009). The present research investigation explores the possibility of using BCNC derived from edible bacterial cellulose as a nano-structured reinforcing constituent in gelatin matrix to form nanocomposite films. This investigation also reports the use of edible materials as both polymer matrix and reinforcing filler to fabricate completely edible nanocomposites exhibiting better properties. The mechanical, barrier, thermal properties and morphology of these nanocomposite films were evaluated and our results suggested that bacterial cellulose nanocrystals are excellent nanomaterials for fabricating high performance edible nanocomposites.

2. Experimental

2.1. Materials

Bacterial cellulose pellicles were produced by *Gluconacetobacter xylinus* as described elsewhere (George, Ramana, Sabapathy, Jagannath, & Bawa, 2005). The cellulose was purified by boiling the pellicles in 0.2 M aqueous NaOH solution for 30 min followed by rinsing with distilled water for several rounds until a neutral pH was attained in the drained water. The purified pellicle was mechanically disintegrated to a cellulosic paste using a laboratory blender (5000–6000 rpm) for 30 min at ambient temperature. The cellulosic paste in the form of microfibrils was further converted into nanocrystals by hydrolyzing with hydrochloric acid (HCl). About 100 g of cellulose paste (wet weight) was added to 500 mL of 4 N HCl (Reagent grade 36%, Fisher Scientific, Mumbai, India) and allowed to hydrolyze. The mixture was stirred continuously at boiling conditions for 4 h. The BCNC suspension formed was then centrifuged at 5000 rpm for 5 min to remove the acid. The BCNC collected were further washed with distilled water for several rounds till a neutral pH was achieved. BCNC suspensions at different concentrations (1, 2, 3, 4 and 5 wt%) were prepared separately in 100 mL distilled water by ultrasonication for 5 min. The ultrasonication provided necessary mechanical agitation to disperse the nanocrystals. Food grade gelatin was procured from Sd Fine Chemicals, Mumbai, India and its solution was prepared by dissolving 10 g of gelatin in 100 ml distilled water at 65 °C for 4 h. Gelatin-BCNC nanocomposites were prepared by the stochiometric addition of BCNC suspension to a calculated quantity of gelatin solution (100 mL) followed by stirring and ultrasonication for 30 min. The nanocomposite solutions were casted in polypropylene petri dishes and dried at 37 °C for 48 h and used for further characterization.

2.2. Methods

TEM images were obtained using JEOL JEM 3010 equipment (Japan) operated at an accelerated voltage of 300 kV. A very dilute cellulose nanocrystal suspension was drop casted on a carbon coated copper grid and used for imaging. The morphology of cellulose nanocrystals were also studied using AFM, using a Solver PRO-M scanning probe microscope from NT-MDT, Ireland. A drop of very dilute cellulose nanocrystal suspension (0.05 wt/wt) was allowed to dry at room temperature overnight on a freshly cleaved mica substrate. The images were acquired using non-contact mode in air with silicon cantilevers (NSG-01), having a tip curvature radius of 10 nm and a force constant of 1.45–15.1 N/m. The resonant frequency of the cantilever was 87–230 kHz. The images were taken on at least three different areas of each sample. The image processing software was used to determine the average diameters and lengths of BCNC by employing ten different independent measurements. Scanning electron micrographs (SEM) of gelatin nanocomposites were obtained using EVO LS10 SEM (Zeiss, UK). The morphological analysis was carried out at high vacuum using an operating voltage of 20 kV. The nanocomposite film samples were briefly immersed in water and rinsed to partially dissolve gelatin on the surface of the sample and to expose the inner structure of film. Infra red spectra of polymer nanocomposite films were recorded using a FTIR (Thermo Nicolet, Model 5700) spectrophotometer.

Mechanical properties of nanocomposite films were evaluated in tensile mode using an Universal Testing Machine (Lloyd Instruments, Model: LRX Plus) with a gauge length of 25 mm and cross head speed of 100 mm/min. Samples were cut into rectangular strips with dimensions of $5'' \times 1''$ and five strips were tested for each sample. Moisture sorption analysis was carried out using a moisture sorption analyzer (Q 5000 SA, TA Instruments, USA) under controlled conditions of temperature and humidity. The stepwise adsorption and desorption studies of the film samples were carried out from 10% RH to 90% RH and back at a step interval of 10% RH at 25 °C. At each RH level, equilibration was stopped when the relative change in sample mass remained below 0.01% for 5 min, and the next RH step was automatically applied. WVP of the nanocomposite films was determined according to ASTM E96-00 method. Water vapor transmission rate (WVTR) was calculated by recording the weight gain of each film samples at 24 h interval for a period of 21 days and plotting it as a function of time. The WVTR was calculated from the slope of weight gain Vs time curve. The WVP of these films were determined using the equation, $WVP = (WVTR \times L)/\Delta P$, where L is the mean film thickness (mm) and ΔP is the partial vapor pressure difference (Pa) across the two sides of the film. Differential scanning calorimeter with a thermal analyst 2100 system (Model DSC2910, TA Instruments, USA) was used to determine the glass transition temperature (T_g) and helix coil transition

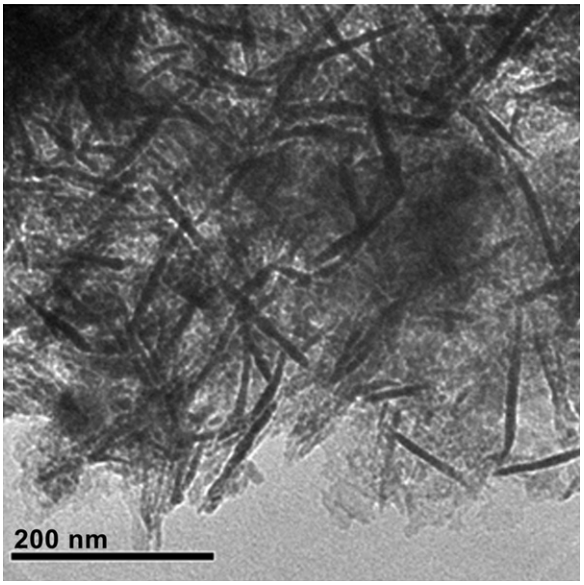

Fig. 1. TEM images of bacterial cellulose nanocrystals obtained after acid hydrolysis treatment.

temperature (T_m) of gelatin nanocomposite films. Samples were scanned at a heating rate of 10 °C/min over a temperature range of −30 °C to 200 °C. Thermogravimetric analyzer (TGA Q50, TA Instruments, USA), a thermal weight change analysis instrument, was used in conjunction with a thermal analysis controller to analyze the thermal degradation aspects of nanocomposite films. The samples were kept in a platinum crucible and heated in a furnace, flushed with N_2 gas at the rate of 40 ml/min, from 30 °C to 700 °C, at a heating rate of 10 °C/min. The derivative weight loss was plotted against temperature for all the samples.

3. Results and discussion

3.1. Bacterial cellulose nanocrystals and their morphology

Bacterial cellulose in the form of long fibers was subjected to acid hydrolysis to obtain nanocrystals. The crystalline regions present in the cellulose are more resistant to acid hydrolysis as compared to the amorphous region. Hence, by employing acid hydrolysis at controlled conditions, crystalline cellulose in the form of rod shaped particles was obtained. TEM analysis was used to confirm the formation of nanocrystals (Fig. 1). TEM image clearly shows that the nanocrystals obtained from bacterial cellulose fibers are rod like units, which is in agreement with earlier reports (Araki & Kuga, 2001). The rod shaped nanocrystals are also having a tendency to aggregate together due to their strong hydrogen bonding tendency between surface hydroxyl groups. Hydrochloric acid hydrolysis unlike sulfuric acid hydrolysis produces cellulose nanocrystals without any surface charges (Hirai et al., 2009). The absence of such surface charges promotes the aggregation of cellulose through hydrogen bonding. AFM analysis was also used to study the morphology of nanocrystals in detail and used to measure its dimensions (Fig. 2a). The AFM image of an individual BCNC was selected to measure the length and diameter of these nanocrystals (Fig. 2b). AFM analysis of ten different nanocrystals, as shown in Fig. 2b was carried out to obtain the mean values of the length and diameter of BCNC. The average diameter and length of these BCNC were found to be 20 ± 5 nm and 290 ± 130 nm respectively. This data was used to calculate the aspect ratio of these nanocrystals, which was found to be 15 ± 8. The standard error found in the

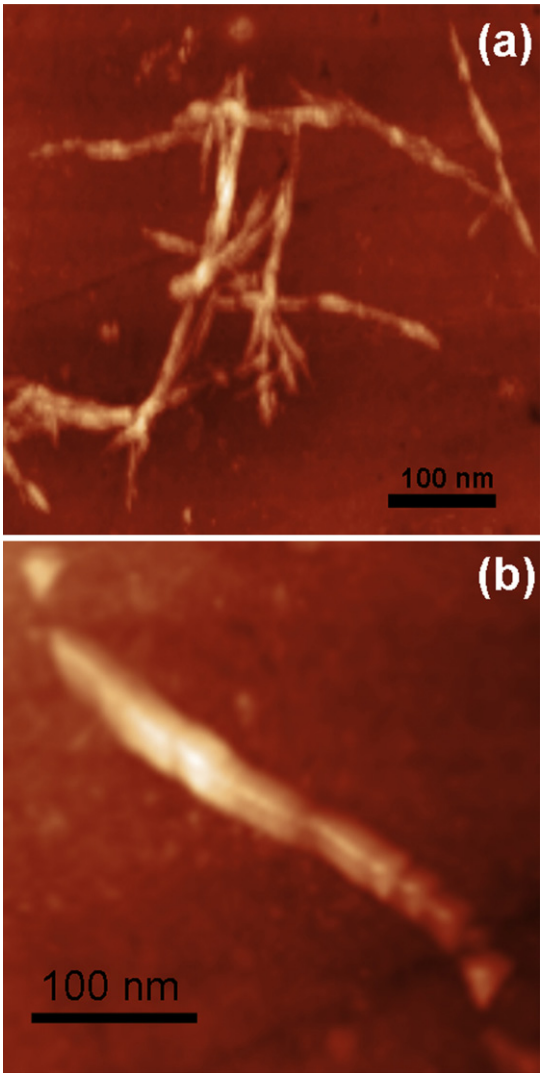

Fig. 2. AFM images of (a) bacterial cellulose nanocrystals obtained after acid hydrolysis treatment and (b) an individual bacterial cellulose nanocrystal.

measurement of aspect ratio (ΔA) of BCNC was calculated using the propagation of error formula,

$$\Delta A = A\sqrt{\left(\frac{\Delta D}{D}\right)^2 + \left(\frac{\Delta L}{L}\right)^2} \quad (1)$$

where A is the aspect ratio, ΔD and ΔL are the errors in the diameters (D) and lengths (L) of the nanocrystals respectively (Rusli, Shanmuganathan, Rowan, Weder, & Eichhorn, 2011). The error in determination of aspect ratio is high due to the difficulty in measuring the exact dimensions of nanocrystals due to overlap and aggregation.

3.2. Characterization of edible nanocomposite films

Gelatin reinforced with edible bacterial cellulose nanocrystals were characterized for elucidating its properties. FTIR spectroscopy was used to confirm the existence of cellulose nanocrystals in gelatin matrix. FTIR spectrum (see the Supporting Information, S-1) shows that gelatin is having two absorption peaks in the range of 1000 and 1100 cm^{-1}, one at 1084 cm^{-1} and another at 1030 cm^{-1} which corresponds to C–O stretching vibrations of gelatin (Muyonga, Cole, & Duodu, 2004). In the case of gelatin BCNC nanocomposites, two more additional bands were observed

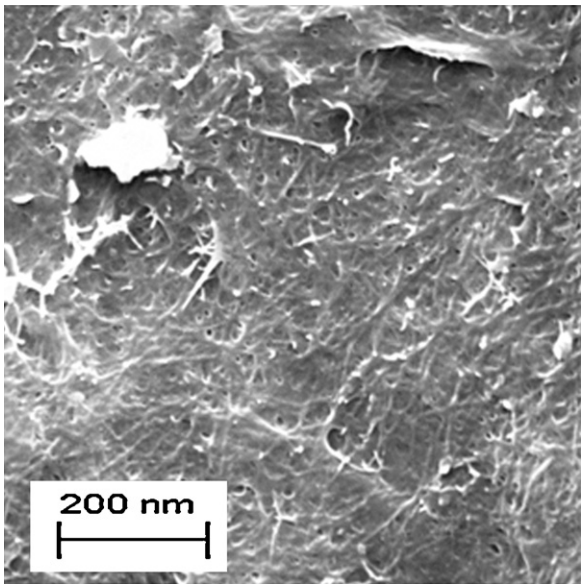

Fig. 3. SEM images of gelatin nanocomposite containing 4 wt% of BCNC.

in this region at 1060 cm^{-1} and 1110 cm^{-1}. The band at 1060 cm^{-1} is mainly due to the C–O stretching vibrations at C_3 position of cellulose and the band at 1110 cm^{-1} is due to ring breathing mode of C–O–C glycosidic linkages of cellulose nanocrystals (Li & Renneckar, 2011). The presence of these two bands along with the characteristic bands of gelatin confirms the existence of cellulose nanocrystals in gelatin matrix. Fig. 3 shows the SEM image of gelatin nanocomposite reinforced with 4 wt% of BCNC. The dispersion and distribution of BCNC seems to be uniform through out the polymer matrix. The cellulose nanocrystals have formed a sheet like network within the polymer matrix. The cellulose nanocrystals are well known for the formation of a percolating network like architecture, due to strong hydrogen bonding existing between the surface hydroxyl groups of cellulose (Capadona, Shanmuganathan, Tyler, Rowan, & Weder, 2008). Even though high intensity ultrasonication was provided before film casting to disperse the cellulose nanocrystals in gelatin matrix, the nanocrystals are showing a tendency to re-aggregate and from a network like structure especially at higher BCNC content in the nanocomposite.

3.3. Mechanical properties

The stress versus strain curves (see the Supporting Information, S-2) of neat gelatin and its nanocomposites reinforced with varying concentrations of BCNC (1–5 wt%) shows that the tensile strength and modulus of gelatin nanocomposites were increased significantly with the addition of nanocrystals. The addition of 4 wt% nanocrystals increased the tensile strength from 83 MPa to 108 MPa, while the elastic modulus increased from 2189 MPa to 2350 MPa (Table 1). Here the cellulose nanocrystals are acting as load bearing components and the effective load transfer from the polymer chains to the nanocrystals resulted in improved tensile strength and modulus. However the addition of nanocrystals beyond 4 wt% reduced the mechanical properties.

The higher aspect ratio of BCNC (~15) is playing an important role in increasing the stress transfer efficiency. An increased aspect ratio will increase the surface area of the reinforcing phase that is effectively in contact with the polymer and facilitates easy stress transfer through filler polymer matrix adhesion. In addition to filler matrix interaction, the filler–filler interactions are also important while studying the reinforcing capability of cellulose nanocrystals. The formation of a percolated network like architecture of cellulose nanocrystals within the polymer matrix that can act as a load bearing components in polymer nanocomposites is reported elsewhere (Capadona et al., 2008). Even though, a good dispersion and distribution of BCNC was achieved in gelatin matrix by sonication, the nanocrystals re-aggregate together to form a percolating network within the polymer matrix upon evaporation of water. This type of network type architecture formed due to strong hydrogen bonding interactions are known to maximize the stress transfer and there with the overall modulus of nanocomposites (Capadona et al., 2007). This claim was supported by the calculations obtained using a percolation model for tensile modulus of the nanocomposites. The theoretical tensile modulus at various volume fractions of cellulose nanocrystals in gelatin matrix were calculated using the following equations (Capadona et al., 2007),

$$E' = \frac{(1 - 2\psi + \psi X_r)E'_s E'_r + (1 - Xr)\psi E'^2_r}{(1 - X_r)E'_r + (Xr - \psi)E'_s} \quad (2)$$

where

$$\psi = X_r \left(\frac{X_r - X_c}{1 - X_c} \right)^{0.4} \quad (3)$$

E'_s and E'_r are the tensile modulus of gelatin and cellulose nanocrystal films, which were experimentally, determined as 2189.5 MPa and 3550 MPa respectively. ψ is the volume fraction of whiskers that participate in the load transfer; X_r is the volume fraction of nanocrystals and X_c is the critical nanocrystal percolation volume fraction. X_c is calculated as $0.7/A$, where A is the aspect ratio of nanocrystals, which was determined using AFM as 15. The value of X_c, which indicates that the percolation threshold or the critical volume fraction needed to reach the geometrical percolation of the nanocrystals was found to be 0.047.

The variation in experimental and theoretical tensile modulus obtained for gelatin nanocomposites was plotted as a function of volume fraction of BCNC (see the Supporting Information, S-3). The results indicate that the experimentally determined modulus is in agreement with the values obtained from Eq. (2) at higher volume fractions. The experimental values were deviated from the predicted values at lower volume fractions like 0.03 which is roughly equivalent to 1 wt%. This behavior supports the hypothesis that percolated networks will be formed only when the volume fraction exceeds the percolation threshold values. The percolation threshold value obtained from the theoretical calculation found to be equivalent to 1.57 wt% of BCNC, which clearly shows that below this concentration, geometrical percolation within the polymer matrix is not favored. However, the experimental modulus values are much higher than the predicted values at lower concentrations (1 wt%), which can be explained on the basis of polymer filler interaction. The significant modulus reinforcement effect without the formation of percolated network is due to the introduction of stiff, nano-sized rod like cellulose nanocrystals with high aspect ratio into soft gelatin matrix. The hydrophilic groups of gelatin like N–H groups can form hydrogen bonds with the surface hydroxyl groups of cellulose nanocrystals. This type of physical interactions can also lead to the enhancement of mechanical properties even if the volume fraction of nanocrystals is less than the percolation threshold values. Similar type of behavior was found in polyurethane (PU) elastomer reinforced with low volume fraction of cellulose nanocrystals (Pei, Malho, Ruokolainen, Zhou, & Berglund, 2011). The stress versus strain plot of gelatin nanocomposites having 1 wt% of BCNC is showing a similar elongation at break values like neat gelatin, whereas a reduction in elongation at break is visible as the BCNC content reaches 2 wt% and above. The formation of rigid cellulose networks above the percolation threshold can stiffen the soft gelatin chains and thereby reduces strain to failure values. This lower stiffness values obtained for gelatin containing 1 wt% BCNC once again supports this claim (Table 1). As the concentration of

Table 1
Mechanical properties of gelatin and its BCNC nanocomposites.

BCNC content in gelatin (wt%)	Tensile strength[a] (MPa)	Tensile modulus[a] (MPa)	Elongation at break[a] (%)	Stiffness[a] (KN/m)
0	83.7 ± 3.2	2189.5 ± 50	33.7 ± 1.6	268.7 ± 11.5
1	88.7 ± 4.8	2225.3 ± 63	33.1 ± 1.8	269.8 ± 12.8
2	95.1 ± 3.9	2272.8 ± 68	29.8 ± 2.2	279.2 ± 12.4
3	103.1 ± 4.7	2335.1 ± 59	27.5 ± 1.9	280.6 ± 13.9
4	108.6 ± 5.1	2350.4 ± 65	23.4 ± 1.8	304.8 ± 13.8
5	89.8 ± 3.5	2321.9 ± 60	20.8 ± 1.6	291.2 ± 14.2

[a] Mean ± SE (n = 10).

cellulose nanocrystal is above 2 wt%, gelatin nanocomposites are showing better tensile strength, modulus and stiffness (Table 1), which is attributed to the combined effect of three dimensional geometrical percolations of cellulose nanocrystals together with the possibility of gelatin cellulose nanocrystal interactions. Moreover both the polymer and reinforcing nanocrystals are hydrophilic in nature and this enhances the miscibility between two phases also. All these factors together contribute to the enhanced mechanical properties of gelatin nanocomposites, which is desirable for the fabrication of high performance edible nanocomposite films.

3.4. Moisture sorption and water vapor permeation analysis

Gelatin consists of a wide range of hydrophilic amino acids, which can absorb large amount of water molecules from the environment. Hence, its moisture resistance has to be improved while using it as an edible food packaging material. Moisture sorption analysis was carried out to evaluate the effect of BCNC on the moisture sorption/desorption parameters of gelatin films at various relative humidity (RH). Moisture sorption curves of gelatin (Fig. 4) display a sigmoid shaped profile, which is very common for hydrophilic polymers. At relative humidity less than 70%, the sorption isotherm is showing a relatively slower increase in equilibrium moisture content, while an exponential increase in moisture content was observed at higher RH. At RH above 70%, a large weight gain is visible even with a small increase in humidity. A similar trend was observed for desorption curves also. The addition of nanocrystals found to reduce the equilibrium moisture content of gelatin at RH above 30%, showing a reduced water binding capacity. This could be due to the interaction between BCNC and the hydrophilic sites of gelatin, which substitutes the gelatin water interaction that predominates in neat hydrophilic polymer. This type of behavior was reported in composite edible films of hydroxy propyl methyl cellulose (HPMC) reinforced with microcrystalline cellulose (Bilbao-Sainz, Avena-Bustillos, Wood, Williams, & McHugh, 2010). The effect of bacterial cellulose nanocrystal content on the WVP of gelatin nanocomposites was also studied (Fig. 5). The addition of BCNC has reduced the WVP of gelatin, which can be attributed to the low hygroscopicity of highly crystalline cellulose nanocrystals. The water vapor transmission preferentially occurs through the amorphous areas of cellulose and the absence of such disordered areas in cellulose nanocrystals can reduce the water vapor permeability. The acid hydrolysis treatment employed during the synthesis of BCNC, leads to the removal of amorphous domains, while leaving the crystalline segments intact. Cellulose nanowhiskers similar to BCNC were reported to have a crystallinity index as high as 91–96% (Siqueira, Bras, & Dufresne, 2009; Siqueira, Abdillahi, Bras, & Dufresne, 2010). The addition of highly crystalline BCNC might have reduced the sorption and diffusion coefficients of gelatin. Moreover the formation of a percolated network of cellulose nanocrystals as visible in the SEM image (Fig. 3) might have formed a denser polymeric matrix, thus hindering the water vapor transmission through the nanocomposite films.

3.5. Thermal properties

DSC curves of gelatin and its nanocomposites (see the Supporting Information, S-4) reveal a soft inflection of the baseline around 65–70 °C, which is the glass transition temperature (T_g) of gelatin, followed by an endothermic peak related to the helix coil transition (T_m) of gelatin. In nanocomposites, with the increase in cellulose nanocrystal content, the T_g becomes broader and shifted towards a higher temperature (Table 2). The glass transition temperatures of polymer nanocomposites are affected by the extent of interaction between nanoparticles and polymer chains (Kropka, Putz, Pryamitsyn, Ganesan, & Green, 2007). Since gelatin and BCNC are hydrophilic in nature, a strong physical interaction between polymer phase and reinforcing filler is quite possible.

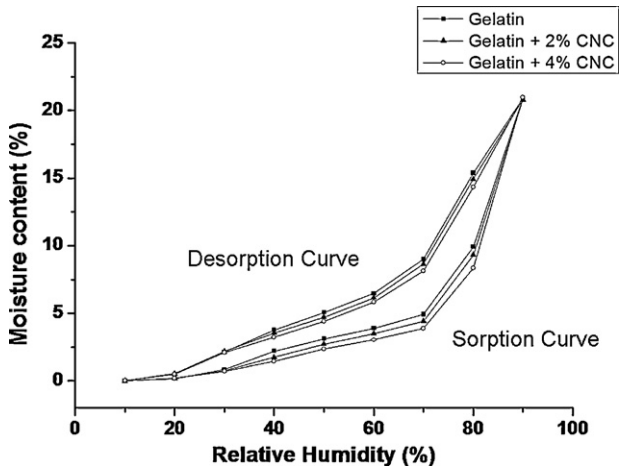

Figure 4. Moisture sorption and desorption analysis of gelatin and its nanocomposites.

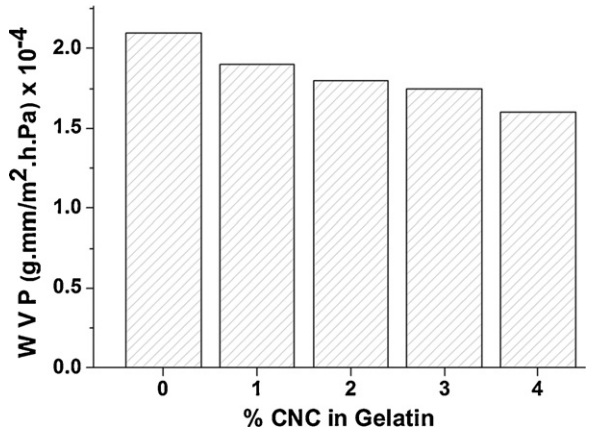

Fig. 5. Water vapor permeation analysis of gelatin and its BCNC nanocomposites.

Table 2
Thermal properties of gelatin and its BCNC nanocomposites.

BCNC content in gelatin (wt%)	Glass transition temperature[a] (T_g, °C)	Helix coil transition temperature[a] (T_m, °C)	Helix coil transition Enthalpy[a] (ΔH_m, J/g)	Peak maximum degradation temperature[a] (T_{Deg}, °C)
0	66.9 ± 2.2	101.5 ± 1.5	163.7 ± 5.6	332.0 ± 2.5
1	67.4 ± 2.3	101.9 ± 1.8	178.4 ± 4.8	333.3 ± 2.8
2	68.1 ± 2.9	103.7 ± 2.4	182.1 ± 5.2	340.8 ± 2.9
3	68.9 ± 2.7	104.1 ± 1.9	188.3 ± 5.9	347.5 ± 1.9
4	69.8 ± 2.1	105.3 ± 2.6	196.8 ± 5.8	348.7 ± 2.4
5	69.9 ± 2.4	109.2 ± 3.1	210.4 ± 4.5	349.9 ± 3.1

[a] Mean ± SE (n = 5).

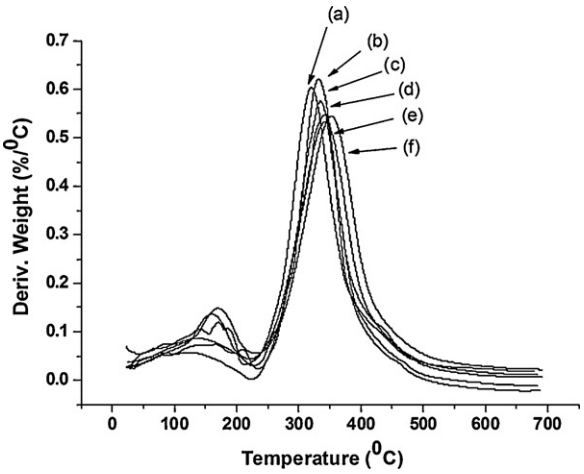

Fig. 6. DTG curves of (a) gelatin and gelatin nanocomposite films reinforced with (b) 1, (c) 2, (d) 3, (e) 4 and (f) 5 wt% of BCNC.

The formation of strong hydrogen bonds between cellulose nanocrystals and gelatin can restrict the segmental mobility of polymer chains and thereby increases T_g. This type of behavior was earlier reported in the case of Poly vinyl alcohol (PVA) reinforced by cellulose nanocrystals (George, Ramana, & Bawa, 2011). The addition of BCNC has also affected the endothermic, denaturation peak associated with the helix-coil transition of gelatin (T_m). An increasing trend shown by the helix coil transition temperature (T_m) as well as the denaturation enthalpy associated with this peak (Table 2) suggests that cellulose nanocrystals can also increase the thermal stability of gelatin, which is confirmed by using TGA.

The differential thermograms of TGA as shown in Fig. 6, indicates that the peak maximum degradation temperature of gelatin was improved with the addition of BCNC. By the addition of 5 wt% of BCNC, the peak maximum degradation temperature of gelatin has increased from 332 °C to 350 °C. Bacterial cellulose nanocrystals synthesized by an enzymatic process was reported to have a thermal stability above 350 °C, which can help in improving the thermal stability of gelatin also (George et al., 2011).

4. Conclusion

Gelatin based edible nanocomposite films were prepared by incorporating bacterial cellulose nanocrystals at various concentrations ranging from 1 to 5 wt%. Nanocrystals of cellulose were isolated from edible bacterial cellulose fibers by acid hydrolysis. AFM and TEM images were used to characterize the morphology and aspect ratio of nanocrystals. Incorporation of BCNC has appreciably reinforced the gelatin matrix and also improved the moisture sorption and WVP behavior. The possibility of the formation of percolated networks of cellulose nanocrystals within the gelatin matrix as well as the possible polymer–filler interactions resulted in easy stress transfer, which attributed to the enhanced mechanical properties. Highly crystalline cellulose nanocrystals reduced the moisture sorption and moisture barrier properties of gelatin by interacting with hydrophilic sites of gelatin and making it reduces its effectiveness in moisture uptake. The addition of BCNC has also affected the segmental mobility of gelatin chains, which in turn resulted in an increased glass transition temperature. High thermal stability of cellulose nanocrystals also contributed to improve the degradation temperature and the dynamic mechanical properties of gelatin. From all these results, cellulose nanocrystals obtained from bacterial cellulose was found to be a better reinforcing agent in improving the properties of edible films.

Acknowledgments

The authors thank Dr. A.S. Bawa, Dr. S.N. Sabapathy, Dr. K.V. Ramana, Dr. V.A. Sajeevkumar and R. Kumar, DFRL, Mysore for their constant support and encouragement.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.10.019.

References

Araki, J. & Kuga, S. (2001). Effect of trace electrolyte on liquid crystal type of cellulose microcrystals. *Langmuir*, *17*, 4493–4496.
Araki, J., Wada, M., Kuga, S. & Okano, T. (1998). Flow properties of microcrystalline cellulose suspension prepared by acid treatment of native cellulose. *Colloids and Surfaces A*, *142*, 75–82.
Azizi Samir, M. A. S., Alloin, F. & Dufresne, A. (2005). Review of recent research into cellulosic whiskers, their properties and their application in nanocomposite field. *Biomacromolecules*, *6*, 612–626.
Bilbao-Sainz, C., Avena-Bustillos, R. J., Wood, D. F., Williams, T. G. & McHugh, T. H. (2010). Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. *Journal of Agriculture and Food Chemistry*, *58*, 3753–3760.
Capadona, J. R., Shanmuganathan, K., Tyler, D. J., Rowan, S. J. & Weder, C. (2008). Stimuli-responsive polymer nanocomposites inspired by the sea cucumber dermis. *Science*, *319*, 1370–1374.
Capadona, J. R., Van Den Berg, O., Capadona, L. A., Schroeter, M., Rowan, S. J., Tyler, D. J., et al. (2007). A versatile approach for the processing of polymer nanocomposites with self assembled nanofiber templates. *Nature Nanotechnology*, *2*, 765–769.
Choi, Y. J., Ahn, Y., Kang, M. S., Jun, H. K., Kim, I. S. & Moon, S. H. (2004). Preparation and characterisation of acrylic acid treated bacterial cellulose cation exchange membrane. *Journal of Chemical Technology and Biotechnology*, *79*, 79–84.
Dogan, N. & McHugh, T. H. (2007). Effects of microcrystalline cellulose on functional properties of hydroxypropyl methyl cellulose microcomposite films. *Journal of Food Science*, *72*, E16–E22.
Dufresne, A. (2006). Comparing the mechanical properties of high performances polymer nanocomposites from biological sources. *Journal of Nanoscience and Nanotechnology*, *6*, 322–330.
Eichhorn, S. J., Dufresne, A., Aranguren, M., Marcovich, N. E., Capadona, J. R., Rowan, S. J., et al. (2010). Review: Current international research into cellulose nanofibres and nanocomposites. *Journal of Material Science*, *45*, 1–33.
Gennadios, A. (2004). Edible films and coatings from proteins. In R. Y. Yada (Ed.), *Proteins in food processing* (pp. 442–467). Woodhead Publishing Inc.: Cambridge.
George, J., Ramana, K. V., Sabapathy, S. N., Jagannath, J. H. & Bawa, A. S. (2005). Characterization of chemically treated bacterial (Acetobacter xylinum) biopolymer: Some thermo-mechanical properties. *International Journal of Biological Macromolecules*, *37*, 189–194.

George, J., Ramana, K. V., Bawa, A. S. & Siddaramaiah. (2011). Bacterial cellulose nanocrystals exhibiting high thermal stability and their polymer nanocomposites. *International Journal of Biological Macromolecules*, *48*, 50–57.

Habibi, Y., Lucia, L. A. & Rojas, O. J. (2010). Cellulose nanocrystals: chemistry, self-assembly, and applications. *Chemical Reviews*, *110*, 3479–3500.

Hirai, A., Inui, O., Horii, F. & Tsuji, M. (2009). Phase separation behavior in aqueous suspensions of bacterial cellulose nanocrystals prepared by sulfuric acid treatment. *Langmuir*, *25*, 497–502.

Iguchi, M., Yamanaka, S. & Budhino, A. (2000). Review bacterial cellulose—a master piece of nature's art. *Journal of Material Science*, *35*, 261–270.

Kropka, J. M., Putz, K. W., Pryamitsyn, V., Ganesan, V. & Green, P. F. (2007). Origin of dynamical properties in PMMA-C_{60} nanocomposites. *Macromolecules*, *40*, 5424–5432.

Li, Q. & Renneckar, S. (2011). Supramolecular structure characterization of molecularly thin cellulose 1 nanoparticles. *Biomacromolecules*, *12*, 650–659.

Lin, K.-W. & Lin, H.-Y. J. (2004). Quality characteristics of Chinese style meatball containing bacterial cellulose (Nata). *Journal of Food Science*, *69*, SNQ107–SNQ111.

Lin, S. B., Chen, L. C. & Chen, H. H. (2011). Physical characteristics of surimi and bacterial cellulose composite gel. *Journal of Food Process Engineering*, *34*, 1363–1379.

Marcuzzo, E., Sensidoni, A., Debeaufort, F. & Voilley, A. (2010). Encapsulation of aroma compounds in biopolymeric emulsion based edible films to control flavour release. *Carbohydrate Polymers*, *80*, 984–988.

Mikkelsen, D., Gidley, M. J. & Williams, B. A. (2011). In vitro fermentation of bacterial cellulose composites as model dietary fibers. *Journal of Agriculture and Food Chemistry*, *59*, 4025–4032.

Muller, C. M. O., Laurindo, J. B. & Yamashita, F. (2009). Effect of cellulose fibers addition on the mechanical properties and water vapour barrier of starch based films. *Food Hydrocolloids*, *23*, 1328–1333.

Muyonga, J. H., Cole, C. G. B. & Duodu, K. G. (2004). Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult nile perch (Lates niloticus). *Food Chemistry*, *86*, 325–332.

Nishi, Y., Uryu, M., Yamanaka, S., Watanabe, K., Kitamura, N., Iguchi, M., et al. (1990). The structure and mechanical properties of sheets prepared from bacterial cellulose. 2. Improvement of the mechanical-properties of sheets and their applicability to diaphragms of electro acoustic transducers. *Journal of Material Science*, *25*, 2997–3001.

Nogi, M. & Yano, H. (2008). Transparent nanocomposites based on cellulose produced by bacteria offer potential innovation in the electronics device industry. *Advanced Materials*, *20*, 1849–1852.

Okiyama, A., Motoki, M. & Yamanaka, S. (1992). Bacterial cellulose II. Processing of the gelatinous cellulose for food materials. *Food Hydrocolloids*, *6*, 479–487.

Pei, A., Malho, J.-M., Ruokolainen, J., Zhou, Q. & Berglund, L. A. (2011). Strong nanocomposite reinforcement effects in polyurethane elastomer with low volume fraction of cellulose nanocrystals. *Macromolecules*, *44*, 4422–4427.

Petersson, L. & Oksman, K. (2006). Biopolymer based nanocomposites: Comparing layered silicates and microcrystaline cellulose as nanoreinforcement. *Composite Science and Technology*, *66*, 2187–2196.

Rusli, R., Shanmuganathan, K., Rowan, S. J., Weder, C. & Eichhorn, S. J. (2011). Stress transfer in cellulose nanowhisker composites—influence of whisker aspect ratio and surface charge. *Biomacromolecules*, *12*, 1363–1369.

Siqueira, G., Abdillahi, H., Bras, J. & Dufresne, A. (2010). High reinforcing capability cellulose nanocrystals extracted from Syngonanthus nitens (Capim Dourado). *Cellulose*, *17*, 289–298.

Siqueira, G., Bras, J. & Dufresne, A. (2009). Cellulose whiskers versus microfibrils: influence of the nature of the nanoparticle and its surface functionalization on the thermal and mechanical properties of nanocomposites. *Biomacromolecules*, *10*, 425–432.

Sobral, P. J. A., Menegalli, F. C., Hubinger, M. D. & Roques, M. A. (2001). Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids*, *15*, 423–432.

Stephens, S. R., Westland, J. A., & Neogi, A. N. (1990). Method of using bacterial cellulose as a dietary fiber component. US patent, 4960763.

Svensson, A., Nicklasson, E., Harrah, T., Panilaitis, B., Kaplan, D. L., Brittberg, M., et al. (2005). Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. *Biomaterials*, *26*, 419–431.

Tharanathan, R. N. (2003). Biodegradable films and composite coating: Past, present and future. *Trends in Food Science and Technology*, *54*, 343–351.

Uddin, A. J., Araki, J. & Gotoh, Y. (2011). Toward strong green nanocomposites: polyvinyl alcohol reinforced with extremely oriented cellulose whiskers. *Biomacromolecules*, *12*, 617–624.

Vandamme, E. J., De Baets, S., Vanbaelen, A., Joris, K. & De Wulf, P. (1998). Improved production of bacterial cellulose and its application potential. *Polymer Degradation and Stability*, *59*, 93–99.